DOI: 10.7860/JCDR/2015/13801.6251

Original Article

Dentistry Section

Effect of Saliva on the Tensile Bond Strength of Different Generation Adhesive Systems: An *In-Vitro* Study

NIMISHA GUPTA[1], ABHAY MANI TRIPATHI[2], SONALI SAHA[3], KAVITA DHINSA[4], AARTI GARG[5]

ABSTRACT

Background: Newer development of bonding agents have gained a better understanding of factors affecting adhesion of interface between composite and dentin surface to improve longevity of restorations.

Objective: The present study evaluated the influence of salivary contamination on the tensile bond strength of different generation adhesive systems (two-step etch-and-rinse, two-step self-etch and one-step self-etch) during different bonding stages to dentin where isolation is not maintained.

Materials and Methods: Superficial dentin surfaces of 90 extracted human molars were randomly divided into three study Groups (Group A: Two-step etch-and-rinse adhesive system; Group B: Two-step self-etch adhesive system and Group C: One-step self-etch adhesive system) according to the different generation of adhesives used. According to treatment conditions in different bonding steps, each Group was further divided into three Subgroups containing ten teeth in each. After adhesive application, resin composite blocks were built on dentin and light cured subsequently. The teeth were then stored in water for 24 hours before sending for testing of tensile bond strength by Universal Testing Machine. The collected data were then statistically analysed using one-way ANOVA and Tukey HSD test.

Results: One-step self-etch adhesive system revealed maximum mean tensile bond strength followed in descending order by Two-step self-etch adhesive system and Two-step etch-and-rinse adhesive system both in uncontaminated and saliva contaminated conditions respectively.

Conclusion: Unlike One-step self-etch adhesive system, saliva contamination could reduce tensile bond strength of the two-step self-etch and two-step etch-and-rinse adhesive system. Furthermore, the step of bonding procedures and the type of adhesive seems to be effective on the bond strength of adhesives contaminated with saliva.

Keywords: Aesthetic dentistry, Adhesion, Composite resin, Saliva

INTRODUCTION

In earlier times, there was a scarcity of materials that adhered to enamel and dentin and caused more removal of tooth structure during cavity preparation [1]. But due to advancements in Aesthetic Dentistry, a conservative approach for cavity preparation has been made possible which has changed the outlook of it. The bonding and bond strength of different adhesive agents are influenced by numerous factors like saliva, gingival fluid, blood, method of preparation of dentin surface and composition of adhesive materials [2]. Evolution of adhesive materials has solved these factors affecting the interface between composites and dentin surface [3].

Thus, the present *in-vitro* study was undertaken to evaluate the influence of salivary contamination on the tensile bond strength of different generation adhesive systems (two-step etch-and-rinse, two-step self-etch and one-step self-etch) during different bonding stages to dentin.

MATERIALS AND METHODS

Preparation of Samples

In this study, 90 extracted human molars were selected (extracted as teeth were periodontally compromised/ for complete denture fabrication/ or impacted) and stored in normal saline until used for the study. Cylindrical moulds were used for mounting the study samples using acrylic resin (self cure) extension till cervical areas of the teeth. The occlusal surface of each sample was grossly reduced with model trimmer followed by a high speed air-rotor handpiece using #245 carbide bur under constant water spray in order to expose the uniform surface of dentin. The dentinal surface of each sample was grinded against the #600 sand grit paper mounted on a mandrel with straight hand piece to obtain a smooth flat dentin surface. The study materials were applied to the different groups according to the manufacturer's recommendation [Table/Fig-1]. And, as per the different treatment conditions a layer of saliva was applied on dentin surface collected from a single individual using brush followed by leaving it undisturbed for 5 seconds. The dentin surfaces of 90 molars teeth were then divided into three study Groups (Group A, B, C) containing thirty samples each (n=30). Study groups were then randomly further subdivided into three Subgroups (Subgroup 1, 2, 3) with ten samples in each subgroup according to the treatment conditions [Table/Fig-2].

