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Etching time effects on the bond strength of three adhesive systems

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Etching Time Effects on the Bond Strength of Three Adhesive Systems

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DEDICATION

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1. INTRODUCTION

Modern dentistry has come a long way since GV Black's time. With the dramatic revolution in materials science in the last twenty years, dentistry has gone from Dr. Black's concept of "extension for prevention" to the modern concept of "prevention of extension" (Van Meerbeek et al., 1998). Rueggeberg et al summarized it best when he described the ideal material as follows: "no longer is a material required merely to 'fill a space', but it must also adhere, seal, provide a durable, long-lasting surface in a very harsh environment, and yet remain biocompatible" (1991). Indeed, the oral environment presents a myriad of challenges to restorative dentistry. The filling material is subjected to chemical degradation, hydrolytic break down, masticatory forces, and thermal stresses (Tam et al., 1994) (Vargas et al., 1997). According to Sturdevant et al., composite and composite bonding systems come very close to fulfilling these criteria, plus they have the additional advantages of esthetics and cost effectiveness (1995). Although composite is used to replace lost tooth structure, the key to the longevity of the restoration is the ability of the dentin bonding system to provide an intimate contact and micromechanical retention between the composite and the underlying tooth structure, thus preventing microleakage and recurrent caries (Salama and Tao, 1991).

While numerous bonding studies have been done on permanent teeth, very little research has focused on bonding to primary teeth (Nor et al., 1996) (Borba de Araujo et al., 1997) (Fritz et al., 1997). Primary teeth are compositionally and morphologically different than permanent teeth, and thus, bonding properties may also differ (Mazzeo et al., 1995). However, the current dentin bonding manufacturers do not have a separate protocol for bonding to primary teeth (Agostini et al., 2001). The small number of

studies that have been done on primary teeth showed mixed shear bond strength (SBS) results. For example, Fagan et al. reported no difference in SBS between primary and permanent dentin (1986). Hosoya et al., in 1996, found that SBS of primary dentin was higher than permanent dentin (1996), and other studies have shown inferior bond strength to primary teeth as compared to permanent teeth (Issao, 1997) (Agostini et al., 2001). Furthermore, many clinicians suggest that primary teeth require longer etching time or have lower SBS than permanent dentin, and thus, modify the etching protocol as they deem appropriate. Thus, the purpose of this study is to evaluate and compare bond strength of primary teeth to permanent teeth and determine whether a separate protocol is needed for etching/bonding to primary teeth using three dentin bonding systems (DBS): **Single Bond** (3M Dental Products, St. Paul, MN), **Clearfil SE** (Kuraray America, INC. New York, NY), and **One Up** (Tokuyama Corp 3-1, Shibuya 3-chome, Shibuya-Ku, Tokyo, Japan).

2. BACKGROUND

2.1 Enamel

Enamel is the most highly calcified tissue in the human body. During its formation and maturation, the organic matrix is resorbed, leaving behind a highly calcified structure with very little organic content (Sturdevant et al., 1995). Enamel is composed of long crystals that are about 40 nm in diameter, radiating from the DEJ and extending the full thickness of enamel. Each individual crystal is enveloped by a thin layer of lipid and/or protein, which potentially function in mineralization. Thousands of these crystals are packed together to form an enamel prism. In between these hexagonal prisms is the organic matrix that serves as a passageway for water and ion movement. (Marshall et al.,

1999) Enamel is a highly calcified tissue, composed of 85 volume percent of mineral, 12 volume percent of water, and only 3 volume percent of proteins and lipids (Sturdevant et al., 1995). Enamel is relatively uniform throughout, except at the cervical region where enamel is thin and aprismatic (Heymann and Bayne, 1993).

Enamel mineral is a calcium deficient, carbonate rich apatite. The pure form of hydroxyapatite is $\{Ca_{10}(PO_4)_6(OH)_2\}$. The calcium in enamel apatite is often substituted by various metal ions while the carbonate substitutes for either the phosphate or hydroxyl group, making this enamel more susceptible to dissolution than pure hydroxyapatite. However, enamel can become much stronger and less soluble by substitution of fluoride ions for the hydroxyl groups, forming fluoroapatite (Marshall et al., 1999).

Unlike permanent enamel, primary enamel is thinner and whiter because it is formed prenatally, and hence, is not subject to environmental factors (Avery, 1987). In addition, primary enamel is more likely to be prismless. This is due to the lack of enamel rod formation in the last 25 μm during amelogenesis as enamel is laid down from the dentin-enamel junction (DEJ) towards the outer enamel surface. For this reason, prismless enamel is usually found on the outer surface of primary enamel, especially near the cervical area (Avery, 1987). It has been found that prismless enamel requires significantly longer etching time to adequately etch its surface (Agostini et al., 2001).

2.2 <u>Dentin</u>

Dentin is the middle layer of the tooth, sandwiched between the highly mineralized outer enamel and the highly vascularized inner pulp (Sturdevant et al., 1995). Unlike enamel, dentin is a hydrated complex composite structure, whose composition and properties vary depending on its location in the tooth (Marshall et al., 1999). For

example, dentin near the DEJ is harder than dentin near the pulp due to differences in mineralization (Marshall et al., 1998). Furthermore, there are several types of dentin, reflecting the degree of mineralization, that form as a result of maturation process or physiologic/aging process (Marshall et al., 1999). Primary dentin is formed during tooth development, and is completed when the root is fully formed. Secondary dentin is formed along the dentin-pulp junction after the cessation of primary dentin and continues to form throughout life. Tertiary dentin, or reactionary dentin, is formed in response to dentin injury (About et al., 2001). Other forms of dentin include demineralized and remineralized dentin, transparent dentin, sclerotic dentin, and carious dentin (Marshall et al., 1999).

The difference in properties between dentin and enamel can be understood when dentin composition and morphology are analyzed. Dentin is 50 volume percent mineral (carbonate rich, calcium deficient apatite), 30 volume percent organic matter (mostly Type I collagen), and the remaining 20 volume percent fluid (Johnson et al., 1991) (Marshall, 1993). The atomic percentages of the defective dentin apatite, from x-ray photo-electron spectrometry (XPS) analysis are as follows:

- 51.5% C
- 8.0% N
- 25.9% O
- 6.7% Ca
- 6.5% P

This is quite close to theoretical atomic percentage composition of dentin (Ruse and Smith, 1991):

- 42% C
- 9% N
- 33% O
- 10% C
- 6% P

Dentin contains tubules that radiate perpendicularly from the pulp chamber to the DEJ. These tubules are remnants of the tracks left by odontoblasts when they laid down the dentin during tooth formation. These tubules are lined by a highly mineralized cuff of peritubular dentin (Marshall, 1993). The space in between these tubules is occupied by an apatite reinforced collagen matrix called intertubular dentin, which is less mineralized (Marshall et al., 1998). Since dentin is much less mineralized and contain twice as much carbonate as enamel, it is much more prone to dissolution by acid (Marshall et al., 1999).

Tubule density is intratooth location dependent. For example, there are more tubules per unit area over pulp horns than over central dentin, and relatively fewer tubules at the cervical area (Tao and Pashley, 1988). Dentinal tubules contain many lateral branches and microchannels that connect neighboring tubules which have important implications in dentin bonding (Marshall, 1993). These micro-channels allow resin to form micro-tags along the inner tubule wall, so the resin tags would not be easily dislodged from the etched, funnel-shaped dentinal tubules (Pioch et al., 1998). These tubules contain fluid and odontoblast processes or extensions that serve as pathway to the pulp (Marshall, 1993). There is a constant outward flow of dentinal fluid via dentinal tubules due to the +10 mm Hg of pulpal pressure, keeping dentin hydrated (Swift et al., 1995) (Vargas et al., 1997). Hence, these tubules are the bases for the “Hydrodynamic Theory”. This

theory describes "tooth pain" as fluid movement within the tubules, depolarizing nerve cells within the pulp to elicit pain sensation (Tay et al., 1995) (Pashley et al., 1978).

The diameter of dentinal tubules increases as they extend from the DEJ to the pulp (Kanca and Sandrik, 1998); the tubules are less dense and narrower near the DEJ, and more dense and wider near the pulp (Koutsi et al., 1994). In fact, several studies looking at tubule density indicated that there are about 20,000 tubules/mm^2 with diameter of 0.8 µm near the DEJ versus about 45,000 tubules/mm^2 with an average diameter of 2.5 µm near the pulp (Marshall, 1993) (Garberoglio and Brannstrom, 1976). Hence, the total area of the tubules varies from 1% near the DEJ to 22% near the pulp, which accounts for the increased permeability as one approaches the pulp (Koutsi et al., 1994). In addition, the surface area occupied by peritubular dentin is 3% near the DEJ versus 60% near the pulp. And finally, the amount of intertubular dentin that is responsible for most of the bond strength is 96% near the DEJ versus only 12% near the pulp (Marshall, 1993). For this reason, dentin bonding is most optimal near the DEJ. Thus, unlike enamel, dentin is a challenging biological substrate due to its heterogeneity and regional differences in composition (Dias da Silva Telles et al., 1998).

Unlike primary and permanent enamel whose compositions are quite similar, primary dentin is different from its permanent counterpart. Using an energy dispersive X-ray spectrometer, Hirayama analyzed the elemental composition of primary and permanent dentin as follows (1990):

DENTIN	Ca	P
Primary Intertubular Dentin	24.9%	12.1%
Primary Peritubular Dentin	30.7%	15.3%
Permanent Intertubular Dentin	25.5%	12.5%
Permanent Peritubular Dentin	34.5%	16.9%

Thus, primary dentin contains less Ca^{2+} and PO_4^{3-}, and therefore, is less mineralized than permanent dentin (Nor et al., 1996). Consequently, Nor and his colleagues advocated shorter etching time for primary dentin (1997). Also, it is of interest to note the higher concentration of Ca^{2+} and PO_4^{3-} in peritubular dentin as compared to intertubular dentin, indicating that peritubular dentin is more mineralized than intertubular dentin in both primary and permanent teeth (Hirayama, 1990). Secondly, there are fewer tubules and the tubule diameters are smaller in primary dentin as compared to permanent dentin (Sumikawa et al., 1999). This may account for the lower permeability seen with primary dentin (Koutsi et al., 1994). Primary dentin contains approximately 26,000 tubules/mm^2 with diameter of about 1.6 μm near the pulp, which is a little over half the number of tubules associated with permanent dentin. Tubule diameter differences are due to the relatively thicker peritubular dentin surrounding primary dentinal tubules (Koutsi et al., 1994). In fact, according to Mazzeo et al., peritubular dentin is 2 to 5 times thicker in primary dentin as compared to permanent dentin (1995). Thirdly, primary dentin contains giant tubules that range in size from 5-70 μm versus the 1 μm diameter of a typical dentinal tubule (Sumikawa et al., 1999). Liu et al. have shown that these tubules are found in approximately 20% of central and lateral incisors and 3% of cuspids (2000).

The restorative implication of these giant tubules is unknown at present (Swift and Bayne, 1997).

2.3 History of Enamel Bonding

Enamel bonding started with Buonocore in 1955 when he first discovered that the paint industry etched the metal surfaces with acid to increase adhesion of paint to metal (Kanca and Sandrik, 1998). Applying this same concept to dentistry, he experimented etching enamel surfaces with 85% phosphoric acid for 30 sec and obtained significantly higher bond strength (Buonocore, 1955). Acid etching, in essence, causes dissolution of enamel prisms, creating micro-porosities within the etched enamel surface. This produces a significant increase in surface area to interlock with the resin tags, forming a micro-mechanical bond (Hayakawa et al., 1998). Thus, the key to enamel bonding is the combination of the low viscosity hydrophobic adhesive and the high surface energy of etched enamel, enabling improved resin flow into the micro-porosities via capillary action (Frey, 2000). Interestingly, increase in surface area from etching has a greater effect on bond strength than the depth of resin penetration (Barkmeier et al., 1986). Gwinnett and Matsui were the first to describe the resin penetration into etched enamel or the concept of hybridization in enamel in 1967 (Tay et al., 1995).

In 1974, Silverstone et al. advocated a 60 sec etch for enamel. Brannstrom and Nordenvall in 1977 compared 15 sec versus 2 min etch on primary and permanent enamel. They did not find any significant difference in topographic appearance using scanning electron microscopy (SEM) analysis between the two time periods. Barkmeier et al. compared 15 sec versus 60 sec enamel etching using 37% phosphoric acid and found no difference in surface morphology nor SBS (1986). Thus, the enamel etching

time has shorten substantially since Buonocore's time. The current recommendation for enamel etching is 30-50% phosphoric acid for 15 sec to minimize excessive mineral loss and still maintain strong reliable bond (Gilpatrick et al., 1991) (Lopes et al., 2002). This is especially true for primary enamel, which contains relatively less mineral than permanent enamel.

2.4 History of Dentin Bonding

Unlike bonding to etched enamel, dentin bonding is much more complicated and unpredictable due to the complexity of dentin as a bonding substrate. Dentin bonding is difficult for the following reasons (Borba de Araujo and Garcia-Godoy, 1997) (Heymann et al., 1993):

- dentin is wet but the older bonding materials are hydrophobic
- degree of mineralization and surface morphology are different, depending on intratooth location
- pulpal biocompatibility
- sensitivity to moisture
    - too much water would dilute the bonding agent and compromise bond strength
    - too little water would cause collapse of collagen and prevent the bonding agent from penetrating into demineralized dentin
- presence of smear layer (0.5-5.0 μm thick) interferes with bonding to underlying dentin

There are four proposed bonding mechanisms to dentin (Kanca and Sandrik, 1998):

1. chemical bonding to organic and/or inorganic components of dentin
2. formation of precipitates on pre-treated dentin substrate to which adhesive may chemically bond
3. micro-mechanical bond created by formation of resin tags in dentinal tubules and lateral canals of etched dentin
4. micro-mechanical bond created by diffusion and polymerization of monomers into the subsurface of etched intertubular dentin, creating a hybrid layer.

Through earlier unsuccessful attempts to chemically bond to calcium and other elements in dentin, we have come to realize that bonding to dentin is similar to enamel. Etching of dentin removes the smear layer and increases the surface area and porosities by selective dissolution of dentin mineral, allowing monomers to infiltrate and obtain a micro-mechanical bond via hybridization and formation of resin tags (Johnson et al., 1991).

Dentin bonding started in 1952 when Kramer and McLean first described an altered layer in dentin using Sevitron Plus dentin bonding system, which probably contained methacrylic acid (Kanca and Sandrik, 1998). In 1965, Bowen unsuccessfully attempted to chemically bond to dentin using NPG-GMA to couple to the calcium in dentin (Kanca and Sandrik, 1998). Another attempt was made by Munksgaard and Asmussen in 1984 to use glutaraldehyde to bond to the nitrogen group in dentin collagen. Again, the bond strengths obtained were only 3-4 MPa (Nakabayashi et al., 1998). Since then, other attempts have been made to incorporate chemicals such as glutaraldehyde, phosphonated acrylic esters, ... etc. into the primer of various dentin bonding systems to promote chemical bonding to dentin with limited success (Leinfelder, 1993) (Johnson et al., 1991). Thus, chemical bonding to dentin is questionable, and at most, provides a modest

contribution to the overall bond strength. In fact, studies using spectroscopy to analyze the nature of the bond for several dentin bonding systems claiming to form chemical bonds to dentin revealed no evidence of such bonds (Eliades et al., 1990) (Johnson et al., 1991).

After unsuccessful attempts at creating a chemical bond to dentin, newer generations of dentin bonding systems were formulated to form chemical or mechanical bonds to the smear layer, modifying the smear layer and partially infiltrating the smear layer, or completely removing the smear layer and bonding to the underlying demineralized dentin (Johnson et al., 1991). Early bonding systems {eg Scotchbond (3M Dental Products, St. Paul, MN)} only penetrated the wet smear layer to a depth of 0.2-0.3 μm due to their hydrophobicity, resulting in poor bond strength of 5-7 MPa (Watanabe et al., 1994). With the development of a mild acidic monomer and an ampiphilic resin, hydroxyethyl methacrylate (HEMA), the smear layer was partially dissolved. An example of this was Prisma Universal Bond 3 (Caulk/Dentsply, Milford, DE). This led to a deeper penetration of resin monomer and an increase in bond strength of 8-12 MPa (Watanabe et al., 1994). Since the early acidic monomers were not strong enough to remove the smear plugs, they limited the depth of resin penetration (Watanabe et al., 1994). This improvement in bond strength was still not sufficient to overcome the polymerization shrinkage associated with curing, leading to early bond failure (Hoelscher et al., 2000).

A turning point in dentin bonding occurred in 1982 when Nakabayashi applied primer on acid conditioned dentin and obtained relatively high bond strength. He observed a layer of resin infiltrated into etched dentin, which he termed the "hybrid layer" (Kato and Nakabayshi, 1996) (Inokoshi et al., 1993). This concept of micromechanical bonding to

dentin was a radical approach, since the existing scientific literature cautioned against etching dentin for fear of opening up dentinal tubules which could serve as pathways for bacterial invasion and pulpal sensitivity (Dickinson et al., 1991). However, more recent research indicated that a short etching time plus rinsing with water afterward will not harm the pulp (Brannstrom, 1984) (Fusayama et al., 1979). In fact, a 15 sec etch results in acid penetration into the dentinal tubules of less than 5 μm (Lopes et al., 2002). This, plus complete infiltration of resin monomers into dentin tubules significantly decreases microleakage and bacterial invasion (Nor et al., 1997).

2.5 Current Concepts in Dentin Bonding

Early dentin bonding failed for a number of reasons. The main reason for failure was bonding a hydrophobic adhesive, mainly halophosphoesters of methacrylate, to a hydrophilic substrate, dentin (Kanca, 1992). Thus, with the addition of an ampiphilic resin monomer such as HEMA incorporated into a water miscible carrier such as ethanol or acetone into the primer, bond strength significantly increased due to enhanced infiltration of this ampiphilic molecule into the wet demineralized dentin (Perdigao et al., 2000). Furthermore, early studies tended to over etch dentin with concentrated acid for as long as 2 min (Kanca, 1992). Over etching dentin can lead to several problems, namely increased incidence of pulpal sensitivity and excessive dissolution of mineral. This leaves a thicker collagen layer that is more vulnerable to collapse since the mineral apatite that supported the collagen network is lost. And finally, the resin monomer may not completely infiltrate the full extent of the demineralized dentin, leaving a weak zone at the bottom of the hybrid layer (Lopes et al., 2002). Thus, Blosser et al. recommended shorter etching times for dentin (1990).

The currently advocated dentin bonding technique is called the "total etch wet bonding technique." Dentin is usually etched for 15 sec with 30-50% phosphoric acid or another comparable acid, blotted to remove excess water, leaving a moist etched dentin surface. This moist surface is critical to the success and longevity of the bond. If dentin is excessively desiccated, the collagen would collapse, closing off all the nanochannels that were once occupied by apatite, preventing the infiltration of resin monomers into the demineralized dentin. Hence, not only is the resin not well infiltrated, the collagen is not encapsulated and protected by the resin and will be subjected to hydrolytic breakdown with time, leading to bond failure (Marshall et al., 1998). Thus, the ideal bond to dentin is complete penetration of monomers to the full depth of demineralized dentin, forming a hybrid layer with collagen completely encapsulated by resin (Nakabayashi et al., 1992). 2/3 of the total dentin bond strength comes from resin infiltration of the collagen network within the intertubular dentin. The other 1/3 comes from resin infiltrating the dentinal tubules and lateral canals within the tubules (Mazzeo et al., 1995). Dentin bond strength should be at least 20 MPa to resist polymerization shrinkage. With current dentin bonding systems, bond strength to dentin has surpassed this 20 MPa requirement. In fact, it is comparable to enamel bond strength (Holtan et al., 1993).

The newest innovation in dentin bonding is the concept of self etching primer (SEP) or self etching adhesive (SEA). The idea behind these dentin bonding systems is incorporating an acidic monomer into the primer so that etching and priming (or etching, priming, and bonding for SEA) can occur simultaneously. Hence, additional steps of acid etching and rinsing are eliminated (Telles et al., 1998). Furthermore, since the acidic monomer etches and infiltrates at the same time, it is possible that no void will be created

due to mismatch between etching depth and extent of infiltration (Toledano et al., 2001). Finally, the difficulty in preparing that critical moist dentin surface after etching is no longer an issue. However, there are concerns about the acidic residue that is not rinsed away and its impact on pulpal response and long term durability of the bond. Although these dentin bonding systems seem promising, long term clinical data are needed to evaluate their success (Lopes et al., 2002).

2.6 Smear Layer

The smear layer was described by Boyde, Switsur and Stewart in 1963 (Cotton, 1984). It is cutting debris left on dentin by cavity preparation (Eick et al., 1970). X-ray photo-electron spectroscopy and secondary ion mass spectroscopy have revealed that the elemental composition of the smear layer is the same as the underlying dentin (Ruse and Smith, 1991), primarily denatured collagen and apatite that is loosely packed on top of the prepared surface. In fact, the smear layer is very weakly attached to underlying dentin, and thus, is responsible for low bond strengths observed with early bonding systems that bond to the smear layer (Gwinnett, 1984). Smear layer is also present in enamel preparations, but it is mostly apatite and very little organic residue, reflecting enamel composition (Bowen et al., 1984). The smear layer of primary teeth has slightly less mineral than in permanent teeth, reflecting the lower mineralization of primary teeth. Thus, the smear layer of primary dentin is more easily removed by etching than that of permanent dentin (Nor et al., 1997).

The thickness of the smear layer varies from 0.4 μm-5 μm, depending on the instrument used, and it is not uniform (Tao and Pashley, 1988) (Pashley, 1984). The dentinal tubule orifices are filled with smear layer debris called smear plugs. The smear

layer is composed of two phases. The solid phase, primarily cutting debris, reportedly is surrounded by a liquid phase made up of tortuous, fluid-filled channels. This makes the smear layer porous and less dense than intact dentin (Pashley et al., 1992).

The advantages of having an intact smear layer are (Nor et al., 1997) (Koutsi et al., 1994) (Brannstrom, 1984):

- protective effect – barrier to prevent restorative materials from getting to the pulp
- decreases post operative sensitivity by decreasing dentin permeability
- decreases the wetness of dentin, thus, facilitating bonding of early hydrophobic bonding systems to dentin
- smear plugs decrease bacterial invasion of the pulp
- buffers the effect of acid and decreases osmotic, thermal and tactile stimuli

The disadvantages of having an intact smear layer are (Inagaki et al., 1989) (Brannstrom, 1984):

- loosely attached to underlying dentin, with cohesive strength of ~ 5 MPa
- restricts resin infiltration into underlying dentin, therefore, limiting bond strength to the cohesive strength of the smear layer
- harbors bacteria and serves as nutrient for bacteria

Early dentin bonding systems were formulated to bond to the smear layer, but resulted in poor bond strength due to the intrinsically weak cohesive strength of the smear layer (Pashley et al., 1992). Improved bond strength was observed in bonding systems that partially removed the smear layer with weak acids, but it was not sufficient to withstand polymerization shrinkage that occurs clinically (Watanabe et al., 1994). With contemporary bonding systems, the smear layer is completely dissolved and removed. This is followed by application of a primer/adhesive to allow better infiltration of the resin monomer into the demineralized dentin, resulting in bond strengths comparable to those to enamel (Hayakawa et al., 1998) (Kanca, 1992). However, with current self-etching systems, for example Clearfil SE Bond, the smear layer is not removed. Instead, it is partially dissolved and incorporated into the hybrid layer. Surprisingly high bond strengths are obtained with Clearfil SE (Toledano et al., 2001). Other self-etching systems, however, have not performed as well (Perdigao et al., 2000). Thus, the absolute need for complete removal of the smear layer for effective bonding still needs further investigation.

2.7 Acid Etching

2.7.1 Enamel

Acid etching of enamel results in selective dissolution of enamel apatite, usually to a depth of 10 μm (Swift et al., 1995). This amount of enamel loss is negligible since the thickness of permanent enamel is approximately 1,500 μm (Sturdevant et al., 1995). Etching of enamel usually results in one of three etching patterns. Type I is the most common etching pattern, and it involves the preferential removal of the prism cores. Type II involves preferential dissolution of the prism peripheries. Type III is a

combination of the first two (Swift et al., 1995). However, Marshall et al. have shown that enamel etching resulted in dissolution of prism cores and peripheries of equal frequency (1975). This etching pattern was also observed in primary enamel (Bozalis and Marshall, 1977).

Acid etching of enamel allows for increased retention of resin. This is due to dissolution of enamel apatite, significantly increasing the surface area and creating a roughened, wettable surface for resin monomer to flow into and to establish micromechanical retention (Malferrari et al., 1994). Since enamel is a high energy substrate that can be wet by hydrophobic monomers, the adhesive can easily flow into and adapt to the etched surface (Lopes et al., 2002). This is clearly seen in studies where after bonding, the enamel is dissolved with acid, leaving behind the resin replication of the etched enamel surface. This intimate adaptation is responsible for the reliable high bond strength associated with enamel bonding (Malferrari et al., 1994).

