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CORRELATION BETWEEN NANOLEAKGE AND SHEAR BOND STRENGTH OF DIFFERENT ADHESIVE SYSTEMS AFTER WATER STORAG

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ABSTRACT

Objective: To evaluate and correlate between shear bond strength and nanoleakage at resin dentin interface of three adhesive systems after 24hours and 6 month of water storage.

Materials & Methods: Twenty four freshly extracted sound human molars were selected for shear bond test. The occlusal surface of each tooth was carefully trimmed to expose a clean flat dentin surface. Each tooth was sectioned bucco-lingually to produce forty eight specimens. The prepared specimens were randomly divided into three groups (16 each): Gp I: Scotchbond MP, Gp II: Clearfil SE bond and Gp III: Futurabond DC and Grandio composite were used for three groups. All specimens were thermocycled. The specimens in each main group were randomly subdivided into two equal subgroups [A and B] (8 samples each) according to storage time either 24 hours or 6 months respectively. The shear bond strength was measured using Universal testing machine at a crosshead speed of 0.5mm/min. For nanoleakage study other twenty four freshly extracted sound human molars were selected. Class V cavities were prepared on buccal and lingual surface, and then specimens were randomly divided into three equal groups eight each, thermocycled. The specimens in each main group were randomly subdivided into two equal subgroups (4 samples each) according to storage time either 24 hours or 6 months respectively. Then specimens were immersed in 50% w/v Silver nitrate solution for 24 hr. and processed for SEM/EDAX.

Results: Clearfil SE bond recorded the highest shear bond strength values (14.019±3.682, 12.826±2.578) at the two different storage periods respectively, followed by Scotchbond MP (12.822±2.136, 12.528 ±2.427) while the lowest shear bond strength values was recorded by Futurabond DC (10.275 ±1.762, 8.949 ±2.669). ANOVA test was used to compare the three tested groups in each subgroup water storage period at a level of significance 0.05. Regarding subgroup B (6 month storage period), a statistical significant difference was recorded (p-value=0.0315*). SEM result was found that all adhesives showing different pattern of nanoleakage.

Conclusion: Under the present situation of this research, it was recorded that there was non-significant inverse correlation between shear bond strength and nanoleakage.

KEYWORDS: total-etch adhesive, self-etch adhesives, shear bond strength, Nanoleakage.

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INTRODUCTION

For the past few years, composite has become current restorative material and today it often replaces amalgam restoration in posterior teeth. Nevertheless, of an adhesive system is always required[1].

The advent of adhesive dentistry has caused a dramatic change in restorative procedures. Current adhesive systems have become more and more acceptable in their clinical performance by enhancing the adhesion of resin to dentin[2]. In order to obtain proper adhesion, it is essential to create a hybrid layer at the resin-dentin interface[3]. The hybrid layer is created by the penetration and polymerization of adhesive monomers after removal and/or modification of the smear layer and superficial demineralization of dentin[4,5].

Bonding to enamel is quite predictable and can be achieved using the acid-etching technique, while bonding to dentin is more difficult due to high organic composition, continuous moist condition, permeability properties and presence of smear layer after cavity preparation[6,7].

Dentin adhesives are currently available as three-step, two-step, and single-step systems, depending on how the three cardinal steps of etching, priming, and bonding to tooth substrates are accomplished or simplified[8]. Two-step systems are subdivided into single-bottle self-priming adhesives that require a separate etching step, and the two-bottle self-etching primers that require an additional bonding step[9]. The recently introduced single-step, self-etch adhesives further combined these three bonding procedures into a single-step application.

Self-etch adhesives are attractive in that prior removal of the smear layer and smear plugs is not required. This reduces the potential for post-operative sensitivity [10] and the bonding problems associated with movement of dentinal fluid through patent dentinal tubules[11].

Nanoleakage was originally used to describe micro porous zones beneath or within hybrid layers that permitted tracer penetration to occur in the absence of interfacial gaps[12]. It occurs through sub micrometer-sized spaces within dentin hybrid layers where disparities existed between the depths of demineralization and monomer diffusion[13].

In vitro shear bond strength testing was commonly used to quantitatively analyze and rank the performance of adhesive systems on enamel and dentin[14]. It was proved adequate and effective for evaluation and comparison of different adhesive systems and restorative materials.

Long term storage of a bonded specimen in water or subjecting it to thermal cycling can give some insight into the temperature dependant degradation of the material[15].

