

INFLUENCE OF DENTIN DESENSITIZERS ON THE MICROTENSILE
BOND STRENGTHS OF SELF-ETCH AND ETCH-AND-RINSE
ADHESIVES TO DENTIN

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A thesis submitted to the faculty of the University of North Carolina at Chapel Hill
in partial fulfillment of the requirements for the degree of Master of Science in the
Department of Operative Dentistry.

Chapel Hill
2012

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ABSTRACT

ROOPWANT KAUR:Influence of Dentin Desensitizers on the Microtensile Bond Strengths of Self-Etch and Etch-and-Rinse Adhesives to Dentin
(Under the direction of Dr. Edward J. Swift, Jr.)

This study evaluated several dentin desensitizers' effects on dentin microtensile bond strengths (MTBS) of various self-etch (SE) and etch-and-rinse (ER) adhesives. For ER, dentin from human molars was phosphoric acid-etched, treated with a desensitizer, and coated with the adhesive. For SE, dentin was treated with the same desensitizers, and coated with the adhesive. Composite build-ups were placed and specimens stored in distilled water at 37°C for 24h. Specimens were sectioned into beams and tested either immediately or after 6 months. Data were analyzed using factorial analysis of variance. At 24h, mean MTBS's ranged from 20.0-46.6 MPa for ER and from 22.0-37.0 MPa for SE. At 6 months, mean MTBS's ranged from 22.0-45.4 MPa for ER and from 20.2-33.4 MPa for SE. The main factors (adhesive, desensitizer) and interaction effects were not statistically significant ($p>0.25$). Use of desensitizers did not affect dentin bond strengths either immediately or after aging.

Dedication

To my 'Heroes' my parents Smt. Amarbir Kaur and S. Devinder Singh

And

To my son -Viraaaj Singh

I love you.

Acknowledgement

Dr. Edward J. Swift, my mentor, Thank you for guiding me and for being a role model. For opening my horizon to research and for accepting me as an advisee. For being patient and for always being there.

Dr. Ricardo Walter, Thank you for helping me understand the concepts of research and for guiding me at every step in my research project and thesis.

Dr. Harald O. Heymann, Thank you for accepting me to the program and for giving me a chance to study operative dentistry at UNC Chapel Hill.

Dr. Andre Ritter, Thank you for the opportunity to learn and explore at UNC Chapel Hill. Your guidance and support helped me immensely.

Dr. Ceib Phillips, Thank you for being on my committee and for guiding me regarding my project and statistics.

Dr. Terry Donovan, Thank you for the literature reviews and for educating me. I learnt a lot about life from you.

Operative Dentistry Faculty for being tremendous role models for me.

Ms. Marie Roberts, Ms. Shannon Tate, Ms. Jamie Desoto, Ms. Ginger Couch, Ms. Barbara Walton, Ms. Dayna McNaught and Ms. Cynthia Lambert for always making me feel part of the operative family. The last three years have been a joy because of your warmth and support.

Operative Dentistry Residents for being supportive colleagues.

My mother and father **Smt. Amarbir Kaur and S. Devinder Singh** for making me who I am today. You are my true inspiration. Without your love and support I wouldn't have made it.

My son **Viraaj** for being the joy of my life. You make me proud each and every moment. You are a true blessing and life is beautiful because of you .I love you.

My husband **Preet** for motivating me to pursue my dreams.

TABLE OF CONTENTS

LIST OF TABLES.....	vii
LIST OF FIGURES.....	viii
LIST OF GRAPHS.....	ix
Chapter	
1. INTRODUCTION.....	1
2. LITERATURE REVIEW.....	3
2.1 Adhesives	
2.1.1 Dentin bonding.....	3
2.1.2 History of adhesives.....	6
2.2. Sensitivity with current adhesives.....	8
2.3. Desensitizers.....	11
2.4. Adhesives and desensitizers.....	14
3. SPECIFIC AIMS and NULL HYPOTHESES.....	15
4. MATERIALS AND METHODS.....	16
5. RESULTS.....	19
6. DISCUSSION.....	21
7. CONCLUSIONS.....	29
8. REFERENCES.....	45

LIST OF TABLES

TABLE 1. Experimental groups	30
TABLE 2. Desensitizers systems evaluated.....	31
TABLE 3. Adhesive systems evaluated.....	32
TABLE 4. Microtensile bond strengths for combinations of Adhesive and Desensitizer at 24 h and 6 months in MPa.....	33
TABLE 5. Microtensile bond strengths by type of adhesive in MPa.....	34
TABLE 6. Microtensile bond strengths by type of desensitizer in MPa.....	35
TABLE 7. Results from linear mixed effects model with and without interactions of explanatory variables and time.....	36
TABLE 8. Least square means for the difference between adhesive groups.....	37

LIST OF FIGURES

FIGURE 1. Experimental design.....	38
FIGURE 2. Isomet 1000 Precision saw	39
FIGURE 3. Ecomet 3 grinder/polisher machine	40
FIGURE 4. Placement of beam on the jig using adhesive.....	41
FIGURE 5. Loading on EZ-Test machine.....	42

LIST OF GRAPHS

GRAPH 1. Means and standard deviations (pooled data) for adhesives at 24 h and 6 months.....	43
GRAPH 2. Means and standard deviations (pooled data) for desensitizers at 24 h and 6 months.....	44

1. INTRODUCTION

Post-operative sensitivity, a well-localized and momentary sensitivity often associated with occlusal loading (e.g., chewing), is an ongoing problem with composite resins. A prevalence of 8-19% has been reported in patients treated with posterior composite resins with the sensitivity lasting several months in some cases. [1,2] Possible causes of post-operative sensitivity are: (a) poor dentin sealing, (b) toxicity of the resin, (c) marginal microleakage induced by polymerization contraction of the resin, and (d) hydraulic stress to odontoblastic processes generated either by the flexure of cusps due to polymerization contraction of the resin or by occlusal loading. [3]

The most accepted mechanism for dentin sensitivity is the hydrodynamic theory described by Brännström, which suggests that rapid movement of fluids within the dentinal tubules produces a deformation of nerve fibers wrapped around the odontoblast cells. [4] Thus, materials that occlude dentin tubules to any extent can potentially reduce fluid filtration across the dentin and decrease sensitivity. [5, 6]

The use of dentin desensitizers prior to adhesive application has proved effective resulting in adequate bond strengths with etch-and-rinse adhesive systems. Several studies have tested the bond strengths of composite resins to dentin after treatment with dentin desensitizers and shown no decrease in bond strengths. [7-10] However, limited literature is available on the use of desensitizers with self-etch adhesives. [11, 12] This thesis studied the effects of different desensitizers on

the microtensile bond strength (MTBS) of four etch-and-rinse and two one-step self-etch adhesives, henceforth called all-in-one, to dentin.

2. LITERATURE REVIEW

Dentin bonding has been the focus of extensive research over the past decades. The reason being is the difficulty in achieving stable long-term bonding to that substrate. The composition of dentin – 50% inorganic material, 30% organic material, and 20% water [13] – complicates the bonding procedure that otherwise is quite simple and predictable in enamel. Bonding to dentin is facilitated by dentin hybridization that can be defined as the penetration of resin monomers into the dentin matrix. [14] Mechanisms of adhesion are (1) mechanical adhesion where interlocking of adhesive with irregularities in the surface of the substrate takes place; (2) adsorption adhesion in which chemical bonding between the adhesive and the substrate takes place; and (3) diffusion adhesion, which is the interlocking between mobile molecules. [15] Some factors that may affect adhesion are effective demineralization of dentin, effective smear layer removal, good wetting of the substrate, dispersion and penetration of the adhesive. [16]

In order to form adequate bonding between dentin and restorative material, dentin bonding agents must condition (etch), prime, and bond the tooth structure. [17-19] Etch-and-rinse systems utilize phosphoric acid-etching, which removes the smear layer, opens dentinal tubules, and decalcifies dentin, as the first step. Hydroxyapatite crystals are dissolved, leaving behind a collagen network that must be kept upright for proper bonding. Over drying the dentin surface after removal of

the etchant will collapse the collagen fibers preventing infiltration of the primer/bonding resin into the collagen mesh. After the etchant is rinsed off, the second step, which is application of primer, follows. The primer contains solvents such as acetone, ethanol and/or water and one or more bifunctional resin monomers such as HEMA. HEMA has two functional groups – a hydrophilic group that will interact with dentin and a hydrophobic group that will interact with the subsequently placed bonding resin. Aside from making the connection between dentin and bonding resin, the primer wets the dentin and helps increasing its surface energy. Once priming is done, the bonding resin is applied. That will make the bridge between primed dentin and resin-based restorative material. Bonding resins contain monomers such as Bis-GMA, HEMA, UDMA, PENTA, and 4-META. In addition, filler particles create thicker resin layers that might be present and can potentially aid in providing stress relief at the tooth-restoration interface. [20] The polymerized primer/bonding resin interlocked with the collagen fibers plays a pivotal role in the bond between composite resin and dentin. This layer is known as the ‘hybrid layer,’ ‘resin reinforced zone,’ or ‘resin-infiltrated layer’ and was first described by Nakabayashi and colleagues in 1982. [21]

Another bonding approach is the self-etch approach. Self-etch adhesive systems have gained popularity in clinical dentistry as they reduce the clinical application time and may be less technique-sensitive than etch-and-rinse adhesives. [22] In two-step self-etch adhesives, the elimination of the separate acid-etching step leads to incorporation of acid monomers and primer in a single solution. The bonding resin is subsequently applied. The hybrid layer formed includes both the smear layer

and the underlying and partially infiltrated dentin. All components – etch, primer, and bonding resin – also are available in one single solution (all-in-one adhesives), although specific components may be packaged in two bottles or two reservoirs. [23, 24] Self-etch adhesives also can be subdivided according to their pH values into three groups: strong (pH 1 or below), moderate (pH 1 to 2), or mild (pH above 2).

