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Influence of Gingival Retraction Agents and Adhesive Bonding Regime on the Bond Strength of Composite to Dentin

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ABSTRACT

The aim of the present study was to evaluate the influence of retraction agents (Expasyl and viscostat) and bonding regimes [Total-etch, Self-etch and Er,Cr:YSGG (ECL) laser] on the shear bond strength (SBS) of resin composite to dentin. One hundred and eighty human mo-lars were prepared for exposure of occlusal dentin. Based on retraction agent treatments of dentin, teeth were equally divided into three main groups, A: Expasyl, B: Viscostat, C: No treatment (Control) (n=60). After dentin cleaning, dentin specimens in each group were bonded to resin composite (Tetric N-Ceram) using three different bonding regimes, 1: Etch & rinse, 2: ECL and 3: Self-etch. These combinations resulted in none study groups (n=20). Bonded specimens were exposed to shear bond strength testing using universal testing ma-chine under a standard load applied at crosshead speed of 1mm/min. Failure mode of the fractured specimens were assessed using stereomicroscope. Data was analysed using analysis of variance and Tukey-Kramer multiple comparisons test.The maximum bond strength was displayed by group C1 (Etch & rinse + Tetric-N- Bond) (24.54± 3.55 MPa) and lowest bond scores were in group B3 (Viscostat + Clearfill SE) (14.52±2.23 MPa). SBS was significantly higher in control s as compared to specimens exposed to Expasyl and viscostat in all corre-sponding groups ($p<0.05$). Different bonding techniques (Etch-rinse, ECL and Self-etch) showed comparable SBS outcomes in all corresponding hemostatic agent groups ($p>0.05$). Most common failure mode among the specimens of study groups was adhesive. The use of haemostatic agents compromised bond integrity of resin composite to dentin irrespective of the bonding regime employed. Post haemostatic agent application, Er,Cr:YSGG, etch-rinse and self-etch conditioning techniques showed comparable bond strength outcomes of resin to dentin.

KEY WORDS: EXPASYL; VISCOSTAT; ETHC-RINSE, SELF-ETCH; BOND STRENGTH; ER,CR:YSGG LASER.

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INTRODUCTION

Dental operative procedures when contaminated with saliva and blood, compromises resin bonding to dentin (Cacciafesta et al., 2004). Rubber dam and other isolation devices, act as a physical barrier against blood and salivary contamination but, its utilization turns out to be impractical and unfeasible in certain clinical situations. Consequently, use of haemostatic agents and retraction cord materials gain importance in these clinical scenarios. These clinical circumstances may range from Class V cavity preparation, impression taking to intra-sulcular restorations and crown cementation (Cochran et al., 1989 Madarati et al 2018). Haemostatic agents are commonly employed for isolation and soft tissue management proce-dure for multiple dental applications. Amongst them, viscostat comprised of 20% ferric sul-phate and Expasyl, containing solution of aluminium chloride are popular (Saati et al., 2018).

Expasyl is formulated in a way to curtail the damage of healthy periodontium avoiding gingival recession or bone resorption. Moreover, viscostat having a pH of ~1.0, stops bleeding, provides isolation and dry field in chairside intra-oral practices. It is gentle on hard and soft tissues and eliminates sulcular fluid contamination for optimal bonding (Ahmad et al., 2015). However, evidence suggests that aluminium chloride and ferric sulphate based haemostatic agents induce changes in dentin and enamel and alters the quality of adhesive hybrid layer, hence compromising the bond quality and strength (Giannini et al., 2015). Different bonding system have been developed to improve bond strength, reduce microleakage, decrease chair side time and minimize contamination (Giannini et al., 2015; Santos et al., 2014). Both the systems have their advantage and disadvantage and their selection varies depending on different clinical scenarios (Migliau, 2017; Santos et al., 2014). Alternatively, laser in the form of Er,Cr:YSGG (ECL) to condition dentin, enamel and lithium disilicate ceramics (LDC) has exhibited exceptional outcomes (Alkhudhairy et al., 2019c, 2019a, 2019b; F. Alkhudhairy et al., 2018a, 2018b; Vohra et al., 2019, 2018).