Etching of primary enamel is a controversial issue. Primary enamel is more likely to have a prismless surface zone than permanent enamel, thus reducing the effectiveness of etching and resulting in a shallower etching pattern (Agostini et al., 2001). This increased resistance to acid etching is due to the close parallel orientation of the primary enamel crystals and the absence of prism boundaries (Malferrari et al., 1994). As a result, some have advocated etching primary enamel as long as 4 minutes (Agostini et al. 2001). Others believe that since there is less mineral in primary teeth as compared to permanent teeth, it follows that the etching time for primary enamel should be shorter (Hirayama, 1990).

2.7.2 ***Dentin***

Acid etching dentin results in removal of the smear layer, demineralizes intertubular and peritubular dentin, and creates funnel-shaped dentinal tubules (Eliades et al., 1997). Etching is most pronounced at the dentin surface and gradually diminishes as it etches deeper into dentin. The mechanism of acid etching dentin is as follows:

$$H^{+} \text{ (acid)} + \text{carbonated apatite} \rightarrow CO_2 + Ca^{2+} + PO_4^{3-}$$

Thus, the diffusion of acid into the dentin subsurface causes dissolution of carbonated apatite, giving off free Ca^{2+} and PO_4^{3-} (Pashley et al., 1992). The following chart compares the elemental composition in atomic percentages of "non-etched dentin" versus "etched dentin", indicating a dramatic loss in Ca^{2+} and PO_4^{3-} after etching.

Bovine Dentin Composition (Atomic %)

SURFACE	C	N	O	Ca	P	Si
NON-ETCHED DENTIN	54.4%	4.7%	24.7%	**7.2%**	**6.9%**	0
ETCHED DENTIN	34.9%	4.7%	39.8%	**0.2%**	**0**	20.5%

A significant increase in Si after etching is due to the phosphoric acid preparation containing silica to make it into a gel for ease of handling (Ruse and Smith, 1991). Etching preferentially dissolves the peritubular dentin, forming funnel-shaped dentinal tubules (Marshall, 1993). Etching also dissolves the mineral of the intertubular dentin, leaving behind an unsupported collagen network that is supported by dentinal fluid. The space that was once occupied by the apatite crystals then serves as channels for resin infiltration, replacing the apatite in providing support for the collagen and forming mechanical interlocks (Swift et al., 1995). The depth of demineralization ranges from 1-

15 μm (Uno and Finger, 1996) (Van Meerbeek et al., 1992) depending on (Watanabe et al., 1994):

- concentration of acid (pH)
- diffusion coefficient of the acid
- application time
- thickness and packing density of the smear layer

For example, 2.5% nitric acid applied for 40 sec resulted in 5 μm of demineralization (Pashley et al., 1992) versus 20% phosphoric acid etched for 30 sec resulting in 10 μm of demineralization (Uno and Finger, 1996). Over etching dentin, however, causes excessive dissolution of apatite, leaving the collagen network very vulnerable to collapse, preventing complete penetration of resin monomer and potentially affecting the pulp (Nor et al., 1997) (Johnson et al., 1991).

Unlike enamel, there is a greater fear of irritating the pulp with etching dentin, especially for prolonged periods of time with a strong acid (Kanca, 1992). Thus, in recent years, different acid etchants have been used including pyruvic acid + glycin, citric acid + calcium chloride, nitric acid + aluminum oxalate, EDTA, maleic acid, oxalic acid, citric acid + ferric chloride, and phosphoric acid in various concentrations and application times to minimize the effect on the pulp while maintaining optimal bond to dentin (Chigira et al., 1994) (Inagaki et al., 1989). Furthermore, divalent cations such as ferric chloride, calcium chloride…etc. were used to help stabalize the collagen network during demineralization to minimize collagen collapse (Inokoshi et al., 1993) (Pashley et al., 1992). However, recent work has shown that ferric chloride addition does not prevent this collapse (Marshall et al., 1999) (Saeki et al., 2001).

Uno and Finger found that the depth of demineralization increases following a logarithmic relationship with both increasing acid concentration and/or etching time. This means at low acid concentration and short etching time, a small change in concentration causes a pronounced change in demineralization. However, as the acid reaches a higher concentration, for example 37% phosphoric acid, very little change in demineralization is observed due to flattening of the curve (1996). For this reason, phosphoric acid etchant usually comes in concentration of 30-40%. This leveling phenomenon can be explained by the buffering capacity of dentin (Pashley et al. 1992). Dissolution of carbonated apatite causes an increase in free Ca^{2+} and PO_4^{3-}, which in turn limit demineralization through the common ion effects (Eliades et al., 1997). Furthermore, the diffusion of the H^+ ions is physically restricted by the collapsed collagen network. And finally, dentinal fluid further neutralizes the acid (Uno and Finger, 1996). Due to the buffering capacity of dentin, brief etching followed by rinsing of the etchant has minimal effect on the pulp (Swift et al., 1995). Thus, recent findings dispel the old myth stemming from research done in the 1970's claiming that acid was harmful to the pulp. In these early studies, they etched the cavity preparation and placed zinc oxide eugenol to fill the cavity. They attributed the adverse pulpal reaction to the effect of the acid. Review of the literature, however, revealed that zinc oxide eugenol is cytotoxic when placed next to the pulp and is responsible for pulpal degeneration (Kanca, 1992). In fact, Cox and his colleagues found that direct acid application on a pulp exposure does not hinder normal healing of the pulp (Pashley et al., 1992).

Even if etching of dentin has minimal effect on the pulp, there are other potential disadvantages of over etching dentin, including (Pashley et al., 1992):

- increasing dentin permeability
- increasing dentin wetness, thereby making bonding more difficult
- denaturing collagen and preventing resin infiltration
- increasing the discrepancy between depth of demineralization and depth of resin penetration

Thus, the current concept in acid etching is "uni-etch" technique, which etches dentin and enamel simultaneously (Malferrari et al., 1994). Although a 7 sec etch with 10% phosphoric acid is sufficient for dentin (Nor et al., 1997), it is inadequate for enamel. Thus in 1996, the recommended uni-etch protocol for permanent teeth is 30 sec etch with 20% phosphoric acid (Uno and Finger). This application time has been cut in half with most current dentin bonding systems utilizing 30-40% phosphoric acid (Pioch et al., 1998) (Barkmeier et al., 1986). This has been shown to produce adequate etching of enamel while not over-etching dentin. With regard to collapsed collagen due to over-etching or desiccation, researchers have taken various routes to overcome this problem with mixed success including (Toledano et al., 2001) (Perdigao and Frankenberger, 2001):

- using water as a solvent in the dentin bonding system to re-expand the collapsed collagen network
- using metal chlorides as mordants to limit collagen denaturation, and thus, collapse
- using acidic monomers to etch and infiltrate dentin simultaneously, preventing collagen collapse

- removing the exposed collagen with sodium hypochlorite

While acid etching has been studied extensively in permanent dentin, there is no separate protocol for etching of primary dentin. Primary dentin contains less mineral than permanent dentin (Hirayama, 1990). Moreover, primary dentin has been shown to have fewer and smaller diameter dentinal tubules than permanent dentin, thus, decreasing the amount of dentinal fluid to neutralize the acid. As a result, primary dentin is more reactive to acid etching, and thus, would require a shorter etching time than permanent dentin (Agostini et al., 2001). In fact, Nor et al. observed that lower bond strengths were obtained when primary dentin was etched according to the manufacturer's recommended time for permanent dentin. Furthermore, they noted a layer of porosity at the bottom of the hybrid layer and attributed this to over etching of primary dentin and subsequent incomplete resin penetration. Thus, they recommended that the etching time for primary dentin should be one half that of permanent dentin (Nor et al., 1997).

2.8 Primer

In 1987, Munksgaard and Asmussen were the first to introduce the concept of "priming" to dentin bonding (Chigira et al., 1994). Primer is a generic name for a methacrylate that contains both hydrophobic and hydrophilic groups. The hydrophobic group has an affinity for the resin while the hydrophilic group has an affinity for the wet dentin surface (Nakabayashi and Takarada, 1992). Examples of these amphiphilic molecules include hydroxyethyl methacrylate (HEMA), bisphenol-glycidyl methacrylate (BPDM), and 4-methacryloxyethyl trimellitate anhydride (4-META) (Swift et al., 1995). Primers became widely used with the introduction of the 3rd generation dentin bonding systems (Dickinson et al., 1991).

The smear layer must be removed before applying the primer since the primer cannot penetrate through the smear layer (Chigira et al., 1994). After dentin is etched and left moist to prevent collagen collapse (Fritz et al., 1997), primer is applied to the moist dentin to increase its surface energy, and hence, wettability (Swift et al., 1995). Thus, primer increases bond strength by promoting interpenetration, impregnation, and entanglement of monomers into dentinal tubules and intertubular dentin (Nakabayashi and Takarada, 1992). A study published in 1995 evaluated bond strengths to primary teeth with and without primer. They found that bonding with primer significantly improved bond strength (Mazzeo et al., 1995). Primer can penetrate laterally through the demineralized inner wall of the dentinal tubules via lateral canals, into the intertubular dentin. This helps to further secure the resin tags within the dentinal tubules, increasing bond strengths (Schupbach et al., 1997).

Primer contains a solvent to facilitate diffusion of monomer into the demineralized dentinal tubules and intertubular dentin by displacing dentinal fluid. The solvent is either water, ethanol, acetone, or a combination thereof (Vargas et al., 1997). Acetone, when added to the primer, causes a decrease in surface tension of the primer and an increase in vapor pressure. The drop in surface tension allows the resin to chase the residual water away and adapt to surface (Kanca and Sandrik, 1998). The increase in vapor pressure makes it easier to evaporate the solvent before applying the adhesive (Kanca, 1992). Water, as a solvent, prevents collagen collapse, or re-hydrates and allows collagen to re-expand, resulting in greater monomer infiltration (Marshall et al., 1998). Ethanol functions in the same way as acetone. Its high vapor pressure allows for expedient evaporation of the solvent (Vargas et al. 1997). Some systems like Single Bond have a

combination of water and acetone as a solvent. A study done in 2001 looked at the effect of solvent on bonding to wet and dry etched dentin. They found that when dentin was wet, all the DBSs resulted in similar SBS. However, when the etched dentin was dry, water based primer tend to result in higher bond strengths than acetone or ethanol based primers due to its ability to rehydrate and re-expand the collapsed collagen (Perdigao and Frankenberger, 2001).

Self-etching primers (SEP) contain acidic monomers that allow for simultaneous demineralization and infiltration of these monomers through the smear layer and into the demineralized dentin (Chigira et al., 1994). Examples of acidic monomers include 2-methacryloyloxyethyl phenyl hydrogen phosphate (Phenyl-P) and 10-methacryloxydecyl dihydrogen phosphate (MDP). Unlike traditional separate etch systems where the acid is rinsed off, the acidic monomers are not washed off with SEPs (Toledano et al., 2001). Hence, the demineralized smear layer is not removed; instead, it gets incorporated into the hybrid layer and becomes part of the bond (Hayakawa et al., 1998). Furthermore, acidic monomers tend to be weaker than traditional acids to prevent over etching of the dentin surface (Perdigao et al., 2000). Even with a weaker acid, SEP causes a modest decrease in dentin hardness. This, however, does not seem to negatively affect bond strength or increase contraction gap (Chigira et al., 1994).

2.9 Hybrid Layer

In 1982, Nakabayshi et al. observed a resin reinforced collagen layer after sectioning a dentin specimen which had been treated with 10% citric acid-3% ferric chloride (Kato and Nakabayashi, 1996). They were the first to coin the term "hybrid layer," describing this resin reinforced interdiffusion zone (Pioch et al., 1998). This zone results from the

infiltration of monomers into etched dentin. More specifically, monomers infiltrate the intertubular dentin, dentinal tubules, and lateral canals within dentinal tubules. This improved flow of monomers allows for stronger mechanical interlocking to intertubular dentin, better resin tag adaptation within dentinal tubules, and micro-hybrid layer formation along the tubule walls, providing additional retention after polymerization (Vargas et al., 1997) (Schupbach et al., 1997). The hybrid layer, therefore, is composed of partially demineralized dentin in intimate contact with polymer (Inai et al., 1998). Thus, the hybrid layer is sandwiched between the adhesive layer and the unaltered dentin (Nakabayashi et al., 1992).

The rate of monomer diffusion through the etched dentin depends on two factors (Nakabayashi et al., 1998):

- permeability of the dentin substrate
- diffusibility of monomer

Etching and use of divalent cations may result in a more permeable dentin substrate and less collagen collapse (Wang and Nakabayashi, 1991). Primers, on the other hand, enhance the diffusibility of the monomers (Inokoshi et al., 1993). The thickness of the hybrid layer ranges from 1-5 μm (Heymann and Bayne, 1993) (Marshall et al., 1998), depending on the degree of etching. The thickness of the hybrid layer may be related to the length of the etching time or the strength of the acid (Pioch et al, 1998). When dentin is over etched, the depth of demineralization exceeds the depth of monomer infiltration, resulting in a void at the bottom of the hybrid layer (Nor et al., 1996). This is due to the fact that as soon as monomers begin to polymerize, the increase in molecular weight decreases their rate of diffusion (Nakabayashi et al., 1998). This void represents

exposed collagen that is not encapsulated by resin or apatite. Thus, this weaken area is prone to hydrolytic breakdown over time, leading to bond failure and microleakage, although initial bond strengths maybe high (Tam and Pilliar, 1994). In fact, Nakabyashi in 1992 cautioned against the unimpregnated band of exposed collagen as being the weak link in bonding in the long run.

With self etching primers (SEP), the acidic monomers are less acidic than traditional separate etch systems. For this reason, SEPs usually result in shallower etching patterns. In fact, SEPs usually result in a hybrid layer of 1-2 μm (Inai et al., 1998) versus 5-10 μm with traditional separate etch systems (Marshall et al., 1998) (Inokoshi et al., 1993). Furthermore, SEPs are not rinsed off after initial substrate demineralization, and the partially dissolved smear layer is incorporated into the hybrid layer (Hayakawa et al., 1998).

Numerous studies have indicated that the thickness of the hybrid layer has no effect on bond strength, as long as the resin infiltrated the full extent of the demineralized dentin (Kanca and Sandrik, 1998) (Pioch et al., 1998). Some investigators even suggested that the hybrid layer is not critical for bonding to dentin. They advocated removal of the unsupported collagen network that is involved in hybrid layer formation with sodium hyphochlorite and bonding directly to dentin mineral (Inai et al., 1998). Results from this effort were mixed. For example, one study examined the effect of complete collagen removal on bond strength using two dentin bonding systems: One Step (Bisco, Itasca, IL) and Scotchbond Multipurpose Plus (3M Dental Products, St. Paul, MN). With One Step, removal of the exposed collagen had no significant effect on bond strengths. With Scotchbond Multipurpose Plus, however, removal of the collagen layer

resulted in significant reduction in bond strengths (Marshall et al., 1998). Still, other studies have shown significantly higher bond strength with sodium hyphochlorite treatment (Inai et al., 1998). Thus, the role of exposed collagen in bonding is still unclear.

Although morphologically similar, the hybrid layer is 25-30% thicker in primary dentin than in permanent dentin (Agostini et al., 2001). In fact, one study indicated that the hybrid layer in primary dentin was 10-12 μm thick (Fritz et al., 1997). This thicker hybrid layer in conjunction with lower mineral content in primary teeth suggests that primary dentin is more reactive to acid etching than permanent dentin (Agostini et al., 2001). Since the same etching protocol for permanent teeth is applied to primary dentin, it is not surprising to see greater demineralization depth and thicker hybrid layers in primary teeth. Increased demineralization depth increases the likelihood of introducing voids at the bottom of the hybrid layer due to incomplete resin infiltration. This is a possible explanation for the observed lower bond strength to primary dentin as compared to permanent dentin (Nor et al., 1996).

2.10 Evolution of Dentin Bonding Systems (DBS)

2.10.1 1st Generation

Buonocore in 1956 developed a glycerophosphoric acid dimethacrylate to bond to the calcium of dentin. In 1965, Bowen developed N-phenylglycine-glycidyl methacrylate (NPG-GMA), followed by Lee in 1971 who developed a polyurethane resin to be used as an adhesive for composite restorations. Thus, the 1st generation of dentin bonding systems were manufactured using one or more of these compounds (Dickinson et al., 1991). These systems were designed to form ionic bond to the apatite and/or covalent bonds to the collagen of the smear layer. Since these systems were hydrophobic and bonded directly to the smear layer, bond strengths were only 2-6 MPa (Heymann and Bayne, 1993).

2.10.2 <u>2nd Generation</u>

2nd generation dentin bonding agents were developed in the early 1980's (Swift et al., 1995). These included Scotchbond (3M Dental Products, St Paul, MN), Dentin Bonding Agent (Johnson and Johnson Dental Products, East Windsor, NJ), Creation Bond Agent (Den-Mat Corp, Santa Maria, CA), Dentin Adheit (Vivadent, Schaan, Liechtenstein), Bondlite (Kerr, Romulus, MI)... (Dickinson et al., 1991) (Swift et al., 1995). Most of these dentin bonding systems contained halophosphorous esters of unfilled resin (eg Bis-GMA). Bis-GMA is a bifunctional molecule containing a phosphate group that can form ionic bonds to the calcium within the smear layer (Swift et al., 1995). Bond strengths of 5-7 MPa were not much improved over the earlier generation since they still bond to the smear layer, reflecting the weak cohesive strength of the smear layer (Pashley et al., 1992).

2.10.3 <u>3rd Generation</u>

3rd generation dentin bonding systems included: Scotchbond 2 (3M Dental Products, St Paul, MN), Gluma (Bayer AG, Leverkusen, Germany), Tenure (Den-Mat Corp, Santa Maria, CA), Syntac (Ivoclar Vivadent, Amherst, NY), Prisma Universal Bond 2 (Caulk/Dentsply, Milford, DE), XR Bond (Kerr/Sybron, Romulus, MI), Mirage Bond (Chameleon Dental Products, Kansas City, MO), All-Bond (BISCO, Inc, Downers Grove, IL)... (Dickinson et al., 1991) (Swift et al., 1995). There were two major improvements in this generation. First, the smear layer was partially or completely

removed with an acidic conditioner to allow better resin penetration. Thus, the bond obtained is predominately a mechanical bond through the smear layer (Swift et al., 1995). Some systems etched enamel and dentin separately, using a stronger acid on enamel and a weaker acid on dentin (eg All-Bond). Others etched enamel and dentin together using a mild acid such as 10% phosphoric acid (eg All-Bond Kanca Technique) (Dickinson et al., 1991). Secondly, in addition to an adhesive which was present in the first two generations, a primer was introduced which facilitated the wetting of dentin, allowing adhesion of a hydrophobic adhesive to the hydrophilic dentin. These bonding systems generally produced higher bond strengths than previous generations. However, they demanded more attention to the elaborate steps and techniques involved and were often very time consuming (Dickinson et al., 1991).

2.10.4 4th Generation

4th generation bonding systems included: All-Bond 2 (Bisco, Inc., Schaumburg, IL), OptiBond FL (Kerr Dental Materials Center, Orange, CA), Scotchbond Multi-Purpose Plus (3M Dental Products, St Paul, MN)… (Perdigao et al., 2000) (Swift and Bayne, 1997). These are the conventional 3 step dentin bonding systems: etching, priming, and adhesive application (Swift and Bayne, 1997). These systems tended to use the total etch technique. The key difference between the 3rd and 4th generation dentin bonding systems is the formation of a hybrid layer (Frey, 2000). 4th generation dentin bonding systems tended to produce much higher bond strengths than their predecessors due to the formation of a high quality hybrid layer. In fact, with the progression from the 2nd through the 4th generations, generally higher bond strengths, lower microleakage, and better clinical performance were seen (Perdigao et al., 2000). 4th generation agents also

usually contained a dual-cured option for indirect restorations and bonded amalgam to expand their versatility. However, the main problem with these systems was that they contained too many bottles, increasing the chance of erring during the bonding procedure. Also, some bottles never got used at all (Perdigao et al., 2000).

2.10.5 5th Generation

5th generation dentin bonding systems included: One Step (Bisco, Inc., Itasca, IL), Prime and Bond 2.1 (Caulk/Dentsply, Milford, DE), Single Bond (3M Dental Products, St Pual, MN), Tenure Quick (Den-Mat Corp, Santa Maria, CA), Bond 1 (Jeneric/Pentron, Wallingford, CT), OptiBond Solo Plus (Kerr Dental Materials Center, Orange, CA), OptiBond Solo (Kerr Dental Materials Center, Orange, CA), Syntac Single-Component (Ivoclar Vivadent, Amherst, NY), Prime & Bond NT (Caulk/Dentsply, Milford, DE), Excite (Ivoclar Vivadent, Amherst, NY), EBS Multi (ESPE America, Plymouth Meeting, PA), PQ1 (Ultradent, South Jordan, Utah)... (Swift and Bayne, 1997) (Perdigao et al., 2000). Their major improvement over their predecessors was simplifying the bonding procedure by combining the primer and adhesive into one (Miyazaki et al., 2000). Thus, the bonding procedure involved etching the tooth, applying the primer/adhesive, and placing the composite (Vargas et al., 1997). Again, these systems allowed for simultaneous and same treatment of dentin and enamel (Kanca, 1997). The hybrid layers obtained with these systems were very similar in morphology and depth as the 4th generation bonding systems. However, the primer/adhesive layer varied in thickness, as thick as 50 μm, depending on the viscosity of the primer/adhesive, whether it is filled or unfilled, and the number of coats applied (Vargas et al., 1997). Although there has been a dramatic improvement in bond strength from the 2nd to the 5th generation (2-5 MPa to

20-30 MPa), 5th generation bond strengths were not higher than the 4th generation (Perdigao et al., 2000). Furthermore, these systems were more technique sensitive than they claimed to be (Swift and Bayne, 1997). For example, acetone based primer/adhesives may lose their efficacy with constant utilization, and thus, may require more coats to prevent occurrence of dry spots (areas not covered with adhesive). With ethanol based primer/adhesive, care must be taken to prevent pooling of the primer/adhesive around the preparation margin. Finally, these systems have not been formulated to be used as dual cure materials (Perdigao et al., 2000).

2.10.6 Self-Etching Primers (SEPs)

SEPs were developed in response to clinicians' increased expectations for a simpler bonding system that is not too technique sensitive (Lopes et al., 2002). The three major concerns with dentin bonding are the multiple steps involved in the bonding procedure (Hasegawa et al., 1989), hydration state of dentin before bonding (Toledano et al.,2001), and the discrepancy between etching depth and resin penetration depth, causing a potential zone of weakness in the bond (Nakabayashi and Saimi, 1996). Thus, SEPs were developed to address these issues. SEP is an aqueous mixture of acidic monomers (ie phosphate ester or carboxylic acid) and HEMA (Toledano et al., 2001). Examples of acidic monomers include monomethacryloxyethyl succinate (MES), dimethacryloxyethyl phosphate (DMEP), tertiary butylacrylamide sulfonic acid (TBAS), 4-methacryloxyethyl trimellitic anhydride (4-META), and 2-methacryloyloxyethyl phenyl hydrogen phosphate (Phenyl-P) (Hasegawa et al., 1989). Phenyl-P is effective in minimizing contraction gap, and thus, microleakage by facilitating monomer diffusion and impregnation into the demineralized dentin substrate (Chigira et al., 1994). Wang and Nakabayashi found that

phenyl-P is better at promoting monomer diffusion than 4-META. In fact, when phenyl-P was taken out of the methyl methacrylate/tri-n-butyl borane (MMA/TBB) system, it did not show any monomer diffusion into the dentin substrate. Thus, phenyl-P is needed to demineralize the dentin substrate and facilitate infiltration and polymerization of MMA. TBB is the catalyst in this reaction (1991). Currently, phenyl-P is the acidic monomer in the Clearfil SE dentin bonding system tested in this study.

SEP simplifies bonding by combining the etching and priming steps. Furthermore, it eliminates the critical etching, rinsing, and drying steps (Toledano et al., 2001). With traditional separate etch systems, the critical step is leaving a "moist" surface after etching. Over-wet surfaces dilute the primer and over-dry surfaces cause collagen collapse and incomplete resin penetration, both resulting in decreased bond strengths (Araujo et al., 1997). Thus, SEP systems resolve this issue by etching and priming at the same time.

The acidic monomers demineralize and infiltrate the micro-channels within the smear layer and continue to demineralize and penetrate the superficial layer of the underlying dentin (Lopes et al., 2002). Since the demineralization and monomer infiltration occur at the same time, dentin collagen does not collapse (Telles et al., 1998). Thus, not only is collagen collapse not an issue, resin should penetrate to the full extent of the demineralized depth, minimizing micro-gap formation (Lopes et al., 2002). SEPs usually contain a mild acid (acidic monomers) which partially remove the smear layer, maintain the smear plugs, and provide a superficial etch of the underlying dentin (Telles et al., 1998). SEPs only demineralize dentin to a depth of 0.5-1 μm, unlike traditional separate etch systems of 4-5 μm (Toledano et al., 2001). Although the hybrid layer produced from SEP systems (~1 μm) is much thinner than separate etch systems (1-5 μm), it can still withstand stresses from polymerization shrinkage. Furthermore, despite the thin hybrid layer, high bond strengths are obtained with some SEPs on dentin and instrumented enamel (Perdigao et al., 2000). Thus, extensive resin tag formation and thick hybrid layer are not crucial for dentin bonding (Wang and Nakabayashi, 1991).

