Effect of Incorporation of Hydroxyapatite Nanorods in Self-Etch Adhesive on Microtensile Bond Strength and Micromorphological Patterns of Resin/Dentin Interface

Hameed K. Musa[1], Hamdi H. Hammama[2], Salah H. Mahmoud[3]

Abstract:

Objective: To evaluate microtensile bond strength (μTBS) of four types of adhesives including the "modified-bioactive" and their effect on micromorphological patterns of resin/dentin interface, immediately after 24hours and after 6months storage, followed by thermocycling. **Material and Methods:** Eighty extracted sound permanent molars were collected from patients seeking extraction in the outpatient clinic oral surgery department, Faculty of Dentistry at Mansoura University, according to the regulation of our institutional ethical committee under # (A03160321). The selected molars were assigned into four groups according to adhesive types: non-modified self-etch adhesive modified self-etch adhesive with hydroxyapatite nanorods, universal adhesive, resin-modified glass ionomer adhesive. The groups according to time of testing were subdivided into two subgroups: (a) was immediately evaluated, (b) was subjected to the 6months storage in artificial saliva followed by thermocycling (2000cycles). **Results:** The Two- way ANOVA test showed that the "adhesive type" and "time" partially affected the μTBS results. There was no significant difference in μTBS between non-modified and modified groups ($p>0.05$). However, there was a significant difference in μTBS between the universal group (Delayed) (D) and the non-modified group (Immediate) (I). There is a high significance of all tested groups with the resin-modified glass ionomer adhesive delayed group ($p<0.05$). **Conclusion:** The incorporation of hydroxyapatite -nanorod to self-etch adhesive improve bond strength with no significant difference. Aging did not affect negatively the bond strength between groups except Riva group. There is a considerable significant difference between the Universal adhesive aged group and the non-modified adhesive immediate group.

Introduction:

Materials improvements and proceeding have made it feasible to mimic the natural look of natural teeth with aesthetic restorations.[1] Many attempts have been made to improve the overall performance of the restoration by addressing difficulties associated with the tooth restoration interface.[2,3] Tooth restoration can be performed using dental adhesive technology, which is relies on the creation of the hybrid layer, a structure made up of demineralized collagen fibrils reinforced by a resin matrix.[4] However, the hybrid layer generated on the variable and dynamic organic dentin phase is not flawless, and it may fail with time, resulting in marginal discolorations, marginal leakage, and later composite restoration retention loss.[5] The problem in conventional resin–dentin adhesive is that includes 30–50% of their volume filled with water instead of resin, these water-filled can deteriorate the bonding interface.[6] So, The displacement of residual water can be achieved via a reaction between water-filled voids and bioactive nano-sized apatite crystals, which are incorporated in self-etch adhesive.[7]

Remineralization of resin/dentin interfaces has been studied aiming to replace water from intrafibrillar gaps, as well as from water-rich, resin-sparse regions of the hybrid layer, with apatite crystallites.[7] So it would be possible to increase the mechanical properties of the dentin-resin interphase and protect the exposed collagen from external challenges and activated matrix metalloproteinase enzymes in the matrix can be inactivated.[7,8] In the current study hydroxyapatite-nanorod fillers were incorporated into the one-step self-etch adhesive. By searching the currently available scientific literature, it was found that there were few studies until now evaluating the effect of aging on hydroxyapatite containing self-etch adhesive on μTBS to dentin.

The null hypothesis was no significant differences in μTBS among four types of adhesives including the "modified-bioactive" adhesive. Also, there were no significant differences in μTBS immediately after 24 hours and after a 6months storage period followed by thermocycling.