Thermal cycling is the in-vitro process of subjecting restoration and tooth to temperature extremes compatible with oral cavity. This simulates the introduction of hot and cold extremes in oral cavity and show the relationship of the linear coefficient of thermal expansion between tooth and restorative material[16].

The international standards organization document "TR 11 450 Dental Materials – guidance for testing of adhesion to tooth structure" (ISO, 1993)[17] have been stated that: longer periods of water storage may be necessary to determine durability of bonds. Contemporary bonding systems usually produce high bond strength to dentin when tested after 24 hours of storage in water. However, several in vitro studies on the durability of resin-dentin bonds have reported reduced bond strength values after only few months of storage, indicating degradation of the bonds over time[18-21].

So the objective of the present study is to evaluate the effectiveness of water storage on shear bond strength and Nanoleakage of composite resin bonded to dentin surface by different adhesive systems total etch and self etch.

MATERIALS AND METHODS

MATERIALS

Materials that have been used in the present study are shown in table 1 including the following:

a) Scotchbond MP 3-Step total etch adhesive*

b) Clearfil SE Bond, 2-Step self etch adhesive**

c) Futurabond Dual Cure 1- Step self etch adhesive*

d) Grandio-SO. Composite resin*

METHODS

Shear bond strength test.

Twenty-four sound, periodontally involved & freshly extracted human molar teeth were selected from patients aging between (35-45) years for this study. Awritten consent was taken from these patients after the study was approved by the Ethics Committee of Tanta University to ensure their agreement to use their teeth in the current study. The teeth were debrided of any remaining tissue

TABLE (1) Composition and Manufacturer's instruction of tested materials used in the present study.

Tested Materials	Composition	Manufacturer's instruction for use
Scotchbond MP 3-Step total etch adhesive	- Etch: 35% Phosphoric Acid (pH=0.2-0.6). - Primer: Water, HEMA, and polycarboxylic acid copolymer (pH=3.3). - Adhesive: BIS-GMA, HEMA, CQ, EDMAB, DHEPT (pH=8.2).	- Apply etch for 10 second then rinse 10 second, leave moist 20s, dry gently. - Apply prime for 20 second, air dry gently - Apply adhesives, air then gently, light cure 10 second
Clearfil SE Bond, 2-Step self etch adhesive	- Primer.10-MDP, HEMA, DHEPT, hydrophilic dimethacrylate, CQ, water. - Adhesive: 10-MDP, HEMA, BIS- GMA, hydropholicdimethacrylate, - CQ, DHEPT, silanated colloidalsilica.	- Apply and allow to stand for 20s Air-dry gently - Apply and gently air then Light cure 10 second
Futurabond Dual Cure 1- Step self etch adhesive	- Liquid A: Water, ethanol, silicon dioxide. - Liquid B: Acid modified methacrylate (methacrylate ester), HEMA, camphorquinone.	- Dispense one drop of Liquid A and one drop of Liquid B into the well and mix for 5 seconds - Apply adhesive with rubbing motion for 15 seconds. –Gentle air-dry for 5 seconds. –Light cure for 20 seconds.
Grandio-SO. Composite resin	- Filler: Glass ceramic filler, functionalized silicon dioxide nano-particles, pigments (iron oxide, titanium dioxide) - Resin: BIS-GMA, BISEMA, TEGDMA In addition, Camphorquinone as a photocatalyst and butylatedhydroxytoluene (BHT) as a stabilizer.	- The PVC tube with internal diameter 0f 2mm and 2mm in height were filled with resin composite and attached to the conditioned dentin surfaces and cured for 40 second.

* 3M-ESPE
** Kuraray Co Ltd
* Voco, Cuxhaven, Germany

tags and cleaned with pumice and water and stored in a solution of 1% chloramine for one week, frozen in distilled water for a maximum period of 2 months until testing[22].

For each tooth, the occlusal surfaces was trimmed until mid coronal dentin was exposed using diamond disc at slow speed under water cooling, then each tooth was sectioned bucco–lingualy to produce forty eight specimens. Each specimen was embedded in acylic resin in PVC ring, with the occlusal dentin surface facing toward the ring base, and was wet ground in a polishing machine, with 400 and 600 grit Silicon carbide papers to achieve a standardize smear layer[22].

The prepared specimens were randomly divided into three groups of sixteen specimens, according to the tested adhesives used:

Group I: Scotchbond MP. Total-etch adhesive (3-steps).

