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IN VITRO MICRO SHEAR BOND STRENGTH OF DIFFERENT ADHESIVE SYSTEMS TO SOUND, CARIES AFFECTED AND SCLEROTIC DENTIN

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ABSTRACT

This in-vitro study compared the Micro Shear bond strength of different adhesive systems to prepared specimens of sound, caries affected and sclerotic dentin.

Materials and methods: A total of 90 freshly extracted human molar teeth were collected, 30 prepared for each type of dentin either sound, caries affected or sclerotic dentin. Every type of the prepared dentin groups was subdivided into five subgroups, each receive one of the tested adhesives (n=6). The teeth were embedded vertically in cylindrical Teflon mold of 2 cm height and 20 mm internal diameter using a chemically cured cross-linked acrylic resin, so as to have the full crown projecting above the surface of acrylic resin. Teeth were ground off using model trimmer with low speed and copious water coolant in direction perpendicular to the long axis of the tooth to expose the dentin surface. All the tested adhesive materials were applied on prepared dentin surface (Sound, carious, and sclerotic) together with resin composite material according to manufactures instructions. The prepared specimens were stored immediately in deionized water for 24 hours at 37°C before testing procedures. Micro shear bond strength (µSBS) was tested using the micro shear bond apparatus (DL200 Emic Sao Jose de Pinhas, PR, and Brazil). µSBS MPA= Shear force (N)/ cross sectional area (mm^2).

Results: ANOVA was used for the effect of type of dentin and adhesive system used. Sound dentin showed the highest µSBS followed by carious dentin and at last came sclerotic dentin. For the adhesive system total etch showed the highest µSBS followed by two step self-etch with etch and rinse and two step self-etch and at last came one step self-etch with etch and rinse and one step self-etch.

Conclusion: Total-etch adhesive revealed higher micro shear bond strength than self-etch adhesive irrespective of the type of dentin tested, in addition, the etch and rinse step improves micro shear bond strength of self-etch adhesive with different type of dentin.

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INTRODUCTION

Bonding of dissimilar materials depends on two main variables, the substrate and the composition of the material that interacts with this substrate at the interface. The interaction and the properties of the material are the major determinants for bond strength achieved. [1]

The fundamental of bonding mechanism of the resin adhesive and tooth structure enamel and dentin is created by the micro-mechanical inter-locking of the pores created by the minerals removed from the hard dental tissues and replaced by resin monomers. This process involves infiltration followed by polymerization of resin within porosities generated on the hard tooth structure and this process is called "hybridization". [2]

Bonding to enamel is a technically straightforward and relatively predictable procedure due to the high mineral content and prismatic pattern. On the other hand, the dentin with its higher organic and water content together with its varying inorganic content and its tubular structure (that varies in quantity and diameter, both of which increase with increasing proximity to the pulp) make bonding to dentin a challenge [3,4, 5]

Dental caries is the most common pathological change of dentin. Fusayama's research demonstrated that carious dentin consists of two distinct layers: an outer layer of bacterially infected dentin, and an inner layer of affected dentin [6]. The outer layer (caries-infected dentin) was characterized as being highly demineralized, physiologically un-reminerazable and showing irreversible denatured collagen fibrils with a virtual disappearance of cross-linkages. The inner layer (caries-affected dentin) is uninfected, partially demineralized and physiologically remineralizable, therefore should be preserved during clinical treatment. [7]

On the other hand, Sclerotic dentin which is a protective response formed either as a reactive process or aging and is seen in the occlusal or cervical lesions, latter being more common. The dentinal tubules are partially or completely obliterated with rod like sclerotic casts via peritubular apposition and minerals in the saliva [8]. These sclerotic plugs are protected by a layer of shiny hyper-mineralized layer which is acid resistant and acts as a diffusion barrier during adhesive procedures [9]. This layer contains denatured collagen with large calcium and phosphate crystals. [10]

In order to produce a superior resin—dentin interface, resin monomers must penetrate into the demineralized dentinal sub-surfaces. However, even though for normal dentin, it has been demonstrated that there are discrepancies between the depths of demineralization and resin monomer penetration.