Preparation of Composite Resin Block

After the bonding procedure, resin composite (Filtek, 3M ESPE) was built up in increments using plastic mould of internal diameter of 4 mm and length of 6 mm, pre-marked at a level of 2 mm, 4 mm and 6 mm respectively. The plastic straw was placed securely on dentin test surface of the samples in such a way so that the centre of all the 3 components of the experimental unit (i.e. the acrylic block, the tooth and the composite resin cylinder) were in straight line. Each single increment of 2 mm was build up with the help of teflon coated carrier and condensed with the help of a teflon coated condenser. Each increment of composite resin was light cured for 30 seconds as per the manufacturer's instructions. After completing the composite resin build-up and curing procedure, a composite resin cylinder of height 6 mm was obtained. The plastic straw was removed from the cured composite by splitting it with a B.P blade and removing with help of a tweezers while applying minimal force so as to avoid any harm to the bonded composite resin cylinder. The extra cured composite resin was removed with the help of composite finishing stone.

S.No.	Material	Composition	Procedures
1.	Two-step etch-and-rinse adhesive system (Adper Single Bond)	Etchant: Orthophosphoric Acid 37% Adhesive: Polyalkenoic acid copolymer Bis - GMA HEMA Dimethacrylates Water Ethanol Photoinitiator	Scotchbond etchant was applied to dentin surface for 15 seconds then rinsed for 10 seconds and blotted dry. Two consecutive coats of adhesive was applied for 15 seconds with gentle agitation followed by drying with air stream for 5 seconds and light cured for 10 seconds.
2.	Two-step self-etch adhesive system (Adper SE Plus)	Primer (Liquid A): Water (80%) HEMA Dye Surfactant Adhesive (Liquid B): Methacrylate resin phosphate (UDMA, TEGDMA,TMPTMA, HEMA) MHP acidic monomers Zirconia Photoinitiators	Liquid A was applied to dentin surface with the applicator tip followed by Liquid B application. The colour disappeared and surface was agitated for 20 seconds followed by drying with air stream for 10 seconds so as to evaporate the excess water. Liquid B (adhesive) was then reapplied and air thinned before it was light cured.
3.	One-step self-etch adhesive system (Single Bond Universal)	MDP Phosphate Monomer Dimethacrylate resins HEMA Vitrebond™ Copolymer Filler Ethanol Water Initiators Silane – for adhesion to glass-ceramic surface	A layer of adhesive was applied to the dentin surface with the help of an applicator tip, scrubbed for 20 seconds, air dried for 5 seconds and light cured for 10 seconds.

[Table/Fig-1]: The utilized materials

CONDITIONS STUDY GROUPS	Uncontaminated Group (Subgroup 1)	Saliva contamination before polymerization (Subgroup 2)	Saliva contamination after polymerization (Subgroup 3)
Group A (Total etch-and-rinse)	Subgroup A1	Subgroup A2	Subgroup A3
Group B (Two step self-etch)	Subgroup B1	Subgroup B2	Subgroup B3
Group C (One step self-etch)	Subgroup C1	Subgroup C2	Subgroup C3

[Table/Fig-2]: Division of Study Groups

Microtensile Bond Strength Testing

The samples were then stored in distilled water for 24 hours before testing the tensile bond strength on Instron Universal Testing Machine where the mechanical loading was applied to the interface of composite and prepared dentin surface (in MPa) until debonding occurred at the interface. The collected data were statistically analysed using two-way ANOVA.

RESULT

[Table/Fig-3] summarizes the mean tensile bond strengths and standard deviation of different groups and subgroups. Tensile bond strength of each adhesive system under different treatment conditions were as follows:

Irrespective of the treatment condition, Group C (one-step self-etch adhesive system) revealed maximum mean tensile bond strength followed in descending order by Group B (two-step self-etch adhesive system) and Group A (two-step etch-and-rinse adhesive system) respectively. The Inter group difference was found to be statistically significant ($p<0.001$).

In the uncontaminated condition, results inferred that maximum difference in mean tensile bond strength existed between Subgroup A1 and Subgroup C1 while minimum difference was between Subgroup A1 and Subgroup B1. Except for difference between Subgroup A1 and Subgroup B1, all the other between Inter Subgroup differences were statistically significant ($p=0.028$).