However, two concerns are raised with SEPs. First, what is the effect of leaving the acidic monomers on dentin and enamel without rinsing them off? And secondly, is the weaker acid able to adequately etch enamel? With regard to the first concern, the weak acid is quickly neutralized by the buffering capacity of dentinal fluid and the common ion effects which limit further dissociation of apatite (Toledano et al., 2001). In fact, because there is little or no discrepancy between demineralization depth and resin penetration depth, there should be no exposed collagen or microgap formation. Hence, there is very little post operative sensitivity compared to traditional separate etch systems (Lopes et al., 2002). As to the adequacy of enamel etching, it has been shown that SEPs generally do not etch uncut enamel efficiently, resulting in lower bond strengths and more microleakage over time when compared to cut enamel (Perdigao et al., 2000). Nakanuma reported that bond strength to enamel was not as strong as to dentin (Hayakawa et al., 1998). Furthermore, the SEPs are more technique sensitive than suggested by the manufacturers. For example, under drying of the SEP results in incomplete evaporation of solvent. This may inhibit polymerization of the monomers. However, over drying of the SEP may saturate the primer with oxygen, which also may inhibit polymerization (Miyazaki et al., 2000). Although in vitro studies look promising for SEPs, long term clinical trials are needed to evaluate the longevity of these bonds (Lopes et al., 2002).

2.10.7 Self-Etching Adhesives (SEAs)

Self-etching adhesives or self-conditioning-primer-adhesives are the next logical step in further simplifying the bonding procedure. These include Etch and Prime 3.0 (Degussa AG, Hanau, Germany), Prompt-L-Pop (ESPE America, Plymouth Meeting, PA), One Up (Tokuyama Corp 3-1, Shibuya 3-chome, Shibuya-Ku, Tokyo, Japan)... (Toledano et al., 2001) (Perdigao et al., 2000). These materials were recently introduced into the market; thus, very few studies have been conducted. These systems combine the etching, priming, and adhesive steps into one (Frey, 2000). Thus, after the tooth is rinsed and gently air dried, the SEA is applied, light cured, and a composite is placed. Thus, all the advantages associated with SEPs are also true for SEAs, except the bonding procedure has been further simplified.

Prompt-L-Pop was among the first of these SEAs to be introduced into the market in 1999. It is conveniently packaged in the form of a lollipop with 3 separate compartments. The first compartment contains methacrylated phosphoric esters, initiators, and stabilizer. The second compartment contains water, fluoride complex, and stabilizers. The third compartment contains a microbrush. The compartments are mixed together by squeezing the 3 pouches, and it is ready for use (Toledano et al., 2001). Although the enamel etching pattern produced by Prompt-L-Pop is very similar to one produced by phosphoric acid (Frey, 2000), bond strength obtained is not as high (Perdigao et al., 2000). Thus, etching pattern by itself, does not determine bond strength. Prompt-L-Pop is known for having minimal post-operative sensitivity due to simultaneous etching and infiltration of monomers through the smear layer and into underlying dentin (Perdigao et al., 2000). However, preliminary studies from our lab

revealed low and inconsistent bond strengths with Prompt-L-Pop (unpublished data). In fact, the few studies that have been done on Prompt-L-Pop revealed a wide range of bond strength values (Perdigao et al., 2000). One Up (Tokuyama), although a SEA, comes in two bottles that must be dispensed and mixed before use. Proper mixing is indicated with a color change of the bonding agent from yellow to red. Again, proper light curing is indicated by further color change to brown. Although these systems are generally simpler to use, they do not result in higher bond strengths when compared to traditional separate etch systems (Lopes et al., 2002).

3. HYPOTHESIS

Compositional and morphological differences between primary and permanent teeth lead to different bonding properties between these two substrates.

3.1 Sub-hypotheses

1. Hole diameter has no effect on SBS
2. Bond to permanent enamel is stronger than bond to primary enamel
3. Bond to permanent dentin is stronger than bond to primary dentin
4. Bond to permanent teeth is stronger than bond to primary teeth
5. Bond to permanent enamel is stronger than to permanent dentin
6. Bond to primary enamel is stronger than to primary dentin
7. Bond to enamel is stronger than bond to dentin (primary and permanent together)

8. Primary teeth require shorter etching time than permanent teeth
9. Dentin requires shorter etching time than enamel
10. Self-etching systems (Clearfil SE and One Up) require longer etching time than separate etch system (Single Bond)
11. With SEP and SEA, the more defined the etching pattern, the stronger the shear bond strength
12. Overall, Clearfil SE has the highest bond strength, followed by Single Bond, and finally One Up.

4. MATERIALS AND METHODS

4.1 Shear Bond Strength (SBS) Test

4.1.1 Teeth Selection

54 extracted noncarious human third molars and 108 primary anterior teeth were used for SBS tests to evaluate 3 dentin bonding systems (DBS) {Single Bond (3M), Clearfil SE (Kuraray), and One Up (Tokuyama)}. Noncarious teeth were selected to eliminate the effect of caries on bonding. These teeth were immediately stored in 0.01% thymol solution after extraction. However, due to various collection dates, the storage time varied before preparation for the bonding procedure. Fortunately, there is no significant effect on enamel or dentin bond strength for teeth stored for 24 hours, 3 months, or 5 years in 0.05% thymol solution (Rueggeberg, 1991). All our teeth were prepared for bonding within 6 months after extraction. SBS was tested using the following 4 substrates: permanent enamel, permanent dentin, primary enamel, and primary dentin. For each substrate, three acid etching time intervals were evaluated: 5 sec, manufacturer recommended time or MRT (15 sec for Single Bond, 20 sec for One Up and Clearfil SE),

and 60 sec. Thus, there were a total of 12 subgroups, each containing 6 samples for each dentin bonding system. The following diagram illustrates sample distribution:

SBS Test (For 1 DBS)

Permanent teeth						**Primary Teeth**					
Permanent Enamel			Permanent Dentin			Primary Enamel			Primary Dentin		
5s	MRT	60s	5s	MRT	60s	5s	MRT	60s	5s	MRT	60s
n=6	n=6	n=6	n=6	n=6	n=6	n=6	n=6	n=6	n=6	n=6	n=6

4.1.2 Preparing surfaces for bonding

4.1.2.1 Permanent Teeth

54 extracted, gamma irradiated human third molars stored in 0.01% thymol solution were sectioned in half buccolingually with a low speed saw (modified Buehler Isomet Low Speed saw, Buehler Ltd, Lake Bluff, IL) to produce 108 samples of teeth with either a mesial or distal surface. Gamma irradiation allows for sterilization of teeth with minimal alteration to the tooth structure (Marshall, 1993). The mesial or distal surface was prepared into enamel or dentin and prepared for bonding as followed:

Permanent enamel surface preparation:

54 of the samples were ground down with water to minimize dessication to produce a flat enamel surface with ~ 3 mm diameter. Polishing was accomplished with 240 grit SiC polishing paper on the Handimet I Strip Grinder (Buehler Ltd, Lake Bluff, IL) and ending with 320 grit to mimic the surface produced with a carbide bur (Tao and Pashley, 1988). The roots were ground off to allow for proper fit of the samples in the Watanabe jig

system. These 54 enamel specimens were then divided into 9 groups of six for various acid treatments.

Permanent dentin surface preparation:

The other 54 specimens were ground down in a similar manner until a flat dentin surface was obtained with ~ 3 mm in diameter. Again, these 54 dentin specimens were then divided into 9 groups of six which were subjected to various acid treatments.

4.1.2.2 Primary Teeth

108 extracted, gamma irradiated human primary anterior teeth stored in 0.01% thymol solution were used for the bonding procedure. These included maxillary centrals, laterals, and canines and mandibular canines. The buccal surface was used for the bonding study.

Primary enamel surface preparation:

The buccal surfaces of 54 primary anterior teeth were ground down and polished to 320 grit, producing a flat surface of ~ 3 mm in diameter. Care was taken to prevent exposure of dentin. These 54 enamel samples were then divided into 9 groups of six.

Primary dentin surface preparation:

Again, the buccal surfaces of the other 54 primary anterior teeth were ground down to 320 grit, exposing a flat surface of dentin with ~ 3 mm diameter. These 54 dentin samples were then divided into 9 groups of 6 for various acid treatments.

Care was taken to insure that the samples were kept moist after surface preparation to prevent surface desiccation, and hence, collagen collapse.

4.1.3 ***Securing Samples to the Single Plane Lap Shear Device (Watanabe System):***

The single plane lap shear device is composed of two plexiglas plates with different diameter tubes. The larger diameter plate houses the sample while the smaller one is packed with composite. A strip of mylar tape with a 2.6 mm diameter hole was taped over the opening of the large plate so that the hole was positioned in the center of the tube. The sample was then placed within the tube, attached to the mylar tape so that the bonding surface was centered over the 2.6 mm diameter hole within the tape and exposed for bonding. The sample was carefully positioned so that the direction of shearing was in the incisogingival direction. Thus, the 2.6 mm diameter hole within the mylar tape provided a constant surface area for bonding. Once the sample was properly positioned in the large plate, dental stone (Die-Keen, Miles, Inc. South Bend, IN) was poured into the tube to further secure the sample. Care was taken to insure that the dental stone did not run onto the bonding surface, which would have interfered with bonding. The samples were ready for etching and bonding.

4.1.4 ***Bonding Procedure***

Three different DBS were tested: Single Bond, Clearfil SE, and One Up.

1. **SINGLE BOND (3M Dental Products, St. Paul, MN):**
   A. ***Acid:*** 35% Phosphoric Acid (Lot #: 9LX, Expiration: December 2001)
   B. Prime/Adhesiver: HEMA, Bis-GMA, Dimethacrylates Pendant Polyalkenoic Acid Copolymer, Ethanol, Water, Photo-initiator
      - ➢ Lot #: 1FM
      - ➢ Expiration: February 2003

2. **CLEARFIL SE (Kuraray America, INC. New York, NY)**
    A. ***Self-etching Primer***: MDP, HEMA, Hydrophilic Dimethacrylate, N,N-Diethanol-p-touidine, Water
        - Lot #: 00141A
        - Expiration: August 2002

    B. ***Adhesive:*** MDP, Bis-GMA, HEMA Hydrophobic Dimethacrylate, Di-Camphorquinone, N,N-Diethanol-p-toluidine, Silanated Silicate, Water
        - Lot #: 00103A
        - Expiration: August 2002

3. **ONE UP (Tokuyama Corp 3-1, Shibuya 3-chome, Shibuya-Ku, Tokyo, Japan)**
    A. ***Self-etching Adhesive:***
        1. **Bottle A**: Methacryloyloxyalkyl Acid Phosphate, MAC-10, Multi-functional Methacrylic Monomers, HEMA, Photo Acid Generator
        2. **Bottle B**: Fluoroaluminosilicate Micro Filler, Monomer, Water, Aryl Borate Derivative, Dye-sensitizer (Coumarin)
        - Lot #: 453050
        - Expiration: November 2002

For each DBS, four substrates were tested (permanent enamel, permanent dentin, primary enamel, and primary dentin) at 3 acid etching time intervals. Thus, for each DBS, there were 12 groups (each containing six samples).

SINGLE BOND (3M)

Manufacturer Recommended Etching Time (MRT)

a. Bonding surface was rinsed with deionized water for 15 sec and blotted dry with a Kimwipe.

b. 35% phosphoric acid etchant was applied to the bonding surface on the large plate and rubbed with a brush for 15 sec.

c. After rinsing off the etchant with deionized water for 15 sec, excess water was blown off and the sample was blotted dry with a Kimwipe, leaving a glistening moist surface. This is critical since dry etched dentin resulted in significantly lower SBS than wet dentin due to collagen collapse (Perdigao and Frankenberger, 2001).

d. A drop of adhesive was placed onto the bonding surface and rubbed with a brush for 2 sec and air thinned for 3-5 sec.

e. Excess adhesive around the bonding surface was removed with Kimwipes and the adhesive was cured for 10 sec using the Optilux visible light curing unit (Demetron, Danbury, CT).

f. The small plate was attached to the large plate with two screws and Z-100 composite (3M Dental Products, St. Paul, MN) (Lot # 1EA; Expiration: November 2003) was added into the tube of the small plate in 2 increments of ~2 mm in depth, curing for 40 sec after each increment.

g. The sample was stored under humid condition at 37°C for 24 hrs before the SBS test. 24 hrs storage was needed because it allowed for complete

polymerization shrinkage of the composite and for the composite to equilibrate with the water (Rueggeberg, 1991).

h. This procedure was repeated for all 4 substrates. (The enamel samples were also kept slightly moist after etching instead of completely dry because it is clinically very difficult to leave a moist dentin surface and a dry enamel surface.)

5 sec Etching Time

A. Same procedure as above, except bonding surface was etched with 35% phosphoric acid etchant for 5 sec.

60 sec Etching Time:

A. Same procedure as above, except bonding surface was etched with 35% phosphoric acid etchant for 60 sec.

CLEARFIL SE (KURARAY)

Manufacturer Recommended Application Time (MRT)

A. With Clearfil SE, there was no separate etchant. Thus, after rinsing the bonding surface with deionized water for 15 sec and blotting the surface dry with a Kimwipe, 1 drop of self etching primer was applied to the bonding surface and rubbed with a brush for 20 sec.

B. Gently air thinned for 3-5 sec to evaporate the solvent.

C. 1 drop of adhesive was applied to the bonding surface and gently rubbed with a brush for 2 sec and air thinned for 3-5 sec.

D. Repeat 1e-1h (Single Bond bonding procedure)

5 sec Application Time

A. Same instruction as above, except the self etching primer was applied for 5 sec prior to air thinning.

60 sec Application Time

A. Same instruction as above, except the self etching primer was applied for 60 sec prior to air thinning.

ONE UP (TOKUYAMA)

Manufacturer Recommended Application Time (MRT)

A. Bonding surface was rinsed with deionized water for 15 sec and blotted dry using a Kimwipe.

B. With One Up, there was no separate etchant. Thus, 1 drop from bottle A was mixed with 1 drop from bottle B and agitated until the color changed to pink, indicating adequate mixing. The resulting self etching adhesive was applied to the bonding surface and brushed gently for 20 sec.

C. Gently air thinned for 3 sec to even out the layer and evaporate the solvent.

D. Excess self etching adhesive was removed with a Kimwipe and light cured for 10 sec.

E. Repeat 1f-1h (Single Bond bonding procedure)

5 sec Application Time

A. Same instruction as above except the self etching adhesive was applied for 5 sec prior to air thinning.

60 sec Application Time

A. Same instruction as above except the self etching adhesive was applied for 60 sec prior to air thinning.

4.1.5 *SBS Test*

After storing the samples in 100% humidity at 37° C for 24 hrs, they were prepared for shear strength testing with a universal mechanical testing machine (Instron Model 1122, Canton, MA) at a cross speed of 5 mm/min. The SBS test was done on all 216 permanent and primary bonded samples. Some samples were excluded due to human errors during the bonding procedure or while running the Instron machine. Shear bond strength data was recorded in kilograms from the testing machine. After SBS test, the hole diameter was recorded by taking an average of two diameter measurements made at 90 degrees to each other using a microscope at 20X. SBS measurements were converted from loads (kilograms) to stress (MPa) by dividing by the bonding surface area for each sample.

4.2 Scanning Electron Microscopy (SEM) Evaluation of the Etched Surface

Etching patterns produced by the 3 DBS's were qualitatively evaluated using the SEM. With Single Bond, only the etching pattern produced by the manufacturer recommended time was evaluated as a control to which the other two DBSs were compared. For Clearfil SE and One Up, etching patterns produced by all three acid etching time intervals were evaluated to determine if a relationship existed between the degree of etching and SBS. Thus, 21 additional irradiated human third molars and 42 primary anterior teeth were needed for this part of the study. Three samples were needed per substrate (permanent enamel, permanent dentin, primary enamel, or primary dentin) per acid etching time (5 sec, MRT, and 60 sec) for Clearfil SE and One Up. For Single Bond, 3 samples were needed per substrate for the manufacturer's recommended time

only. Thus, for Clearfil SE and One Up, there were 12 groups of 3 samples each as shown below:

Sample Preparation for SEM evaluation (Clearfil SE and One Up only)

Permanent teeth						Primary Teeth					
Permanent Enamel			Permanent Dentin			Primary Enamel			Primary Dentin		
5s	MRT	60s	5s	MRT	60s	5s	MRT	60s	5s	MRT	60s
n=3	n=3	n=3	n=3	n=3	n=3	n=3	n=3	n=3	n=3	n=3	n=3

Sample Preparation for SEM evaluation (Single Bond only)

Permanent teeth		Primary Teeth	
Permanent Enamel	Permanent Dentin	Primary Enamel	Primary Dentin
MRT	MRT	MRT	MRT
n=3	n=3	n=3	n=3

4.2.1 Etching and Sample Preparation for SEM Analysis

The samples were initially prepared in the same way as for bonding--grinding and polishing the surface to 320 grit. Once again, the samples were kept moist to prevent drying. Unlike the bonding procedure, the samples were not mounted onto the plexiglas plates.

SINGLE BOND (3M)

A. After rinsing the sample with deionized water for 15 sec, the sample was blotted dry with a Kimwipe.

B. 35% phosphoric acid was applied in the same manner as for the bonding procedure for 15 sec and rinsed with deionized water.

C. The above procedure was repeated for 5 sec and 60 sec etch.

D. After etching and rinsing, the samples were fixed, dehydrated with ethanol, and sputtered with Au-Pd in preparation for SEM analysis.

CLEARFIL SE (Kuraray)

A. After rinsing the sample with deionized water for 15 sec, the sample was blotted dry with a Kimwipe.

B. Self etching primer was applied in the same manner as for bonding procedure for 20 sec. Since SEP is usually not rinsed away, the monomer in the SEP must be dissolved and removed to better visualize the etching pattern produced by the SEP. Monomer was removed by submerging the sample in 100% alcohol for 5 min, followed by 5 min in deionized water (Oliveira et al., 2002).

C. After removing the monomer, the samples were fixed, dehydrated and sputter-coated.

D. The procedure was repeated for the 5 sec and 60 sec SEP applications.

ONE UP (Tokuyama)

A. The same procedure for Clearfil was used for One Up, except the self etching adhesive (mixing one drop from bottle A and one drop from bottle B) was applied for 20 sec.

B. The procedure was repeated for the 5 sec and 60 sec SEA applications.

4.2.2 Sample Fixation and Dehydration

A. The samples were fixed to minimize collagen denaturation and surface distortion during SEM analysis.

B. The samples were fixed in 2.5% gluteraldehyde in a 0.1M sodium cacodylate buffer (pH = 7.4) for 12 hrs at 4° C.

C. The samples were then rinsed with 0.2M sodium cacodylate for 1 hr, replacing the solution every 20 min.

D. The samples were rinsed with deionized water for 1 min.

E. The samples were then dehydrated in ascending grades of ethanol starting with:

25% for 20 min

50% for 20 min

75% for 20 min

95% for 30 min

100% for 60 min

F. The samples were dried in HMDS for 10 min and placed in a glass vial to air dry over night (Perdigao et al., 1995).

4.2.3 Sputtering

A. After fixing and dehydrating, the samples were sputter coated with a 200 nm layer of gold/palladium to increase the conductivity of the sample to allow better visualization of the etched surface in the secondary mode. Sputtering was

accomplished using the Hummer VII sputtering system (Anatech LTD, Alexandria, VA).

B. After sputtering, a layer of conductive carbon paint (SPI Supplies, West Chester, PA) was applied to the sample edges to provide additional conductivity to the metal mount.

4.2.4 SEM Evaluation

A. SEM evaluation was done in the secondary mode.

B. 2 samples were randomly selected from each group (eg One Up, 15 sec etch, permanent enamel) were analyzed and photographed at 500x and 2000x.

C. These images were then transferred to a computer and saved on a disk using the Advance Imaging software system (Kevex Corp., San Carlos, CA).

D. Samples etched with Single Bond 35% phosphoric acid etchant for 15 sec were used as controls to compare to the etching patterns of the other two DBS's.

5. RESULTS

5.1 Shear Bond Strength Test

Below is a summary of the shear bond strength values obtained for the substrates tested (permanent enamel, permanent dentin, primary enamel, and primary dentin) using 3 different DBS: Single Bond, Clearfil SE, and One Up. Single Bond is a fifth generation dentin bonding system where etching is done as a separate step. Clearfil SE is a self-etching primer. One Up is a self-etching adhesive. For each substrate, 3 different acid etching time intervals were tested to determine if etching time has an effect on SBS.

PERMANENT ENAMEL

TIME (SEC)		SINGLE BOND (MPa)	CLEARFIL (MPa)	ONE UP (MPa)
5	ENAMEL	30.9 +/- 1.6	25.6 +/- 6.0	16.1 +/- 6.2
MR TIME	ENAMEL	31.6 +/- 2.5	35.5 +/- 4.3	23.8 +/- 1.2
60	ENAMEL	27.9 +/- 2.4	31.7 +/- 5.7	24.6 +/- 4.2

PERMANENT DENTIN

TIME (SEC)		SINGLE BOND (MPa)	CLEARFIL (MPa)	ONE UP (MPa)
5	DENTIN	35.3 +/- 3.7	30.3 +/- 6.8	10.0 +/- 6.9
MR TIME	DENTIN	28.9 +/- 2.9	35.3 +/- 2.9	10.7 +/- 3.9
60	DENTIN	27.3 +/- 5.0	33.1 +/- 5.8	16.9 +/- 3.2

PRIMARY ENAMEL

TIME (SEC)		SINGLE BOND (MPa)	CLEARFIL (MPa)	ONE UP (MPa)
5	ENAMEL	26.6 +/- 2.4	27.8 +/- 4.8	5.8 +/- 4.7
MR TIME	ENAMEL	26.0 +/- 3.8	31.0 +/- 1.6	8.4 +/- 8.3
60	ENAMEL	26.4 +/- 4.4	30.6 +/- 1.8	14.3 +/- 5.5

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PRIMARY DENTIN

TIME (SEC)		SINGLE BOND (MPa)	CLEARFIL (MPa)	ONE UP (MPa)
5	DENTIN	33.9 +/- 4.3	35.7 +/- 4.4	7.6 +/- 2.7
MR TIME	DENTIN	31.9 +/- 3.9	36.0 +/- 5.2	9.0 +/- 3.9
60	DENTIN	30.9 +/- 1.6	34.9 +/- 5.8	9.9 +/- 2.3

The following statistical analyses were performed: Two way ANOVA and adjustment for multiple comparisons: Tukey-Kramer.

5.2 Hole Diameter has no Effect on SBS

Based on the graphs of SBS versus hole diameter ***(Figures 5.2.1-5.2.4),*** minor variations in hole diameter, reflecting the bonding surface area, has no effect on SBS.