Materials and Methods:

Four different types of adhesives including the "modified-bioactive" adhesive being evaluated; 1S-SE adhesive (Opti Bond™ All-In-One) (Kerr, Orange, CA, USA PVPA Italia, s.r. l) (non-Modified),1S-SE adhesive (Opti Bond™, All-In-One) with HAP-NR (Modified), Universal adhesive (Scotchbond universal adhesive) (3M-ESPE, MN, St Paul, USA), Resin Modified Glass Ionomer bonding agent with Riva conditioner (Riva Bond LC, SDI, Australlia,). As well as Filtek (Z250 XT), a nanohybrid resin composite (3M-ESPE, St. Paul, MN, USA) restorative material, hydroxyapatite -nanorod fillers are used in this in vitro study and artificial saliva which was prepared in Faculty of Pharmacy, Mansoura University. It composed of Methyl-p hydroxybenzoate, KCL Sodium C carboxymethyl, cellulose, calcium phosphate, $MgCl_2.6H_2o$, $CaCl_2.2H_2o$, K_2po_4, kH_2po_4, and pH adjusted to 6.75with KOH.

Preparation of the specimens for microtensile test

Fourteen molars from each subgroup were prepared with a total no. of 56molars from all groups were obtained

[1]*Postgraduate MSc student, Department of Operative Dentistry, Faculty of Dentistry, Mansoura University, Egypt.* hameedkhalefa5@gmail.com
[2]*Associate Professor, Department of Operative Dentistry, Faculty of Dentistry, Mansoura University, Egypt.*
[3]*Professor, Department of Operative Dentistry, Faculty of Dentistry, Mansoura University, Egypt.*

(n=56). After the samples were embedded in an acrylic resin block (Acrostone, Cairo, Egypt), the occlusal enamel and superficial dentin were removed for each tooth, exposing the mid-dentin area. Using a precision saw of diamond (IsoMet 4000 saw, USA). All tested adhesives were applied according to the manufacturer's recommendations and curing with a light-emitting diode (LED) light-curing unit (Eli par ™ Deep Cure-S LED Curing Light). The intensity of LED unit was monitored via a radiometer (Demeter LC, Kerr, Germany) 1300mW/cm^2 with a wavelength between 350-520nm.

A nanohybrid resin composite restorative material (Filtek, Z250 XT) was used to cover the bonded surface in increment technique. Using a light emitted diode (LED) light-curing unit, each increment was light-cured for 20s. Then the teeth were kept in distilled water for 24hours at room temperature until the time of testing for the immediate subgroup (n=28) and delayed subgroup stored in artificial saliva for 6month in incubator at 37°c and then they subjected to thermocycling (n=28) (2000 cycles) in a thermocycler. The samples were immersed into two water baths, the cold was 5°c and the hot one was 55°c corresponding to thermal changes in clinical use following the ISO Standard TR 11450. The dwell period was 15s, with a 5s transfer time from one bath to another.[9] Continuous checking for water temperature had occurred to achieve a reliable thermocycling effect.[10]

The bonded specimen was receiving a series of cutting perpendicular to the longitudinal axis in X and Y directions using a low-speed automated saw made of diamond (IsoMetTM 4000, USA) underwater coolant to produce dentin-resin composite beams with a surface area of (1mm x 1mm).

The beams are stuck by their ends in the middle groove of Geraldeli's jig using cyanoacrylate-based glue. Then, the beams were mounted onto a universal testing machine (Instron model 3345, England) using Geraldeli's jig to apply tensile load to the specimen with a cross-head speed of 0.5mm/min until the bond through the specimen failed. After deboning, the fractured sites of all the specimens were observed under a stereomicroscope (Nikon MA 100, Tokyo, Japan) at 30X magnification to identify the mode of failure.

Preparation of the specimens for micromorphological observation

Three extra molars from each subgroup were prepared with a total no. of 24 molars from all groups were obtained (n=24). The samples are fixed and restored in the same manner for µTBS specimen preparation. The restored teeth were then sectioned into two semi-equal halves using a water-cooled diamond disc at low speed along the long axis of the teeth in a direction perpendicular to the resin-dentin interface (IsoMetTM 4000, USA). Each half was polished with silicon carbide paper with grits of 600, 1000, 1200-and 2000. Specimens were gold - sputtered (SPI Module - Sputter Carbon/Gold Coater, EDEN instruments, Japan) and observed in secondary electron detection mode under an SEM (JSM6510LV, JEOL, Japan) at magnification (X500, X1000, and X2000).[11]

Statistical analysis:

All the collected µTBS data were tabulated and statistically analyzed using a statistical package (SPSS TM Software, V.21, IBM, NY, USA). Data were checked for normal distribution according to the Shapiro Wilk test.