Adhesive Agent	Mean tensile bond strength among different Study Groups			
	Irrespective of treatment conditions	No contamination with saliva	Saliva contamination before polymerization	Saliva contamination after polymerization
Group A Two-step etch-and-rinse adhesive system	19.57	Subgroup A1 22.98	Subgroup A2 17.80	Subgroup A3 17.95
Standard Deviation	1.26	1.99	1.63	1.26
Group B Two-step self-etch adhesive system	22.92	sub Group B1 25.31	Subgroup B2 22.62	Subgroup B3 20.85
Standard Deviation	2.03	2.14	2.65	2.03
Group C One -step self-etch adhesive system	25.60	Subgroup C1 27.91	Subgroup C2 25.30	Subgroup C3 23.60
Standard Deviation	2.44	2.24	2.36	2.24

[Table/Fig-3]: Overall comparison of mean tensile bond strength among different study Groups

When saliva contamination occurred before adhesive polymerization, maximum difference in mean tensile bond strength existed between Subgroup A2 and Subgroup C2 while minimum difference was between Subgroup B2 and Subgroup C2 and Inter Subgroup comparison was found to be statistically significant ($p<0.05$).

When saliva contamination occurred after adhesive polymerization maximum difference existed between Subgroup A3 and Subgroup C3 while minimum difference was between Subgroup B3 and Subgroup C3 and Inter Subgroup comparison was found to be statistically significant ($p<0.05$).

DISCUSSION

Beginning in the 1950s, research on dentin bonding continued at a slow pace through 1960s, 1970s, 1980s & late 1980s culminated in the introduction of the first, second, third and fourth generation of dentin bonding agents respectively. Due to their very poor clinical results, multiple applications and more time consumption, manufacturers attempted to simplify this system, and the so called "one-bottle system" (fifth generation) evolved [3].

The one-bottle systems generally demonstrated good performance to dentin bond strength and marginal seal. A major problem faced in day to day practice with total etch adhesives was postoperative sensitivity, that made clinician's to practice with self-etching systems which included initially a two step applications. But advancements in self-etching systems (sixth and seventh generation) have led to one step application containing etching, primer and bonding agent in one bottle [3].

Also, with increased demand and use of aesthetic restoration, contamination control has become important. Saliva is a very dilute solution composed of more than 99% water as well as immunoglobulins, glycoproteins, enzymes, mucins, nitrogenous products and a variety of electrolytes. Saliva contaminated dentin causes the reduction in bond strength due to adsorption of glycoproteins that act as a barrier that prevents monomers from penetrating the collagen network of dentin [4].

In as little as one second, salivary exposure does compromise bonding of resin to enamel and to screen adhesive's bond strength several tests can be performed by Munck et al., but the most frequently used test methods are bond strength tests and commonly used are the shear and microtensile bond strength test [5]. Shear bond strength test has been criticized and therefore, a new methodology called microtensile bond strength test was developed by Sano et al to screen adhesive's bond strength [6]. Microtensile bond strength is inversely related to the bonded surface area, with other advantages that they test the regional bond strengths and bonding effectiveness to clinically relevant tooth substrates such as carious and sclerotic dentin [7]. Thus in the present study, microtensile bond strength of different generation adhesive systems was evaluated on extracted human molars.

In a study by Dhawan et al., Molars were preferred for the study as flat dentin surface provided a wider area of dentin to be treated [8].

In a study by Neelagiri et al., to evaluate the influence of salivary contamination on the tensile bond strength of different generation adhesive systems in the present study, early morning saliva was considered an acceptable material to be used for saliva contamination testing as artificial saliva may confound results [4].

Additionally, composite and adhesive systems were taken up from the same manufacturer (3M ESPE) as it achieved the maximum effect of dentin bonding procedure. According to Roh & Chung differences in the chemical composition might lead to unexpected chemical reactions that would be hazardous to bonding [9].

Results of the present study showed that one-step self-etch adhesive system (Group C) revealed maximum mean tensile bond strength when compared to two-step self-etch (Group B) and two-step etch-and-rinse adhesive systems (Group A). The Inter group difference was found to be statistically significant. This may be attributed to the fact that one-step self-etch adhesive systems are technically simple with good biological tissue responses and in addition, this group of adhesives contain Phenyl-P or MDP (10-Methacryloxydecyl Dihydrogen Phosphate) which exhibits good adhesion to dentin and enamel, than the self-etching adhesive systems which do not contain MDP as stated by Da Silva et al., [10].

Additionally, two-step etch-and-rinse adhesive system (Group A) showed lowest mean tensile bond strength when compared to self-etching adhesive systems. This may be due to technical complexity, mainly related to the time of dentin conditioning, humidity and the degree of demineralization which results in voids together with a demineralised zone which is non-hybridized and ultimately leads to decrease in bond strength and microleakage as stated by Knobloch et al., [11].