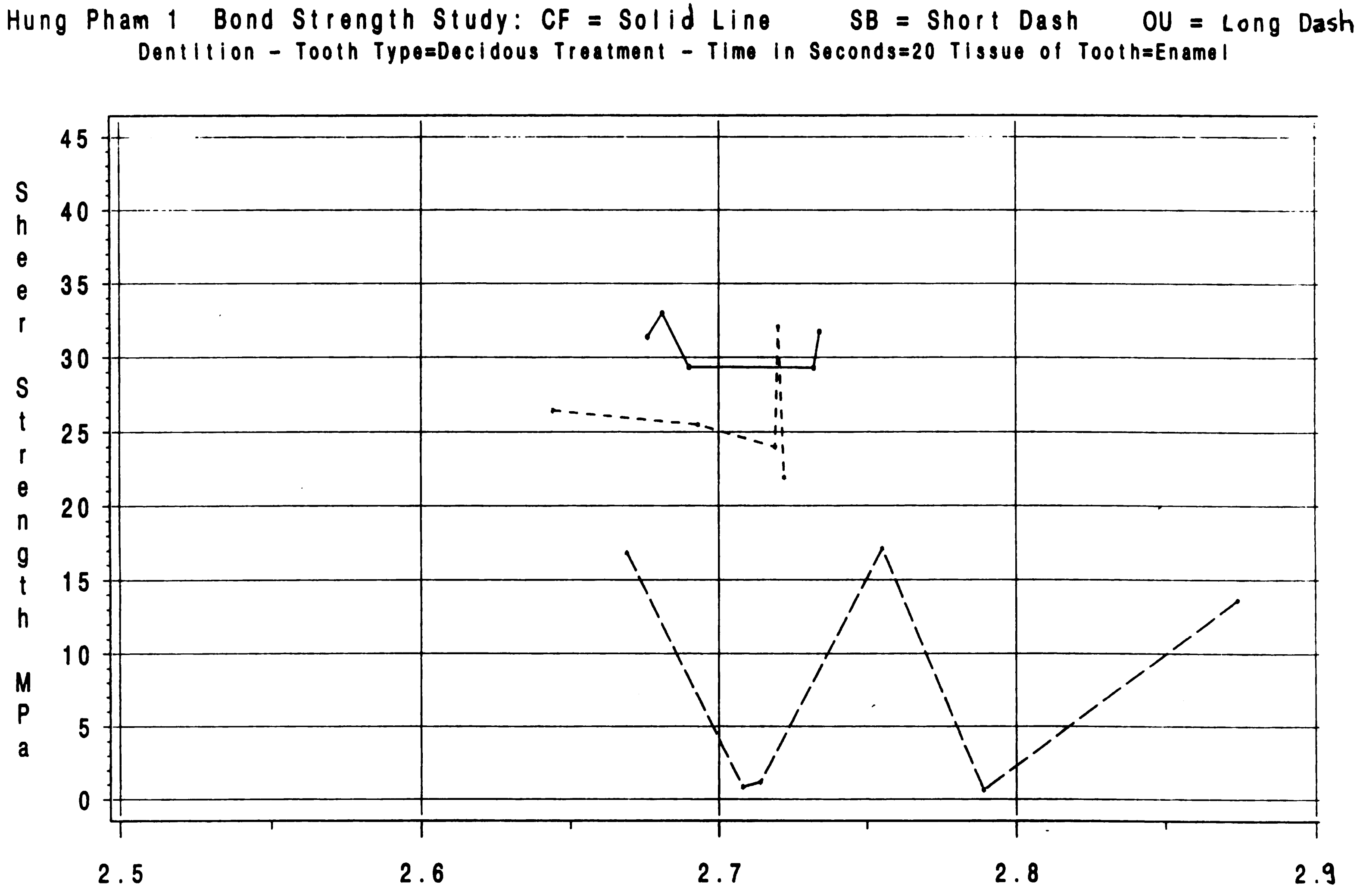

Fig. 5.2.1

Program: c:\Hung Pham 1\read_excel.sas Programmer: Robert S. Wilson Run Date: Friday, 3 May 2002 at 18:23:27

Hung Pham 1 Bond Strength Study: CF = Solid Line SB = Short Dash OU = Long Dash
Dentition - Tooth Type=Decidous Treatment - Time in Seconds=20 Tissue of Tooth=Dentin

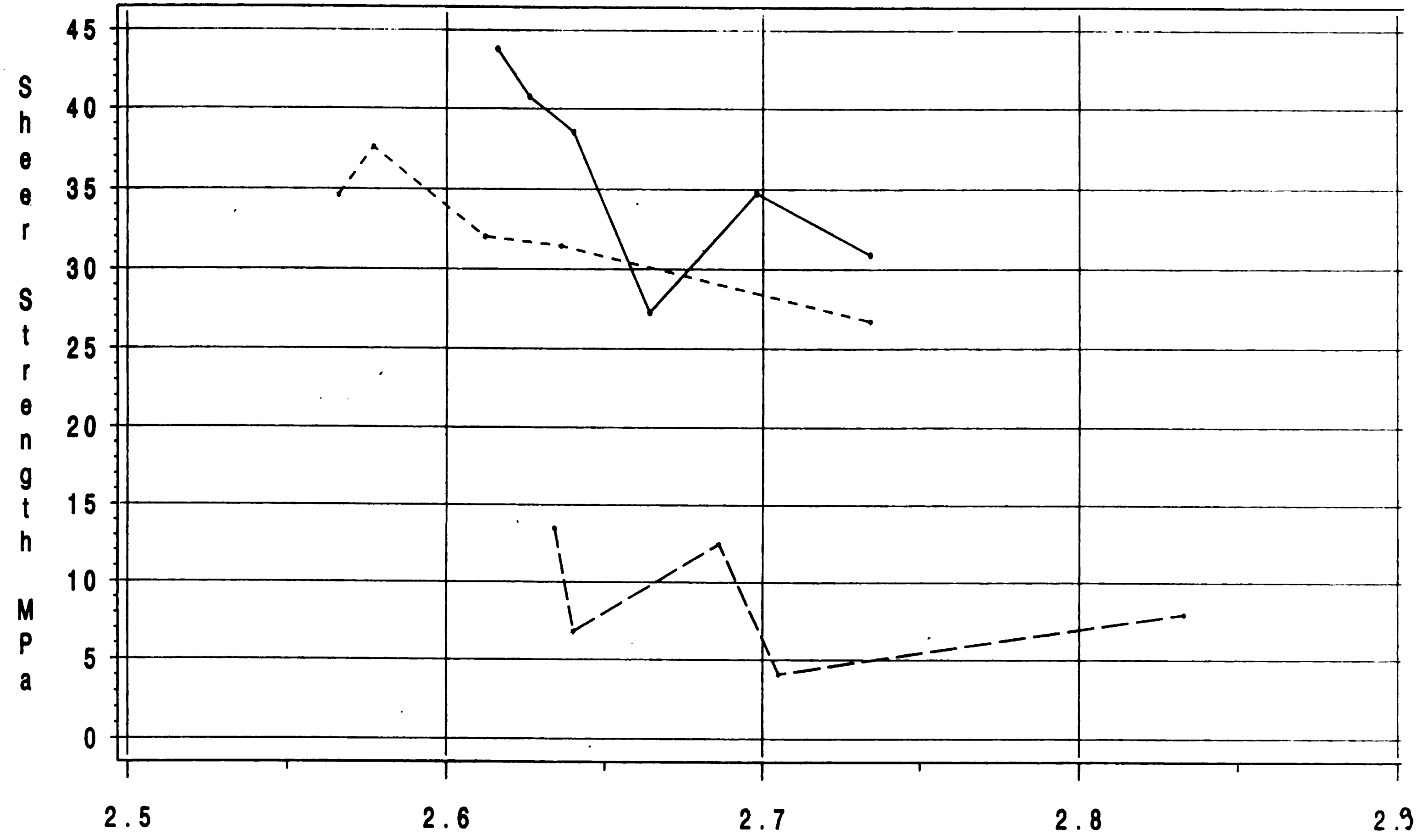

Fig. 5.2.2

Program: e:\Hung Pham 1\read_excel.sas Programmer: Robert S. Wilson Run Date: Friday, 3 May 2002 at 10:11:24

Hung Pham 1 Bond Strength Study: CF = Solid Line SB = Short Dash OU = Long Dash

Dentition - Tooth Type=Permanent Treatment - Time in Seconds=20 Tissue of Tooth=Enamel

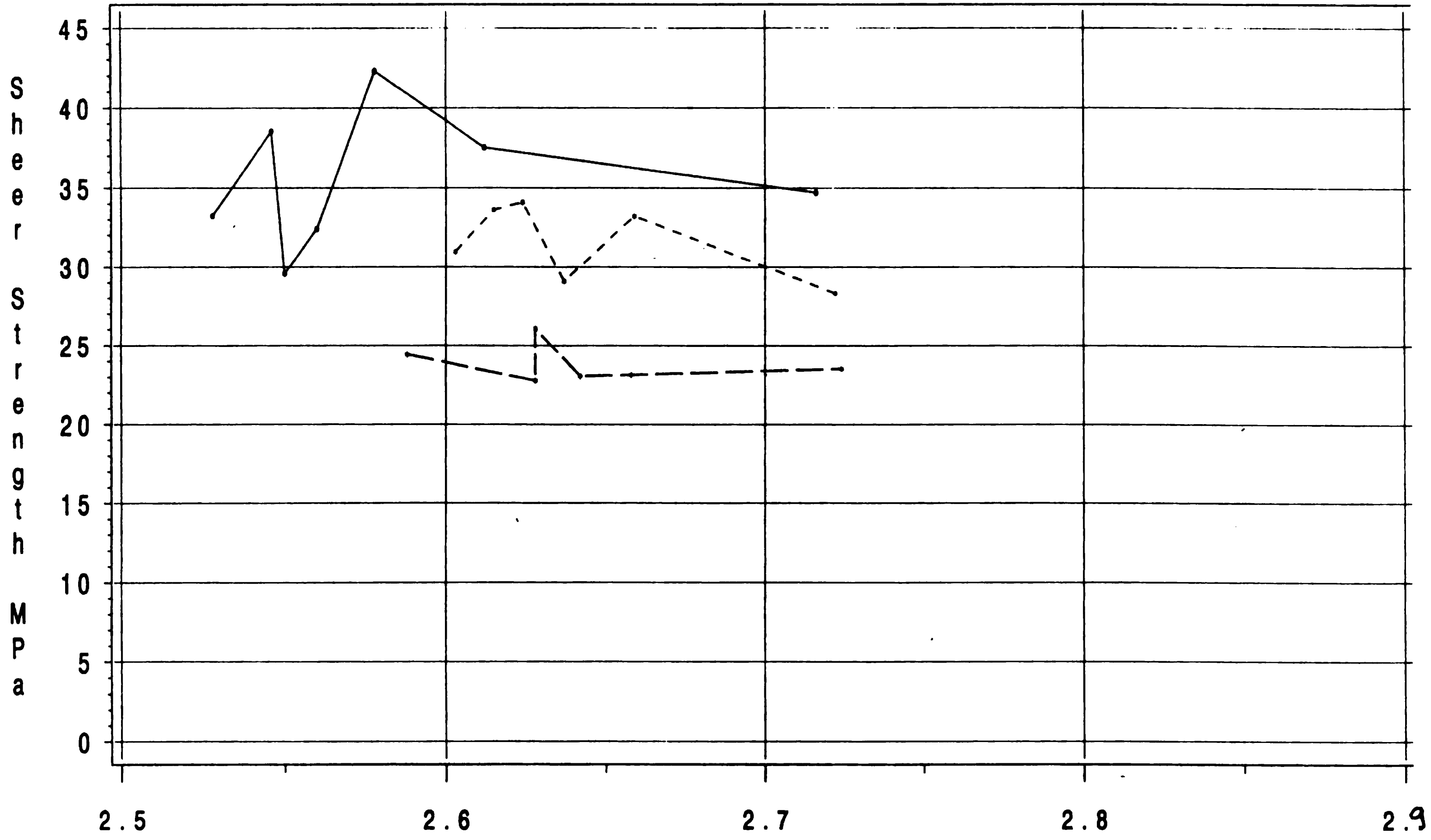

Fig. 5.2.3

Program: c:\Hung Pham 1\read_excel.sas Programmer: Robert S. Wilson Run Date: Friday, 3 May 2002 at 18:23:27

Hung Pham 1 Bond Strength Study: CF = Solid Line SB = Short Dash OU = Long Dash

Dentition - Tooth Type=Permanent Treatment - Time in Seconds=20 Tissue of Tooth=Dentin

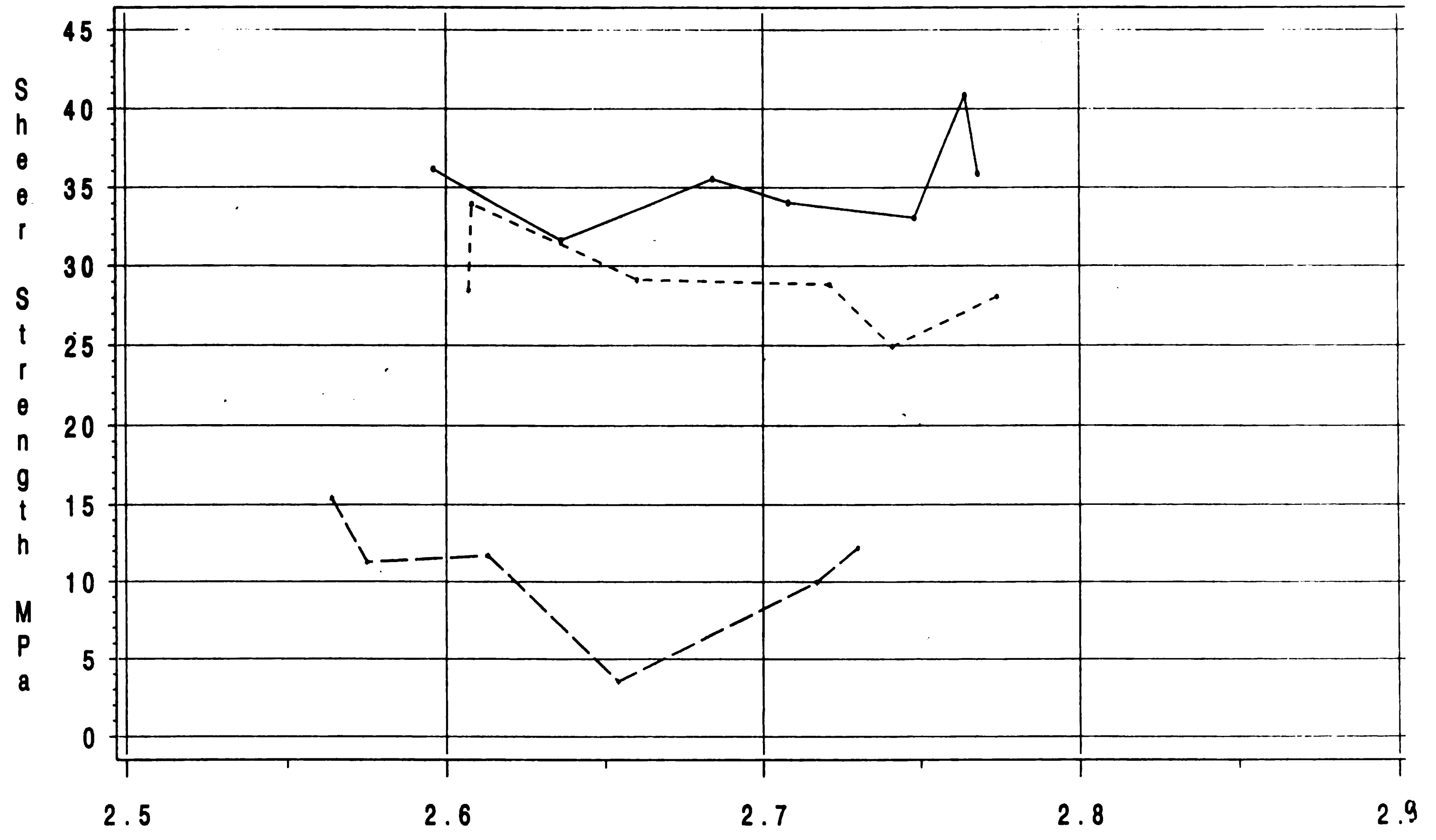

Fig. 5.2.4

Program: c:\Hung Pham 1\read_excel.sas Programmer: Robert S. Wilson Run Date: Friday, 3 May 2002 at 18:23:27

5.3 SBS to Permanent Enamel is Comparable to Primary Enamel with Single Bond and Clearfil SE DBS

***Table 5.3.1:* Single Bond, Clearfil SE, and One Up (all 3 Etching Times)**

SUBSTRATE	SBS (MPa) -LSMEAN
Primary Enamel	21.7*
Permanent Enamel	27.7*

* *Significantly different ($p < 0.0001$)*

***Table 5.3.2:* Single Bond, Clearfil SE, and One Up (MRT only)**

SUBSTRATE	SBS (MPa) -LSMEAN
Primary Enamel	21.7*
Permanent Enamel	30.2*

* *Significantly different ($p < 0.0001$)*

***Table 5.3.3:* Single Bond and Clearfil SE only (MRT only)**

SUBSTRATE	SBS (MPa) -LSMEAN
Primary Enamel	28.3^
Permanent Enamel	30.7^

^ *Not significantly different ($p = 0.0742$)*

When comparing enamel bond strengths between permanent and primary teeth with respect to the 3 DBS, permanent enamel bond strength of 27.7 MPa was significantly higher than 21.7 MPa of primary enamel. At the MRT, again permanent enamel SBS of 30.2 MPa was still greater than primary enamel SBS of 21.7 MPa. However, since One Up yielded inconsistent bond strength, it makes more sense to analyze Single Bond and Clearfil SE only. With just these two DBS, permanent enamel SBS of 30.7 MPa was not significantly different than 28.3 MPa for primary enamel.

5.4 Bond to Primary Dentin is just as Strong if not Stronger than Bond to Permanent Dentin

***Table 5.4.1:* Single Bond, Clearfil SE, & One Up (all 3 Etching Times)**

SUBSTRATE	SBS (MPa) -LSMEAN
Primary Dentin	25.0^
Permanent Dentin	25.3^

^ *Not Significantly different (p = 0.7380)*

***Table 5.4.2:* Single Bond, Clearfil SE, & One Up (MRT only)**

SUBSTRATE	SBS (MPa) -LSMEAN
Primary Dentin	25.6^
Permanent Dentin	25.0^

^ *Not Significantly different (p = 0.7064)*

***Table 5.4.3:* Single Bond & Clearfil SE only (MRT only)**

SUBSTRATE	SBS (MPa) -LSMEAN
Primary Dentin	34.0*
Permanent Dentin	31.3*

* *Significantly different (p = 0.0497)*

Most studies indicate that the bond to permanent dentin is stronger than to primary dentin. In this study, when the SBS of all 3 DBSs were analyzed, the SBS for permanent dentin was 25.3 MPa, which was not significantly higher than 25.0 MPa for primary dentin. For a more clinically applicable comparison, the SBS of the 3 DBSs were evaluated at the MRT. Again, SBS of 25.0 MPa for permanent dentin was not significant different from 25.6 MPa for primary dentin. To further confirm this finding, the SBS of

only Single Bond and Clearfil SE were analyzed. One Up data was not used since its SBS values have high standard deviations and it does not yield a reliable bond. With these 2 DBSs, the SBS of permanent dentin (31.2 MPa) was significantly lower than the SBS to primary dentin (34.0 MPa). Thus, bond strength to primary dentin was as strong if not stronger than to permanent dentin for these systems.

5.5 Bond to Permanent Teeth is Comparable to Bond to Primary Teeth

Table 5.5.1:* *Single Bond, Clearfil SE, & One Up (all 3 Etching Times)

Single Bond

SUBSTRATE	SBS (MPa) -LSMEAN
Primary Teeth	28.8^
Permanent Teeth	30.3^

^ *Not Significantly different (p = 0.1599)*

Clearfil SE

SUBSTRATE	SBS (MPa) -LSMEAN
Primary Teeth	32.6^
Permanent Teeth	32.2^

^ *Not Significantly different (p = 0.7069)*

One Up

SUBSTRATE	SBS (MPa) -LSMEAN
Primary Teeth	9.1*
Permanent Teeth	17.0*

* *Significantly different ($p < 0.0001$)*

Table 5.5.2: Single Bond, Clearfil SE, & One Up (MRT only)

Single Bond

SUBSTRATE	SBS (MPa) –LSMEAN
Primary Teeth	28.9^
Permanent Teeth	30.2^

^ *Not Significantly different* ($p = 0.4761$)

Clearfil SE

SUBSTRATE	SBS (MPa) –LSMEAN
Primary Teeth	33.4^
Permanent Teeth	35.4^

^ *Not Significantly different* ($p = 0.2489$)

One Up

SUBSTRATE	SBS (MPa) –LSMEAN
Primary Teeth	8.6*
Permanent Teeth	17.3*

* *Significantly different* ($p < 0.0001$)

When the average SBS values for the 3 different etching times were evaluated, the SBS of Single Bond permanent teeth (30.3 MPa) was not significantly higher than 28.8 MPa for primary teeth. Similarly for Clearfil SE, permanent teeth yielded SBS of 32.2 MPa, which was not significantly lower than 32.6 MPa for primary teeth. Thus, for Single Bond and Clearfil SE, SBS to primary teeth was comparable to that of permanent teeth. With One Up, however, permanent teeth yielded SBS of 17.0 MPa, which was significantly higher than 9.1 MPa for primary teeth. Hence, bond strength to permanent teeth was significantly stronger than to primary teeth with One Up.

When looking specifically at the MRT to see if bond strength to primary teeth was comparable to permanent teeth, the results were as follows. At the MRT for Single Bond, SBS for permanent teeth of 30.2 MPa was comparable to that for primary teeth of 28.9 MPa. Likewise, for Clearfil SE, SBS of 35.4 MPa for permanent teeth was not significantly different from 33.4 MPa for primary teeth. However, for One Up, the SBS for permanent teeth at the MRT was 17.3 MPa, which was significantly higher than 8.6 MPa for primary teeth. Thus, this finding corroborates with previous findings for all three etching times that for Single Bond and Clearfil SE, the bond strength to primary teeth was as strong as to permanent teeth. With One Up, however, bond strength to permanent teeth was stronger than to primary teeth.

5.6 Bond to Primary Enamel is Comparable to Bond to Primary Dentin

***Table 5.6.1:* Single Bond & Clearfil SE only (all 3 etching times)**

SUBSTRATE	SBS (MPa) –LSMEAN
Primary Enamel	28.2*
Primary Dentin	33.2*

** Significantly different ($p < 0.0001$)*

***Table 5.6.2:* Single Bond & Clearfil SE only (MRT only)**

SUBSTRATE	SBS (MPa) –LSMEAN
Primary Enamel	28.5*
Primary Dentin	34.0*

** Significantly different ($p = 0.0013$)*

Few studies have been done on primary teeth. Most studies have shown that bonding to primary enamel is stronger than to primary dentin. In this study, the opposite was true.

When looking specifically at the 2 DBSs, Single Bond and Clearfil SE, primary enamel SBS of 28.2 MPa was significantly lower than 33.2 MPa of primary dentin.

Specifically evaluating SBS of primary enamel versus primary dentin at the MRT, it was also found that primary dentin had a significantly higher SBS of 34.0 MPa compared to 28.5 MPa for primary enamel. Thus, bond to primary dentin was stronger than bond to primary enamel.

5.7 Bond to Permanent Dentin is Comparable to Bond to Permanent Enamel

***Table 5.7.1:* Single Bond & Clearfil SE only (all 3 etching times)**

SUBSTRATE	**SBS (MPa) –LSMEAN**
Permanent Enamel	30.8^
Permanent Dentin	31.7^

^ *Not significantly different ($p < 0.3474$)*

***Table 5.7.2:* Single Bond & Clearfil SE only (MRT only)**

SUBSTRATE	**SBS (MPa) –LSMEAN**
Permanent Enamel	33.5^
Permanent Dentin	32.2^

^ *Not significantly different ($p < 0.3776$)*

Similar statistical analysis was carried out on permanent teeth to compare enamel bond strength to dentin bond strength. For permanent teeth (looking at all 3 etching times), enamel SBS of 30.8 MPa was not significantly different from dentin SBS of 31.7 MPa. This was further confirmed by looking at the MRT for Single Bond and Clearfil SE. Again, permanent enamel bond strength of 33.5 MPa was not significantly different fr

st

from permanent dentin bond strength of 32.2 MPa. Hence, unlike primary teeth, bond strength to dentin was comparable to that of enamel with permanent teeth.

5.8 Bond to Dentin (Primary and Permanent) is Stronger than Bond to Enamel (Primary and Permanent) with Single Bond and Clearfil SE

Table 5.8.1: ***Single Bond, Clearfil SE, & One Up (All 3 etching times)***

Single Bond

SUBSTRATE	SBS (MPa) –LSMEAN
Enamel	28.5*
Dentin	30.6*

* *Significantly different ($p = 0.0499$)*

Clearfil SE

SUBSTRATE	SBS (MPa) –LSMEAN
Enamel	30.5*
Dentin	34.3*

* *Significantly different ($p = 0.0003$)*

One Up

SUBSTRATE	SBS (MPa) –LSMEAN
Enamel	15.5*
Dentin	10.6*

* *Significantly different ($p < 0.0001$)*

***Table 5.8.2*: Single Bond & Clearfil SE only**

5s Etch:

SUBSTRATE	SBS (MPa) -LSMEAN
Enamel	27.6*
Dentin	33.8*

* *Significantly different* ($p < 0.0001$)

60s Etch:

SUBSTRATE	SBS (MPa) -LSMEAN
Enamel	33.5^
Dentin	32.2^

^ *Not significantly different* ($p < 0.6877$)

Most studies have shown that the bond to enamel is stronger than the bond to dentin. In this part of the study, enamel bonding (permanent and primary enamel) was compared to dentin bonding (permanent and primary dentin). For Single Bond, SBS to enamel of 28.5 MPa was significantly lower than 30.6 MPa of dentin. Similarly, for Clearfil SE, enamel SBS of 30.5 MPa was also significantly lower than 34.3 MPa for dentin. However, the reverse was seen for One Up. With One Up, enamel SBS of 15.5 MPa was significantly higher than 10.6 MPa of dentin. Thus, with Single Bond and Clearfil SE, bond obtained with dentin was significantly higher than with enamel. In contrast, the bond to enamel was stronger than to dentin with One Up.

To further confirm the stronger bond obtained with dentin, SBS for dentin and enamel were evaluated at 5 sec and 60 sec etching times using Single Bond and Clearfil SE. At 5 sec, the enamel SBS of 27.6 MPa was significantly less than 33.8 MPa for dentin. At 60 sec, the enamel SBS of 29.9 MPa was not significantly different from 30.4 MPa for

dentin. Thus, for Single Bond and Clearfil SE, dentin bonding is as strong if not stronger than enamel bonding when these substrates were etched for 5 sec or 60 sec.