Group II: Clearfil SE Bond. Self-etch adhesive (2-step).

Group III: Futurabond DC. Self-etch adhesive (1-step).

Each adhesive system was applied according to the manufacturer's instruction as shown in Table 1 a PVC tube with internal diameter of 2 mm and 2 mm in height were firmly attached to conditioned dentin and filled with resin composite and then cured for 20 seconds using halogen light curing at an intensity of 600 mW/cm^2 to form a resin composite cylinder of approximately $2\times2mm^2$ height. After 1 h, the PVC tube was carefully removed with scalpel and resin composite cylinder was checked under stereomicroscope to identify the presence of air bubbles.

All specimens were thermocycled in thermocycling apparatus* for 800 cycles between 5 c° o 55 c° with 30 sec. dwell time and 20 sec transfer time, this corresponding to 9 month of clinical services[23].

Each group was divided into two subgroups (8 specimens for each) according to water storage period.

The prepared specimens were stored in incubator at 37˚C for 24 hours and six months in distilled water before testing and the water was changed periodically to minimize risk of bacterial growth.

All specimens were subjected to shear bond strength using Universal testing machine** with a load cell of 5 KN at cross head speed of 0.5mm/min, until failure occurred and data were recorded using computer software. A 0.5 mm diameter stainless steel orthodontic wire was looped flush between the load cell projection and the resin cylinder making contact with the lower half-circle of the cylinder and touching the tooth surface. The maximum load at the time of failure was recorded and the bond strength expressed in MPa was calculated from the cross-sectional area of the resin composite cylinder according to the following equation T=P/II r2.

Where T is the shear bond strength (MPa), P is the load of failure (N), II is 3.14 and r is the radius of a composite micro-cylinder (mm).

The values of shear bond strength data will be calculated and statistically analyzed using one way ANOVA test.

Nanoleakage observation.

Twenty four extracted non-carious human molar teeth were used. For each tooth a standardized Class V cavities was prepared with gingival margin above cemento-enamel junction. Cavities were prepared in the mid coronal (2.0 mm depth 2.0 mm diameter) on buccal and lingual surfaces to produce forty eight cavities. A round carbide burs in a high-speed hand

* Petrotest Bath used for temperature 55˚C, Julabo Bath used for temperature 5oc
** Nexygen, model LRX-plus; Lloyd instruments ltd, Fareham, Uk
* FEI Quanta 200 SEM, France

piece used to prepare the cavities. Hand cutting instruments was used to provide adequate cavity finishing.

The specimens then divided into three groups (eight specimens for each) according to tested adhesives being used.

The cavities were submitted to the same bonding protocols as previously mentioned. Then composite was packed in increments in the cavity and light-cured for 40 second.

All specimens for nanoleakage were thermocycled in the thermocycling device as mention. The specimens of each group were divided into two subgroups C&D four specimens each according to water storage periods.

After storage in water at 37 c° for 24 h, the restorations were finished using Super Snap disks. Root apices were sealed with a cyanoacrylate adhesive and the teeth were coated with two layers of nail varnish up to 1 mm from restoration margins.[7] Then specimens were immersed in a 50% (w/v) silver nitrate solution in complete darkness for 24 h, rinsed in running water for 5 min, and immersed in photo developing solution, and exposed to a fluorescent light for 8 hour in order to reduce the silver ions to metallic silver. After removed from the photo developing solution, the specimens were rinsed in running water for 5 min, and sectioned in a bucco-lingual direction through the center of the restoration. The specimens were embedded in epoxy resin and the cut surface was polished with increasingly fine diamond pastes. The specimens were ultrasonicated in distillated water for 5 minutes, air dried, mounted on aluminum stubs, and sputter-coated with Au–Pd. The nanoleakage patterns were observed using scanning electron microscope/energy dispersive analytical dentin interfaces were analyzed in an scanning electron microscope (SEM)*, operated with backscattered electron mode at 1500x magnification.

Ultramorphological nanoleakage patterns were detected first then the amount of silver nitrate penetration (wt %) were analyzed using the EDAX (Energy Dispersive Analytical x-ray).

RESULT

Shear bond strength result.

The mean values of shear bond strength results of adhesive systems for both groups after 24 hour and 6 months of water storage were shown in Table (2).

It was found that ClearFil SE adhesive system recorded statistically non-significant ($p > 0.0957$) higher Shear bond strength mean value (14.019± 3.682 MPa) followed by Scotchbond MP adhesive system (12.822±2.136 MPa) while the lowest mean value was recorded by Futurabond DC adhesive system (10.275±1.762 MP) after 24hr of water storage.