2.1.2 History of adhesives

Since enamel was first phosphoric acid etched by Buonocore in the 1950's, bonding to the tooth structures has been focus of much research. In 1975, the first generation of adhesives was introduced but poor clinical results were found when used to restore cervical lesions without mechanical retention. [25] Developments led to a second generation of adhesives that was launched in the early 1980's and consisted of halophosphorous esters of monomers such as Bis-GMA or HEMA. The mechanism of action was interaction of phosphate groups in the adhesive and calcium ions in the smear layer along with wetting which led to bond formation. [26] However, a major shortcoming was that these adhesives bonded to smear layer, which bonds only weakly to the underlying dentin. [27] Combined with polymerization shrinkage, bond strengths of only 1-10 MPa [26] were likely to result in clinical failure. [28] Better clinical performance was noted with third-generation adhesives in the mid 1980's. [28, 29] These systems removed or modified the smear layer to cause effective penetration of resin into dentin.

Total-etch systems, currently known as etch-and-rinse, were introduced in the United States in the late 1980's. Fusayama's original concept [30] led to a technique proposed by Kanca and Bertolotti. With this technique, phosphoric acid-etching of dentin and enamel is done. [31, 32] At the time, potential pulpal damage was feared.[33]. Etching of dentin was considered taboo, but new research led to new concepts, old ideas were revisited, and the total-etch technique was accepted and

remains in use today with products such as All-Bond 2 (Bisco, Inc), OptiBond FL (Kerr) and Scotchbond Multi-Purpose (3M ESPE).

Not long after, self-etch adhesives were introduced to the market. While two-step self-etch adhesives have been available since the early 1990's, all-in-one adhesives were launched in the late 1990's. In all-in-one materials, all functional steps (i.e. conditioning, priming, and bonding) are combined in a single final solution. Through generations, various advancements in chemistry and application methods have occurred. Steps in the technique have been shortened allowing easier application and theoretically less technique sensitivity. However, post-operative sensitivity remains a concern and many clinicians use desensitizers to prevent it.

2.2. SENSITIVITY WITH CURRENT ADHESIVES

Etch-and-rinse adhesives require dentin to be left moist to allow proper bonding to occur. That will avoid collapse of the exposed collagen mesh. With two-step etch-and-rinse systems, the primer and the bonding resin are applied in a single solution simultaneously infiltrating the exposed collagen network leading to formation of the hybrid layer. [34-36] Even though that is a simplification of the technique, some two-step etch-and-rinse systems are technique-sensitive because of the difficulty in achieving adequate surface moisture, which may result in less-than-ideal bonds when the dentin is excessively wet [19, 37] or dry [38-41]. Difficulty in bonding is faced due to the intrinsic wetness of dentin after removal of the smear layer. [42,43] Incomplete sealing and continuous transudation of dentinal fluid through open dentinal tubules before polymerization of the adhesive may result in entrapment of water-filled blisters along the adhesive interface. [44] Compression of these blisters during mastication may cause, within the dentinal tubules [45], rapid fluid movement that activates the intradental A-delta nerve fibers [46] resulting in post-operative sensitivity.

In all-in-one adhesives, various acidic monomers are part of the composition and are responsible for conditioning of the tooth structures. Resultant adhesives are classified according to their pH and consequently their ability to demineralize enamel and dentin. [22, 47-49] Owing to their mild acidic nature, some have low pH and therefore can remove the smear layer and open the dentinal tubules [50]. This can lead to post-operative sensitivity may occur. [51]

Post-operative sensitivity secondary to recently placed restorations can be a result of number of factors like polymerization shrinkage related to the C-factor, aggressive tooth preparation leading to overheating, microleakage, poor adhesion protocol and contamination of the substrate during bonding etc. One of the clinical strategies used to mitigate post-operative sensitivity is dentin desensitizers. Many studies show that use of desensitizers under a restoration helps reduce sensitivity. [52-54]

Three common theories found in the literature related to sensitivity are – direct innervation theory, odontoblast receptor and fluid movement/hydrodynamic theory. [55] According to the direct innervation theory, nerve endings penetrate dentine and extend to the dentinoenamel junction (DEJ). [55] Action potential is initiated by direct mechanical stimulation of the dentinal receptors. Some shortcomings of this theory are highlighted by the fact that outer part of dentin is most sensitive yet is not innervated which does not seem to be in agreement with this theory. Newly erupted teeth also show sensitivity where the intratubular receptors don't develop until tooth has erupted itself.

The odontoblastic theory proposed by Rapp et al. states that odontoblasts act as receptors and relay the signal to nerve terminal. [56] Studies show that odontoblasts are scaffold/matrix-forming cells and are hence not capable of forming synapses between odontoblasts and nerve terminals. [56] This theory is generally lacking proof and is inconclusive.

The hydrodynamic mechanism proposed by Brännström states that pain is due to the hydrodynamic mechanism or fluid force. [4] Presence of open dentinal

tubules has been seen when SEM was done on hypersensitive dentin. [57] Presence and movement of dentinal fluid inside dentinal tubules is the basis of this theory. Centrifugal fluid movement activates the nerve endings at the end of the dentinal tubules or at the pulp dentine complex. [58] Response of the pulpal nerves is dependent on the pressure applied, i.e., intensity of stimulus applied. [58] Cooling, drying, application of hypertonic substances, acidic fruits, tooth brushing, etc. can also aggravate sensitivity [59].

The many treatment strategies for management of sensitivity are nerve desensitization using potassium nitrate, covering or plugging of dentinal tubules which includes plugging dentinal tubules using ions/salts like aluminum, aluminum hexafluorosilicate, calcium hydroxide, calcium carbonate, calcium phosphate, calcium silicate, dibasic sodium citrate, fluorosilicate, potassium oxalate, silicate, sodium monofluorophosphate, sodium fluoride, sodium fluoride/stannous fluoride combination, strontium acetate with fluoride and strontium chloride. Use of protein precipitants like formaldehyde, glutaraldehyde, silver nitrate, and strontium chloride hexahydrate and zinc chloride have proven beneficial. Phytocomplexes Rhubarb rhaipanicum and Spinacia oleracea have also been used. [60] Use of dentinal sealers such as glass ionomer cements, composites, adhesives, resin-based desensitizers, varnishes, methyl methacrylate and sealants has also been suggested. [61]

2.3. DESENSITIZERS

Dentin is a tissue containing dentinal tubules with an approximate diameter of 0.6-2.0 μm . [58] More than 2 million dentinal tubules can be exposed per cm^2 , which once exposed can provide pathways to and from the pulp. How to seal the dentinal tubules is a controversial topic.

The two main methods of treatment of sensitivity are tubular occlusion of exposed dentin and blockage of nerve activity by means of direct ionic diffusion, which leads to an increase of the concentration of potassium ions acting on pulpal nerve sensorial activity. [62]

Use of oxalate particles has been studied. Liberation of calcium from dentin occurs when acidic oxalates are applied to the dentin surface which produces insoluble calcium oxalate crystals that block dentinal tubules. It has been shown that if the dentin surface is treated with an oxalate solution, the hydraulic conductance is decreased, which can efficiently prevent sensitivity. [63]

Dentinal tubule sealers are used and common examples are adhesive restorative materials and dentinal adhesives. Dentin desensitizers have been advocated as part of the bonding procedures for many years. Potential benefits suggested in *in vitro* studies are disinfection of cavity preparations [64, 65], desensitization, and re-wetting of cavity preparations when used in conjunction with etch-and-rinse adhesives. [66, 67] Desensitizers are HEMA-containing products that may contain glutaraldehyde and chlorhexidine, for instance. Some desensitizers have shown no negative effects on bonding. [68-71]

Glutaraldehyde which is present in Gluma Desensitizer, Gluma Power Gel and MicroPrime G, all of which are used in this study, reacts with serum albumin in the dentinal fluid and coagulates the plasma proteins hence leading to reduction of dentin permeability, ultimately counteracting the hydrodynamic mechanism of dentin sensitivity. [71-74] Glutaraldehyde forms crosslinks with serum collagen and albumin [64]. After topical application of glutaraldehyde to the dentin surface, multiple transverse septa occur in the lumen of the dentinal tubules down to a depth of 200 μm , effectively creating a barrier that eliminates the hydrodynamic mechanism of dentin sensitivity. [75-78] After albumin precipitation, HEMA polymerization takes place and may be aid in sensitivity prevention. [65]

HEMA or hydroxyethylmethacrylate, which is present in Gluma Desensitizer, Gluma Power Gel, Hemaseal & Cide, and MicroPrime G, is an important component as it plays the role of physically blocking the dentinal tubules. It can be absorbed by dentin and collagen. [64] The glutaraldehyde/HEMA combination (Gluma Desensitizer, Gluma Power Gel and MicroPrime G) is an antimicrobial, a flocculating agent that strengthens collagen, and an agent that creates tubular occlusion, thereby reducing post-operative sensitivity by limiting fluid movement without affecting the strength of bonding or adhesive cements.

Other desensitizers may contain chlorhexidine or benzalkonium chloride. Chlorhexidine is present in Hemaseal & Cide which acts by protein precipitation and tubule occlusion. It can be chemisorbed on hydroxyapatite of tooth or can act as an ion to form an insoluble compound with phosphate ions in plaque, saliva and hydroxyapatite. Benzalkonium chloride is an effective antimicrobial agent present in

HurriSeal and MicroPrime B. It helps in crosslinking with HEMA to form a temporary coating hence helping in dentinal bonding. HurriSeal also contains HEMA and sodium fluoride and water. Studies have investigated the role of these materials on the exposed dentin of cervical lesions and results show an acceptable durability. [79]

2.4. ADHESIVES AND DESENSITIZERS

Adhesives and desensitizer are often used together in clinical dentistry to reduce post-operative sensitivity. Many studies have been done in the past to analyze whether there is any impact, positive or negative, and if any variables associated make a difference. Recent studies show favorable results indicating either increased bond strengths or no adverse effect.