5.9 Primary Teeth Require Shorter Etching Time than Permanent Teeth

***Table 5.9.1:* Single Bond, Clearfil SE, & One Up**

Primary Teeth:

ETCHING TIME	SBS (MPa) –LSMEAN
5 sec	22.8^
MRT	23.7^
60 sec	24.0^

^ Not significantly different ($p < 0.4742$)

Permanent Teeth:

ETCHING TIME	SBS (MPa) –LSMEAN
5 sec	24.7*
MRT	27.7^
60 sec	27.2^

** Significantly different* ($p < 0.0084$)
^ Not significantly different

***Table 5.9.2:* Single Bond & Clearfil SE Only**

Primary Teeth:

ETCHING TIME	SBS (MPa) –LSMEAN
5 sec	31.0^
MRT	31.4^
60 sec	29.9^

^ Not significantly different ($p < 0.5201$)

Permanent Teeth:

ETCHING TIME	SBS (MPa) -LSMEAN
5 sec	30.4^
MRT	32.8^
60 sec	30.4^

^ *Not significantly different ($p < 0.0784$)*

Primary teeth are less mineralized than permanent teeth, and thus, it is logical that they would require shorter etching time. When testing all 3 DBSs, there was no significant difference in SBS for primary teeth when the substrates were etched for 5 sec, MRT, or 60 sec. For permanent teeth, however, the MRT and 60 sec etch both resulted in significantly higher SBS than the 5 sec etch. This suggests that primary teeth can be etched for as short as 5 sec and still achieve high bond strength. For permanent teeth, MRT is needed to obtain optimum bond strength.

When only Single Bond and Clearfil SE systems were analyzed, the same trend was obtained. Even though both primary and permanent teeth did not show a significant difference in SBS between the 5 sec, MRT, and 60 sec etch, the trend was still evident. Permanent teeth tended to have higher SBS at the MRT compared to the 5 sec etch, suggesting that etching of permanent teeth should be at least for the MRT. Primary teeth, on the other hand, had comparable SBS at 5 sec and MRT, indicating that shorter etching time for primary teeth will not compromise bond strength.

5.10 Dentin Requires Shorter Etching Time than Enamel

***Table 5.10.1*: Single Bond & Clearfil SE (All 3 etching times)**

5s Etch

SUBSTRATE	SBS (MPa) -LSMEAN
Enamel	27.5*
Dentin	33.8*

* *Significantly different (p = 0.0001)*

MRT

SUBSTRATE	SBS (MPa) -LSMEAN
Enamel	31.1^
Dentin	33.1^

^ *Not significantly different (p = 0.1214)*

60s Etch

SUBSTRATE	SBS (MPa) -LSMEAN
Enamel	29.9^
Dentin	30.5^

^ *Not significantly different (p = 0.6341)*

***Table 5.10.2*: Single Bond & Clearfil SE (5s and 60s only)**

5s Etch

SUBSTRATE	SBS (MPa) -LSMEAN
Enamel	27.6*
Dentin	33.8*

* *Significantly different (p = 0.0001)*

60s Etch

SUBSTRATE	SBS (MPa) –LSMEAN
Enamel	29.9^
Dentin	30.4^

^ *Not significantly different (p = 0.6877)*

Dentin has less mineral content than enamel, and thus, would theoretically require shorter etching time than enamel. Etching time effects on enamel and dentin were evaluated with Single Bond and Clearfil SE. With both of these dentin bonding systems, enamel SBS of 27.5 MPa was significantly weaker than dentin SBS of 33.8 MPa when the substrates were etched for 5 sec. At the MRT and 60 sec etch, however, there was no difference in SBS between enamel and dentin. This finding that a shorter etching time may be effective for dentin bonding was confirmed when SBS of enamel and dentin were compared at 5 sec versus 60 sec etch. For enamel, 5 sec etch resulted in SBS of 27.6 MPa, which was not significantly different from 60 sec etch of 29.9 MPa. However, dentin etched for 5 sec resulted in SBS of 33.8 MPa, which was significantly stronger than SBS of 30.4 MPa obtained with 60 sec etch. Thus, enamel required a longer etching time to adequately etch the surface while dentin could be etched for a shorter period of time.

5.11 Self Etching Systems (Clearfil SE and One Up) Require Longer Etching Time than Separate Etch System (Single Bond)

***Table 5.11.1:** Single Bond, Clearfil SE, & One Up (5 sec & 60 sec Only))*

Single Bond

ETCHING TIME	SBS (MPa) -LSMEAN
5 sec	31.8*
60 sec	27.3*

* *Significantly different* ($p = 0.0011$)

Clearfil SE

ETCHING TIME	SBS (MPa) -LSMEAN
5 sec	29.7*
60 sec	33.0*

* *Significantly different* ($p = 0.0123$)

One Up

ETCHING TIME	SBS (MPa) -LSMEAN
5 sec	9.9*
60 sec	16.4*

* *Significantly different* ($p < 0.0001$)

Table 5.11.2: Single Bond, Clearfil SE, & One Up (5 sec, MRT, & 60 sec)

Single Bond

ETCHING TIME	SBS (MPa) –LSMEAN
5 sec	31.7*
MRT	29.6
60 sec	27.4*

** Significantly different ($p = 0.0053$)*

Clearfil SE

ETCHING TIME	SBS (MPa) –LSMEAN
5 sec	29.5*
MRT	34.6^
60 sec	33.0^

** Significantly different ($p = 0.0003$)*
^ Not significantly different

One Up

SUBSTRATE	SBS (MPa) –LSMEAN
5 sec	9.9*
MRT	12.9*
60 sec	16.4*

** Significantly different ($p < 0.0001$)*

In this section of the study, all four substrates' SBS values (permanent enamel, permanent dentin, primary enamel, and primary dentin) were collapsed to examine the etching efficiency of self-etching systems (Clearfil SE and One Up) versus a separate etch system (Single Bond). All 3 DBS tested were acid etch-time dependent. For Single

Bond, the SBS decreased with increasing etching time. With Clearfil SE and One Up, the SBS increased with prolonged etching time. This suggests that Single Bond, being a separate etch system, can etch much more efficiently than the SEP or SEA system, which contained a milder acid. This trend was confirmed when SBS of 5 sec etch were compared with the 60 sec etch. For Single Bond, 5 sec etch resulted in 31.8 MPa, which was significantly higher than the 60 sec etch of 27.3 MPa. The reverse was seen with the other 2 materials. For Clearfil SE, the 5 sec etch resulted in 29.7 MPa, which was significantly lower than the 60 sec etch of 33.0 MPa. Likewise, One Up had SBS of 9.8 MPa for 5 sec etch as compared to 16.4 MPa for 60 sec etch.

When all 3 DBSs were evaluated at all three acid etching times, a similar trend was observed. For Single Bond, highest bond strength was obtained with a 5 sec etch, and it progressively decreased with increasing etching time. For Clearfil SE, the MRT and 60 sec etch resulted in significantly higher SBS than the 5 sec etch, indicating that at least the MRT is needed for optimal bonding with this DBS. With One Up, SBS increased with longer etching time, with the highest SBS at 60 sec etch. Interestingly, although both Clearfil SE and One Up are self-etching systems, Clearfil SE resulted in the highest bond strength at the MRT while One Up did so at the 60 sec etch. This illustrates that the etching efficiency differs among different self-etching systems.

5.12 With SEP and SEA, the More Defined the Etching Pattern, the Stronger the SBS

With One Up, the highest and most consistent bond was obtained with permanent enamel, with an average bond strength of 21.5 MPa. This was followed by permanent dentin with a bond strength of 12.5 MPa, primary enamel with a bond strength of 9.5

MPa, and primary dentin with a bond strength of 8.8 MPa. With One Up, bond strength increased with increasing etching time with the highest bond strength at the 60 sec etch with all four substrates. When analyzing the etched surfaces of primary and permanent enamel, a more distinct etching pattern was seen with longer etching time. (Refer to ***Fig. 5.12.16 – Fig. 5.12.18*** for permanent enamel etching pattern, and ***Fig. 5.12.19 – Fig. 5.12.21*** for primary enamel etching pattern). Furthermore, the etched primary enamel appeared to have about the same roughness as the etched permanent enamel. As for dentin, enlarged dentinal tubules were seen with increasing etching time. However, the smear plugs were virtually intact, even at 60 sec SEA application time, as seen in ***Fig. 5.12.24***. Etched primary dentin appeared to have larger diameter tubules than etched permanent dentin (***Fig. 5.12.27***).

When analyzing the overall etching pattern of Clearfil SE versus One Up, Clearfil SE had a much more pronounced etching pattern than One Up. Unlike One Up, the etched enamel was much more defined, with a predominantly Type I enamel etching pattern, the most common enamel etching pattern according to Swift et al. (1995). Interestingly, the 5 sec enamel etch with Clearfil SE (***Fig. 5.12.7***) produced a similar etching pattern as a 60 sec etch with One Up (***Fig. 5.12.21***). Secondly, the etched dentin surface had larger diameter tubules with more of the smear plugs removed with Clearfil SE (***Fig. 5.12.14***) as compared to One Up (***Fig. 5.12.26***), indicating a stronger etch with Clearfil SE. Thus, the more distinct etching pattern associated with Clearfil SE was probably related to the significantly higher SBS as compared to One Up.

The etching pattern of Clearfil SE was specifically compared at the three etching times. When the 5 sec enamel etching pattern was analyzed (***Fig. 5.12.4***), the surface

was not as rough and it was not as well defined as with the 20 sec or 60 sec etch. The 60 sec enamel etch (***Fig. 5.12.6***) appeared to have the most pronounced etching pattern, although not markedly different from the 20 sec etching pattern (***Fig. 5.12.5***). Dentin etched for 5 sec still retained almost all of its dentin plugs, especially with permanent dentin (***Fig. 5.12.10***). At 20 sec (***Fig. 5.12.11***) and 60 sec (***Fig. 5.12.12***) etch, most of dentin plugs were removed with increased dissolution of the inner peritubular dentin wall, resulting in enlarged dentinal tubules. The etching pattern produced from the 20 sec etch was not markedly different from the 60 sec etch, although the 60 sec produced the most pronounced etching pattern. Thus, 20 sec etch with Clearfil SE seems to be the ideal time for three of the four substrates tested. Only primary dentin achieved optimal SBS with a 5 sec etch (***Fig. 5.12.13***) even though most of the smear plugs remained intact, which was not statistically different from the 20 sec etch (***Fig. 5.12.14***)

SINGLE BOND

Permanent & Primary Teeth

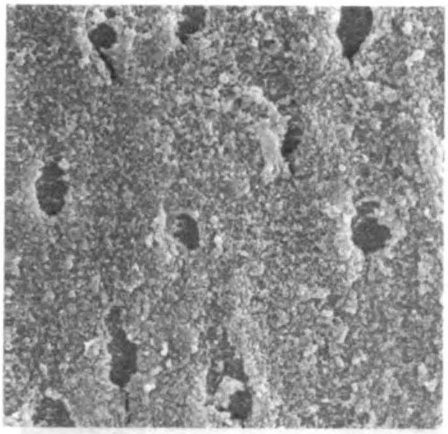

FIG. 5.12.1
SINGLE BOND, 15 SEC
PERMANENT DENTIN

Fig. 5.12.1 SEM image of permanent dentin etched with 35% phosphoric acid for the MRT (15 sec). Note the enlarged dentinal tubules due to dissolution of the peritubular dentin and the complete removal of the smear plugs.

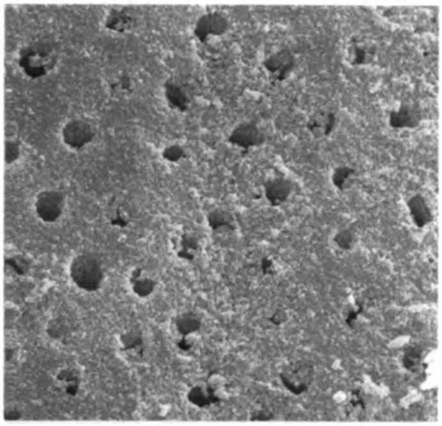

FIG. 5.12.2
SINGLE BOND, 15 SEC
PRIMARY DENTIN

Fig. 5.12.2 SEM image of primary dentin etched with 35% phosphoric acid for the MRT (15 sec). Again, note the enlarged dentinal tubules and the absence of the smear plugs.

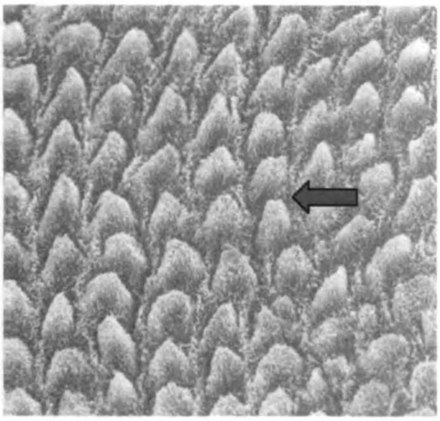

FIG. 5.12.3
SINGLE BOND, 15 SEC
PRIMARY ENAMEL

Fig. 5.12.3 SEM image of primary enamel etched with 35% phosphoric acid for the MRT (15 sec). Note the Type II enamel etching pattern -- preferential dissolution of the enamel prism peripheries as noted by the dark areas around the prism cores (green arrow).

CLEARFIL SE

Permanent Enamel

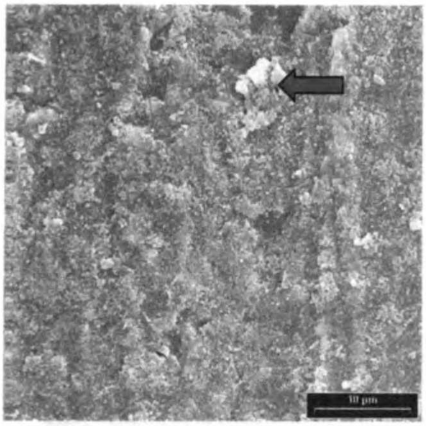

FIG. 5.12.4
CLEARFIL SE, 5 SEC
PERMANENT ENAMEL

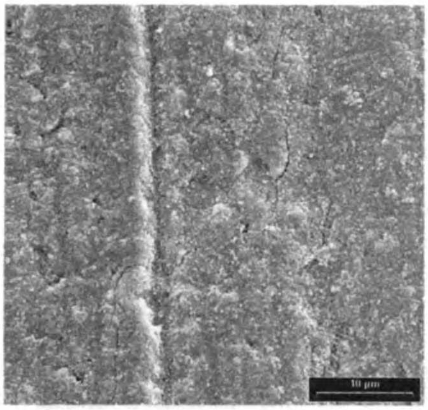

FIG. 5.12.5
CLEARFIL SE, 20 SEC
PERMANENT ENAMEL

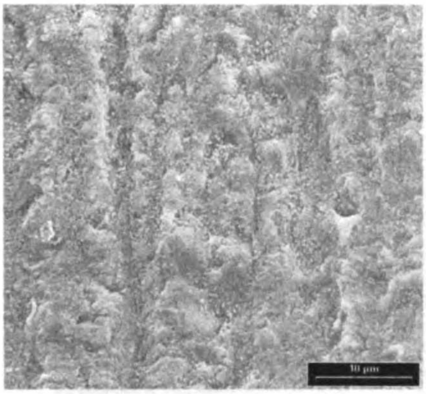

FIG. 5.12.6
CLEARFIL SE, 60 SEC
PERMANENT ENAMEL

Fig. 5.12.4-5.12.6 SEM images of permanent enamel etched with Clearfil SE SEP for 5 sec, MRT (20 sec), and 60 sec, respectively. When the SEP was applied for 5 sec, the etching pattern appeared indistinct with some smear debris still left as indicated by the arrow in **Fig. 5.12.4**. The enamel prisms were not visible. At the 20 sec SEP application time, the smear debris was dissolved, and the etching pattern was more distinct. At the 60 sec SEP application time, the etching pattern was the most pronounced of the 3 images. The enamel prisms could be identified. Overall, permanent enamel was not as reactive to the SEP application as primary enamel (as noted in **Fig. 5.12.5** {permanent enamel} and **Fig. 5.12.8** {primary enamel}).

FIG. 5.12.7
CLEARFIL SE, 5 SEC
PRIMARY ENAMEL

FIG. 5.12.8
CLEARFIL SE, 20 SEC
PRIMARY ENAMEL

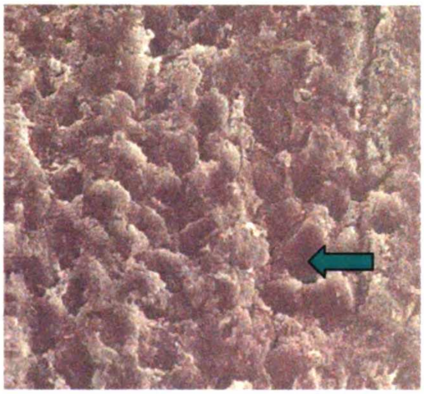

FIG. 5.12.9
CLEARFIL SE, 60 SEC
PRIMARY ENAMEL

CLEARFIL SE

Primary Enamel

Fig. 5.12.7-5.12.9 SEM images of primary enamel etched with Clearfil SE SEP for 5 sec, MRT (20 sec), and 60 sec, respectively. With the 5 sec SEP application time, the etching pattern was not very distinct; the enamel prisms were barely visible. At the MRT, the etching pattern was well defined with a Type I etching pattern (preferential dissolution of the enamel prism cores as indicated by the arrow in **Fig. 5.12.9**). At the 60 sec application time, the etching pattern was most pronounced, further confirming the Type I etching pattern. Overall, primary enamel was more reactive to the SEP application than permanent enamel.

CLEARFIL SE

Permanent Dentin

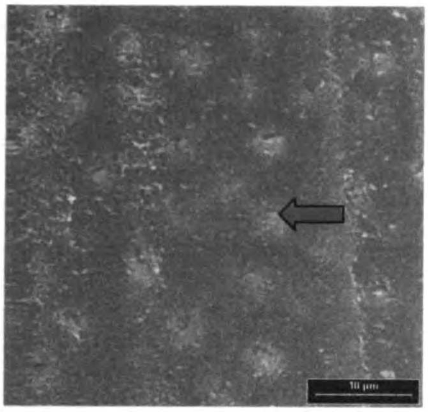

FIG. 5.12.10
CLEARFIL SE, 5 SEC
PERMANENT DENTIN

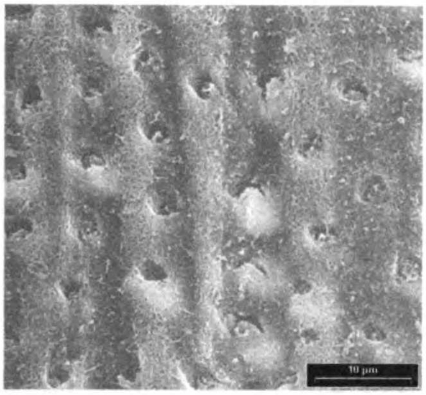

FIG. 5.12.11
CLEARFIL SE, 20 SEC
PERMANENT DENTIN

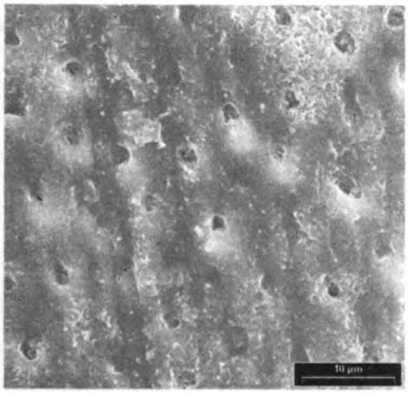

FIG. 5.12.12
CLEARFIL SE, 60 SEC
PERMANENT DENTIN

Fig. 5.12.10-5.12.12 SEM images of permanent dentin etched with Clearfil SE SEP for 5 sec, MRT (20 sec), and 60 sec, respectively. At the 5 sec application time, the dentinal tubules could be seen, although they were obliterated by smear plugs (as indicated by the arrow in the **Fig. 5.12.10**). At the 20 sec application time, the dentinal tubules were enlarged and more of the smear plugs were dissolved. At the 60 sec application time, most of the smear plugs were removed.

CLEARFIL SE

Primary Dentin

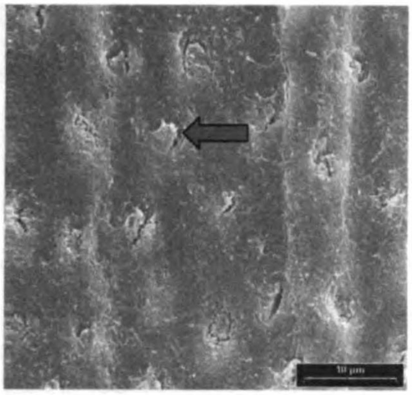

FIG. 5.12.13
CLEARFIL SE, 5 SEC
PRIMARY DENTIN

Fig. 5.12.13-Fig. 5.12.15 SEM images of primary dentin etched with Clearfil SE SEP for 5 sec, MRT (20 sec), and 60 sec, respectively. At the 5 sec application time, the dentinal tubules could be clearly seen with the smear plugs intact as indicated by the arrow in **Fig. 5.12.13**. At the 20 sec application time, the dentinal tubules were enlarged, and the smear plugs were completely removed. At the 60 sec application time, the dentinal tubules were further enlarged. Overall, primary dentin was more reactive to the Clearfil SE SEP than permanent dentin.

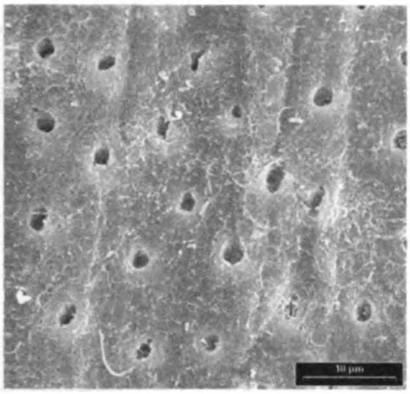

FIG. 5.12.14
CLEARFIL SE, 20 SEC
PRIMARY DENTIN

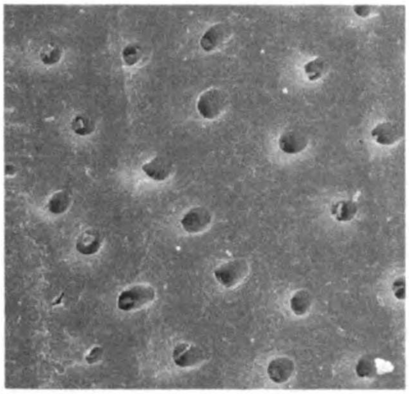

FIG. 5.12.15
CLEARFIL SE, 60 SEC
PRIMARY DENTIN

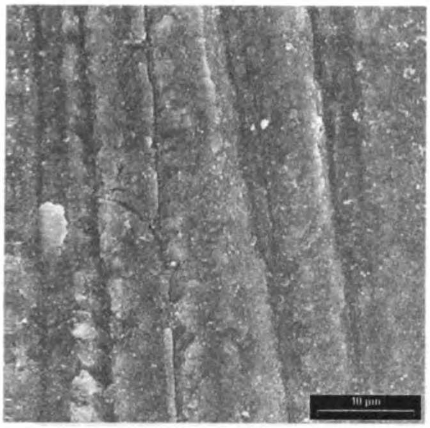

FIG. 5.12.16
ONE UP, 5 SEC
PERMANENT ENAMEL

ONE UP

Permanent Enamel

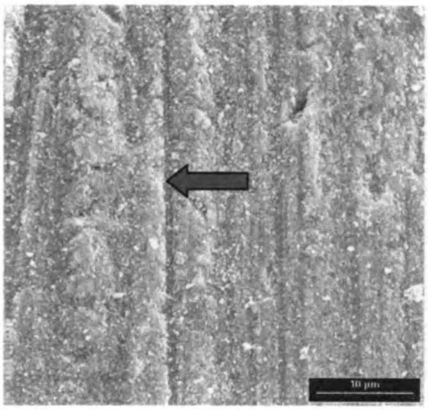

FIG. 5.12.17
ONE UP, 20 SEC
PERMANENT ENAMEL

Fig. 5.12.16-Fig.5.12.18 SEM images of permanent enamel etched with One Up SEA for 5 sec, MRT (20 sec), and 60 sec, respectively. At the 5 sec application time, the etching pattern appeared very indistinct; the enamel prisms were not visible. Even though the etching pattern was progressively more pronounced with the 20 sec and the 60 sec application times, the enamel prisms could not be visualized even at the 60 sec application time. Overall, One Up SEA etched less effectively than Clearfil SE SEP and Single Bond 35% phosphoric acid etchant.