On the other hand, It was found that ClearFil SE adhesive system recorded statistically significant ($p < 0.0315$) higher Shear bond strength mean value (12.826±2.578 MPa) followed by Scotchbond MP adhesive group (12.528±2.427 MPa) while the lowest mean value was recorded by Futurabond DC adhesive system (8.949± 2.669MPa) after 6 month.

It was found that water storage aging time have a negative effect on shear bond strength irrespective of adhesive system.

Amount of silver nitrate penetration at dentin/ restoration interface

It was found that Futurabond DC adhesive system recorded statistically non-significant ($p > 0.2344$) higher nanoleakage % mean value

* FEI Quanta 200 SEM, France

followed by ClearFil SE adhesive system while the lowest mean value was recorded by Scotchbond adhesive system (10±1.721, 9,17±2.459, and 7,12 ±1.262) Respectively as in table (3).

It was found that Scotchbond MP adhesive system recorded statistically significant ($p < 0.0057$) higher nanoleakage % mean value followed by Futurabond adhesive group while the lowest mean value was recorded by ClearFil SE adhesive group (18.28±4.999, 16.61±3.427 and 10.29±2.0578 Respectively).

Correlation between shear bond strength and nanoleakage.

There was non-significant inverse correlation between bond and nanoleakage as revealed by regression statistics ($r=-0.0549$, $r^2=0.003$, $p>0.05$) as shown in table (4).

TABLE (2) Mean values of the mean shear bond strength values of the tested adhesives after 24 hour and 6 months of water storage

Adhesive system	Mean±SD	
	Aging24 Hr	**Aging6 Months**
Scotchbond MP	12.8222.136	12.528
Clearfil SE bond	14.019	12.582
Futurabond DC	10.275	8.949

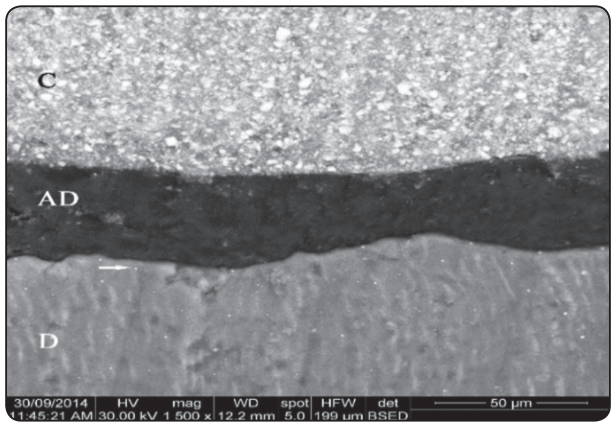
TABLE (3) Silver nitrate percentage of nanoleakage wt% results of all tested adhesive after 24 hour and 6 months of water storage.

Adhesive system	Mean±SD	
	Aging24 Hr	**Aging6 Months**
Scotchbond MP	7.12	18.28
Clearfil SE bond	9.17	10.29
Futurabond DC	10	16.61

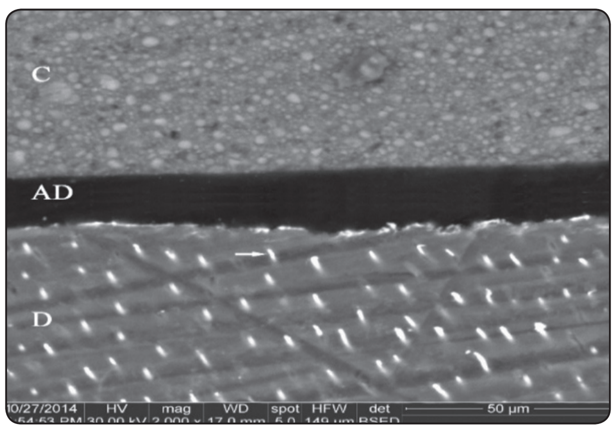
TABLE (4) Correlation between different shear bond strength and nanoleakage for all groups.