No difference in bond strengths was found when the effect of Gluma desensitization on dentin bond strengths was evaluated. [80] No interference on behalf of desensitizers with resin cement to dentin was found when use of two HEMA-containing dentin desensitizing agents was analysed with resins. [81]

Al Qahtani and colleagues studied the effect on shear bond strength of rewetting dry dentin with two desensitizers and three etch-and-rinse dentin bonding agents (Syntac Single-Component, OptiBond Solo Plus, and Prime & Bond NT). After application to moist dentin and dry dentin, high bond strengths were noted in the HurriSeal and Prime & Bond NT group. [66-68]

On the other hand, some studies have also shown decreased bond strengths when desensitizers were used.[68,82,83] It is important to keep in mind that the type of desensitizer used may impact the bond strength and is an important factor affecting bond strength to dentin. [84] We also know that use of desensitizers under restorations has been advocated for many years. [85]

3. SPECIFIC AIMS and NULL HYPOTHESES

1. To determine whether the use of dentin desensitizers has an adverse effect on the dentin bond strengths of resin-based adhesives.
2. To determine whether time affects the dentin bond strengths of resin-based adhesives.

The null hypotheses to be tested are that desensitizers do not adversely affect dentin bond strengths of two-step etch-and-rinse and one-step self-etch adhesives to dentin and that time does not affect bond strengths of these resin-based adhesives.

4. MATERIALS AND METHODS

One hundred and fifty extracted human third molars were used in this study. As shown in Table 1, each tooth was randomly assigned to one of six adhesive groups (Excite F, iBond Total Etch, OptiBond Solo Plus, Prime & Bond NT, Adper Prompt L-Pop, Xeno IV). Teeth in each adhesive group were further assigned to one of six desensitizer groups (Gluma Desensitizer, Gluma Power Gel, Hemaseal & Cide, HurriSeal, MicroPrime B, MicroPrime G) and control. Approximately four teeth were tested per group. The control group consisted of teeth in which no desensitizers were used. Study was designed as per Figure 1.

After disinfection with Chloramine-T solution, the crowns and roots were separated using a low speed diamond disk (see Figure 3). Occlusal surfaces were ground wet with 600-grit silicon carbide paper using a mechanical grinder under to obtain flat dentin (see Figure 2).

For the etch-and-rinse adhesive groups – iBond Total Etch, Excite F, Prime & Bond NT and OptiBond Solo Plus - Ultra-Etch (35% phosphoric acid, Ultradent Products, Inc, Jordan, UT.) was applied for 15 s and then thoroughly rinsed off for 10 s. Specimens for all groups were blot using KimWipes (Kimberly-Clark, Texas, USA) prior to application of the desensitizers using micro brushes. The desensitizers were applied after etching in the etch-and-rinse groups. Desensitizers and their application are described in Table 2. Adhesives and their application are shown in Table 3.

After application of the adhesives, Venus Diamond composite resin build-ups were placed in three 2-mm increments. Each composite increment was light-cured for 40 s with a Spectrum 800 halogen light-curing unit (Dentsply Caulk) at a minimum intensity of 550 mW/cm². Specimens were stored in distilled water at 37°C and sectioned into beams using an Isomet 1000 Precision saw (Buehler, Lake Bluff, IL) after 24 h. Beams were approximately 1 x 1 mm with half of each beam consisting of composite resin and the other half of dentin. On average, each tooth yielded about 15 beams. One-third of the beams were tested at 24 h and one-third at six months aging. Remaining beams are scheduled for testing at one year; data yielded will be presented in another report.

The beams were carefully positioned in a custom notched jig so the force applied would be perpendicular to dentin-composite interface (see Figure 4). Scotch Super Glue was used to fix the beams to the jig. The adhesive was sprayed with Zap-it Accelerator Spray (Dental Ventures of America, Inc Corona, CA) to accelerate its setting process. Each beam was loaded to failure in an EZ-Test (Shimadzu, Tokyo, Japan) (see Figure 5). The MTBS was calculated dividing the peak break by the bonded area (mm²) and was expressed in megapascals (MPa). The failure mode was determined by visual inspection and classified as interfacial, cohesive in dentin, or cohesive in composite resin.

Because of the expected correlation between beams tested at 24 h and 6 months, linear mixed effects model was used with compound symmetry covariance. Because of the small sample size, Kenward-Roger method was used to compute DF (degrees of freedom) for the linear mixed effects model. [86] Adhesive, desensitizer

and time were considered as main effects, and the pairwise interactions between adhesive and time, and desensitizer and time were also considered. Pairwise contrasts among adhesives were conducted using least square means. All analyses were conducted using teeth as unit of analysis in SAS 9.2 at a significance level of 0.05.

5. RESULTS

Descriptive statistics for each adhesive/desensitizer combination at each time point are provided in Table 4. Adhesive and desensitizer MTBS at both time points are provided in Table 5 and Table 6. 3 teeth were excluded from data as they did not meet requirements.

At 24 h, the mean MTBS of the adhesives ranged from 29.9 MPa for Xeno IV to 34.0 MPa for Excite F. At 6 months, the mean MTBS ranged from 24.1 MPa for Adper Prompt L-Pop to 33.2 MPa for OptiBond Solo Plus (Table 5).

At 24 h, the mean MTBS for desensitizer groups ranged from 29.0 MPa for HurriSeal to 36.8 MPa for MicroPrime B. At 6 months, the mean MTBS ranged from 26.2 MPa for Gluma Desensitizer and HurriSeal to 31.3 for MicroPrime B (Table 6).

The interactions between adhesive and time ($p = 0.59$) and desensitizer and time ($p = 0.29$) were not statistically significant, indicating that the patterns over time were similar for all adhesives and desensitizers. After removing the interactions, there was a statistically significant difference among the means of the adhesive groups ($p = 0.046$) but not among the desensitizers ($p = 0.16$) (Table 7). There was a statistically significant difference in overall mean MTBS from 24 h to 6 months ($p=0.001$), as the microtensile bond strengths decreased over time.

The pairwise contrasts among the adhesive groups indicated statistically significant differences in the mean MTBS for four pairs of adhesives (Table 8). The average MTBS was higher in Excite F, OptiBond Solo Plus, Prime & Bond NT than

Adper Prompt L-Pop. OptiBond Solo Plus also had a higher MTBS than Xeno IV (Table 8).

6. DISCUSSION

Results of this study revealed that dentin desensitizers did not adversely affect dentin bond strengths of etch-and-rinse and self-etch adhesives. However, time did affect the findings of this study, as bond strengths were lower at six months than at 24 hours.

The problem of post-operative sensitivity commonly presents to the restorative dentist as a transient pain that cannot be related to any other dental defect or pathology. It can be a result of number of factors such as polymerization shrinkage related to the C-factor, aggressive tooth preparation leading to overheating, microleakage, poor adhesion protocol, or contamination of the substrate during bonding. The hydrodynamic (fluid movement) theory proposed by Brännström is an important theory related to understanding of sensitivity. Dentin is a complex structure and has different characteristics and variations. Procedures such as root planing, cavity preparation, or crown preparation involves stripping of cementum or the enamel layer leading to exposure of dentinal tubules. This may lead to post-operative sensitivity.

Many methods have been advocated to prevent post-operative sensitivity, including use of oxalate particles, dentinal tubule sealers and dentin desensitizers. It is known that use of certain adhesives requires conditioning which leads to post-operative sensitivity. In the current market, both self-etch and etch-and-rinse adhesives are quite popular. Application of etch-and-rinse adhesives can be

technique-sensitive, since wet bonding is required for full expansion and optimal hybridization of the demineralized collagen matrix. [87,88] Intrinsic wetness of vital deep dentin after removal of the smear layer [42] leads to difficulty in bonding, and the increased permeability associated with the simplified version of these adhesives. [43]Incomplete sealing and continuous transudation of dentinal fluid through open dentinal tubules before polymerization of the adhesive may result in entrapment of water-filled blisters along the adhesive interface. [43]Compression of these blisters during mastication may cause, within the dentinal tubules[45], rapid fluid movement that activates the intradental A-delta nerve fibers [46], which results in post-operative sensitivity.

In etch-and-rinse adhesives, currently three steps or two steps are used. Three-step etch-and-rinse adhesives have three components – etchant, primer and adhesive resin. Two-step etch-and-rinse systems include an etchant and combined primer and adhesive resin. Etch-and-rinse adhesives form an excellent bond to enamel with excellent marginal integrity. For the self-etch adhesives, two-step and one-step products are available. Two-step systems contain a self-etching primer and an adhesive resin. One-step products combine all three functional steps. According to Ernst, they may not be the first choice in all cases but might be a better idea in a variety of indications due to easier application, less chance of post-operative sensitivity and easy hybridization of dentin.

In the present study, several etch-and-rinse adhesive groups – iBond Total Etch, Excite F, OptiBond Solo Plus, and Prime & Bond NT – were tested and the one-step self-etch adhesives Adper Prompt L-Pop and Xeno IV also were evaluated.

Adper Prompt L-Pop (3M ESPE) is a strong water-based, self-etch adhesive that contains acidic methacrylated phosphoric esters. Xeno IV (Dentsply Caulk) is a milder self-etch adhesive containing mono, di, tri methacrylates and PENTA.

Results at 24 hours and 6 months for adhesives indicate that the overall mean was highest for OptiBond Solo Plus which is an etch-and-rinse adhesive, followed by ExciTE F which behaved similar or greater than Prime & Bond NT. The lowest mean MTBS was observed in the self-etch adhesives where in Xeno IV and Adper Prompt L-Pop were comparable.

Beams were evaluated at 24 hours and 6 months of storage could have impacted the bond strengths. It is known that adhesives exhibit evidence of mechanical and morphological degradation which leads to decrease in bond. [89]

It should be noted that number of teeth varied at 24 hours and 6 months. Some of the samples at 24 hours broke before MTBS testing. Hence, data for 3 teeth was excluded from data because they did not meet requirements.

The desensitizers used were Gluma Power Gel, Gluma Desensitizer, Hemaseal & Cide, HurriSeal, MicroPrime B, or MicroPrime G. Desensitizers serve the role of disinfection of cavity preparations, desensitization and rewetting on the cavity. Commonly used contain glutaraldehyde, HEMA, sodium fluoride, benzalkonium chloride and combinations. In this study, glutaraldehyde was present in Gluma, Gluma Power Gel and MicroPrime G. It reacts with serum albumin in the dentinal fluid, coagulates with plasma protein and then cross links with bovine serum collagen and albumin. HEMA present in Gluma, Gluma Power Gel, Hemaseal and Cide and MicroPrime G blocks the dentinal tubules and is absorbed by dentin and

collagen. Glutaraldehyde and HEMA are present in Gluma, Gluma Power Gel and MicroPrime G. In addition to being an effective antimicrobial agent, it is also a flocculating agent providing strength to collagen and creates tubular occlusion.