Results:

Microtensile Bond Strength (µTBS) results

The effect of the two investigated parameters (adhesive types and time) and their interaction on µTBS was determined using a two-way ANOVA test. The "adhesive type" and "time" both had a partial impact on the µTBS outcomes, according to the two-way ANOVA test (Table 1). In time, one-way ANOVA and Tukey's post-hoc multiple comparison tests were utilized to compare the mean values of µTBS results groups. Tukey post-hoc multiple comparison test showed that the dentin bonded with universal methacryloyloxydecyl dihydrogen phosphate containing adhesive system had considerably greater bond strength values ($p < 0.05$), while dentin bonded with Riva bond LC system had significantly lowest bond strength values (Table 2). Regarding adhesives for all immediate groups, the difference was non- significance (Table 2). For all first seven groups for both immediate and delayed showed highly significant differences with the last group (RMGI-based adhesive). Among the tested adhesives, all specimens prepared with resin- modified glass ionomer–based adhesive (Riva Bond LC) failed prior to µTBS-testing (pre-testing failures). Moreover, the universal adhesive –delayed group showed a low significance with the non-modified–immediate group (P=0.045).

Analysis of Failures Mode

Adhesive failure types were the most common in all groups (Table 3). Furthermore, there is a decrease in the percentage of adhesive failure in the Universal adhesive-delayed group compared to other groups. The adhesive failure mode was commonly observed in resin modified glass ionomer-based adhesive (Riva -delayed group).

Micromorphological observation of resin-dentin interface under SEM

All adhesives tested in the resin-based adhesive exhibit considerable tubular infiltration with no creation of interfacial gaps, indicating a thin uniform hybrid layer formation, except the non-modified delayed group which shows lower resin tags penetrating dentin surface comparable to other tested groups. However, hybrid-like layer formation beneath the bonding interface or an acid-base resistant layer for dentin areas treated with resin modified glass ionomer-based adhesive with typical pattern of hybrid like layer.

Table (1): Two-way ANOVA test

Dependent Variable: MTBS					
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	3652.960[a]	7	521.851	13.414	.000
Intercept	20503.581	1	20503.581	527.018	.000
Adhesive	2509.633	3	836.544	21.502	.000
Time	225.957	1	225.957	5.808	.024
Adhesive * Time	917.370	3	305.790	7.860	.001
Error	933.717	24	38.905		
Total	25090.257	32			
Corrected Total	4586.677	31			
a. R Squared = .796 (Adjusted R Squared = .737)					

Table (2): The mean µTBS values (MPa) of the tested groups

Groups (I, D)	N	Mean of µTBS± Std.
Non-modified adhesive -I	7	19.77±4.78 [b*]
Non-modified adhesive -D	7	22.25±4.03 [a, b]
Modified adhesive -I	7	33.81±12.10 [a, b]
Modified adhesive -D	7	32.78±2.40 [a ,b]
Universal adhesive-I	7	33.54±3.90 [a, b]
Universal adhesive-D	7	34.58±5.86 [a*]
Riva adhesive-I	7	24.73±4.95 [a, b]
Riva adhesive-D	7	1.000 [c*]

Data are expressed as a mean and Standard Deviation. *Superscript represents significance ≤ 0.05.
Abbreviation: I, immediate; D, delayed groups; Superscript small letters; a, represent highest level of significance; b, represent 2nd highest level of significance; c, represent lowest level of significance. ab, Superscripts represent non significance difference. a* Superscript represent significance difference. b* Superscript represent significance difference. c* Superscript represent significance difference. Test used: one- way ANOVA followed by Post-hoc Tuckey.