Acid etching of dentin may cause excessive exposure of the collagen fiber meshwork and thus limit the capacity for monomer in penetration to its fullest extent [11, 12]. Collagen not embedded in monomers is susceptible to degradation, which can culminate in bond failure and reduce clinical longevity [13-14]. De Munck et al 2003; state that, while acid conditioning of enamel is effective, stable, and durable, the same cannot be said for dentin. [15]

Within this context, self-etch adhesive systems were developed. The key characteristic of these systems is that they skip the acid etching stage prior to application [16] , greatly decreasing the level of technical sensitivity required [17] , particularly because they obviate the need for optimal dentin moisture, which is required for total-etch systems [18-19]. Unlike conventional adhesive systems, self-etch systems have monomers added to their composition that ensure etching of the dental structure, so that, as soon as an area of the tooth is etched (or decalcified), it is immediately occupied by the resin monomer [12,20] .

Due to the limited conditioning ability of self-etch adhesive systems, the hybrid layer they form is thinner (21-22) as compared to that of conventional adhesive systems. However, the quality of this layer has been noted as the most important factor (19), despite the persistence of the smear layer in the hybrid layer. (23-24)

All the approaches whether etch and rinse or self-etching have different ways of applications and dealing with tooth structure and both are performing successfully in vivo and in vitro. (3)

Many studies on dentin bonding have used sound dentin as bonding substrate, which have contributed to the dramatic development of dentin bonding agents during the past two decades. On the other hand, there are fewer studies about bonding to caries-affected or sclerotic dentin, in which the bond strength is lower than those of normal dentin. The inferior bonding efficacy of caries-affected or sclerotic dentin would affect the clinical performance of resin composite restorations.

In view of the foregoing, this study aimed to conduct an *in vitro* comparison, by means of the micro-shear bond strength (μSBS) of five different dentin bonding systems to sound, caries affected, and sclerotic dentin.

MATERIALS AND METHOD

Total of 90 freshly extracted human molar teeth were collected and stored in 0.1% Thymol solution at room temperature. Teeth were washed under running water and extrinsic deposits were removed using hand scalar.

The roots were removed 3mm below the cemento-enamel junction and the teeth were embedded vertically in cylindrical Teflon mold of 2 cm height and 20 mm internal diameter using a chemically cured cross-linked acrylic resin (Meadway Dental supplies, LTD, UK) so as to have the full crown projecting above the surface of acrylic resin.

After complete setting of acrylic resin the prepared tooth with acrylic removed from Teflon mold and stored in saline solution till grinding. Teeth were divided into 3 groups according to the type of dentin tested interaction of all variables were illustrated in table (1)

Preparation of sound dentin samples:

A total of 30 samples of the sound molar teeth were ground off using model trimmer with low speed and copious water coolant in direction perpendicular to the long axis of the tooth to expose the first layer of dentin.

Preparation of the carious dentin samples:

A total of 30 samples of molar teeth having occlusal carious lesion extending into the dentin were assessed with dental operating microscope (carl Zeiss OPMI PICO surgical microscope, Germany). Grinding was processed the same like the sound teeth till exposure of the first layer of dentin. Caries was removed using round steel burs (sizes 3 and 5) in a slow-speed hand-piece. Caries removal was verified visually using magnifying loupes (x3.5) and tactually using explorer to verify sound dentin and staining using caries detector selection CDS, Kurary CO Osaka, Japan as well.

The produced occlusal surface was ground flat perpendicular to the long axis of the tooth. The produced dentin surface was hard; caries affected, stained or not stained.

Preparation of the sclerotic dentin samples:

A total of 30 molar specimens representing sclerotic dentin were selected. Selection of sclerotic dentin can be assessed using the magnification of (30X) which make the dentin area to appear transparent, whitish, glassy and translucent. The occlusal enamel of 30 molar samples representing sclerotic dentin were ground with the same technique of other tested samples.

The dentinal surface was abraded with 320, 500, and 600-grit SIC paper under water coolant to create standardized smear layer before adhesive application. The dentin surface was then thoroughly rinsed and air-dried.

A specially designed stainless-steal holder was fabricated to hold and stabilize the acrylic block and the tooth during bonding procedures. The holder was designed to create room for split Teflon ring of 19mm external diameter 2mm thickness and 5 mm internal diameter. The stainless-steel holder was supplied with screw to act as seat and stabilize of acrylic block and this screw in moving upwards and downwards to hold the acrylic block to the required level.

The area of the Holder which is toward the ground occlusal surface was designed to create a room for split Teflon ring of 19m external diameter, 2mm thickness and 0.97mm internal diameter in which the tested material will be packed. Every type of tested dentin sound, carious and scale sclerotic receive the tested adhesive.

All the tested adhesive materials were applied on prepared the dentin surface (Sound, carious, and sclerotic) together with the resin composite material according to manufactures instructions and it is listed in table (2). The prepared specimens were stored immediately in deionized water for 24 hours at 37°C before testing procedures.