At 24 hours, highest mean MTBS was noted for MicroPrime B at 37 MPa to lowest at 29 MPa for HurriSeal whereas at six months, it ranged from 31.3 MPa for MicroPrime B to 26.2 MPa for HurriSeal. Overall, there was a decrease in MTBS over time. Lower trend of mean MTBS was noted. Desensitizers containing HEMA/Glutaraldehyde performed well.

We wanted to account for the main effects in this study – i.e. time, desensitizer and adhesive – and their interactions to determine whether any combination of factors affected bond strengths. Time was a significant factor when interactions were accounted for and remained significant even when interactions were removed. Adhesive also showed marginal significance at 0.0457 but was rounded off to 0.05 hence, not statistically significant. What can be interpreted from this finding is that time impacted the findings of this study. Lower bond strengths were obtained after specimens were stored for six months.

Many studies have been done in the past showing varied effects of use of the desensitizer on MTBS of adhesives. Although, many have shown adverse effects, some have shown increased bond strengths. Studies that have shown no effect of the use of desensitizer on MTBS are relatively few. Most studies show that there is no harm in using GLUMA which contains HEMA and glutaraldehyde.

A study investigated the influence of three dentin hypersensitivity treating agents (Gluma CPS, MS Coat and Saforide) on bond strength to dentin of two luting

agents (Panavia Fluoro Cement and Super-Bond C & B). What they found was that the use of the Gluma desensitizer did not affect bond strength of any of the three adhesive systems, and the bond strength of the Panavia cement with the AD Gel conditioning was not reduced by application of any of the three desensitizers. [90]

Another study evaluated the influence of a dentin desensitizer and ozone application on the bond strength to dentin of a composite resin material. The dentin desensitizing agent and ozone treatment were applied on the cervical dentin surfaces of extracted, caries-free, erupted third molars. Statistical analysis showed no significant influence of the different hypersensitivity treatments on shear bond strength to dentin. The authors concluded that short-term use of dentin hypersensitivity treatments like ozone and dentin desensitizers containing glutaraldehyde did not affect the shear bond strength to dentin of subsequent composite resin restorations. [91]

Investigation was done to see whether a desensitizing agent (GLUMA Desensitizer) containing glutaraldehyde and HEMA improved the bond strength and bonding durability of a self-etching primer adhesive to Er:YAG-irradiated dentine. Tensile bond strengths (TBS) of 10 specimens of each treatment group were measured after 24-h water storage, 6 months water storage and 12 months water storage, respectively, and the failure modes were analyzed. They concluded that application of GLUMA Desensitizer to Er:YAG-irradiated dentine increased the bond strength and durability of the self-etching priming adhesive used. [92]

Bond strength of a self-etching dentin adhesive was evaluated for its ability to bond to caries-affected and sound dentin after applying three desensitizers to the

gingival walls. The application of MicroPrime and Gluma Desensitizer to caries-affected dentin did not show any effect on bond strength testing. According to this study, desensitizer application on sound dentin is recommended with self-etch bonding systems.[93]

This study evaluated the effect of rewetting dried dentin with two commercial desensitizing agents (Protect and HurriSeal) on the dentin shear bond strength of three total-etch dentin bonding agents (Syntac Single-Component, OptiBond Solo Plus and Prime & Bond NT) and compared both to applying these same bonding agents to moist dentin and dry dentin. The specimens were stored in distilled water at 37 degrees C for 24 hours prior to thermocycling 2,500 times. In most groups, no significant difference in shear bond strength between the control and others was noted. In the Prime & Bond NT bonding agent groups, the shear bond strength of the HurriSeal group (20.7 MPa) was significantly higher than the mean of the other groups: control (8.0 MPa), dry (5.7 MPa) and Protect (5.5 MPa). [66]

Main aim of this study was to determine whether the use of two HEMA-containing dentin desensitizing agents [Health-Dent Desensitizer with fluoride (H) or Gluma Desensitizer (G)], when applied at simulated "cavity preparation" and "cementation" appointments, affected the bond strength of lab processed resin composite restorations cemented to dentin. The treatments were applied in two sessions in order to simulate cavity preparation and cementation appointments. Results of this in vitro study indicated that the use of desensitizers at the preparation and/or cementation appointment does not interfere with bond strengths of resin cement to dentin. [81]

In our study, the microtensile bond strength method was used to assess the bond strength. Several advantages have been advocated for use of the MTBS method developed by Sano in 1994 [94] e.g., better stress distribution at the bonding area, improved comparison of data from peripheral central dentin and ability for collection of multiple micro specimens from each tooth.

Many MTBS studies have been done in the past but due to lack of standardization, important comparisons cannot be made. Many limitations also impacted the findings of this study since sound teeth were used which may not reproduce most of the cases encountered clinically. Mostly when used clinically, desensitizers are acting on more thicker and denser smear layer which can affect how the adhesives perform.

Many factors like pulpal pressure, dentinal fluid movement and tooth dynamics cannot be mimicked in the lab. It is well known that hydraulic conductance also has an important role in adhesion. We were unable to determine the age of patient or time of extraction. Young dentin might respond differently to bonding than mature dentin. Rods within the tooth play an important role and since in our study, molars were used owing to the fact that bond strengths may be different due to increased intertubular dentin. An SEM analysis could be done to compare the formation of hybrid layer with etch-and-rinse and self-etch adhesive systems.

Within the limitations of this study, we conclude that all the desensitizers and adhesives performed equally and that time was the only factor that affected the findings of the study. A trend of lower dentin bond strengths was noted over time. Use of a desensitizer did not improve or reduce dentin bond strengths either

immediately or after aging. Clinically, it is safe to use a desensitizer along with adhesives. However, further testing needs to be done allowing more aging of samples with thermo cycling and artificially aging the teeth.

7. CONCLUSIONS

Within the limitations of our study, one can conclude that:

1. In general, the microtensile bond strengths of the various adhesive and desensitizer combinations were not significantly different.
2. Time was the only significant factor affecting dentin bond strengths, as these significantly decreased over time
3. Use of desensitizers did not significantly reduce or improve dentin bond strengths either immediately or after aging.

This study failed to reject the first null hypotheses, as the desensitizers had no adverse effect on dentin bond strengths of two-step etch-and-rinse or one-step self-etch adhesives. The second null hypothesis was rejected, as time did affect dentin bond strengths.

TABLE 1. Experimental groups

Group	Adhesive	Desensitizer
1	Excite F	Control*
2		Gluma
3		Gluma Power Gel
4		Hemaseal & Cide
5		HurriSeal
6		MicroPrime B
7		MicroPrime G
8	iBond Total Etch	Control*
9		Gluma
10		Gluma Power Gel
11		Hemaseal & Cide
12		HurriSeal
13		MicroPrime B
14		MicroPrime G
15	OptiBond Solo Plus	Control*
16		Gluma
17		Gluma Power Gel
18		Hemaseal & Cide
19		HurriSeal
20		MicroPrime B
21		MicroPrime G
22	Prime & Bond NT	Control*
23		Gluma
24		Gluma Power Gel
25		Hemaseal & Cide
26		HurriSeal
27		MicroPrime B
28		MicroPrime G
29	Adper Prompt L-Pop	Control*
30		Gluma
31		Gluma Power Gel
32		Hemaseal & Cide
33		HurriSeal
34		MicroPrime B
35		MicroPrime G
36	Xeno IV	Control*
37		Gluma
38		Gluma Power Gel
39		Hemaseal & Cide
40		HurriSeal
41		MicroPrime B
42		MicroPrime G

*No desensitizer used

TABLE 2. Desensitizers evaluated

Desensitizer	Composition	Application method
Gluma (Heraeus Kulzer)	Glutaraldehyde 5% HEMA 35% Water	1.Rubbed onto the dentin surface for 40 s 2.Rinsed thoroughly 3.Excess material was removed with an air-syringe
Gluma Power Gel (Heraeus Kulzer)	Glutaraldehyde 2-HEMA Silica	1.Applied for 40 seconds 2.Rinsed thoroughly 3.Dried with light air stream
Hemaseal&Cide (Advantage Dental Products)	4% chlorhexidine digluconate 2-hydroxyethylmethacrylate Water	1.Scrubbed onto the dentin surface for 15 s 2.Whisked away excess with high-volume suction and microbrush leaving dentin slightly wet prior to bonding procedures
HurriSeal (Beutlich)	Benzalkonium chloride HEMA Sodium fluoride Water	1.Rubbed desensitizer (2 coats) onto the dentin surface for 20 s 2.Blotted with Kim wipe 3.Air syringe was not used
MicroPrime B (Danville)	Benzethonium chloride Sodium fluoride	1.Rubbed onto the dentin surface for 30 s 2.Rinsed thoroughly 3.Excess material was removed with an air-syringe
MicroPrime G (Danville)	Glutaraldehyde HEMA Sodium Fluoride Water	1.Rubbed onto the dentin surface for 40 s 2.Rinsed thoroughly 3.Excess material was removed with an air-syringe

Bis GMA - bisphenol A-glycidyl methacrylate; HEMA - hydroxyethylmethacrylate; UDMA - urethane dimethacrylate; 4-META - methacryloyloxyethyl trimellitate anhydride; PENTA - dipentaerythritolpentaacrylate monophosphate

TABLE 3. Adhesive systems evaluated

Adhesives	Composition	Application method
Adper Prompt L-Pop (3M ESPE)	Bis GMA 2- (HEMA) Methacrylated phosphoric esters Polyalkenoic acid Initiators based on camphorquinine Stabilizers Water	1. Rubbed onto the dentin surface with moderate pressure for 15 s. 2. Adhesive was air thinned until no movement could be detected 3. Adhesive was light-cured for 10 s
iBond Total Etch (Heraeus Kulzer)	UDMA 4-META HEMA Glutaraldehyde Polymeric monomers + nano sized amorphous silica in ethanol	1. Applied to the dentin surface with a microbrush and allowed to set for 15 s 2. Ethanol solvent was removed with a gentle air stream. 3. Adhesive was light-cured for 20 s.
Excite F (Ivoclar Vivadent)	Catalysts Stabilizers Fluoride Dimethacrylate HEMA Highly dispersed silica Ethanol	1. Saturated with the adhesive using the pre-mounted applicator brush. 2. Adhesive was agitated for 10 s and dispersed to a thin layer with a weak stream of air. 3. Adhesive was light-cured for 10 s.
OptiBond Solo Plus (Kerr)	Alkyl dimethacrylate resins Barium aluminoborosilicate glass Ethyl alcohol Fumed silica (silicon dioxide) Sodium hexafluorosilicate	1. Treated with light brushing motion for 15 s. 2. Adhesive was air-thinned for 3 s 3. Adhesive was light-cured for 20 s
Prime & Bond NT (DENTSPLY Caulk)	Acetone Cetylamine hydrofluoride Di and trimethacrylate resins PENTA Nano fillers - amorphous silicon dioxide Photoinitiators Stabilizers	1. Left undisturbed for 20 s 2. Gently thinned with an air stream for 5 seconds. 3. Adhesive was light-cured for 1
Xeno IV (DENTSPLY Caulk)	Mono/di/trimethacrylate resins; PENTA Photoinitiators; Stabilizers; Cetylamine hydro fluoride; Acetone; Water	1. Scrubbed onto the dentin surface twice for 15 s. 2. Applied and lightly scrubbed for 15 seconds, 3. Applied another coat and lightly scrubbed for 15 seconds. 4. Adhesive was then light-cured for 10 s.