ECL works on the principal of ablation and destruction of dentinal surface by hydration of organic component and water from the dentinal tissues working at a wavelength of 2. 78mm. This method of dentin condi-tioning has gained exceptional appraisal due to less technique sensitivity, predictable out-come, controlled conditioning and safety (Alkhudhairy et al., 2019c, 2019a). To our knowledge from indexed literature, evidence related to ECL as dentin conditioner af-ter using different haemostatic material is scarce. Moreover, limited evidence on comparative studies between self-etch and total etch bonding materials after using haemostatic agents is available. It is hypothesized that pre-application of haemostatic agent and dentin conditioning with ECL will exhibit comparable outcomes to dentin conditioned with etch and rinse after haemostatic agent application. Therefore, the aim of the study was to evaluate the influence of haemostatic agents (Expasyl and viscostat) and bonding regimes [Total-etch, Self-etch and Er,Cr:YSGG (ECL) laser] on the shear bond strength (SBS) of resin composite to dentin.

MATERIAL AND METHODS

One hundred and eighty non-fractured, permanent non-carious, intact, third molars were iso-lated and cleaned from inorganic remnants and debris with the help of periodontal curette and scaler (Superior Instruments Co, New York, USA). The teeth were stored in thymol solution (0.5%) for two weeks to disinfect and then were kept in distilled water at 4°C. Within the sections of polyvinyl pipes (8mm diameter), teeth were placed vertically in acrylic resin (Ortho-Jet, Lang Dental MFG, IL, USA) up to the cementoenamel junction. To maintain uniformity the dentin surface was exposed by cutting with Diamond saw on slow speed machine and polished with silicon carbide paper (Buehler, Lake Bluff, IL, USA) under water irrigation for 10 sec at 250rpm using a polishing machine (Aropol 2V, Arotec). Based on gingival retraction materials used, samples were divided into three groups.

Group A (n=60): Application of Expasyl (Acteon Pharma) gingival retraction paste. Five minutes after application the material became visible owing to its colour change. The paste was washed off by air water spray for 30 sec and air dried.

Group B(n=60): ViscoStat (Ultradent Product, USA) was applied on dentin with a micro-brush and removed with running water spray after two minute and air dried.

Group C (n=60): (No treatment control): There was no application of gingival retraction ma-terial (Table 1). Now based on surface conditioning protocol each group was further classified into three sub-groups as A1, A2, A3; B1, B2, B3; C1, C2, C3 with 20 samples in each group.

Subgroup A1, B1 and C1: Samples were conditioned with 35% phosphoric acid (Ultra-Etch; Ultradent Products, Inc., South Jordan, UT, USA) for 15sec and rinsed for 10sec. A universal bonding agent (Tetric N-Bond Universal, Ivoclar-Vivadent) was applied and light cured (Bluephase G2, Ivoclar,Vivadent) for 10 sec.

Subgroup A2, B2 and C2: Dentinal surface of each given sample was conditioned by ECL (Waterlase C100, BioLase Tech Inc., California, USA) power 4.5W and frequency 30Hz in a non-contact mode from a distance 2mm using tip (MZ=8) for a duration of 60 sec. after con-ditioning procedure a universal bong agent was applied as discussed in previous subgroups.

Subgroup A3, B3 and C3: Clearfill SE (two step self-etch) was applied on all samples. Priming for 20 seconds (no mixing was done). Bond application and light curing for 10 sec using Bluephase G2 (Ivoclar,Vivadent).Surface conditioning of dentin was followed by ap-plication of Tetric N-Ceram (Ivoclar Vivadent). The bonding of composite was performed in cylindrical block of 4.5mm diameter and 2.45mm height. The application of composite was in line with the instructions of the manufacturer. To simulate oral conditions thermocycling of all the samples was done between 5°C to 55°C for 10000 cycles.