Note: polishing scratches/lines were seen as vertical lines in these images as indicated by the arrow in **Fig. 5.12.17.**

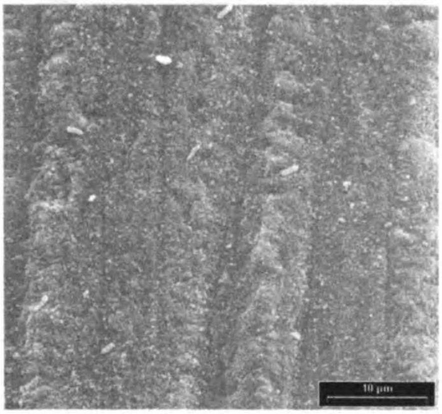

FIG. 5.12.18
ONE UP, 60 SEC
PERMANENT ENAMEL

FIG. 5.12.19
ONE UP, 5 SEC
PRIMARY ENAMEL

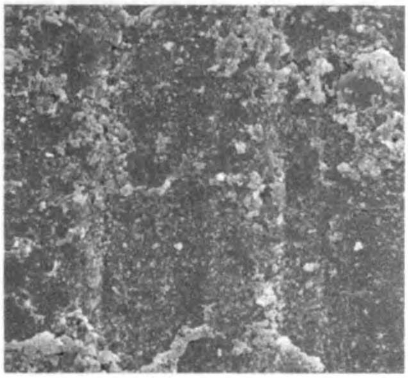

FIG. 5.12.20
ONE UP, 20 SEC
PRIMARY ENAMEL

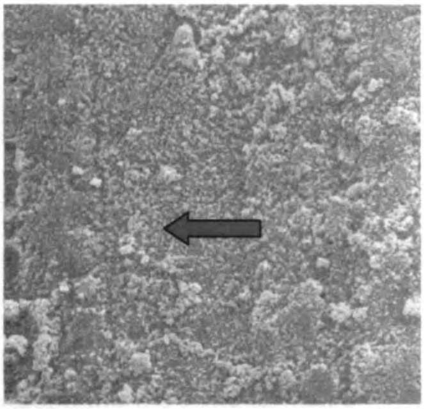

FIG. 5.12.21
ONE UP, 60 SEC
PRIMARY ENAMEL

ONE UP

Primary Enamel

Fig. 5.12.19-Fig.5.12.21 SEM images of primary enamel etched with One Up SEA for 5 sec, MRT (20 sec), and 60 sec, respectively. At the 5 sec application time, the etching pattern was indistinct. The 20 sec application time resulted in a more pronounced etching pattern, although the enamel prisms could not be seen until the 60 sec application time (as indicated by the arrow in **Fig. 5.12.21**). Overall, primary enamel was more reactive to the One Up SEA than permanent enamel.

ONE UP

Permanent Dentin

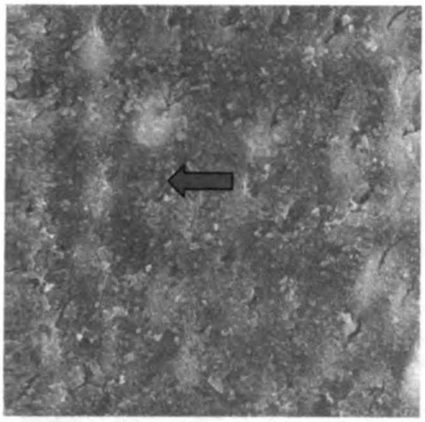

FIG. 5.12.22
ONE UP, 5 SEC
PERMANENT DENTIN

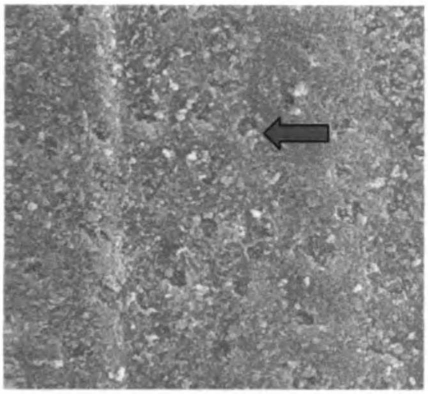

FIG. 5.12.23
ONE UP, 20 SEC
PERMANENT DENTIN

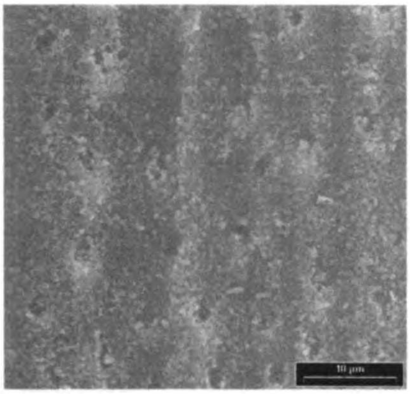

FIG. 5.12.24
ONE UP, 60 SEC
PERMANENT DENTIN

Fig. 5.12.22-Fig. 5.12.24 SEM images of permanent dentin etched with One Up SEA for 5 sec, MRT (20 sec), and 60 sec, respectively. With the 5 sec application time, the dentinal tubules could be seen, although they were mostly obliterated by dentinal smear plugs, as indicated by the arrow in **Fig. 5.12.22**. At the 20 sec application time, more of the peritubular dentin was etched, resulting in enlarged dentinal tubules as indicated by the arrow in **Fig. 5.12.23**. However, the etching pattern was still not very distinct. At the 60 sec application time, the etching pattern was the most pronounced of the 3 etching times. However, smear plugs were still partially present in the dentinal tubules, indicating the inefficient etching property of the One Up SEA. Overall, permanent dentin was more reactive to the Clearfil SEP than to the One Up SEA.

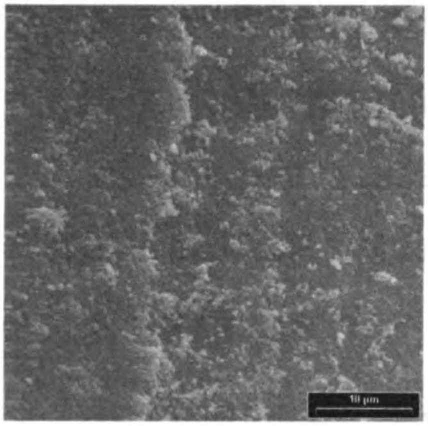

FIG. 5.12.25
ONE UP, 5 SEC
PRIMARY DENTIN

ONE UP

Primary Dentin

Fig. 5.12.25-Fig.5.12.27 SEM images of primary dentin etched with One Up SEA for 5 sec, MRT (20 sec), and 60 sec, respectively. At the 5 sec application time, the etching pattern was not clear; the dentinal tubules could not be seen. The dentinal tubules were more apparent at the 20 sec application time as indicated by the arrow in **Fig. 5.12.26**. At the 60 sec application time, the dentinal tubules were much more enlarged and most of the smear plugs were removed. Overall, primary dentin was more reactive to One Up SEA than permanent dentin as noted by **Fig. 5.12.24** (permanent dentin) and **Fig. 5.12.27** (primary dentin).

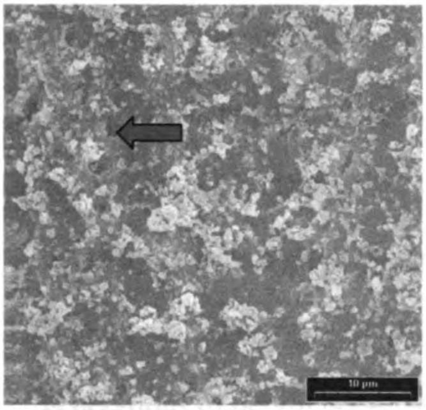

FIG. 5.12.26
ONE UP, 20 SEC
PRIMARY DENTIN

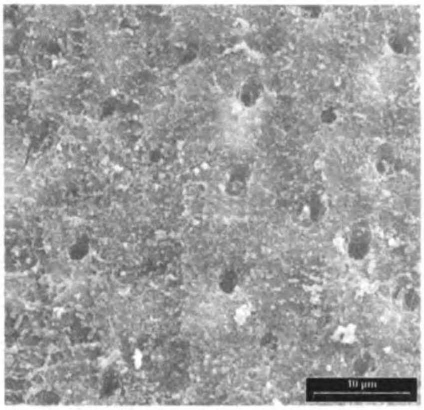

FIG. 5.12.27
ONE UP, 60 SEC
PRIMARY DENTIN

5.13 Overall, Clearfil SE has the Highest SBS, Followed by Single Bond and Finally One Up

Overall, Clearfil SE with its average SBS of 32.4 MPa, had the highest bond strength. This was followed by Single Bond with 29.6 MPa and One Up with 13.1 MPa.

6. DISCUSSION

Traditional amalgam and cast restorations have gradually grown out of favor as health concerns over chronic mercury exposure and demand for esthetic, conservative dentistry increased. Thus, composite and bonding technology allowing for conservation of tooth structure, micromechanical bonding property, and most importantly, esthetics, are quickly replacing traditional dentistry (Sturdevant et al., 1995). The bonding technology has improved tremendously since Buonocore's time. This wave of the future for dentistry creates a great impetus for newer and better bonding systems. Thus, since Buonocore's discovery of enamel etching (Buonocore, 1955), there have been 5 generations of dentin bonding system, self-etching primer system, and the latest all-in-one self-etching adhesive system (Lopes et al., 2002). These newer dentin bonding systems generally result in improved bond strength, decreased microleakage, and are simpler to use (Perdigao et al., 2000).

With all the focus on dentin bonding research, it is surprising to note that very few studies have been done on bonding to primary teeth (Nor et al., 1996). In fact, most of the bonding research (in vitro as well as clinical studies) has been done on permanent teeth (Araujo et al., 1997). Even the dentin bonding manufacturers only include bonding instructions for permanent teeth (Agostini et al., 2001). When looking at the compositional and morphological differences between primary and permanent teeth, it is

reasonable to assume that a different bonding protocol, especially etching time, may be needed for primary teeth. First of all, primary enamel tends to be thinner and more aprismatic than permanent enamel (Agostini et al., 2001). Secondly, primary dentin tends to be less mineralized and have fewer and smaller diameter dentinal tubules than permanent teeth (Nor et al., 1997). Thirdly, primary dentin can have giant tubules that have not been reported in permanent dentin (Liu et al., 2000). These differences suggest that a differentiated etching protocol may be necessary for primary teeth. Thus, the purpose of this study was to evaluate the SBS to primary and permanent teeth and determine whether the etching protocol for permanent teeth should be applied to primary teeth.

The discussion section is divided into 6 major categories as follows:

- The effect of hole diameter on SBS
- SBS for permanent teeth versus primary teeth
- SBS for enamel versus dentin
- The effect of etching time on SBS
- The effect of etching pattern on SBS
- Ranking of the dentin bonding systems tested

6.1 Hole Diameter has no Effect on SBS

Holes were punched in mylar tape to create a standardized area for shear bond strength tests. Hole diameter ranged from 2.5 mm to 2.8 mm, due to difficulty in creating exactly the same size hole each time with a hole punch. Thus, the concern was whether hole size made a difference in shear bond strength results. To account for the difference in hole diameter, after the SBS tests, diameter values were established by taking an

average of two measurements of the hole diameter at 90 degrees to each other, using the microscope. The variation in hole diameter was taken into account when failure load measurements were converted to SBS values. Furthermore, when the SBS values obtained were graphed against the hole diameters, it was found that there was no relationship between hole diameters and shear bond strength ***(Refer to Figures 5.2.1-5.2.4)***. This is an important observation because any differences in shear bond strength were either due to differences in substrates tested or dentin bonding systems used, and not because of variation in hole diameter. Thus, this study was conducted on the premise that the small variation in hole diameter has no effect on shear bond strength result.

6.2 SBS of permanent versus primary teeth

Key points:

1. Bond to primary enamel was not significantly different from bond to permanent enamel
2. Bond to primary dentin was at least as strong as to permanent dentin
3. Bond to primary teeth was not significantly different from bond to permanent teeth

6.2.1 Enamel Bonding

The concept that bonding to primary teeth is inferior to permanent teeth is mostly supported by studies done with earlier generations of DBS; newer generations are less likely to show significant difference in SBS between primary and permanent teeth (Araujo et al., 1997). When looking at previous studies specifically on primary versus permanent enamel bond strengths, most studies indicated that primary enamel bond strength was comparable to permanent enamel (Malferrari et al., 1994) (Gwinnett and

Garcia-Godoy, 1992). In fact, when sealants were placed on primary second molars versus permanent first molars in vivo, 6 and 12 month observations showed no difference in retention rate between primary and permanent molars (Duggal et al., 1997). In this study, permanent enamel SBS was significantly higher than primary enamel SBS when all three DBSs were evaluated. However, this result was skewed by One Up's abnormally low SBS data. One Up had low, erratic bond values to all three substrates tested (permanent dentin, primary enamel and primary dentin) except for permanent enamel. Since One Up performed very poorly in this study, it was dropped from the statistical analysis. When Single Bond and Clearfil SE were subsequently analyzed at the MRT, primary enamel SBS was still lower than permanent enamel SBS, although it was not statistically significant. Hence, although primary smooth enamel contains an outer layer of prismless enamel that is more resistant to etching (Agostini et al., 2001), as long as this layer was removed, etching property and bond strength were similar to those of permanent enamel (Malferrari et al., 1994).

It is interesting to note that One Up provides strong bond to permanent enamel, but not to any other substrates, including primary enamel. This suggests that either there is a significant difference in the structure of primary versus permanent enamel or that primary enamel is more difficult to etch. Primary teeth have less mineral than permanent teeth (Hirayama, 1990). Therefore, primary teeth should etch more efficiently than permanent teeth (Nor et al., 1997). However, primary enamel tends to have more prismless enamel, which makes acid etching less effective, producing a shallower etching pattern (Meola and Papaccio, 1986). In the present study, however, the superficial layer of primary enamel containing most of the prismless enamel was ground off to create a flat surface

for bonding. Thus, the problem of prismless enamel interfering with acid etching is not an issue. When the etching patterns of primary and permanent enamel produced by One Up were compared, the etched primary enamel seemed to have about the same or more pronounced etching pattern than the etched permanent enamel. Yet, bond strength to primary enamel was significantly lower than that to permanent enamel. This indicates that SBS is related to some other factors other than the roughness or the depth of the etched surface.

6.2.2 Dentin Bonding

With respect to dentin bonding, this study showed that bond strength to primary dentin was equal to or stronger than to permanent dentin. When all 3 DBSs were evaluated at the 3 acid etching times or the MRT alone, SBS of primary dentin was comparable to that of permanent dentin. This finding is in agreement with Burrow et al. who found no significant difference between the SBS of Single Bond to primary versus permanent dentin. Their bond values of 18.2+/-4.3 MPa for primary dentin and 21.6+/-4.5 MPa for permanent dentin reflect the higher bond strengths usually obtained with newer DBSs (2002).

However, when One Up was dropped from the analysis due to its low, erratic bond values, leaving Single Bond and Clearfil SE, SBS of primary dentin was significantly higher than that of permanent dentin. This is in contrast to findings from most studies. (Nor et al., 1997) (Nor et al., 1996) (Salama and Tao, 1991) (Bordin-Aykroyd et al., 1992) (Burrow et al., 2002). A study done in 1991 using Gluma DBS compared bond strength between primary and permanent dentin. The occlusal surfaces of primary molars and permanent premolars were prepared into dentin before bonding. Bond strength of

8.4+/-4.6 MPa to primary dentin was significantly lower than 12.1+/-3.2 MPa to permanent dentin. The author attributed this difference in SBS between primary and permanent dentin partly to the dentin preparation depth to achieve a flat bonding surface. Primary molars have different occlusal groove depth and thinner dentin thickness than permanent premolars (Salama and Tao, 1991). Furthermore, it is known that dentin structure and composition varies depending on how far the bonding surface is from the pulp (Marshall, 1993). In other words, dentin structure and composition are related to the remaining dentin thickness. Thus, variation in bond strength between primary dentin samples or between primary and permanent dentin samples can be due to variation in the intratooth locations of the specimens. Most studies suggest that bond strength is reduced as the pulp is approached (Kanca, 1997). However, one study comparing bond strength to superficial versus deep dentin using Clearfil SE found that dentin depth did not influence SBS (Toledano et al., 2001). In the present study, the buccal surface of primary anterior teeth and the mesial or distal surface of permanent third molars were prepared for bonding. Thus, we were able to avoid the problem of occlusal groove depth as described above. However, we do not have a constant intratooth location, especially for primary teeth. This is because primary anterior teeth have a very small surface area. It is technically challenging if not impossible to create an adequate flat surface area for bonding and still maintain the same distance from the pulp for each sample.

Differences in bond strength between primary and permanent dentin may also be explained by hybrid layer thickness. Studies looking at hybrid layer thickness generally found a thicker hybrid layer in primary dentin as compared to permanent dentin when they were etched for the same period of time. This suggests that primary dentin is more

reactive to acidic conditioning than permanent dentin (Nor et al., 1996) (Nor et al., 1997). In addition, Nor et al. noted a higher incidence of incomplete resin infiltration to the full extent of the demineralized depth when primary dentin was etched per MRT. This gap, resulting from incomplete infiltration of monomers, is basically a demineralized area at the bottom of the hybrid layer with exposed collagen that is not supported by the mineral or resin encapsulation. Thus, Nor postulated that premature failure of this weak zone may be responsible for the lower bond strength to primary dentin. Hence, he recommended shorter etching time for primary dentin to obtain similar hybrid layer thickness as permanent dentin, and accordingly, higher bond strength (1996). In this study with primary dentin, the short etching time of 5 sec seemed to result in higher bond strength for Single Bond but not for Clearfil SE, although both were not significantly different from SBS obtained at MRT. This makes sense since Single Bond has a stronger etchant (37% phosphoric acid), which can etch the substrate much more efficiently than the milder acidic monomer of Clearfil SE in the 5 sec allotted. However, there was no significant drop in SBS value when primary dentin was over-etched for 60 sec with Single Bond and Clearfil SE, suggesting that over-etching and incomplete resin infiltration may not be a problem with the newer DBSs. Perhaps, the improved formulation of newer DBSs allows for complete infiltration of monomers to the full demineralized depth.

6.2.3 Primary versus Permanent SBS

The majority of the studies in the past only looked at one type of substrate (enamel or dentin) when comparing bond strength between primary and permanent teeth (Fagan et al., 1986) (Nor et al., 1996) (Salama and Tao, 1991) (Telles et al., 1998). Thus, they

could not conclude whether bonding to primary teeth, in general, was weaker than bonding to permanent teeth. In this study, bonding was done on all four substrates (permanent enamel, permanent dentin, primary enamel, and primary dentin). Thus, a generalization can be made about SBS of primary teeth versus permanent teeth. When looking at all 3 acid etching time intervals with the 3 DBSs studied, Single Bond and Clearfil SE showed no significant difference in SBS between permanent and primary teeth. One Up, however, showed a significantly higher bond strength to permanent teeth as compared to primary teeth. When the MRT was specifically evaluated, the same trend was observed. This finding is in agreement with the Malferrari et al. study done in 1994 with Gluma 2000 (Bayer AG), demonstrating that bonding to primary teeth was just as strong as bonding to permanent teeth. Hence, we concluded that bond strength to primary teeth is comparable to that of permanent teeth with Single Bond and Clearfil SE. On the other hand, bonding to permanent teeth was superior to primary teeth with One Up. Thus, One Up DBS should not be recommended for use on primary dentition.

6.3 SBS of Enamel versus Dentin

Key points:

1. Bond to primary enamel is weaker than bond to primary dentin
2. Bond to permanent dentin is comparable to permanent enamel
3. Bond to enamel (in general) is weaker than bond to dentin

Enamel bonding has always been the gold standard in bonding technology. A study that compared the SBS to primary enamel to that of primary dentin using four self-etching primer systems (Prime and Bond NT, Clearfil SE, Prompt-L-Pop, and Etch and Prime), found that, with the exception of Clearfil SE, the SEP systems tested revealed

significantly higher bond strength to enamel than to dentin (Agostini et al., 2001). In fact, most studies supported this (Fritz et al., 1997). Furthermore, all four SEP systems resulted in enamel bond strength between 18-26 MPa, which is sufficient to resist polymerization shrinkage. Surprisingly with Clearfil SE, bond strength to primary dentin of 39.0+/-8.5 MPa is twice as much as enamel bond strength of 18.8+/-4.1 MPa (Agostini et al., 2001). In contrast, Fritz et al. found superior bond strengths with primary enamel (18.7+/-1.8 MPa) versus primary dentin (14.9+/-2.6 MPa) with Gluma CPS (1997). However, another study done in 1995 comparing primary enamel to primary dentin SBS with 3 DBS (Optibond Multi-use Bonding Agent, Prisma Universal Bond 3 Multi-Purpose Bonding System, and Scotchbond Multi-purpose Dental Adhesive) showed no difference in SBS between these two substrates. When bond values were analyzed, their SBS value for primary enamel of ~10 MPa is relatively low for enamel bond strength. Thus, they were not able to show a difference in SBS between primary enamel and primary dentin (Mazzeo et al., 1995).

As for permanent teeth, Kanca compared permanent enamel SBS with that of dentin with One Step and found that bonding to the two substrates was comparable (1997). Another study done in 2001 with 3 DBSs (Scotchbond Multi-Purpose, Clearfil SE, and Etch & Prime 3.0) showed significantly higher SBS to permanent enamel than to permanent dentin. Although enamel bonding was not significantly higher than dentin bonding with Clearfil SE in this study, enamel SBS of 19.6+/-6.2 MPa was still higher than dentin SBS of 15.4+/-5.9 MPa (Toledano et al., 2001). In a different bonding study using All Bond 2, Kanca showed that permanent dentin SBS of 36.5+/-1.6 MPa was significantly higher than enamel SBS of 31.9+/-4.8 MPa (1992). Although enamel

bonding is considered the "gold standard" with newer systems, dentin bonding has caught up and sometimes is superior. This is highly dependent on the materials used.

When comparing primary enamel bonding to primary dentin bonding, results from the present study indicate that with Single Bond and Clearfil SE, primary dentin bonds were significantly stronger than primary enamel bonds. This is true when all three acid etching times were evaluated or specifically at the MRT. This is in contrast to most studies that showed bond strength to primary enamel was stronger than to primary dentin as mentioned above (Fritz et al., 1997) (Agostini et al., 1991). In fact, at the MRT, the collapsed SBS value for Single Bond and Clearfil SE was 34.0 MPa for primary dentin versus 28.5 MPa for primary enamel. The reason why SBS to primary dentin is greater than to primary enamel is not clear. Most studies have indicated that with either substrate, the mode of bond failure is predominantly cohesive failure within the adhesive (Cadroy et al., 1997) (Fritz et al., 1997) (Malferrari et al., 1994). This means that the bond to both of these substrates with these newer DBSs is so strong that the weak link is the cohesive strength of the adhesive layer. As mentioned previously, primary dentin contains less mineral, and thus, is softer than primary enamel (Hirayama, 1990). Therefore, the stronger dentin bond could theoretically be due to dentin deforming more readily under shear stress and absorbing more of the shear load before fracturing. Enamel, on the other hand, is a much harder substance due to higher mineral content and relatively little collagen (Sturdevant et al., 1995). Thus, it does not deform readily. Consequently, the adhesive layer in the case of the enamel sample would receive the bulk of the shear load and is more likely to fracture under lighter force. Why the same trend is not observed in permanent teeth is not clear.

Unlike primary teeth, there was no significant difference in bond strength between permanent enamel and permanent dentin in the present study. This was true when all three acid etching times or just the MRT were analyzed with Single Bond and Clearfil SE. When the SBS of the three acid etching times were averaged, permanent enamel SBS of 30.8 MPa was not significantly different from dentin SBS of 31.7 MPa. For more relevant clinical application, the MRT was specifically analyzed. Again, permanent enamel SBS of 33.5 MPa was not significantly different from permanent dentin of 32.2 MPa. This finding is in agreement with Kanca's findings. They found that permanent dentin SBS ranged from 23-29 MPa, which was comparable to permanent enamel bond strength of 24-28 MPa (1997). However, the majority of early studies indicate that enamel bonding is superior to dentin bonding (Swift et al., 1995). In one particular study with Clearfil SE, enamel SBS of 19.6+/-6.2 MPa was significantly higher than dentin SBS of 15.4+/-5.9 MPa (Toledano et al., 2001). This is especially true with earlier dentin bonding systems where a primer was not included or the etchant was not strong enough to remove the smear layer and/or smear plugs. As a result, the earlier generation of DBSs tend to have higher bond strengths to enamel while the newer DBSs tend to have comparable bond strengths to enamel and dentin or even stronger dentin SBS (Kanca, 1992). This marked improvement in dentin bonding is mostly due to the introduction of primers in DBSs. The application of a primer, an ampiphilic molecule, allows for infiltration of a hydrophobic monomer into the hydrophilic etched dentin. This improved monomer flow provides the needed intimate mechanical interlocking and adaptation to the substrate that is responsible for the improvement in dentin bond strength. Enamel, on the other hand, is naturally hydrophobic. Thus, adhesive can readily flow into the rough

etched enamel surface to provide high bond strength without the help of a primer. In fact, it has been found that application of primer onto etched enamel may reduce enamel bond strength by 31-44% (Toledano et al., 2001). Some even suggest limiting the water-based primer to dentin only (Woronko et al., 1996). This, however, is clinically non-feasible.