Bis-GMA - bisphenol A-glycidyl methacrylate; HEMA - hydroxyethyl methacrylate; UDMA - urethane dimethacrylate; 4-META - methacryloyloxyethyl trimellitate anhydride; PENTA - dipentaerythritolpentaacrylate monophosphate

TABLE 4. Microtensile bond strengths for combinations of adhesive and desensitizer at 24 h and 6 months in MPa*

Adhesive	Desensitizer	24 hours		6 months	
		Mean	S.D.	Mean	S.D.
ExcITE F	Control	28.9	3.8	24.1	5.9
	Gluma	33.1	9.2	28.1	8.5
	Gluma Power Gel	35.8	11.7	45.4	31.2
	Hemaseal & Cide	46.5	8.3	26.7	2.3
	HurriSeal	22.6	9.5	26.1	5.6
	MicroPrime B	43.6	18.9	28.5	11.0
	MicroPrime G	34.3	7.5	36.0	5.6
iBond Total Etch	Control	36.6	5.1	34.6	7.6
	Gluma	38.9	16.9	27.0	4.6
	Gluma Power Gel	19.9	9.8	24.9	10.1
	Hemaseal & Cide	30.0	5.3	30.0	7.5
	HurriSeal	37.2	3.5	26.4	3.2
	MicroPrime B	37.4	7.5	26.9	5.8
	MicroPrime G	42.4	2.2	24.1	3.7
OptiBond Solo Plus	Control	42.3	18.2	38.0	9.1
	Gluma	26.6	6.6	32.9	21.1
	Gluma Power Gel	34.5	4.8	37.8	11.4
	Hemaseal & Cide	33.1	6.8	29.4	5.12
	HurriSeal	28.1	12.1	28.0	4.0
	MicroPrime B	35.5	12.7	30.8	11.4
	MicroPrime G	30.7	12.8	32.6	10.4
Prime & Bond NT	Control	30.3	6.6	25.1	10.8
	Gluma	32.6	6.5	23.2	6.5
	Gluma Power Gel	30.8	7.4	30.4	10.4
	Hemaseal & Cide	39.2	5.3	29.5	6.5
	HurriSeal	27.1	6.2	21.8	6.1
	MicroPrime B	40.2	1.9	38.3	7.5
	MicroPrime G	35.0	9.5	36.0	10.0
Adper Prompt L-Pop	Control	21.9	8.1	21.7	6.0
	Gluma	36.8	16.4	23.7	9.5
	Gluma Power Gel	31.0	3.8	23.1	11.0
	Hemaseal & Cide	28.4	7.9	23.6	6.0
	HurriSeal	27.1	11.9	30.2	3.3
	MicroPrime B	31.3	9.3	28.3	1.7
	MicroPrime G	37.0	3.0	20.2	6.8
Xeno IV	Control	28.1	14.4	29.2	7.9
	Gluma	27.4	8.4	24.8	7.5
	Gluma Power Gel	28.6	11.1	29.0	18.9
	Hemaseal & Cide	34.2	6.4	26.7	7.4
	HurriSeal	35.9	17.6	24.7	4.2
	MicroPrime B	34.4	18.9	33.4	6.2
	MicroPrime G	23.6	5.8	23.5	5.2

TABLE 5. Microtensile bond strengths by type of adhesive in MPa

Adhesive	24 hours			6 months		
	# of teeth *	Mean	SD	# of teeth *	Mean	SD
Excite F	24	34.0	12.2	25	30.9	14.5
Prime & Bond NT	24	33.6	7.4	25	29.6	9.7
iBond Total Etch	23	33.4	10.9	22	27.1	6.6
OptiBond Solo Plus	21	33.0	10.8	24	33.2	10.3
Adper Prompt L-Pop	28	30.2	10.4	28	24.1	7.1
Xeno IV	30	29.9	12.2	31	27.6	9.4

*Number of teeth varied at 24 hours and 6 months. Some of the specimens at 24 hours broke before MTBS testing. Data for those specimens (3 teeth) were not included.

TABLE 6. Microtensile bond strengths by type of desensitizer in MPa

24 hours				6 months		
Desensitizer	# of teeth*	Mean	SD	# of teeth*	Mean	SD
MicroPrime B	20	36.8	12.7	20	31.3	7.7
Hemaseal & Cide	20	34.6	8.7	20	27.3	5.8
MicroPrime G	21	33.5	9.0	23	29.1	9.3
Gluma	22	32.5	11.4	23	26.2	9.7
control	22	30.2	11.6	24	28.6	9.5
Gluma Power Gel	25	29.4	9.8	25	31.1	17.2
HurriSeal	20	29.0	10.8	20	26.2	4.8

*Number of teeth varied at 24 hours and 6 months. Some of the specimens at 24 hours broke before MTBS testing. Data for those specimens (3 teeth) were not included.

TABLE 7. Results from linear mixed effects model with and without interactions of explanatory variables and time

Effect	Numerator DF*	Denominator DF*	F Value*	P*	F Value	P
Adhesive	5	149	2.26	0.05	2.32	0.046
Desensitizer	6	176	1.59	0.15	1.57	0.15
Time	1	142	11.67	0.0008	10.94	.001
Adhesive*Time	5	146	0.75	0.59	N/A	N/A
Desensitizer*Time	6	163	1.25	0.28	N/A	N/A

*with variables evaluated i.e. adhesive *time and desensitizer*time

TABLE 8. Least square means for the global difference between adhesive groups

Group 1	Group 2	Difference (Group 1–Group 2)	S.E.	DF	t Value	P
iBond Total Etch	Excite F	-2.56	2.30	146	-1.11	0.27
	OptiBond Solo Plus	-3.05	2.32	164	-1.31	0.19
	Prime & Bond NT	-1.33	2.28	151	-0.58	0.57
	Adper Prompt L-Pop	3.03	2.23	148	1.36	0.18
	Xeno IV	1.41	2.18	150	0.65	0.52
Excite F	OptiBond Solo Plus	-0.49	2.30	149	-0.21	0.83
	Prime & Bond NT	1.23	2.25	148	0.55	0.58
	Adper Prompt L-Pop	5.59	2.20	144	2.54	0.01
	Xeno IV	3.97	2.14	146	1.85	0.06
OptiBond Solo Plus	Prime & Bond NT	1.73	2.27	160	0.76	0.44
	Adper Prompt L-Pop	6.08	2.22	151	2.74	0.0069
	Xeno IV	4.46	2.17	152	2.06	0.04
Prime & Bond NT	Adper Prompt L-Pop	4.35	2.18	151	2.00	0.04
	Xeno IV	2.73	2.11	160	1.29	0.19
Adper Prompt L-Pop	Xeno IV	-1.62	2.06	147	-0.78	0.43