Shear Bond Strength (SBS) testing: In a Universal testing machine (Instron Santam, model STM-20, Riyadh, KSA) 20 samples from all nine subgroups were placed under known static loads at a cross head speed of 1mm/min. The force applied by universal testing machine was kept parallel to bonded surface. The force to debond sample was calculated in megapascal (MPa).

Failure mode Analysis: Two examiners performed fracture analysis using stereomicroscope at 40x magnification (SZX7, Olympus, Hamburg, Germany). Failure mode was classified into three categories i.e., admixed, cohesive, adhesive. Statistical Analysis:Data was tabulated using statistical program for social science (SPSS version 21, Inc., Chicago, US) for bond strength

Table 1. Materials used in this study

Material	Composition	Batch #	Manufacturer
Viscostat	20% Ferric Sulfate equivalent solution	B57QB	Ultradent
Expasyl	Aluminum chloride 15% equivalent solution	3293	Acteon Pharma
Tetric N-Ceram	Dimethacrylates, Polymer filler, barium glass filler, Initiator, stabilizer pigments.	S14434	Ivoclar Vivadent
Tetric-N-Bond	BISGMA, 2-hydroxyethyl methacrylate, phosphonic acid acrylate, Urethane Dimethacrylate	584747	Ivoclar, Vivadent
Clear fill SE Bond	MDP, HEMA, Water, Camphorquinone, NN-di-ethanol p-touloudine	125478925	Kuraray America Inc

testing outcomes. Normality of the data obtained was evaluated using Kolmogorov-Smirnov test. Using analysis of variance (ANOVA) and Tukey's post hoc test at a significance level of p = 0.05 means and standard deviations (SD) were compared.

RESULTS AND DISCUSSION

Data in the present study were normally distributed. Table 2 demonstrates SBS values among experimental groups. The maximum bond strength was displayed by group C1 (Etch & rinse + Tetric-N- Bond) (24.54± 3.55 MPa) and lowest bond scores were in group B3 (Viscostat + Clearfill SE) (14.52±2.23 MPa). Different superscript alphabets denote statistically significant difference within same row and column.! Showing significant difference among study group (ANOVA). Based on application of hemostatic agents, bond strength values among subgroups A1 (17.55±3.85) and B1 (16.54±2.46) were found to be comparable p>0.01. Moreover, subgroup C1 (24.54± 3.55) exhibited significantly higher SBS compared to A1 and B1 (p<0.01). Simi-larly, SBS among A2 (16.21±3.41) and B2 (15.25±3.27) specimens were comparable (p>0.05), however lower than C2 (22.25± 4.78) specimens. Furthermore, subgroup C3 (21.35±3.24) showed higher SBS compared to A3 (15.62±2.39) and B3 (14.52±2.23) (p<0.01).

Overall, application of hemostatic agents (Expasyl and viscostat) significantly re-duced SBS values. Moreover, based on dentin conditioning, bond strength scores among groups C1 (Etch & rinse + Tetric-N- Bond) (24.54± 3.55), C2 (ECL + Tetric -N- Bond) (22.25± 4.78) and C3 (Clearfill SE) (21.35±3.24) were comparable (p >0.05).. Overall, different conditioning re-gimes exhibited comparable outcomes for SBS values among the specimens of groups A1,A2,A3,B1,B2,B3 (p>0.05).. For bond strength values, analysis of variance (ANOVA) showed significant difference among all study groups (p<0.05). Failure modes observed among the de-bonded specimens are presented in table 3. Most of the failures in viscostat group were adhesive (B1 55%), (B2 65%) and (B3 80%). Moreover, in lased specimens ad-mixed failure was more frequent (A2 50%) and (C2 70%). Cohesive failure was common among specimens of groups A1 and C1. Overall, in all groups adhesive failures were com-monly observed.