Bond vs. Nanoleakage	**Correlation coefficient (R)**	**(R^2)**	**p value**
Scotchbond	-0.3598	0.1295	0.4835 ns
ClearFil SE	0.3281	0.1077	0.5255 ns
Futurabond	0.6797	0.4619	0.1375 ns
Total	-0.0549	0.003	0.8287 ns

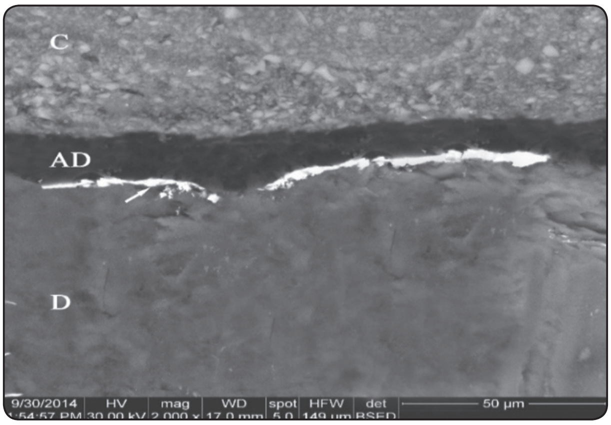
Nanoleakage SEM/EDEX observation



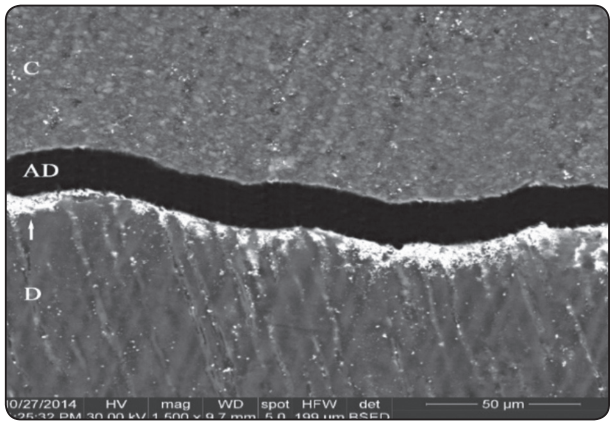
SEM photomicrograph for Scotchbond MP after 24 hr aging where faint silver deposit observed beneath the hybrid layer.



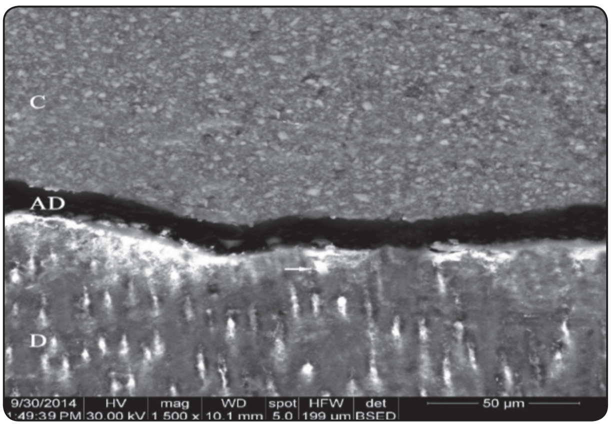
SEM photomicrograph for Scotchbond MP after 6 months aging where water tree or finger-like extensions appear as silver deposit take form of small trees that extend from the base of the hybrid layer toward the dentin. (C: Composite, AD: Adhesive layer, D: Dentin & Arrow: refer to silver deposit)



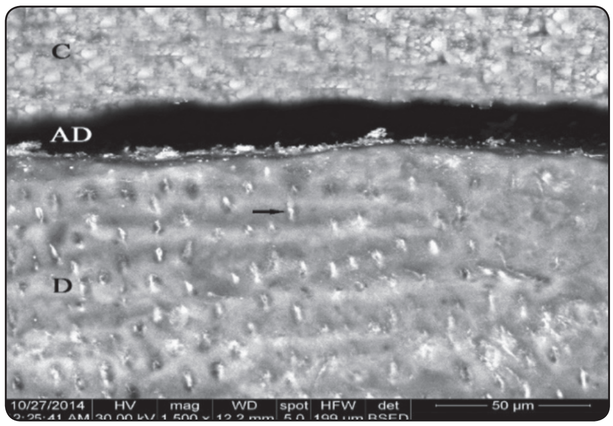
SEM photomicrograph for clearfil SE bond after 24hr showing reticular pattern as silver deposit appear interconnecting horizontal particle at base of hybrid layer.



SEM photomicrograph for Clearfil SE bond after 6 months aging. Reticular pattern Silver deposit appear as interconnecting horizontal particles at base of hybrid layer and spotted pattern appear as isolated silver deposit throughout the dentin.