FIGURE 1. Experimental design

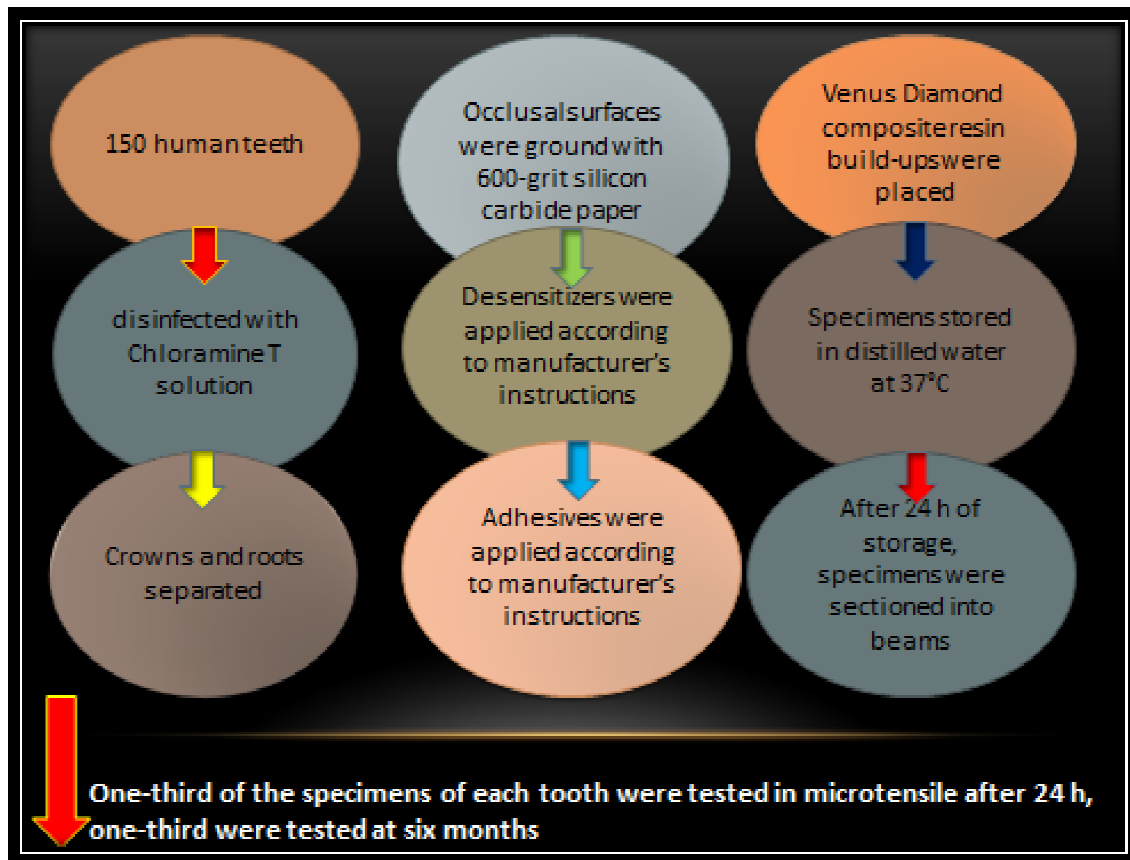


FIGURE 2. Isomet 1000 Precision saw

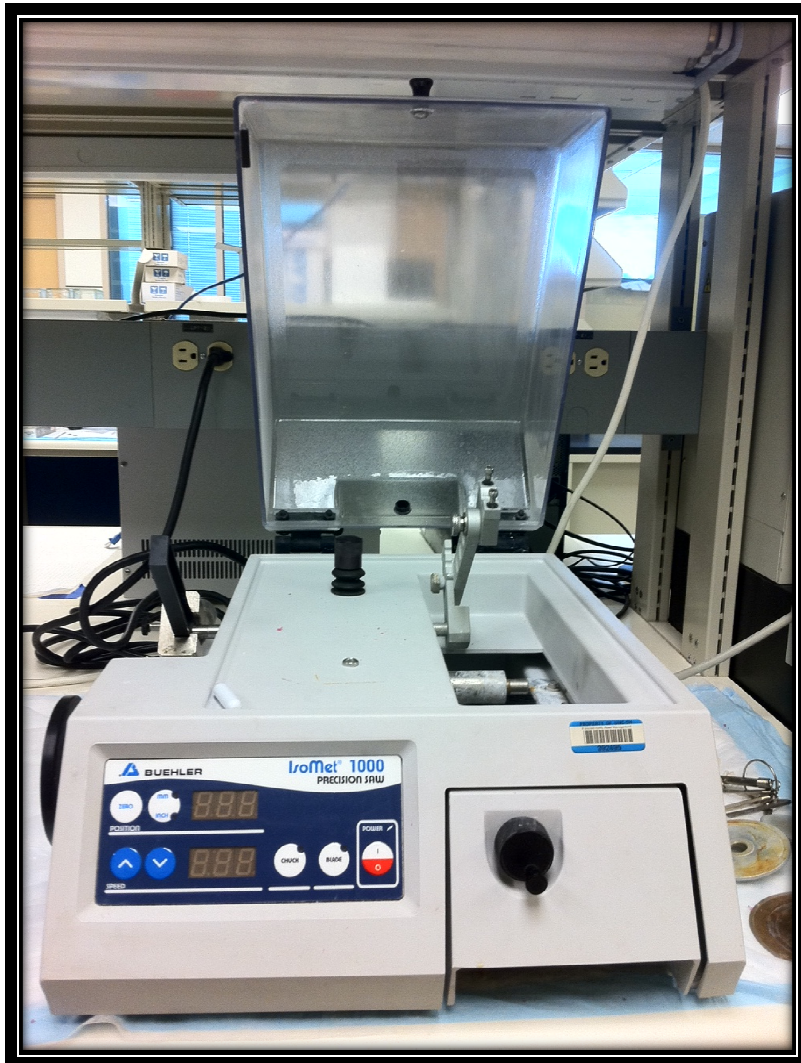


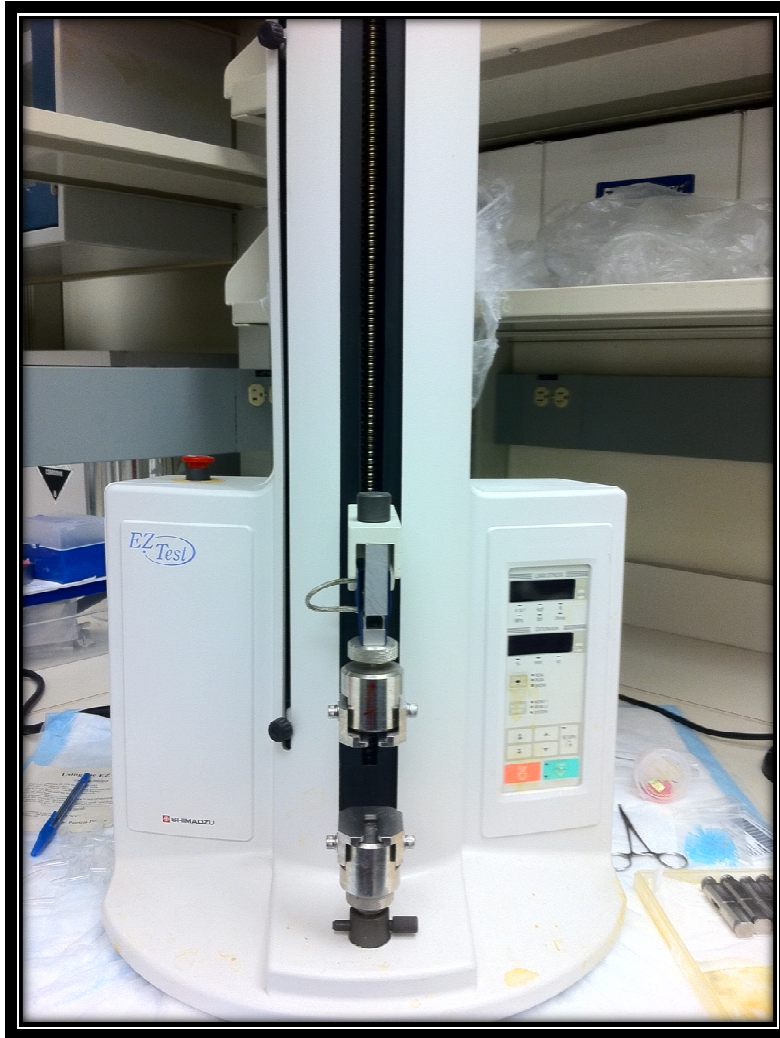
FIGURE 3. Ecomet 3 grinder/polisher machine



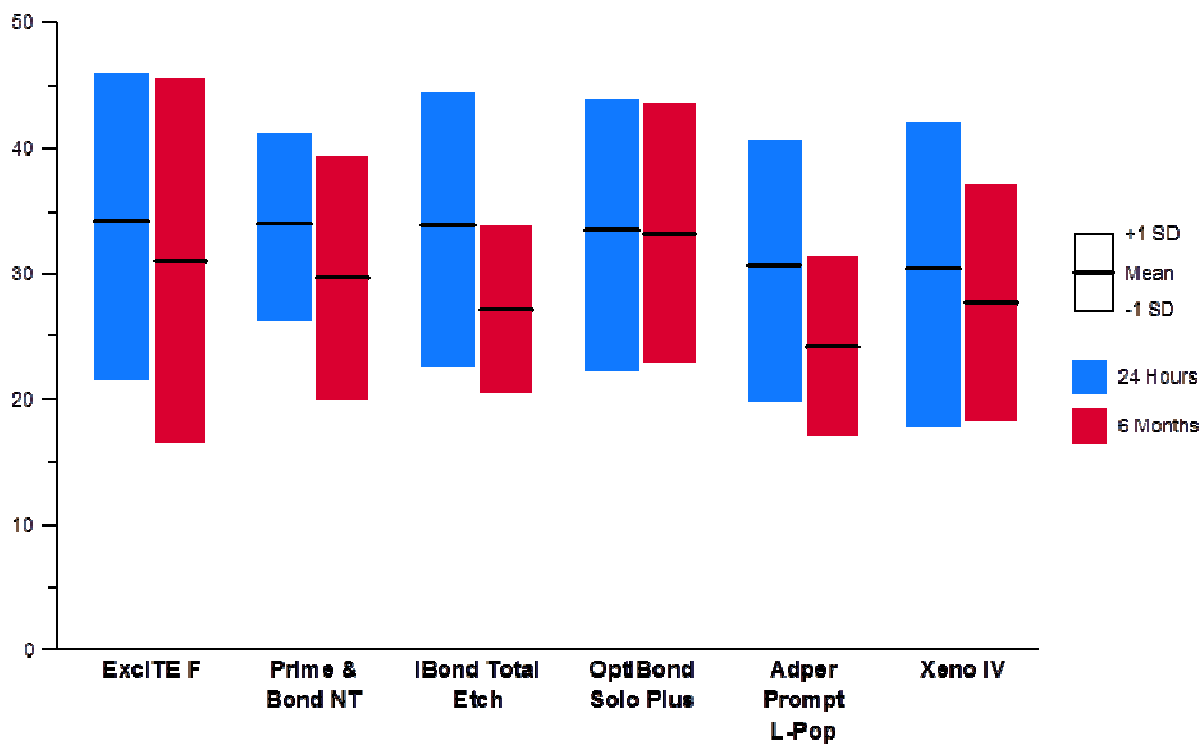
FIGURE 4. Placement of beam on the jig using adhesive