The present study was based on the hypothesis that pre-application of haemostatic agent and conditioning of dentin with ECL will exhibit comparable outcomes to dentin, preconditioned with total etch and rinse after application of haemostatic agents. Subsequently, the hypothesis was rejected as application of haemostatic agent in combination with ECL and etch & rinse compromised bond strength values. Control group specimen without application of haemo-static agents displayed better bond integrity compared to specimens treated with haemostatic agents. In the present study bond integrity was evaluated using universal testing machine. The meth-od has low technique sensitivity and gives comparative analysis between groups. Moreover, this reliable and precise test is homogenized, standardized

Table 2. Comparison of means and SD for bond strength values among study groups using ANOVA and Tukey multiple comparisons test.

Experimental group	Etch & rinse + Tetric-N- Bond (1)	ECL + Tetric -N- Bond (2)	Clearfill SE (self-etch) (3)	P value!
Expasyl (A)	17.55±3.85 a	16.21±3.41a	15.62±2.39 a	<0.01
Viscostat (B)	16.54±2.46 a	15.25±3.27 a	14.52±2.23 a	
No treatment Control (C)	24.54± 3.55 b	22.25± 4.78 b	21.35±3.24 b	

A1: Expasyl + Etch & rinse + Tetric-N- Bon, A2: Expasyl + ECL + Tetric -N- Bond, A3: Expasyl + Clearfill SE (2 step self-etch) B1: Viscostat + Etch & rinse + Tetric-N-Bond, B2: Viscostat + ECL + Tetric -N- Bond, B3: Viscostat + Clearfill SE (2 step self-etch),C1: Etch & rinse + Tetric-N- Bond, C2: ECL + Tetric -N- Bond, C3: Clearfill SE (2 step self-etch) and is consistent with other studies (Sirisha et al., 2014a, 2014b). Adhesives are sensitive to moisture and blood contaminants. For optimum bond strength operating field free of moisture is inevitable (Ahmad et al., 2015). Presently, haemostatic material Viscostat, containing 20% ferric sulphate displayed the lowest SBS scores (14.52±2.23) compared to all other experimental groups, though the results were statistically insignificant. These findings were found to be in harmony with studies by (Unlu et al., 2016 and Ebrahimi et al., 2013). A possible explanation to low bond scores can be attributed to viscous nature of viscostat making its removal difficult after etch and rinse and ECL conditioning of dentin (Pucci et al., 2016). Moreover, the composition of viscostat promotes coagulation of proteins in dentine resulting in poor penetration of adhesives. Furthermore, a study, by Kimmes et al., (2006) proclaims viscostat application on dentin does not alter SBS values. Different types of study methodologies, nature of adhesives, form of dentin (superficial and deep) and curing time may attribute to varied results.

Amongst the different conditioning regimes used in the present study, Clearfill SE (2 step self-etch) displayed low bond integrity scores with both haemostatic agents Expasyl (15.62±2.39) and Viscostat (14.52±1.23). Clearfill SE comprises of maleic acid (weak acid) which is not able to penetrate the haemostatic material and deeper areas of dentin, compro-mising bond values (Margvelashvili et al., 2010). Moreover, low pH of haemostatic agents (between 1.5 to 3.86) and poor penetration of HEMA monomer in Clearfill SE obturates and plugs the dental tubules forming amorphous layer over the dentin and altering the quality of smear layer hence hindering the penetration of 2 step self-adhesive (Ayo-Yusuf et al., 2005; Margvelashvili et al., 2010).