TABLE (1) Interaction of different variables

D / A	D_1	D_2	D_3	
A_1	A_1D_1	A_1D_2	A_1D_3	18
A_2	A_2D_1	A_2D_2	A_2D_3	18
A_3	A_3D_1	A_3D_2	A_3D_3	18
A_4	A_4D_1	A_4D_2	A_4D_4	18
A_5	A_5D_1	A_5D_5	A_5D_5	18
	30	30	30	Total= 90
n=6				

- **D_1** =Sound dentin samples.
- **$D_{2=}$**Carious dentin samples.
- **$D_{3=}$**Sclerotic dentin samples.
- **$A_{1=}$**One step self-etch adhesive.
- **$A_{2=}$**One step self-etch adhesive with total acid etch and rinse.
- **$A_{3=}$**Total etch and rinse adhesive.
- **A_4** =Two steps self-etch adhesive.
- **A_5** =Two steps self-etch adhesive with total acid etch and rinse.

Table (2) Types and Technique of application as recommended by manufacturer of different adhesive system

Prime and bond active DENTSPLY DE trey GMBH, 78467 Konstanz, Germany	Self-etch one step	Phosphoric acid modified acrylate resin, Multifunctional acrylate, Bi-functional acrylate, Acidic acrylate, Isopropanol, Water, Initiator, Stabilizer	Apply Prime and bond active to wet cavity surfaces uniformly then gentle agitate for 20 $_{sec.}$ then evaporate by air for 5 $_{sec.}$ then light cure for 10 $_{sec.}$
Conditioner 36 Etching Gel	36% Phosphoric acid, Highly dispersed silicon dioxide, Detergent, Pigment, Water		Applied on the tooth for 15 $_{sec.}$ then rinsed with water for 10$_{sec.}$ Then then the dentin surface was blotted from extra water
Prime and bond N.T DENTSPLY DE trey GMBH, , 78467 Konstanz, Germany	Total etching and rinsing adhesive.	Di- and trimethacrylate resins, Functionalised amorphous silica, PENTA (dipentaerythritol penta acrylate monophosphate), Photoinitiators, Stabilisers, Cetylamine hydrofluoride, Acetone	35% H_3PO_4 applied for 15 $_{sec.}$ then rinsed with water for 10 $_{sec}$. then the dentin surface was blotted from extra water using paper point to appear shiny without pooling water then 2-3 consecutive coat of adhesive for 15 $_{sec}$, with gentle air thinning 5 $_{sec.}$ to evaporate the solvent then light cure for 10 $_{sec}$.

Adper SE plus, 3M ESPE, St. Paul. MN. 55144-1000 USA	Self-etch Two-steps	Liquid A Water,HEMA,Polyethylene-Polypropylene Liquid B UDMA (di-urethane dimethacrylate), TEGDMA (triethylene glycol dimethacrylate), TMPTMA (hydrophobic trimethylopropane trimethacrylate), HEMA phosphates,MHP(6-methacryloxyexacryloxy phosphate, Bonded Zirconia nanofiller,Initiator system based on camphorquinone.	Liquid A is applied using a micro brush; to cover all dentin surface, followed by application of Liquid B. The pink color of liquid A disappear quickly. This color change indicates the activation of the acidic monomers in liquid B and the beginning of the etching process. Agitation of Liquid B on the surface for 20_{sec} to ensure a proper etch, then air-dried for 10_{sec} to remove water. Liquid B (adhesive) is re-applied to provide a hydrophobic overcoat then air thinned for 10_{sec} and light cured for 10_{sec} using Eli par Free Light2.
Adper Single Bond 2, 3M ESPE, St.Paul, MN, 55144-1000,USA	Total-etch Two-steps	BisGMA (biphenyl a diglycidyl ether di methacrylate), HEMA (2-hydroxyethyl methacrylate), dimethacrylates, ethanol, water, a novel photo initiator system and a methacrylate functional copolymer of polyacrylic and polyitaconic acids.	Apply Scotchbond™ Etchant to dentin, Wait 15_S and Rinse for 10_s. Blot excess water using a cotton pellet, the dentin surface should appear shiny without pooling of water. Immediately after blotting, apply 2-3 consecutive coats of adhesive for 15_s with gentle agitation using a fully saturated micro brush .Gently air thin for 5_s to evaporate solvent. Light-cure for 10_s using Elipar Free Light2.
Filtek Supreme XT, 3M ESPE, St.paul, MN,55144-1000,USA	Methacrylate -based Nano-hybrid	Bisphenol A glycol dimethacrylate, bisphenol A ethoxylated, methacrylate, urethane dimethacrylate, Triethylene glycol dimethacrylate. Fillers: Agglomerated 20 nm Nano silica filler, Zirconia/Silica Nano cluster 5-20nm,cluster particle size 0.6-1.4μm/78.5 wt.%	Composites was light polymerized for 20 sec. using LED *at intensity of 1000 mw/cm^2

Elipar Free Light 2 (3M/ESPE, ST. Paul, N, USA).