The results of the present study in uncontaminated condition suggested that, maximum difference in mean tensile bond strength existed between Subgroup A1 (two-step etch-and-rinse adhesive system) and Subgroup C1 (one-step self-etch adhesive system) while minimum difference was observed between Subgroup A1 and Subgroup B1 (two-step self-etch adhesive system). Except for difference between Subgroup A1 and Subgroup B1, all the other Inter Subgroup differences were statistically significant. The results of the present study were in accordance to previous study conducted by Pegado et al., [12]. This may be attributed to the fact that conditioning and rinsing steps involved in two-step etch-and-rinse adhesive system, may lead to operating errors and presence of HEMA in this adhesive system can lower the vapour pressure of water, with water being more difficult to remove from demineralised deep dentin, thus impairing the diffusion of Bisphenol A Glycidyl Methacrylate (Bis-GMA) resin monomer as stated by Tolendo et al., study [13].

When dentin surfaces was contaminated with saliva before polymerization of the adhesive system, the results of the study suggested that, maximum difference in mean tensile bond strength existed between Subgroup A2 (two-step etch-and-rinse adhesive system) and Subgroup C2 (one-step self-etch adhesive system) while minimum difference was between Subgroup B2 (two-step self-etch adhesive system) and Subgroup C2. The Subgroup comparison was significant statistically between all the comparisons. This result may be attributed to the fact that reduction of bond strength due to saliva contamination may be related to the type of resin adhesive used and the stage of bonding procedures involved [14]. According to Sheikh et al self-etch adhesives may be less sensitive to salivary contamination compared to previous generation of adhesive systems because of their hydrophilic feature and possibly due to the inherited acidity of self-etch adhesive systems which allows them not only to modify/penetrate the smear layer but also break through the muco-polysaccharides in the saliva that develop bond strengths [15].

After the polymerization of the adhesive system, when dentin surfaces was contaminated with saliva the results of the present study suggested that, maximum difference in mean tensile bond strength existed between Subgroup A3 (two-step etch-and-rinse adhesive system) and Subgroup C3 (one-step self-etch adhesive system) while minimum difference was between Subgroup B3 (two-step self-etch adhesive system) and Subgroup C3 respectively. For all the comparisons, the between Subgroup comparison was statistically significant. This may be due to the fact that when surfaces are contaminated with saliva after light curing, adsorption of glycoproteins to the polymerized air inhibited surface occurs, which prevented complete infiltration of the resin layer that resulted in reduction of bond strength as stated by Hiraishi et al, Kermanshah et al., [14,16]. In a previous *in-vitro* study conducted by Yalcin et al., who evaluated the effect of saliva contamination on the micro-tensile bond strength of self-etching adhesive systems stated that neither two step self-etch nor one step self-etch adhesive systems showed a significant decrease in the bond strength under contaminated conditions [17].

Hence, with newer technologies and approaches in the field of bonding agents the factors affecting the adhesion can be minimized which depends upon the composition and properties of the bonding agent [18].

CONCLUSION

In the uncontaminated and contaminated condition, the result of the present study revealed that one-step self-etch adhesive system get

least influenced by salivary contamination followed by descending order two-step self-etch adhesive system and two-step etch-and-rinse adhesive system respectively.