Table 3. Modes of failure among different experimental groups

Experimental groups	Adhesive (%)	Cohesive (%)	Admixed (%)
A1	20	70	10
A2	20	30	50
A3	70	20	10
B1	55	25	20
B2	65	15	20
B3	80	10	10
C1	20	50	30
C2	25	5	70
C3	5	30	65

A1: Expasyl + Etch & rinse + Tetric-N- Bon, A2: Expasyl + ECL + Tetric -N- Bond, A3: Expasyl + Clearfill SE (2 step self-etch) B1: Viscostat + Etch & rinse + Tetric-N-Bond, B2: Viscostat + ECL + Tetric -N- Bond, B3: Viscostat + Clearfill SE (2 step self-etch),C1: Etch & rinse + Tetric-N- Bond, C2: ECL + Tetric -N- Bond, C3: Clearfill SE (2 step self-etch)

To our surprise conditioning of dentin with ECL and etch and rinse showed comparable SBS outcome both with and without haemostatic agents. ECL when used at 4.5W and 30Hz working at a wavelength of 2780nm exhibits strong affinity with hydroxyapatite and water, is well absorbed by the dentin structure itself resulting in increase size in dental tubule orifice, forming irregular rugged appearance free from smear layer and disposal of both organic and inorganic structure. Hence, improving dentin permeability and receptiveness to bond (Alkhudhairy et al., 2019c; Alkhudhairy et al., 2018). Moreover, conventional etch and rinse method of dentin conditioning. Showed highest SBS values amongst all groups both in the presence (17.55±3.85) (16.54±2.46) and absence (24.54± 3.55) of haemostatic agents. A probable description to this outcome is 37% phosphoric acids in etch and rinse, this creates micro porosities and better mechanical retention between dentin surface and adhesive pro-moting better fluid movement. Furthermore, phosphoric acid completely removes smear layer and peritubular dentin for better bond integrity (Bertolotti, 1991; Turp et al., 2013).

Further-more, the authors speculate that ECL and etch and rinse conditioning along with added ad-vantage of HEMA in ethanol based Tetric-N-bond enhances wettability with its low hydro-philic nature promoting adhesion (Kumari et al., 2015). Interestingly, Expasyl haemostatic agent, showed better bond integrity compared to viscostat. Primarily, reason for better result is less acidic pH of expasyl (pH-3.86) compared to viscostat (pH-1.78). Hence, expasyl being gentler on the dentin (Tarighi and Khoroushi, 2014). Moreover, study by Lahoti, (2016) ad-vocates that expasyl does not alter the smear layer resulting in better SBS values. Further-more, a study by Harnirattisai et al., (2009) proclaimed that expasyl on dentine causes no change in dentinal contents compared to viscostat resulting in better bond quality.

Majority of the failures in Clearfill SE groups in combination with expasyl and viscostat were adhesive. The type of failure is favourable as it results in less iatrogenic damage to the tooth structure (Henkin et al., 2016). Likewise, this failure type corresponds to low SBS in self etch group. Similarly, in lased and only Clearfill SE group admixed failure type was frequent. The type of failure corresponds to high SBS values. Admixed type of failure results from stress and fracture within the material itself. Factors, contributing to this failure type may include type of debonding procedure, lateral forces and nature of conditioning pattern (Almoammar, 2019).

Likewise, high incidence of cohesive bond failure was experienced in groups condi-tioned with etch and rinse. Application of haemostatic agents not just results in chemical interaction but also physical topographical modifications in dentin. However, this was not assessed in the present study. In addition, contemporary resin based bioactive materials are able to chemically interact with dentinal surface and continuously allow for release and recharge of ions, potentially improv-ing bond integrity. Therefore, the use of these materials as an alternate to conventional resin for restorations in combination with haemostatic agents should be investigated. Furthermore, scanning electron microscopy and surface profilometry studies on dentinal surfaces after ap-plication of haemostatic agent and ECL to develop insights in topographical changes are rec-ommended.

CONCLUSION

The use of haemostatic agents compromised bond integrity of resin composite to dentin irre-spective of the bonding regime employed. Post haemostatic agent application, Er,Cr:YSGG, etch-rinse and self-etch conditioning techniques showed comparable bond strength outcomes of resin to dentin.

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