The tooth slice with 2mm height cured composite resin cylinder bonded perpendicular to the dentin adhered to the micro shear bond apparatus (DL200 Emic Sao Jose de Pinhas, PR, Brazil), with loop of ligature wire (Unitex, diameter 0.09 inches, TP orthodontics, leads UK). The wire was loop around the resin composite cylinder making contact through half of its circumference and was gently held flush against the resin dentin interface. A shear force was applied parallel to the bonded surface at cross head spread of 1.0 mm/min until failure occurred. The resin composite interface, the wire loop and the center of the load cell were aligned to be straight to secure proper orientation of shear test force. Micro shear bond strength was calculated accordingly to the formula.

μSBS MPa= Shear force (N)/cross sectional area (mm2).

RESULTS

The mean micro shear bond strength and standard deviation of all the tested groups is presented in the table (3) and figure (1). Two-way ANOVA was used to test the two main effects namely, types of dentin and types of bonding systems. A significant interaction was noticed between the two main effects therefore, one-way ANOVA was used to test each main effect separately. One-way ANOVA for the effect of type of dentin showed statistical significant difference between all types of dentin (P= 0.001). Post-hoc test to test the difference between groups revealed that sound dentin showed the highest mSBS, followed by carious dentin and at the last came sclerotic dentin. Meanwhile, One-way ANOVA for the effect of type of bonding system showed statistical significant difference between all types of bonding systems (P= 0.001). The post-hoc test revealed that total etch system has significantly higher mSBS than all other systems (P= 0.001). Moreover, two-steps self-etch and two-steps self-etch with etch & resin had significantly higher mSBS than that of one-step self-etch systems (P= 0.001). No mSBS significant differences were revealed between the two types of two-steps self-etch (P= 0.289) or between the two types of one-step self-etch (P= 0.073).

TABLE (3) Mean micro shear bond strength and standard deviation of all the tested groups (Different shading indicates significance)

A	D	D1	D2	D3	Sig*
A1	N	6	6	6	0.001
	Mean	27.51283	21.40433	12.56367	
	Std. Deviation	0.340617	0.251227	0.341232	
A2	N	6	6	6	0.001
	Mean	27.93250	20.89983	14.25683	
	Std. Deviation	0.252658	0.046705	0.232810	
A3	N	6	6	6	0.001
	Mean	30.65900	23.97600	16.69167	
	Std. Deviation	0.108370	0.300237	0.419080	
A4	N	6	6	6	0.001
	Mean	28.55317	22.55883	14.50933	
	Std. Deviation	0.302442	0.564424	0.390138	
A5	N	6	6	6	0.001
	Mean	28.87933	22.64917	15.17533	
	Std. Deviation	0.371569	0.372664	0.227736	
		0.001	0.001	0.001	