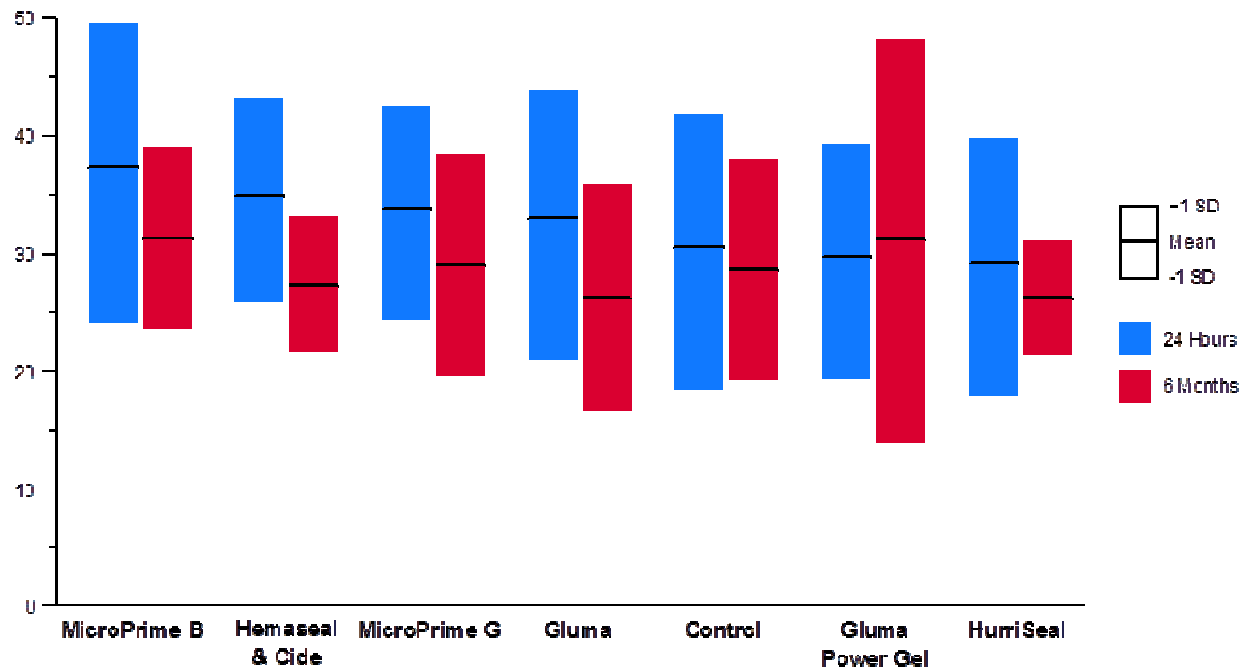
FIGURE 5. Loading on EZ-Test machine



GRAPH 1. Means and standard deviations (pooled data) for adhesives at 24 h and 6 months



GRAPH 2. Means and standard deviations (pooled data) for desensitizers at 24 h and 6 months



REFERENCES

1. Leinfelder KF. *Wear patterns and failure mechanisms*. In: Posterior Composites – Proceedings of International Symposium on Resins 1982; Taylor DF, ed., Chapel Hill, NC: The University of North Carolina Press, 329-357.
2. Johnson GH, Gordon GE, Bales DJ. *Postoperative sensitivity associated with posterior composite and amalgam restorations*. Oper Dent 1988; 13:66-73.
3. Suzuki M, Jordan RE, Boksman L. *Posterior composite restorations - clinical considerations*. In: Posterior Composite Resin Dental Restorative Materials 1985; Vanherle G and Smith DC, Ed., St. Paul, MN: 3M Co., 455-464.
4. Brännström M, Aström A. *The hydrodynamics of the dentine; its possible relationship to dentinal pain*. Int Dent J 1972; 22:219-227.
5. Jain P, Vargas MA, Denehy GE, Boyer DB. *Dentin desensitizing agents: SEM and X-ray microanalysis assessment*. Am J Dent 1997; 10:21-26.
6. Pashley DH, Livingston MJ, Greenhill JD. *Regional resistances to fluid flow in human dentine in vitro*. Arch Oral Biol 1978; 23:807-810.
7. Dondi dall'Orologio G, Malferrari S. *Desensitizing effects of Gluma and Gluma 2000 on hypersensitive dentin*. Am J Dent 1993; 6:283-286.
8. Felton DA, Bergenholtz G, Kanoy BE. *Evaluation of the desensitizing effect of Gluma Dentin Bond on teeth prepared for complete-coverage restorations*. Int J Prosthodont 1991; 4:292-298.
9. Soeno K, Taira Y, Matsumura H, Atsuta M. *Effect of desensitizers on bond strength of adhesive luting agents to dentin*. J Oral Rehabil 2001; 28:1122-1128.
10. Cobb DS, Reinhardt JW, Vargas MA. *Effect of HEMA-containing dentin desensitizers on shear bond strength of a resin cement*. Am J Dent 1997; 10:62-65.
11. Sengun A, Koyuturk AE, Sener Y, Ozer F. *Effect of desensitizers on the bond strength of a self-etching adhesive system to caries-affected dentin on the gingival wall*. Oper Dent 2005; 30:430-435.

12. Yu X, Liang B, Jin X, Fu B, Hannig M. *Comparative in vivo study on the desensitizing efficacy of dentin desensitizers and one-bottle self-etching adhesives*. Oper Dent 2010; 35:279-286.
13. Marshall GW, Inai N, Magidi IW, Balooch M, Kinney JH, Tagami J, Marshall SJ. *Dentin demineralization effects of dentin depth, pH and different acids*. Dent Mater 1997;13:338-343.
14. Söderholm, KJ. *Correlation of in vivo and in vitro performance of adhesive restorative materials: a report of the ASC MD156 Task Group on Test Methods for the Adhesion of Restorative Materials*. Dent Mater 1991; 7:74-83.
15. Allen KW: *Theories of adhesion*. In Packham DE, editor, Handbook of adhesion, 1992, 473-475.
16. Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, Coutinho E, Suzuki K, Lambrechts P, Van Meerbeek B. *Systematic review of the chemical composition of contemporary dental adhesives*. Biomaterials 2007; 28:3757-3785.
17. Van Meerbeek B, Inokoshi S, Braem M, Lambrechts P, Vanherle G. *Aspects of the resin-dentin interdiffusion zone with different dentin adhesive systems*. J Dent Res 1992; 71:1530-1540.
18. Inokoshi S, Hosoda H, Harnirattisai C, Shimada Y. *Interfacial structure between dentin and seven dentin bonding systems revealed using argon ion beam etching*. Oper Dent 1993; 18:8-16.
19. Swift EJ, Heymann HO, Perdigão J. *Bonding to enamel and dentin: A brief history and state of the art*. Quintessence Int 1995; 26:95-110.
20. Condon J.R,J.L Ferracane. *Reduction of composite contraction stress through non-bonded microfiller particles*. Dental Materials 1998,14:256-260.
21. Nakabayashi N, Kojima K, Masuhara E. *The promotion of adhesion by the infiltration of monomers into tooth substrates*. J Biomed Mater Res 1982; 16:265-273.
22. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, Van Landuyt K, Lambrechts P, Vanherle G. *Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges*. Oper Dent 2003; 28:215-235.

23. Eick JD, Gwinnett AJ, Pashley DH, Robinson SJ. *Current concepts on adhesion to dentin*. Crit Rev Oral Biol Med 1997; 8:306-335.
24. Albaladejo A, Osorio R, Toledano M, Ferrari M. *Hybrid layers of etch-and-rinse versus self-etching adhesive systems*. Med Oral Patol Oral Cir Bucal 2010; 15:112-118.
25. Jendresen MD. *Clinical performance of a new composite resin for Class V erosion* (abstract 1057). J Dent Res 1978; 57:339.
26. Eliades GC, Caputo AA, Vougiouklakis GJ. *Composition, wetting properties and bond strength with dentin of 6 new dentin adhesives*. Dent Mater 1985; 1:170-176.
27. Davidson CL, de Gee AJ, Feilzer A. *The competition between the composite-dentin bond strength and the polymerization contraction stress*. J Dent Res 1984; 63:1396-1399.
28. Van Meerbeek B, Peumans M, Verschueren M, Gladys S, Braem M, Lambrechts P. *Clinical status of ten dentin adhesive systems*. J Dent Res 1994; 73:1690-1702.
29. Van Meerbeek B, Peumans M, Gladys S, Braem M, Lambrechts P, Vanherle G. *Three-year clinical effectiveness of four total-etch systems in cervical lesions*. Quintessence Int 1996; 27:775-784.
30. Fusayama T, Nakamura M, Kurosaki N, Iwaku M. *Non-pressure adhesion of a new adhesive restorative resin*. J Dent Res 1979; 58:1364-1370.
31. Kanca J. *Bonding to tooth structure: A rational rationale for a clinical protocol*. J Esthet Dent 1989; 1:135-138.
32. Bertolotti RL. *Total-etch—the rational dentin bonding protocol*. J Esthet Dent 1991; 3:1-6.
33. Macko DJ, Rutberg M, Langeland. *Pulpal response to the application of phosphoric acid to dentin*. Oral Surg Oral Med Oral Pathol 1978; 45:930-946.
34. Kanca J. *A method for bonding to tooth structure using phosphoric acid as a dentin-enamel conditioner*. Quintessence Int 1991; 22:285-290.
35. Gwinnett AJ. *Quantitative contribution of resin infiltration/hybridization to dentin bonding*. Am J Dent 1993; 6:7-9.

36. Shimono M, Maeda T, Suda H, Takayashi K, eds. *Dentin/pulp complex*. Tokyo: Quintessence; 1996; 359-363.
37. Tay FR, Gwinnett AJ, Wei SH. *The overwet phenomenon: an optical, micromorphological study of surface moisture in the acid-conditioned, resin-dentin interface*. Am J Dent 1996; 9:43-48.
38. Nakabayashi N, Pashley DH. *Hybridization of dental hard tissues*. Tokyo: Quintessence; 1998.
39. Kanca J 3rd. *Improving bond strength through acid etching of dentin and bonding to wet dentin surfaces*. J Am Dent Assoc 1992; 123:35-43.
40. Kanca J 3rd. *Wet bonding: effect of drying time and distance*. Am J Dent 1996; 9:273-276.
41. Manso AP, Marquezini L Jr, Silva SM, Pashley DH, Tay FR, Carvalho RM. *Stability of wet versus dry bonding with different solvent-based adhesives*. Dent Mater 2008; 24:476-482.
42. Itthagarun A, Tay FR. *Self-contamination of deep dentin by dentin fluid*. Am J Dent 2000; 13:195-200.
43. Tay FR, Gwinnett AJ, Wei SH. *The overwet phenomenon: a transmission electron microscopic study of surface moisture in the acid-conditioned, resin-dentin interface*. Am J Dent 1996; 9:161-166.
44. Tay FR, Suh BI, Pashley DH, Prati C, Chuang SF, Li F. *Factors contributing to the incompatibility between simplified-step self-cured or dual-cured composites. Part II. Single-bottle, total-etch adhesive*. J Adhes Dent 2003; 5:91-105.
45. Brännström M, Johnson G. *Movements of the dentine and pulp liquids on application of thermal stimuli. An in vitro study*. Acta Odontol Scand 1970; 28:59-70.
46. Närhi M, Yamamoto H, Ngassapa D, Hirvonen T. *The neurophysiological basis and the role of inflammatory reactions in dentine hypersensitivity*. Arch Oral Biol 39:23S-30S.
47. Yoshida Y, Nagakane K, Fukuda R, Nakayama Y, Okazaki M, Shintani H, Inoue S, Tagawa Y, Suzuki K, De Munck J, Van Meerbeek B. *Comparative study on adhesive performance of functional monomers*. J Dent Res 2004; 83:454-458.