And finally, when we combined primary and permanent teeth SBS values to look at the overall enamel versus dentin bond strength, it was observed that with Single Bond and Clearfil SE, dentin bond strength was significantly higher than enamel bond strength. However, with One-Up, enamel bond strength was significantly higher than dentin bond strength. When bond values for One-Up were analyzed, however, strong reliable bonds were obtained only for permanent enamel; very poor bond values with large standard deviations were seen with permanent dentin and primary enamel and dentin. Furthermore, One-Up has significantly lower SBS than the other two DBSs, and its bonds were unpredictable. Thus, bond strength is dependant on many factors including the DBS used.

6.4 The Effect of Acid Etching Time on SBS

Enamel, composed of 97% apatite by weight, is much more mineralized than dentin. Thus, it takes more time or a stronger acid to etch or dissolve away the mineral on an enamel substrate than dentin (Malferrari et al., 1994). The degree of etching is critical for optimal bonding to the substrate. For enamel, inadequate etching would result in a shallow etch pattern with less surface area which decreases the micromechanical retention of the resin to the tooth. On the other hand, over etching of the enamel surface would result in excessive unnecessary mineral loss (Agostini et al., 2001). For dentin, under etching would result in a shallow etched surface with possibly incomplete smear

layer removal, and thus, poor resin infiltration. On the contrary, over-etching would result in excessive mineral loss and collagen denaturation and collapse. This could result in incomplete resin infiltration to the etched depth, thus creating a weak link in the bond (Nor et al., 1997). For this reason, under or over etching is not indicated for optimal bonding.

Permanent enamel is the most mineral dense of the four substrates tested (permanent enamel and dentin, and primary enamel and dentin). Thus, it follows that permanent enamel would require the longest acid etching time. Results from Barkmeier's study of etching time effects on enamel SBS suggested that the optimal etching time for permanent enamel was 15 sec with 37% phosphoric acid (1986). In fact, another study done in 1991 by Gilpatrick et al. indicated that 5 sec etch with 37% phosphoric acid was sufficient to allow for adequate bond to permanent enamel. However, they recommended longer etching time because of the concern for long term microleakage (1991). Again, our finding that 5 sec etch was sufficient for both enamel and dentin with Single Bond and Clearfil SE support this finding. Another study evaluating SBS of permanent dentin using 32% phosphoric acid for 20 sec showed the highest bond strength when compared to other times and conditions tested (Kanca, 1992). Furthermore, Kanca has shown that etching of permanent dentin for 10 sec with 37% phosphoric acid resulted in bond strength of ~ 30 MPa (1997). Thus, a 15-20 sec etch with 35-40% phosphoric acid appears to be adequate for producing optimal SBS to permanent enamel and dentin substrates.

Primary enamel etching was originally carried out for 1 to 2 min because the smooth primary enamel was aprismatic, and therefore, required longer etching time. Garcia-

Godoy and Gwinnett examined the effect of etching on smooth versus ground primary enamel. They found that ground etched enamel produced a uniform distribution of prismatic structure with as short an etching time as 15 sec. In contrast, smooth etched enamel surface had an ill-defined prismatic structure – even with 120 sec etch (1991). These findings are reasonable since primary enamel has a peripheral layer of prismless enamel, which is more resistant to etching. For this reason, we ground our primary enamel surface down to 320 grit to mimic a surface created with a carbide bur. This also provided us with a consistent flat surface for bonding and enhanced the etching efficiency of primary enamel. Thus, the old thinking that primary enamel should be etched for 1-2 min is no longer true as long as the enamel is ground before etching. Thus, like permanent enamel, primary enamel should be etched for 15-20 sec with 35-40% phosphoric acid for optimal etching patterns and bond strengths.

Primary dentin has less mineral (Hirayama, 1990) and fewer dentinal tubules than permanent dentin. Therefore, there is less outflow of dentinal fluid to dilute the acid during the etching process, suggesting that primary dentin is more susceptible to demineralization (Agostini et al., 2001). Thus, etching time for primary dentin should be shorter than for permanent dentin. Nor et al. supported this notion of shorter etching time for primary dentin when they evaluated the reactivity of primary versus permanent dentin to 10% phosphoric acid. They concluded that primary dentin was more reactive to acidic conditioning than permanent dentin since the smear layer was more easily removed from primary dentin. In fact, they found that the peritubular dentin of primary teeth was affected by as short an etching time as 7 sec with 10% phosphoric acid, resulting in funnel-shaped opening of the tubules. Hence, they recommended that the etching time of

primary dentin should be 50% that of permanent dentin to obtain an etched dentin surface morphology similar to its permanent counterpart (1997). Thus, primary dentin should be etched between 7-15 sec with 35-40% phosphoric acid for optimal etching pattern and SBS.

6.4.1 Primary versus Permanent Teeth

From the literature review, permanent enamel, permanent dentin, and primary enamel should be etched for 15-30 sec with 35-40% phosphoric acid for optimal etching pattern and SBS. Primary dentin, however, should be etched for an even shorter period of time since it has less mineral and dentinal tubules, making it more reactive to acidic conditioning. In the present study, all three DBSs were included initially to evaluate etching time effect on SBS. Our results indicated that there was no significant difference in SBS when primary teeth were etched for 5 sec, MRT, or 60 sec. On the other hand, etching of permanent teeth for 5 sec resulted in significantly lower SBS as compared to the MRT or 60 sec. This suggests that primary teeth, due to their lower mineral content, are more reactive to acidic conditioning than permanent teeth. In fact, Garcia-Godoy and Gwinnett studied the effect of 15 sec, 30 sec, 60 sec, and 120 sec etches on smooth versus ground primary enamel. They found that etched ground primary enamel produced a uniform distribution of prismatic structure regardless of etching time (1997). Thus, it is possible that ground primary enamel could be adequately etched with as short an etching time as 5 sec, as demonstrated in this study. Likewise, it has been found that the peritubular dentin of primary teeth is affected by as short an etching time as 7 sec with 10% phosphoric acid (Nor et al., 1997). Together, these findings suggested that adequate etching of primary teeth could be obtained in a shorter period of time than the MRT.

This was reflected in the comparable bond strengths to primary teeth obtained with the 5 sec, MRT, or 60 sec etch in this study. Permanent teeth, on the other hand, require longer etching time (at least MRT) to obtain a strong bond. This trend for permanent teeth requiring longer etching time than primary teeth was further confirmed when One Up was dropped from the analysis due to low, erratic bond values. In fact, it was recommended in 1996 that both permanent enamel and dentin be etched for 30 sec with 20% phosphoric acid since this is sufficient to produce a 10 µm thick hybrid layer in dentin and a chalky appearance in etched enamel (Uno and Finger, 1996). Thus, our findings suggest that primary teeth can be etched for as short as 5 sec and still obtain strong short term bonds. Permanent teeth, on the other hand, should be etched for at least the MRT for optimal bond strength. However, there is insufficient long term data at this time to support 5 sec etch of primary teeth. Thus, the MRT may be the best etching time to use for both primary and permanent teeth.

6.4.2 Enamel Versus Dentin

Significantly higher mineral content in enamel versus dentin would theoretically require longer etching times for enamel for optimal SBS. In our study, data for Single Bond and Clearfil SE were collapsed to compare the optimal acid etching time for enamel versus dentin. One Up was not included in this analysis due to its poor, erratic bond values, possibly obscuring data interpretation. The results indicated that dentin SBS was significantly higher than enamel SBS at 5 sec. However, enamel and dentin SBS were comparable at the MRT and 60 sec. This is in agreement with Kanca's study who found that the bond strength to etched dentin was comparable to etched enamel at the MRT with One-Step (Bisco). Furthermore, similar to results obtained with Single Bond from the

present study, Kanca had shown that SBS obtained with a 60 sec etch of dentin (24.5+/-6.8 MPa) was significantly lower than the SBS obtained at the MRT of 30.7+/-1.1 MPa (1997). This is not surprising since both Single Bond and One Step are separate etch systems. When dentin is etched for a prolonged period of time (60 sec), it is probably over-etched. This means that an excessive amount of mineral was removed from the dentin substrate, leaving a thicker layer of collagen that is more prone to collapse and thus prevents the complete infiltration of the monomers into the demineralized substrate. Furthermore, if prolonged etching resulted in greater etching depth than the monomer can infiltrate, it can result in a zone of exposed collagen vulnerable to collapse and hydrolytic breakdown over time since it lacks resin encapsulation (Nor et al., 1997). In either case, this leaves a weak zone at the bottom of the hybrid layer that can lead to premature bond failure.

For enamel, the opposite trend is seen, whereby higher bond strength was achieved with longer etching time. Although Kanca did not examine the effect of etching on enamel SBS at 60 sec, he did show that enamel SBS was not adversely affected by a 5 sec etch (27.9+/-2.7 MPa) as compared to the SBS obtained at the MRT (26.4+/-4.9 MPa) (1997). Again, these findings are in agreement with this study's findings with Single Bond, showing no significant differences in SBS between the 5 sec etch and the MRT for enamel. This same trend was not observed with Clearfil SE because the milder action of the self etching primer requires more time to adequately etch the enamel substrate (Toledano et al., 2001). In another study, Barkmeier et al. compared permanent enamel SBSs obtained with a 15 sec etch with a 60 sec etch using 37% phosphoric acid. They found that the bond strengths obtained between these two etching times were

comparable, suggesting that enamel SBS was not adversely affected with a prolonged etching time of 60 sec (1986). These findings, taken together, support the concept that since dentin is less mineralized, it is more reactive to acidic conditioning. Enamel, on the other hand, is a highly mineralized substrate, and thus, requires a longer period of time to adequately etch its surface. From our study, optimal SBS was achieved with as short an etching time as 5 sec for dentin and 15 sec for enamel.

6.5 Self Etching Systems (Clearfil SE and One Up) Require Longer Etching Time than Separate Etch System (Single Bond)

When looking at our 3 different generations of bonding agent, Single Bond (5th generation), Clearfil SE (self-etching primer), and One Up (self-etching adhesive), the etching efficiency varied. Single Bond has a separate etchant that is washed off after a specified time, and thus, the etching time can be accurately controlled. Clearfil SE and One Up, on the other hand, are both self-etching agents, and thus, the etchants are not removed. Instead, after applying the self-etching agent for a specified amount of time, the material is blown thin, and the adhesive is applied (in the case of Clearfil SE) or light cured (in the case of One Up). Thus, instead of referring to the etching period as the "etching time", it is more accurately termed "application time".

In our study, all 3 DBS's (Single Bond, Clearfil SE, and One Up) were acid etch-time dependent. However, the etching efficiency was different for the 3 systems. For Single Bond, the highest bond strength was obtained at the 5 sec etching time; the lowest bond strength was obtained at the 60 sec time. Even with the enamel substrate that is highly mineralized compared to dentin, there was no significant difference in SBS between 5 sec etch and 60 sec etch with Single Bond, suggesting that 5 sec etch was sufficient for even

the enamel substrate. This indicates that Single Bond, being a separate etch system, can adequately etch enamel and dentin in as short an etching time as 5 sec. This is in agreement with Gilpatrick et al. who also found that 5 sec etch with 37% phosphoric acid was sufficient for permanent enamel (1991). Over etching of the dentin substrate can result with Single Bond if the etching time is significantly longer than the MRT, resulting in decreased SBS.

One the other hand, Clearfil SE and One Up resulted in higher bond strength with increasing self-etching application time, with the highest bond strength obtained with 60 sec application for One Up and the MRT for Clearfil SE. Although the highest SBS was obtained at the MRT for Clearfil SE, it was not significantly different from the SBS obtained with the 60 sec application time. Since these 2 systems are self-etching systems, they probably contain a milder acid that takes a longer period of time to adequately etch the substrate (Toledano et al., 2001). This is evident in the etching patterns produced by the 3 systems. ***Figures 5.12.2, 5.12.14***, and ***5.12.26*** show 3 SEM images of primary dentin etched for the MRT using Single Bond, Clearfil SE, and One Up, respectively. Note the difference in the degree of etching associated with these 3 systems. Single Bond produced the most pronounced etching pattern, with enlarged dentinal tubules and all the smear plugs completely removed. The intertubular dentin also appeared to be well etched as seen by its rough appearance. Clearfil SE also produced a distinct etching pattern, characterized by enlarged dentinal tubules with almost all the smear plug removed. The intertubular dentin also showed a moderate degree of etching as seen by the roughened surface, although not to the same extent as with Single Bond. Unlike the first two, One

Up produced an indistinct etching pattern whereby the dentinal tubules were still obliterated by the smear plugs, and thus, barely visible.

However, between these 2 self-etching systems, there was a difference in the efficiency of the etching. For Clearfil SE, although it is a milder acid as reflected by its higher pH of 2.0 (Agostini et al., 2001), it etched more efficiently than One Up (with a pH of 1 – unpublished data from our lab) as demonstrated by the etching images. ***Figures 5.12.9 and 5.12.21*** are two SEM images of primary enamel etched for 60s using Clearfil SE and One Up, respectively. Clearfil SE etched much more efficiently than One up as noted from these images. Moreover, this difference in etching efficiency may also be responsible for the significantly higher bond strength associated with Clearfil SE as compared to One Up. Another important point to address is that although Clearfil SE is a self-etching agent that performs best at longer application time, it etches adequately to produce strong reliable bonds even at 5 sec application time. One Up, on the other hand, performed poorly at the 5 sec application time, and did better at the MRT and 60 sec application times. Hence, Single Bond etches very aggressively and could over-etch, especially dentin substrate, if the etchant is left on the tooth beyond the MRT. In contrast, with Clearfil SE and One Up, the self-etching primer or self-etching adhesive can be applied longer than the MRT without significantly affecting bond strength.

6.6 With SEP and SEA, the More Defined the Etching Pattern, the Stronger the SBS

Additional samples were prepared to study the etching pattern of the SEP and SEA systems. SEM images were taken of the etched surface. Since SEP and SEA contain an acidic monomer, the monomer must be removed for better visualization of the etched

surface. For comparison, 37% phosphoric acid (from Single Bond) was used to etch the four substrates studied to see the effect of the traditional etchant on these substrates. For enamel, there are three possible enamel acid etching patterns due to selective demineralization of enamel prisms: (1) preferential dissolution of prism cores, (2) preferential dissolution of prism peripheries, or (3) combination of the first two types (Swift et al., 1995). For dentin, traditional etchants (i.e. 35% phosphoric acid) will completely remove the smear layer, smear plugs, and demineralize both intertubular and peritubular dentin, resulting in enlarged, funnel shaped tubules. With SEP and SEA systems, however, the etching pattern is not as distinct since the acidic monomers are not rinsed away. Thus, the smear layer is dissolved but not completely removed, smear plugs maybe partially intact, and the dentinal tubules maybe partially open. The degree of etching depends on the acidity and permeability of these acidic monomers.

With One Up, a SEA, the highest and most consistent bond was obtained with permanent enamel, with an average bond strength of 21.5 MPa. This was significantly higher than the erratic SBS obtained with the other three substrates (permanent dentin, primary enamel, and primary dentin) which averaged about 8-12 MPa. With One Up, bond strength improved with longer etching times. In fact, the highest bond strength was obtained at the 60 sec etch with all four substrates. A possible explanation for this difference in bond strength is the degree of etching before the SEA is light cured. With One Up, the bonding protocol specified curing the SEA after 20 sec of application. Thus, the etching capability stops when it is cured as opposed to a SEP like Clearfil SE, which has a longer etching time because of the additional step of adhesive application before curing. Thus, when looking at the etched surfaces of primary and permanent enamel,

increased roughness was seen with longer SEA application time. However, the etching pattern was not clear; the enamel prisms could not be identified, even with 60 sec etch. As for the etched dentin, enlarged dentinal tubules were seen with increasing etching time. However, the smear plugs were intact at all three application times. The significantly higher bond strength between permanent and primary enamel could not be explained on the basis of the degree of roughness of the etched surface, since the etched primary enamel surface appeared to have the same roughness as the permanent etched enamel surface. As for dentin, the etched primary dentin surface had larger diameter tubules than the etched permanent dentin surface. Resin infiltration into dentinal tubules contributes very little to the total SBS. The majority of the resin retention to dentin is through the resin infiltration into the etched intertubular dentin (Mazzeo et al., 1995). Hence, the enlarged dentinal tubules in primary dentin do not help in resin retention as reflected by the lower SBS of primary dentin as compared to permanent dentin. Thus, why permanent dentin had significantly higher SBS than primary dentin is not clear from examination of the SEM images.

With Clearfil SE, bond strength to dentin was generally higher than enamel. Moreover, bond strength to primary teeth was comparable to that of permanent teeth. Unlike One Up, the highest bond strength was obtained with the MRT for all four substrates. The MRT and the 60 sec application times both resulted in significantly higher bond strength than the 5 sec etch, although the 2 former etching times were not significantly different from each other. Overall, Clearfil SE had significantly higher bond strength than One Up.

When analyzing the overall etching pattern of these two DBSs, Clearfil SE had a much more pronounced etching pattern than One Up. Unlike One Up, the enamel prisms could be identified with Clearfil SE etched samples, especially with the 60 sec etch of primary enamel. In fact, it had predominantly a type I etching pattern whereby the prism core was preferentially demineralized (Swift et al., 1995). Interestingly, this is in contrast to the Type II etching pattern seen with Single Bond (37% phosphoric acid) whereby the prism peripheries were preferentially etched. However, these patterns can change with time and the different patterns can be found on the same tooth (Marshall et al., 1975). Furthermore, the primary enamel etching pattern was more defined than the permanent enamel, possibly suggesting that primary enamel is less mineralized, and thus, more readily etched than permanent enamel. As for dentin, the etched dentin surface had larger diameter tubules with more of the smear plugs removed with Clearfil SE as compared to One Up. (Refer to SEM images of dentin with Clearfil SE ***{Fig. 5.12.15}*** and One Up ***{Fig. 5.12.27}***) In fact, the 60 sec etch of primary dentin resulted in almost complete removal of the smear plugs. Like enamel, the etched primary dentin had a more distinct etching pattern than permanent dentin, indicating a possible difference in the degree of mineralization between these two substrates. Thus, when comparing the etching pattern between Clearfil SE and One Up, a possible conclusion is that the more pronounced etching pattern produced by Clearfil SE contributes to the higher SBS.

Like One Up, the etching pattern of Clearfil SE was compared at the following etching times (5 sec, MRT (20 sec), and 60 sec). For both enamel and dentin bonding, the optimal bond strength was at the MRT. When the etching pattern of enamel was analyzed at the 5 sec etch, the surface was not as rough and it was not as well defined as

the 20 sec and 60 sec etches. The 60 sec enamel etch appeared to have the most pronounced etching pattern, although its SBS was not significantly different from the 20 sec etch. Likewise, dentin etched for 5 sec still retained almost all of its dentin plugs, especially with permanent dentin. At 20 sec and 60 sec etch, the dentin tubules were larger and most of dentin plugs were removed. The etching pattern produced from the 20 sec etch was not significantly different from the 60 sec etch although the 60 sec produced the most pronounced etching pattern. Thus, 20 sec etch with Clearfil SE seems to be sufficient for producing an optimal etching pattern and SBS for permanent enamel and dentin and primary enamel. Primary dentin can achieve optimal bond strength even with a 5 sec etch. However, in a clinical situation, it is impractical to etch primary enamel for one time and dentin another. Thus, permanent and primary teeth should ideally be etched for 20 sec with Clearfil SE bonding system for optimal bond strength.

6.7 Overall, Clearfil SE has the Highest SBS, Followed by Single Bond, and Finally One Up

The three DBSs tested represent three approaches to dentin and enamel bonding. Clearfil SE, a self etching primer, achieved the highest bond strength, with comparable bond strength to primary and permanent teeth. It provided high bond strength to both dentin and enamel for the 3 time intervals tested. In fact, its bond to dentin averaged 34.3 MPa and to enamel, 30.5 MPa. Since Clearfil SE is a self etching system, it contains a mild etchant that is not washed off (Toledano et al., 2001). Thus, although a strong bond was achieved with a 5 sec application time, stronger bonds were obtained with the MRT and 60 sec. Although Clearfil SE looks very promising short term, long term results are not known (Lopes et al., 2002). There are studies reporting increased microleakage with

time with self-etching systems. Furthermore, self-etching systems do not etch smooth enamel surfaces as well as a separate etch systems, due to the milder nature of the acid (Toledano et al., 2001).

Single Bond, a conventional separate etch system, performed very well overall to both primary and permanent teeth. More specifically, its average SBS to dentin was 30.6 MPa and 28.5 MPa to enamel. Unlike Clearfil SE, it has a strong acid (37% phosphoric acid) that is able to adequately etch both enamel and dentin in as short an etching time as 5 sec. This is seen in its SBS, being highest at the 5 sec etch and progressively lower with longer etching time. Lengthening the etching time to 60 sec probably resulted in over-etching of the substrate (especially dentin), resulting in a non-ideal surface for bonding. Thus, this study suggests that, with Single Bond, etching primary and permanent teeth for 5-15 sec is sufficient for optimal bonding.

And finally, One Up performed the poorest of the three. Its SBS values were very low and erratic. Surprisingly, the only substrate to which it bonded well, was permanent enamel, with an average bond strength of 21.5 MPa. It bonded very poorly to permanent dentin, primary enamel and primary dentin. In fact, its average SBS to enamel was 15.5 MPa and dentin was 10.6 MPa. Because of its low erratic bond values, we decided to drop One Up from our statistical analysis and focus our investigation on Clearfil SE and Single Bond. Like Clearfil SE, One Up is a self-etching system and seemed to provide higher bond strengths with longer etching times. Ironically, One Up contains a more acidic self-etching agent than Clearfil SE, and yet, it did not etch as well as Clearfil SE, as shown in the etching images (see ***Fig. 5.12.14*** {Clearfil SE} and ***Fig. 5.12.26*** {One Up}). Thus, there must be other factors other than acidity (e.g. permeability of the acidic

monomers) that is responsible for etching efficiency, and SBS. One factor could be that the SEA was polymerized after the specified time, thus preventing further etching, whereas the SEP was air thinned but not polymerized prior to adhesive application, giving more time for etching. Another possible explanation is offered by Heymann et al. who stated that although there are compositional differences between different DBSs, SBS is most closely related to the DBS ability to intimately adapt to the etched surface (1993). Perhaps, Clearfil SE contains different monomers or components that are more closely adapted and create micromechanical bonds to the etched surface than One Up.

7. CONCLUSION

In this study, the 3 DBS's were intentionally selected to represent 3 of the most current generations of dentin bonding agents: Single Bond (5th generation), Clearfil SE (self-etching primer), and One Up (self-etching adhesive). Nowadays, advertisements of newer dentin bonding agents appeal to practitioners' need for efficiency, ease of use, and long term durability. Self etching systems were developed to address these problems by simplifying the steps, eliminating the critical step of washing away the etchant, leaving behind the crucial moist dentin surface. Furthermore, these self-etching systems theoretically resolve the other problem of collagen collapse since they simultaneously demineralize and infiltrate with monomers. However, although both Clearfil SE and One Up are self-etching systems, Clearfil SE provided exceptional SBS, while One Up provided low erratic bond values. As of now, very few studies are available on the long term durability of these bonds. Furthermore, other unresolved questions with regard to self-etching systems include effect of the unrinsed acidic primer, consequence of multiple application of acidic primer…etc (Toledano et al., 2001). Single Bond, with its separate

etch system, still proves to be a reliable system to use for both primary and permanent teeth.

More specifically, we are able to conclude the following:

1. Bond to primary teeth was just as strong as to permanent teeth
2. Bond to dentin (primary and permanent) was just as strong as to enamel
3. Bond to primary dentin was at least as strong if not stronger than to primary enamel
4. Bond to permanent dentin was just as strong as to permanent enamel
5. Dentin required shorter etching times than enamel
6. Bond to primary dentin was just as strong as to permanent dentin
7. Bond to primary enamel was just as strong as to permanent enamel
8. Enamel bond strength was not compromised with prolonged etching time (60 sec), especially with SEP and SEA
9. Dentin bond strength was significantly compromised with prolonged etching time (60 sec) with Single Bond
10. Primary teeth could be etched for a shorter period of time (5 sec) without compromising the short term bond. However, etching for the MRT is still recommended to minimize long term microleakage.
11. With self-etching systems, the more defined the etching pattern (up to a limit), the stronger the SBS
12. Overall, Clearfil SE provided the strongest SBS, followed by Single Bond, and lastly, One Up

Thus, from our bonding study of both primary and permanent dentition, we can conclude that short term bonding of primary teeth was comparable to permanent teeth. Secondly, the same protocol used for permanent teeth could be used for primary teeth without compromising its SBS with these 3 DBS tested. However, long term bonding properties such as microleakage, post-op sensitivities, recurrent caries… were not within the scope of this study. Moreover, rapid evolution of newer DBSs and fierce competition among dental materials manufacturers resulted in the release of these bonding systems into clinical use without thorough tests to confirm their clinical performance (Van Meerbeek et al., 1998). Hence, long term study should be carried out before a definite conclusion about durability of these bonds can be reached. Thus, although bench top studies such as this are invaluable pre-clinical tests to determine performance of DBS's, they are at best, simplistic predictions of actual clinical performance (Van Meerbeek et al., 1998) (Vargas et al., 1997).