Table (3): Percentage of fracture mode.

Groups	Fracture pattern			
	A	CD	CC	A/M
Non-modified -I	17 (48.5%)	2 (5.8%)	-	16 (45.7%)
Non-modified -D	12 (34.3%)	12 (34.3%)	2 (5.8%)	9 (25.6%)
Modified adhesive -I	7 (20%)	2 (5.8%)	21 (60%)	5 (14.2%)
Modified adhesive -D	7 (20%)	7 (20%)	9 (25.6 %)	12 (34.3%)
Universal -I	12 (34.3%)	4 (11.4%)	19 (54.3%)	-
Universal -D	4 (11.4%)	10 (28.7%)	16 (45.7%)	5 (14.2%)
Riva-I	17 (48.5%)	_	9 (25.6%)	9 (25.6%)
Riva-D	35 (100%)	_	_	_

Abbreviation: I, immediate; D, delayed groups; A, adhesive fracture mode; CD, Cohesive failure mode within dentin; CC, Cohesive failure mode within composite; A/M, Adhesive/ Mixed failure mode.

Discussion:

The fact that nanofillers can enhance the adhesive layer at the resin–dentin contact could explain this improvement in µTBS.[12] It was discovered that using hydroxyapatite nanorod in very little amounts increased the bond strength of adhesives. This is evidenced by the tiny percentage of added fillers that have no effect on the modified adhesive viscosity or flow.[13] Furthermore, the bond strength was not affected by the storage period in artificial saliva followed by thermocycling. On the other hand, the significant difference of the bond strength in the current study between the universal group (UG) and the non-modified group (NMG) may not be due to the thickness of the hybrid layer and penetration of resin tags as several previous studies informed there was no association between the bond strength and the resin tag length.[14] The hydroxyapatite surface calcium phosphate is more capable of ionic interaction with the functional groups of methacryloyloxydecyl dihydrogen phosphate monomers in universal adhesive, strengthening the chemical bonding between adhesive and dentin substrate.[15]

Campos et al.[16] finding was in agreement with these findings. They studied the performance of self-etch adhesive systems including various functional monomers in vitro (methacryloyloxydecyl dihydrogen phosphate monomers and Glycerol Phosphate Dimethacrylate Monomers). Their findings showed that the different functional monomers have a direct impact on the adhesive bond effectiveness, with the 10- methacryloyloxydecyl dihydrogen phosphate monomer (10-MDP) being highlighted by greater µTBS values. Wang et al.[17] findings found similar results. The studies reported that there was no relationship between the bond strength and the resin tag length.[14] Despite the antagonistic issue of the relationship

between resin tag length and bond strength, the resin-modified glass ionomer-based adhesive make a chemical bond with calcium ions in dentin, which is regarded as the primary binding mechanism of this material. The creation of an acid-base resistant layer is caused by the chemical interaction of adhesives with hydroxyapatite crystals, which results in less soluble calcium might enhance the bond strength of these materials.[18]

Conclusion:

Within the limitations and based on the outcome of the present study, the μTBS test supported by analysis of failure mode and micromorphological observation of adhesive\dentin interface, the following conclusions could be drowned:

1. The incorporation of bioactive HAP-NR to one-step self-etch adhesive improve the bond strength and produce more reliable and durable bonding to dentin than non-modified adhesive systems

2. Aging did not affect negatively the bond strength between groups except the Riva group.

3. There is a considerable significant difference between the Universal adhesive and the immediate non-modified adhesive.

4. The resin-modified glass ionomer-based adhesive provided inferior bond strength compared to resin-based adhesives after aging, while the Universal adhesive provided superior bond strength.

References:

1.Kugel G, Ferrari M. THE SCIENCE OF BONDING: FROM FIRST TO SIXTH GENERATION. J Am Dent Assoc. 2000;131:20S-5S.

2.Sultan M, ElKorashy M, Fawzy A. Bonding Effectiveness of Bioactive Self-Adhesive Restorative Material to Enamel and Dentin Using Different Bonding Protocols. Egypt Dent J. 2020;66:1915-1924.