48. Ernst, CP. *Options for dentin bonding*. J Esthet Restor Dent 2006; 18:61-67.
49. Peumans M, Kanumilli P, De Munck J, et al. Clinical effectiveness of contemporary adhesives: a systematic review of current clinical trials. Dent Mater 2005; 21:864-881
50. Skupien JA, Susin AH, Angst PD, Anesi R, Machado P, Bortolotto T, Krejci I. *Micromorphological effects and the thickness of the hybrid layer – a comparison of current adhesive systems*. J Adhes Dent 2010; 12:435-442.
51. Perdigão J, Dutra-Correa M, Anauate-Netto C, Castilhos N, Carmo AR, Lewgoy HR, Amore R, Cordeiro HJ. *Two-year clinical evaluation of self-etching adhesives in posterior restorations*. J Adhes Dent 2009; 11:149-159.
52. Jalalian E, Meraji N, Mirzaei M. *A comparison of the efficacy of potassium nitrate and Gluma desensitizer in the reduction of hypersensitivity in teeth with full-crown preparations*. J Contemp Dent Pract 2009; 10:66-73.
53. Ozen T, Orhan K, Avsever H, Tunca YM, Ulker AE, Akyol M. *Dentin hypersensitivity: a randomized clinical comparison of three different agents in a short-term treatment period*. Oper Dent 2009; 34:392-398.
54. Yu X, Liang B, Jin X, Fu B, Hannig M. *Comparative in vivo study on the desensitizing efficacy of dentin desensitizers and one-bottle self-etching adhesives*. Oper Dent 2010; 35:279-286.
55. Irvine JH. *Root surface sensitivity: A review of aetiology and management*. J N Z Soc Periodontol 1988; 66:15-18.
56. Rapp R, Avery JK, Strachan DS. *Possible role of the acetylcholinesterase in neural conduction within the dental pulp*. In: Finn SB, editor. Biology of the dental pulp organ. Birmingham: University of Alabama Press; 1968:309-331.
57. Absi EG, Addy M, Adams D. *Dentine hypersensitivity: A study of the patency of dentinal tubules in sensitive and non-sensitive cervical dentine*. J Clin Periodontol 1987; 14:280-284.
58. Pashley DH. *Dynamics of the pulpo-dentinal complex*. Crit Rev Oral Biol Med 1996; 7:104-133.

59. Chidchuangchai W, Vongsavan N, Matthews B. *Sensory transduction mechanisms responsible for pain caused by cold stimulation of dentine in man*. Arch Oral Biol 2007; 52:154-160.
60. Miglani S. *Dentin hypersensitivity: recent trends in management*. J Conserv Dent 2010; 13:218-224.
61. Pashley DH. *Dentin permeability, dentin sensitivity and treatment through tubule occlusion*. J Endod 1986; 12:465-474.
62. Brännström M. *The hydrodynamic theory of dentinal pain: sensation in preparations, caries, and the dentinal crack syndrome*. J Endod 1986; 12:453-457.
63. Pashley DH, Galloway SE. *The effect of oxalate treatment on the smear layer of ground surfaces of human dentin*. Arch Oral Biol 1985; 30:731-737.
64. Dondi dall'Orologio G, Lone A, Finger WJ. *Clinical evaluation of the role of glutardialdehyde in a one-bottle adhesive*. Am J Dent 2002; 15:330-334.
65. Qin C, Xu J, Zhang Y. *Spectroscopic investigation of the function of aqueous 2-hydroxyethylmethacrylate/glutaraldehyde solution as a dentin desensitizer*. Eur J Oral Sci 2006; 114:354-359.
66. Al Qahtani MQ, Platt JA, Moore BK, Cochran MA. *The effect on shear bond strength of rewetting dry dentin with two desensitizers*. Oper Dent 2003; 28:287-296.
67. Walter R, Miguez PA, Swift EJ Jr, Pereira PN. *Long-term bond strength to dentin treated with different re-wetting solutions*. Am J Dent 2008; 21:143-147.
68. Aranha AC, Siqueira Junior A de S, Cavalcante LM, Pimenta LA, Marchi GM. *Microtensile bond strengths of composites to dentin treated with desensitizer products*. J Adhes Dent 2006; 8:85-90.
69. Kobler A, Schaller HG, Gernhardt CR. *Effects of the desensitizing agents Gluma and Hyposen on the tensile bond strength of dentin adhesives*. Am J Dent 2008; 21:388-392.
70. Walter R, Swift EJ Jr, Ritter AV, Bartholomew WW, Gibson CG. *Dentin bonding of an etch-and-rinse adhesive using self- and light-cured composites*. Am J Dent 2009; 22:215-218.

71. Bergenholtz G, Jontell M, Tuttle A, Knutsson G. *Inhibition of serum albumin flux across exposed dentine following conditioning with GLUMA primer, glutaraldehyde or potassium oxalates.* J Dent 1993; 21:220-227.
72. Suh BI, Feng L, Pashley DH, Tay FR. *Factors contributing to the incompatibility between simplified-step adhesives and chemically-cured or dual-cured composites. Part III. Effect of acidic resin monomers.* J Adhes Dent 2003; 5:267-282.
73. Swift EJ Jr, Lloyd AH, Felton DA. *The effect of resin desensitizing agents on crown retention.* J Am Dent Assoc 1997; 128:195-200.
74. Swift EJ Jr, Perdigão J, Combe EC, Simpson CH 3rd, Nunes MF. *Effects of restorative and adhesive curing methods on dentin bond strengths.* Am J Dent 2001; 14:137-140.
75. Takahashi A, Sato Y, Uno S, Pereira PN, Sano H. *Effects of mechanical properties of adhesive resins on bond strength to dentin.* Dent Mater 2002; 18:263-268.
76. Tay FR, Pashley DH, Mak YF, Carvalho RM, Lai SC, Suh BI. *Integrating oxalate desensitizers with total-etch two-step adhesive.* J Dent Res 2003; 82:703-707.
77. Tay FR, Pashley DH, Suh BI, Carvalho RM, Itthagarun A. *Single-step adhesives are permeable membranes.* J Dent 2002; 30:371-382.
78. Tay FR, Pashley DH. *Water treeing--a potential mechanism for degradation of dentin adhesives.* Am J Dent 2003; 16:6-12.
79. Ling TY, Gillam DG. *The effectiveness of desensitizing agents for the treatment of cervical dentine sensitivity (CDS)--a review.* J West Soc Periodontol Periodontal Abstr 1996; 44:5-12.
80. Reinhardt JW, Stephens NH, Fortin D. *Effect of Gluma desensitization on dentin bond strength.* Am J Dent 1995; 8:170-172.
81. Cobb DS, Reinhardt JW, Vargas MA. *Effect of HEMA-containing dentin desensitizers on shear bond strength of a resin cement.* Am J Dent 1997; 10:62-65.
82. Seara SF, Erthal BS, Ribeiro M, Kroll L, Pereira GD. *The influence of a dentin desensitizer on the microtensile bond strength of two bonding systems.* Oper Dent. 2002;2:154-160.

83. Silva S, Malacarne-Zanon J, Carvalho R, Alves M, De Goes M, Anido-Anido A, Carrilho M. *Effects of Potassium Oxalate on Knoop Hardness of Etch-and-Rinse Adhesives*. Oper Dent. 2012. E publication.
84. Külünk S, Saraç D, Külünk T, Karakaş O. *The effects of different desensitizing agents on the shear bond strength of adhesive resin cement to dentin*. J Esthet Restor Dent 2011; 23:380-387.
85. Ravikumar N, Shankar P, Indira R. *Shear bond strengths of two dentin bonding agents with two desensitizers: An in vitro study*. J Conserv Dent 2011; 14:247-251.
86. Kenward MG, Roger JH. *Small sample inference for fixed effects from restricted maximum likelihood*. Biometrics 1997; 53:983-997.
87. Gwinnett AJ. *Moist versus dry dentin: its effect on shear bond strength*. Am J Dent 1992; 5:127-129.
88. Kanca J. *Resin bonding to wet substrate. I. Bonding to dentin*. Quintessence Int 1992; 23:39-41
89. Perdigão J. *Dentin bonding-variables related to the clinical situation and the substrate treatment*. Dent Mater 2010; 26:24-37.
90. Soeno K, Taira Y, Matsumura H, Atsuta M. *Effect of desensitizers on bond strength of adhesive luting agents to dentin*. J Oral Rehabil 2001; 28:1122-1128.
91. Can-Karabulut DC, Karabulut B. *The effect of dentin hypersensitivity treatments on the shear bond strength to dentin of a composite material*. Gen Dent 2011; 59:12-17.
92. Omae M, Inoue M, Itota T, et al. *Effect of a desensitizing agent containing glutaraldehyde and HEMA on bond strength to Er:YAG laser-irradiated dentine*. J Dent 2007; 35:398-402.
93. Sengun A, Koyuturk AE, Sener Y, Ozer F. *Effect of desensitizers on the bond strength of a self-etching adhesive system to caries-affected dentin on the gingival wall*. Oper Dent 2005; 30:430-435.
94. Sano H. *Relationship between surface area for adhesion and tensile bond strength--evaluation of a micro-tensile bond test*. Dent Mater 1994; 10:236-240.