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SINGLE BOND

PRIMARY TEETH

SINGLE BOND 5 S: PRIMARY ENAMEL

Average hole diameter 2.675

sample	kg	dia.(in mm)	radii (in cm)	area (in cm2)	kg/cm2	Mpa	
1	13.3	2.689	0.13445	0.0568	234.32	**22.972**	
2	16.1	2.703	0.13515	0.0574	280.71	**27.521**	
3	14.6	2.663	0.13315	0.0557	262.27	**25.712**	**26.64 average**
4	16.1	2.643	0.13215	0.0548	293.6	**28.785**	**2.35 stdev**
5	16.2	2.678	0.1339	0.0563	287.76	**28.211**	

SINGLE BOND: PRIMARY DENTIN: 5 SEC

Average hole diameter 2.665

sample	kg	dia.(in mm)	radii (in cm)	area (in cm2)	kg/cm2	Mpa	
1	17.5	2.673	0.13365	0.0561	312.01	**30.589**	
2	21	2.673	0.13365	0.0561	374.41	**36.707**	
3	20.5	2.733	0.13665	0.0586	349.63	**34.277**	
4	14.7	2.571	0.12855	0.0519	283.3	**27.774**	
5	17.4	2.646	0.1323	0.055	316.59	**31.038**	**33.88 average**
6	21.5	2.593	0.12965	0.0528	407.35	**39.936**	**4.27 stdev**
7	22.5	2.763	0.13815	0.0599	375.45	**36.809**	

SINGLE BOND 15 S: PRIMARY ENAMEL

Average hole diameter 2.700

sample	kg	dia.(in mm)	radii (in cm)	area (in cm2)	kg/cm2	Mpa	
1	14.8	2.644	0.1322	0.0549	269.69	**26.44**	
2	13	2.722	0.1361	0.0582	223.51	**21.913**	
3	19	2.72	0.136	0.0581	327.15	**32.073**	**25.98 average**
4	14.8	2.693	0.13465	0.0569	259.97	**25.487**	**3.81 stdev**
5	14.2	2.719	0.13595	0.058	244.68	**23.988**	

SINGLE BOND 15S: PRIMARY DENTIN

Average hole diameter 2.596

sample	kg	dia.(in mm)	radii (in cm)	area (in cm2)	kg/cm2	Mpa	
1	20	2.577	0.12885	0.0521	383.65	37.612	
2	17.5	2.612	0.1306	0.0536	326.75	32.035	
3	14	2.449	0.12245	0.0471	297.36	29.153	
4	16	2.734	0.1367	0.0587	272.68	26.733	
5	17.5	2.636	0.1318	0.0545	320.83	31.454	**31.93 average**
6	*18.25*	*2.566*	*0.1283*	0.0517	353.09	34.616	**3.86 stdev**

SINGLE BOND 60S: PRIMARY ENAMEL

Average hole diameter 2.673

sample	kg	dia.(in mm)	radii (in cm)	area (in cm2)	kg/cm2	Mpa	
1	18.9	2.704	0.1352	0.0574	329.29	**32.283**	
2	15.8	2.62	0.131	0.0539	293.21	**28.746**	
3	14.1	2.637	0.13185	0.0546	258.3	**25.324**	**27.78 average**
4	14.1	2.714	0.1357	0.0578	243.85	**23.907**	**2.92 stdev**
5	16.3	2.679	0.13395	0.0563	289.32	**28.364**	
6	16.2	2.686	0.1343	0.0566	286.04	**28.044**	

SINGLE BOND 60S: PRIMARY DENTIN

Average hole diameter 2.741

sample	kg	dia.(in mm)	radii (in cm)	area (in cm2)	kg/cm2	Mpa	
1	14	2.726	0.1363	0.0583	240	**23.529**	
2	15	2.733	0.13665	0.0586	255.82	**25.081**	
3	20.5	2.749	0.13745	0.0593	345.57	**33.879**	**26.24 average**
4	14	2.732	0.1366	0.0586	238.94	**23.426**	**4.36 stdev**
5	15.5	2.767	0.13835	0.0601	257.9	**25.284**	

PERMANENT TEETH

SINGLE BOND 5S: PERMANENT ENAMEL

Average hole diameter 2.651

sample	**kg**	**dia.(in mm)**	**radii (in cm)**	**area (in cm2)**	**kg/cm2**	**Mpa**	
1	18.5	2.766	0.1383	0.0601	308.03	**30.199**	
2	16.5	2.627	0.13135	0.0542	304.57	**29.86**	
3	18.5	2.676	0.1338	0.0562	329.1	**32.265**	
4	17.5	2.585	0.12925	0.0525	333.62	**32.707**	
5	15.5	2.596	0.1298	0.0529	292.99	**28.725**	**30.94 average**
6	18	2.655	0.13275	0.0553	325.29	**31.891**	**1.58 stdev**

SINGLE BOND 5S: PERMANENT DENTIN

Average hole diameter 2.728

sample	**kg**	**dia.(in mm)**	**radii (in cm)**	**area (in cm2)**	**kg/cm2**	**Mpa**	
1	23.5	2.67	0.1335	0.056	419.93	**41.169**	
2	18.4	2.759	0.13795	0.0598	307.92	**30.189**	
3	19.7	2.64	0.132	0.0547	360.07	**35.301**	
4	23	2.77	0.1385	0.0602	381.86	**37.437**	
5	22	2.822	0.1411	0.0625	351.92	**34.502**	**35.31 average**
6	19.5	2.707	0.13535	0.0575	338.99	**33.234**	**3.74 stdev**

SINGLE BOND 15S: PERMANENT ENAMEL

Average hole diameter 2.643

sample	**kg**	**dia.(in mm)**	**radii (in cm)**	**area (in cm2)**	**kg/cm2**	**Mpa**	
1	18.4	2.615	0.13075	0.0537	342.77	**33.605**	
2	18.8	2.624	0.1312	0.0541	347.82	**34.1**	
3	16.8	2.603	0.13015	0.0532	315.86	**30.966**	
4	16.2	2.637	0.13185	0.0546	296.77	**29.095**	
5	18.8	2.659	0.13295	0.0555	338.73	**33.209**	**31.55 average**
6	16.8	2.722	0.1361	0.0582	288.84	**28.318**	**2.46 stdev**

SINGLE BOND 15S: PERMANENT DENTIN

Average hole diameter 2.685

sample	**kg**	**dia.(in mm)**	**radii (in cm)**	**area (in cm2)**	**kg/cm2**	**Mpa**	
1	16.5	2.66	0.133	0.0555	297.06	**29.124**	
2	15	2.741	0.13705	0.059	254.33	**24.935**	
3	18.5	2.608	0.1304	0.0534	346.49	**33.969**	
4	17.3	2.774	0.1387	0.0604	286.39	**28.078**	
5	15.5	2.607	0.13035	0.0534	290.52	**28.483**	**28.91 average**
6	17.1	2.721	0.13605	0.0581	294.22	**28.845**	**2.91 stdev**

SINGLE BOND 60S: PERMANENT ENAMEL

Average hole diameter 2.684

sample	**kg**	**dia.(in mm)**	**radii (in cm)**	**area (in cm2)**	**kg/cm2**	**Mpa**	
1	17.9	2.769	0.13845	0.0602	297.4	**29.157**	
2	15.8	2.651	0.13255	0.0552	286.4	**28.078**	
3	17.6	2.669	0.13345	0.0559	314.74	**30.856**	
4	16	2.636	0.1318	0.0545	293.33	**28.758**	
5	15	2.671	0.13355	0.056	267.84	**26.259**	**27.88 average**
6	14.2	2.708	0.1354	0.0576	246.67	**24.184**	**2.35 stdev**

SINGLE BOND 60S: PERMANENT DENTIN

Average hole diameter 2.703

sample	**kg**	**dia.(in mm)**	**radii (in cm)**	**area (in cm2)**	**kg/cm2**	**Mpa**	
1	15.7	2.689	0.13445	0.0568	276.6	**27.117**	
2	12.9	2.745	0.13725	0.0591	218.09	**21.381**	
3	18.2	2.61	0.1305	0.0535	340.35	**33.367**	
4	13.3	2.777	0.13885	0.0605	219.7	**21.539**	
5	16.6	2.675	0.13375	0.0562	295.52	**28.973**	**27.28 average**
6	18.6	2.724	0.1362	0.0582	319.32	**31.306**	**4.98 stdev**

CLEARFIL SE BOND

PRIMARY TEETH

CLEARFIL 5S: PRIMARY ENAMEL

Average hole diameter 2.733

sample	**kg**	**dia.(in mm)**	**radii (in cm)**	**area (in cm2**	**kg/cm2**	**Mpa**	
1	12.5	2.698	0.1349	0.0571	218.75	21.446	
2	19.5	2.744	0.1372	0.0591	329.91	32.344	
3	17.5	2.777	0.1389	0.0605	289.08	28.341	
4	*19.5*	*2.815*	*0.14075*	*0.062205*	313.48	30.733	**27.18 average**
5	12.75	2.629	0.1315	0.0543	235	23.039	**4.76 stdev**

CLEARFIL 5S: PRIMARY DENTIN

Average hole diameter 2.686

sample	**kg**	**dia.(in mm)**	**radii (in cm)**	**area (in cm2**	**kg/cm2**	**Mpa**	
1	22	2.664	0.1332	0.0557	394.9	38.715	
2	23	2.782	0.1391	0.0608	378.57	37.115	
3	19.5	2.718	0.1359	0.058	336.25	32.966	
4	*21*	*2.7*	*0.135*	*0.057227*	366.96	35.977	**35.72 average**
5	19.5	2.584	0.1292	0.0524	372.03	36.474	**4.36 stdev**
6	16	2.688	0.1344	0.0567	282.09	27.656	
7	23.5	2.67	0.1335	0.056	419.93	41.169	

CLEARFIL 20S: PRIMARY ENAMEL

Average hole diameter 2.703

sample	**kg**	**dia.(in mm)**	**radii (in cm)**	**area (in cm2**	**kg/cm2**	**Mpa**	
1	19	2.734	0.1367	0.0587	323.81	31.746	
2	19	2.681	0.1341	0.0564	336.74	33.013	
3	17.5	2.732	0.1366	0.0586	298.68	29.282	
4	*17*	*2.69*	*0.1345*	*0.056803*	299.28	29.341	**30.96 average**
5	18	2.676	0.1338	0.0562	320.21	31.393	**1.62 stdev**

CLEARFIL 20S: PRIMARY DENTIN

Average hole diameter 2.677

sample	kg	dia.(in mm)	radii (in cm)	area (in cm2	kg/cm2	Mpa	
1	21.5	2.64	0.132	0.0547	392.97	38.527	
2	15.5	2.664	0.1332	0.0557	278.22	27.277	
4	20.25	2.698	0.1349	0.0571	354.38	34.743	
5	*22.5*	*2.626*	*0.1313*	*0.054133*	415.65	40.75	**36.00 average**
6	24	2.616	0.1308	0.0537	446.75	43.799	**5.18 stdev**
7	18.5	2.734	0.1367	0.0587	315.29	30.91	

CLEARFIL: PRIMARY ENAMEL: 60 SEC

Average hole diameter 2.691

sample	kg	dia.(in mm)	radii (in cm)	area (in cm2	kg/cm2	Mpa	
1	18.5	2.657	0.1329	0.0554	333.83	32.728	
2	17.6	2.663	0.1332	0.0557	316.16	30.996	
3	17.6	2.652	0.1326	0.0552	318.78	31.253	
4	*18.2*	*2.711*	*0.13555*	*0.057694*	315.46	30.927	**30.63 average**
5	15.3	2.639	0.132	0.0547	279.86	27.437	**1.75 stdev**
6	19.4	2.821	0.1411	0.0625	310.55	30.446	

CLEARFIL 60S: PRIMARY DENTIN

Average hole diameter 2.690

sample	kg	dia.(in mm)	radii (in cm)	area (in cm2	kg/cm2	Mpa	
1	22.75	2.773	0.1387	0.0604	376.89	36.95	
2	22.75	2.635	0.1318	0.0545	417.4	40.921	
3	16	2.668	0.1334	0.0559	286.34	28.072	
4	*20.5*	*2.654*	*0.1327*	*0.055293*	370.75	36.348	**34.91 average**
5	16.5	2.735	0.1368	0.0587	281	27.549	**5.76 stdev**
6	22.75	2.677	0.1339	0.0563	404.4	39.647	

PERMANENT TEETH

CLEARFIL 5S: PERMANENT ENAMEL

Average hole diameter 2.641

sample	**kg**	**dia.(in mm)**	**radii (in cm)**	**area (in cm2**	**kg/cm2**	**Mpa**	
1	13.8	2.514	0.1257	0.0496	278.15	27.27	
2	15.4	2.602	0.1301	0.0531	289.76	28.408	
3	17.2	2.684	0.1342	0.0566	304.15	29.819	
4	*16.2*	*2.782*	*0.1391*	*0.060755*	266.64	26.142	**25.64 average**
5	16.8	2.552	0.1276	0.0511	328.61	32.216	**6.04 stdev**
6	7.8	2.612	0.1306	0.0536	145.64	14.278	
7	12.8	2.738	0.1369	0.0588	217.51	21.324	

CLEARFIL 5S: PERMANENT DENTIN

Average hole diameter 2.678

sample	**kg**	**dia.(in mm)**	**radii (in cm)**	**area (in cm2**	**kg/cm2**	**Mpa**	
1	21.75	2.638	0.1319	0.0546	398.14	39.034	
2	18.25	2.674	0.1337	0.0561	325.14	31.876	
3	14	2.81	0.1405	0.062	225.86	22.143	
4	*16.4*	*2.662*	*0.1331*	*0.055627*	294.82	28.904	**30.29 average**
5	12.8	2.744	0.1372	0.0591	216.56	21.231	**6.79 stdev**
6	20	2.6	0.13	0.0531	376.89	36.95	
7	17.5	2.618	0.1309	0.0538	325.26	31.888	

CLEARFIL 20S: PERMANENT ENAMEL

Average hole diameter 2.584

sample	**kg**	**dia.(in mm)**	**radii (in cm)**	**area (in cm2**	**kg/cm2**	**Mpa**	
1	17	2.528	0.1264	0.0502	338.86	33.222	
2	20.5	2.612	0.1306	0.0536	382.77	37.526	
3	15.4	2.55	0.1275	0.051	301.7	29.578	
4	*22.5*	*2.578*	*0.1289*	*0.052172*	431.27	42.281	**35.46 average**
5	20.5	2.716	0.1358	0.0579	354.02	34.708	**4.28 stdev**
6	17	2.56	0.128	0.0514	330.45	32.397	
7	20	2.546	0.1273	0.0509	393.05	38.534	

CLEARFIL 20S: PERMANENT DENTIN

			Average hole diameter		2.701		
sample	**kg**	**dia.(in mm)**	**radii (in cm)**	**area (in cm2**	**kg/cm2**	**Mpa**	
1	20.5	2.684	0.1342	0.0566	362.51	35.54	
2	22	2.768	0.1384	0.0601	365.78	35.861	
3	25	2.764	0.1382	0.06	416.86	40.869	
4	*19.5*	*2.596*	*0.1298*	*0.052903*	368.6	36.137	**35.31 average**
5	20	2.708	0.1354	0.0576	347.43	34.061	**2.94 stdev**
6	17.6	2.636	0.1318	0.0545	322.67	31.634	
7	20	2.748	0.1374	0.0593	337.39	33.077	

CLEARFIL 60S: PERMANENT ENAMEL

			Average hole diameter		2.666		
sample	**kg**	**dia.(in mm)**	**radii (in cm)**	**area (in cm2**	**kg/cm2**	**Mpa**	
1	19.5	2.626	0.1313	0.0541	360.23	35.316	
2	17.6	2.746	0.1373	0.0592	297.33	29.15	
3	20.25	2.726	0.1363	0.0583	347.14	34.033	
5	21	2.556	0.1278	0.0513	409.48	40.145	
6	17	2.68	0.134	0.0564	301.52	29.56	**33.31 average**
7	18	2.664	0.1332	0.0557	323.1	31.676	**4.13 stdev**

CLEARFIL 60S: PERMANENT DENTIN

			Average hole diameter		2.699		
sample	**kg**	**dia.(in mm)**	**radii (in cm)**	**area (in cm2**	**kg/cm2**	**Mpa**	
1	15	2.666	0.1333	0.0558	268.84	26.357	
2	23.25	2.652	0.1326	0.0552	421.12	41.286	
3	21	2.69	0.1345	0.0568	369.7	36.245	
4	20.5	2.706	0.1353	0.057481	356.64	34.965	**33.08 average**
5	19.6	2.784	0.1392	0.0608	322.14	31.583	**5.77 stdev**
6	21	2.7	0.135	0.0572	366.96	35.977	
7	14.6	2.694	0.1347	0.057	256.26	25.124	

ONE-UP

PRIMARY TEETH

ONE UP 5 SEC: PRIMARY ENAMEL

sample	kg	dia.(in mm)	radii (in cm)	area (in cm2	kg/cm2	Mpa	
			Average hole diameter		2.734		
1	0.9	2.682	0.1341	0.0565	15.939	**1.5626**	
2	7.4	2.854	0.1427	0.0639	115.73	**11.346**	
3	5.5	2.665	0.1333	0.0558	98.65	**9.6716**	
4	*1*	*2.68*	*0.134*	*0.056382*	17.736	**1.7388**	**5.79 average**
5	0.8	2.75	0.1375	0.0594	13.476	**1.3212**	**4.71 stdev**
6	5.6	2.774	0.1387	0.0604	92.705	**9.0888**	

ONE-UP 5 SEC: PRIMARY DENTIN

sample	kg	dia.(in mm)	radii (in cm)	area (in cm2	kg/cm2	Mpa	
			Average hole diameter		2.673		
1	2.6	2.717	0.1359	0.0579	44.867	**4.3987**	
2	5.5	2.584	0.1292	0.0524	104.93	**10.287**	
3	3	2.74	0.137	0.0589	50.904	**4.9906**	
4	*5.2*	*2.69*	*0.1345*	*0.056803*	91.544	**8.9749**	**7.58 average**
5	3.6	2.64	0.132	0.0547	65.8	**6.451**	**2.65 stdev**
6	5.9	2.667	0.1334	0.0558	105.67	**10.359**	

ONE UP 20 SEC: PRIMARY ENAMEL

sample	kg	dia.(in mm)	radii (in cm)	area (in cm2	kg/cm2	Mpa	
			Average hole diameter		2.752		
1	9.6	2.669	0.1335	0.0559	171.67	**16.831**	
2	0.4	2.789	0.1395	0.0611	6.5508	**0.6422**	
3	9	2.874	0.1437	0.0648	138.8	**13.606**	
4	*0.7*	*2.714*	*0.1357*	*0.057821*	12.106	**1.1869**	**8.37 average**
5	*0.5*	*2.708*	*0.1354*	*0.057566*	8.6857	**0.8515**	**8.29 stdev**
6	10.4	2.755	0.1378	0.0596	174.55	**17.113**	

ONE-UP 20 SEC: PRIMARY DENTIN

Average hole diameter 2.700

sample	kg	dia.(in mm)	radii (in cm)	area (in cm2	kg/cm2	Mpa	
1	2.4	2.705	0.1353	0.0574	41.784	**4.0964**	
2	5.1	2.833	0.1417	0.063	80.948	**7.9361**	
3	3.8	2.64	0.132	0.0547	69.455	**6.8094**	
4	*7.2*	*2.686*	*0.1343*	*0.056635*	127.13	**12.464**	**8.96 average**
5	7.5	2.634	0.1317	0.0545	137.71	**13.501**	**3.94 stdev**

ONE UP 60 SEC: PRIMARY ENAMEL

Average hole diameter 2.724

sample	kg	dia.(in mm)	radii (in cm)	area (in cm2	kg/cm2	Mpa	
1	5.6	2.784	0.1392	0.0608	92.041	**9.0236**	
2	9.6	2.644	0.1322	0.0549	174.94	**17.151**	
3	6.5	2.707	0.1354	0.0575	113	**11.078**	
4	*12.8*	*2.747*	*0.13735*	*0.059236*	216.08	**21.185**	**14.30 average**
5	5	2.741	0.1371	0.059	84.778	**8.3116**	**5.52 stdev**
6	11.3	2.721	0.1361	0.0581	194.42	**19.061**	

ONE UP 60S: PRIMARY DENTIN

Average hole diameter 2.730

sample	kg	dia.(in mm)	radii (in cm)	area (in cm2	kg/cm2	Mpa	
1	7.2	2.713	0.1357	0.0578	124.61	**12.217**	
2	4.7	2.79	0.1395	0.0611	76.917	**7.5408**	
3	6.9	2.677	0.1339	0.0563	122.65	**12.025**	
4	*4.4*	*2.68*	*0.134*	*0.056382*	78.039	**7.6509**	**9.88 average**
5	6.2	2.788	0.1394	0.061	101.61	**9.9618**	**2.26 stdev**

PERMANENT TEETH

ONE UP 5S: PERMANENT ENAMEL

Average hole diameter 2.627

sample	kg	dia.(in mm)	radii (in cm)	area (in cm2	kg/cm2	Mpa	
1	9.8	2.629	0.1315	0.0543	180.62	**17.708**	
2	2.4	2.641	0.1321	0.0548	43.833	**4.2974**	
3	11.6	2.588	0.1294	0.0526	220.63	**21.63**	
4	*11*	*2.658*	*0.1329*	*0.05546*	198.34	**19.445**	**16.10 average**
5	8.3	2.656	0.1328	0.0554	149.88	**14.694**	**6.21 stdev**
6	10.1	2.59	0.1295	0.0527	191.8	**18.804**	

ONE UP 5S: PERMANENT DENTIN

Average hole diameter 2.649

sample	kg	dia.(in mm)	radii (in cm)	area (in cm2	kg/cm2	Mpa	
1	5.8	2.637	0.1319	0.0546	106.25	**10.417**	
2	11.4	2.684	0.1342	0.0566	201.59	**19.764**	
3	3	2.681	0.1341	0.0564	53.169	**5.2126**	
4	*9.2*	*2.695*	*0.13475*	*0.057015*	161.36	**15.82**	**10.02 average**
5	*0.6*	*2.674*	*0.1337*	*0.05613*	10.69	**1.048**	**6.89 stdev**
6	4	2.522	0.1261	0.0499	80.112	**7.8542**	

ONE UP 20S: PERMANENT ENAMEL

Average hole diameter 2.645

sample	kg	dia.(in mm)	radii (in cm)	area (in cm2	kg/cm2	Mpa	
1	14	2.724	0.1362	0.0582	240.35	**23.564**	
2	12.6	2.628	0.1314	0.0542	232.41	**22.785**	
3	13.1	2.658	0.1329	0.0555	236.21	**23.157**	
4	*13.1*	*2.588*	*0.1294*	*0.052577*	249.16	**24.427**	**23.84 average**
5	12.9	2.642	0.1321	0.0548	235.43	**23.081**	**1.22 stdev**
6	14.4	2.628	0.1314	0.0542	265.61	**26.04**	

ONE UP 20S: PERMANENT DENTIN

Average hole diameter 3.309

sample	kg	dia.(in mm)	radii (in cm)	area (in cm2	kg/cm2	Mpa	
1	6	6.575	0.3288	0.3394	17.68	**1.7334**	
2	8.1	2.564	0.1282	0.0516	156.96	**15.388**	
3	5.9	2.717	0.1359	0.0579	101.81	**9.9817**	
4	*6.4*	*2.613*	*0.13065*	*0.053598*	119.41	**11.707**	**9.10 average**
5	2	2.654	0.1327	0.0553	36.171	**3.5462**	**5.33 stdev**
6	7.3	2.73	0.1365	0.0585	124.78	**12.233**	

ONE UP 60S: PERMANENT ENAMEL

Average hole diameter 2.657

sample	kg	dia.(in mm)	radii (in cm)	area (in cm2	kg/cm2	Mpa	
1	16.5	2.584	0.1292	0.0524	314.8	**30.862**	
2	12.4	2.681	0.1341	0.0564	219.76	**21.546**	
3	16	2.628	0.1314	0.0542	295.12	**28.933**	
4	*13.1*	*2.701*	*0.13505*	*0.057269*	228.75	**22.426**	**24.56 average**
5	13.1	2.726	0.1363	0.0583	224.57	**22.017**	**4.19 stdev**
6	11.9	2.624	0.1312	0.0541	220.17	**21.585**	

ONE UP 60S: PERMANENT DENTIN

Average hole diameter 2.690

sample	kg	dia.(in mm)	radii (in cm)	area (in cm2	kg/cm2	Mpa	
1	12.4	2.786	0.1393	0.0609	203.51	**19.952**	
2	6.3	2.605	0.1303	0.0533	118.26	**11.595**	
3	10.5	2.613	0.1307	0.0536	195.9	**19.206**	
4	*8.5*	*2.697*	*0.13485*	*0.057099*	148.86	**14.594**	**16.85 average**
5	10	2.714	0.1357	0.0578	172.95	**16.955**	**3.22 stdev**
6	11.2	2.727	0.1364	0.0584	191.86	**18.81**	