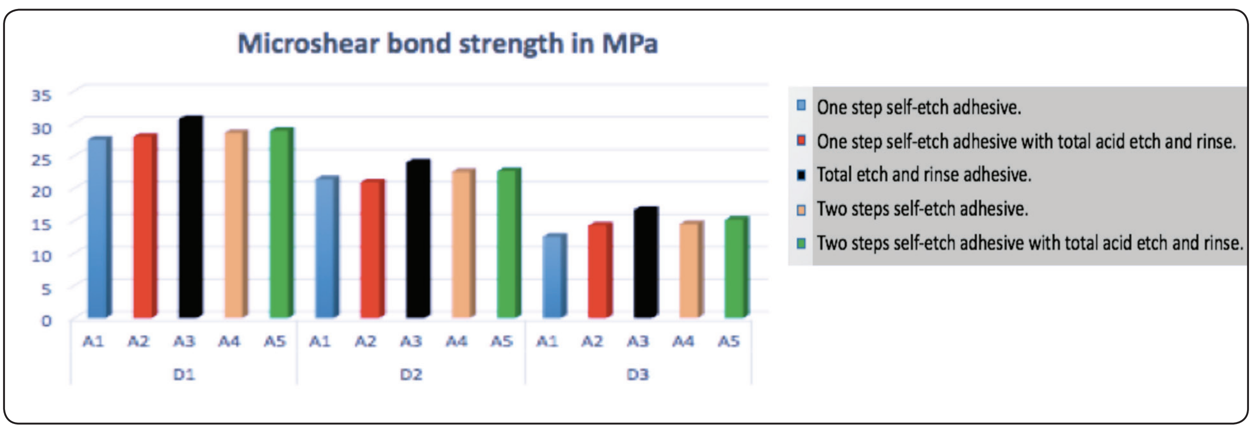

Fig. (1) Mean micro shear bond strength

DISCUSSION

Acid-etching is increasing the permeability of enamel to resins and dentin and evolution of hybrid layer in dentin [25] . Acid etching to dentin will create monometer-sized porosities in the intertubular dentin matrix exposing the collagen fibrillar matrix.

The self-etch adhesive contain acidic monomer which simultaneously condition and prime the dentin substrate and introducing the adhesive monomer at the same time as well with the same depth of demineralization to ensure complete penetration of adhesive [26].

In case of caries effected or sclerotic dentin the dentinal tubules are partially or totally obliterated by whitlockite crystals which cause an inconsistent resin-dentin hybridization [27].

The use of self-etching weather two step or one step by the manufacturers have been made to be more acidic and more hydrophilic and it is instructed to be used with agitation in order to remove smear layer and smear plug and dispersed to thicker hybrid layer[28].

The difficulty of water evaporation from these system is big disadvantage and if the water is evaporated it is easy to be diffused back from banded dentin which lower the mechanical properties of the polymer [29].

The hydrophilic monomer will cluster together before polymerization forming microscopic water filled chandelles called “Water trees” [30]. Which permit water movement from under lying dentin through the hybrid layer and weakening adhesive composite interface [31].

The self-etching approach considered to be less technique sensitive which makes more reliable clinical result especially in the in vivo studies but the performance of these self-etching adhesives is highly product dependent.

The results of the present study showed that each adhesive system used resulted in different micro shear bond strength. This is due to that penetration ability of an adhesive monomer and hybridization quality with prepared dentin substrate is the key for achieving reliable bond strength and hermetic seal to dentin surface[32, 33]

Caries affected dentin showed reduced bond strengths in comparison to sound dentin, regardless of the adhesive systems used. This may be attributed to the claim that the disorganized collagen and/or the mineral trapped within the gelatinized collagen cannot be easily removed even during the etching step[25]. The disorganized collagen and the gelatinous layer within the smear layer may hinder resin monomer infiltration and prevent a perfect seal at the resin—dentin interface. Therefore, the caries-affected dentin smear layer enriched with organic components would contribute to the inferior adhesion to the caries-affected dentin[34, 35,36].

Furthermore, bonded inter-faces of caries-affected dentin are more prone to hydrolytic degradation than those of normal dentin. Pashley et al. 2004, demonstrated that host-derived matrix metallo-proteinases (MMPs) enzymes in the dentin matrix promote the degradation of exposed, unprotected collagen within incompletely resin-infiltrated acid-etched dentin[37].

But in samples of sclerotic hyper mineralized dentin bond strength dropped significantly as the hybrid layer formed was claimed to be so thin that it is almost non-existent. Any discontinuity in the bonding interface act as a weak link and subsequently initiate adhesive failures in the sclerotic dentin and reduces its durability even when best restorative material is used. The eccentric hybrid layer formed differs from that of sound freshly cut dentin due to the absence of type I collagen [8].

When total-etch approach is used, higher bond strength was obtained, and within the same dentin substrate, the use of a separate etching step increased the mSBS. Also separate etching step increased the mSBS within the same adhesive monomer used. This may be attributed to that penetration ability of an adhesive monomer and hybridization quality with prepared dentin substrate is the key for achieving reliable bond strength and hermetic seal to dentin surface. Our results was in agreement with the observations of many studies which prove that etch and rinse adhesives produce a thicker hybrid layer with funnel-shaped resin tags due to phosphoric acid etching (pH-0.1) While the self-etching adhesives showed a continuous and thinner hybrid layer with cylindrical resin tags due to mild pH[11, 37,38].

CONCLUSION

Total-etching adhesive revealed higher micro shear bond strength than self-etch adhesive whatever the type of dentin tested, in addition, the etch and rinse step improves micro shear bond strength of self-etch adhesive with different type of dentin.

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