REFERENCES

[1] Chandki R, Kala M. Total-etch vs self-etch: still a controversy in the science of bonding. *AOSR.* 2011;1(1):38-42.
[2] Hegde MN, Hegde P, Shetty SK. The influence of salivary contamination on the shear bond strength of two newer generation dentin bonding agents – An in vitro study. *J Conserv Dent*. 2008;11(3):127–30.
[3] Chopra V, Sharma H, Prasad SD. A comparative evaluation of the bonding efficacy of two-step vs all-in-one bonding agents – An in-vitro study. *J Conserv Dent*. 2009;12(3):101-4.
[4] Neelagiri K, Kundabala M, Shashi RA, Thomas MS, Parolia A. Effects of saliva contamination and decontamination procedures on shear bond strength of self-etch dentine bonding systems: An in vitro study. *J Conserv Dent*. 2010;13(2):71-75.
[5] De Munck J, Landuyt KV, Peumans M, Poitevin A, Lambrechts P, Braem M, et al. A Critical Review of the Durability of Adhesion to Tooth Tissue: Methods and Results. *J Dent Res*. 2005;84(2):118-32.
[6] Sano H, Shono T, Sonoda H, Takatsu T, Ciucchi B, Carvalho R, et al. Relationship between surface area for adhesion and tensile bond strength-evaluation of a micro-tensile bond test. *Dent Mater J*. 1994;10(4):236-40.
[7] Meerbeek BV, De Munck J, Yoshido Y, Inoue S, Vargas M, Vijay P, et al. Adhesion to Enamel and Dentin: Current Status and Future Challenges. *OPER DENT*. 2003;28(3):215-35.
[8] Dhawan R, Indira R, Dhawan S. A Comparative Evaluation of Tensile Bond Strength and Hybrid Layer of Three Generation Bonding Agents by Scanning Electron Microscope (An In-vitro study). *JOHCD.* 2008;2(2):36-41.
[9] Roh OD, Chung JH. Micro-shear bond strength of five resin-based composites to dentin with five different dentin adhesives. *Am J Dent*. 2005;18(6):333-37.
[10] Da Silva JM, Rodrigues JR, Camargo CH, Fernandes VV, Hiller KA, Schweikl H, et al. Effectiveness and biological compatibility of different generations of dentin adhesives. *Clin Oral Investig*. 2014;18(2):607-13.
[11] Knobloch LA, Gailey D, Azer S, Johnston WM, Clelland N, Kerby RE. Bond strengths of one and two-step self-etch adhesive systems. *J Prosthet Dent*. 2007;97(4):216-22.
[12] Pegado REF, Do Amaral FLB, Florio FM, Basting RT. Effect of Different Bonding Strategies on Adhesion to Deep and Superficial Permanent Dentin. *Eur J Dent*. 2010;4:110-17.
[13] Toledano M, Proenca JP, Erhardt MC, Osorio E, Aguilera FS, Osorio R, et al. Increase in dentin-bond strength if doubling application time of an acetone-containing one-step adhesive. *OPER DENT*. 2007;32(2):133-37.
[14] Hiraishi N, Kitasako Y, Nikaido T, Nomura S, Burrow MF, Tagami J. Effect of artificial saliva contamination on pH value change and dentin bond strength. *Dent Mater J*. 2003;19:429-34.
[15] Sheikh H, Heymann HO, Swift EJ, Ziemiecki TL, Ritter AV. Effect of Saliva Contamination and Cleansing Solutions on the Bond Strengths of Self-Etch Adhesives to Dentin. *J Esthet Restor Dent*. 2010;22(6):402-10.
[16] Kermanshah H, Ghabraei SH, Bitaraf T. Effect of salivary contamination during different bonding stages on shear dentin bond strength of one-step self-etch and total etch adhesive. *Journal of Dentistry.* 2010;7(3):132-38.
[17] Yalçin M, Simsek N, Keles A, Ahmetoglu F, Dundar A, Umar I. Effect of salivary contamination on micro-tensile bond strength of self-etch adhesives systems after bonding procedure. *J Dent Res*. 2013;1:55-59.
[18] Singh S, Adhlakha V, Babaji P, Chandna P, Thomas AM, Chopra S. A comparative evaluation of the effect of bonding agent on the tensile bond strength of two pit and fissure sealants using invasive and non invasive techniques: An in-vitro study. *J Clin Diagn Res*. 2013;7(10):2343–47.

PARTICULARS OF CONTRIBUTORS:
1. P.G Student, Department of Pedodontics & Preventive Dentistry, Sardar Patel Post Graduate Institute of Dental & Medical Sciences, Lucknow, India.
2. Professor & Head, Department Of Pedodontics & Preventive Dentistry, Sardar Patel Post Graduate Institute of Dental & Medical Sciences, Lucknow, India.
3. Reader, Department of Pedodontics & Preventive Dentistry, Sardar Patel Post Graduate Institute of Dental & Medical Sciences, Lucknow, India.
4. Senior Lecturer, Department of Pedodontics & Preventive Dentistry, Sardar Patel Post Graduate Institute of Dental & Medical Sciences, Lucknow, India.
5. Senior Lecturer, Department of Pedodontics & Preventive Dentistry, Jaipur Dental College, Jaipur, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Nimisha Gupta,
P.G Student, Department of Pedodontics & Preventive Dentistry, Sardar Patel Post Graduate Institute of Dental & Medical Sciences, Lucknow, India.
E-mail : nimisha.doc@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Mar 12, 2015**
Date of Peer Review: **Apr 22, 2015**
Date of Acceptance: **May 15, 2015**
Date of Publishing: **Jul 01, 2015**