3.Ahmed AA, Hassan MM, Abdalla AI. Microshear bond strength of universal adhesives to dentin used in total-etch and self-etch modes. Tanta Dent J. 2018;15:91.

4.Gjorgievska E, Nicholson JW, Iljovska S, Slipper IJ. Marginal adaptation and performance of bioactive dental restorative materials in deciduous and young permanent teeth. J Appl Oral Sci. 2008;16:1-6.

5.Tjäderhane L. Dentin Bonding: Can We Make it Last? Operative Dentistry. 2015;40:4-18.

6.Magne D, Weiss P, Bouler J-M, Laboux O, Daculsi G. Study of the Maturation of the Organic (Type I Collagen) and Mineral (Nonstoichiometric Apatite) Constituents of a Calcified Tissue (Dentin) as a Function of Location: A Fourier Transform Infrared Microspectroscopic Investigation. J Bone Miner Res. 2001;16:750-757.

7.He L, Hao Y, Zhen L, Liu H, Shao M, Xu X, et al. Biomineralization of dentin. J Struc Biology. 2019;207:115-122.

8.Kim YK, Mai S, Mazzoni A, Liu Y, Tezvergil-Mutluay A, Takahashi K, et al. Biomimetic remineralization as a progressive dehydration mechanism of collagen matrices--implications in the aging of resin-dentin bonds. Acta Biomater. 2010;6:3729-3739.

9.Shafiei F, Akbarian S, Karim Etminan M. Effect of Adhesive Pretreatments on Marginal Sealing of Aged Nano-ionomer Restorations. J Dent Res Dent Clin Dent Prospects. 2015;9:144-150.

10. De Caluwé T, Vercruysse CWJ, Ladik I, Convents R, Declercq H, Martens LC, et al. Addition of bioactive glass to glass ionomer cements: Effect on the physico-chemical properties and biocompatibility. Academy Dent Material. 2017;33:e186-e203.

11. Hamama HHH, Yiu CKY, Burrow MF. Effect of chemomechanical caries removal on bonding of resin-modified glass ionomer cement adhesives to caries-affected dentine. Australian Dent J. 2015;60:190-199.

12. Sadat-Shojai M, Atai M, Nodehi A, Khanlar LN. Hydroxyapatite nanorods as novel fillers for improving the properties of dental adhesives: Synthesis and application. Dent Materials. 2010;26:471-482.

13. Abdelaziz EM, Al-Wakeel E, El-Morsy F, Sheha RR, Enan ET. Viscosity and micro-tensile bond strength of total-etch adhesive system reinforced with hydroxyapatite nano-particles. . Mans J Dent. 2014;1:115-118.

14. Peumans M, De Munck J, Van Landuyt K, Van Meerbeek B. Thirteen-year randomized controlled clinical trial of a two-step self-etch adhesive in non-carious cervical lesions. Dent Materials. 2015;31:308-314.

15. Chersoni S, Suppa P, Grandini S, Goracci C, Monticelli F, Yiu C, et al. In vivo and in vitro Permeability of One-step Self-etch Adhesives. J Dent Research. 2004;83:459-464.

16. Campos VS, Taques LV, Gomes GM, Reis GEdS, Lacerda WF, Araujo FdO, et al. Evaluation of The Bond Strength at The Resin-Dentin Interface Using Self-Etching Adhesive Systems Containing Different Functional Monomers Brazilian J Dev. 2020;6:103635-103646.

17. Wang R, Shi Y, Li T, Pan Y, Cui Y, Xia W. Adhesive interfacial characteristics and the related bonding performance of four self-etching adhesives with different functional monomers applied to dentin. J Dent. 2017;62:72-80.

18. Inoue S, Abe Y, Yoshida Y, De Munck J, Sano H, Suzuki K, et al. Effect of conditioner on bond strength of glass-ionomer adhesive to dentin/enamel with and without smear layer interposition. Oper Dent. 2004;29:685-692.