SEM photomicrograph for Futurabond after 24 hr aging. Water tree pattern was observed and reticular pattern appears as interconnected horizontal particle beneath hybrid layer.



SEM photomicrograph for Futurabond after 6 months aging. Water tree nanoleakage could be observed as silver deposit take form of small tree that extend from the base of the hybrid layer toward the dentin and reticular pattern was observed where silver deposit appear as interconnecting horizontal particles at base of hybrid layer.

DISCUSSION

The preference for conventional shear test is justified because they are easy to perform, requiring minimal equipment and specimen preparation[24].

In the current study, we found that Clearfil SE Bond water-based two-step mild self-etching adhesive (pH=2) recorded the highest significant value of shear bond strength followed by Scotchbond MP three-step total-etch adhesives (pH=0.2-0.6 for etch, pH=3.3 for primer) while the lowest significant shear bond value was in Futurabond DC water-ethanol (one-step self-etch adhesives) pH=1.2-1.4 after 24hour and 6 month of water storage.

The reason that Clearfil SE bond recorded the highest mean values of shear bond is due to chemical interaction is achieved through specific functional monomers, such as 10-MDP

(10-methacryloxydecyl dihydrogen phosphate), 4-MET (4-methacryloxyethyl trimellitic acid) and phenyl-P, The ionic bond formation of the carboxylic/phosphate groups of these functional monomers to Ca of hydroxyapatite was first proven. In this sense, the chemical bonding promoted by 10-MDP is not only more effective, but also more stable in water than that provided by 4-MET and phenyl-P, in this order[25].

The current findings about Clearfil SE Bond agreed with **Van Meerbeek et al., and others** [26-33] as they reported that, Clearfil SE bond has been proven to yield reliable results in terms of bonding effectiveness and durability when compared to other commercially available self-etch adhesives,

Futurabond DC is one-step self-etch adhesive recorded the lowest statistically significant shear bond strength, as the high concentration of HEMA has been recently recognized to lower vapor pressure of water and so prevent its complete removal from the adhesive during bonding and promote water to be bonded in an unstable soft hydrogels within both hybrid and adhesive layers[34-36]. Beside there was a differential infiltration gradient established as a consequence of phase separation within the adhesive, and due to differences in molecular weight or affinity to dentin of the infiltrating compounds of the adhesive system[37]. Another reason was, a relatively high concentration of solvent is required to keep these adhesives blended in solution, and air-drying is not able to accomplish significant solvent evaporation[38]. This created water filled channels within the adhesive[39].

The result of the present study showed that mean shear bond strength of adhesives after 6 month storage in water recorded statically non-significant lower shear bond strength than 24hr months, which is also in agreement with result studies conducted by **Sano et al.,** [40] **Okuda et a l.,** [41] **and Armstrong et al.,** [42] which showed a decrease in bond strength of adhesives after long-term water storage. Most of which hypothesized that a decrease in bond strength over the time is due to hydrolytic degradation of ester bonds of polymerized resin within the hybrid layer which gradually increase as water diffused through nanoleakage channels which become larger over the time, resulting in lower bond strengths and interfacial failure .

Nanoleakage assessment became one of the most commonly used tools by the researchers to evaluate the quality of the bond between the adhesive and the substrate[43].

In the current study, we found that Futurabond DC (one-step self-etching adhesive) recorded the highest significant value of Silver nitrate % followed by Clearfil SE Bond (two-step self-etch adhesives) while the lowest silver nitrate % value was in Scotch bond MP (three-step total-etch adhesives) after 24 hr of water storage in water. As Futurabond DC one-step self-etch adhesive represented the highest significant silver deposition along the interface through 24hr water storage, with water-tree protruding from the hybrid layer into the adhesive layer and significantly massive silver deposition was observed after 6 months of water-storage. Silver deposition within resin dentin interfaces is not solely caused by incomplete resin infiltration into demineralized dentin. Also represent area within the polymerized resin matrix where residual water remains, resulting in regions of incomplete polymerization and/or hydrogel formation, or hydrophilic domains of acidic monomers that are more prone to water sorption[44].

The reason that ScotchbondMP bond recorded the lowest non-significant percentage of silver nitrate of nanoleakage was that it contained polyalkenoic acid copolymer. The polyalkenoic acid copolymer can form Ca-polyalkenoate complexes at the superficial region of the hybrid layer and within the superficial three micrometers of dentinal tubules, which might stabilize the bonded interface by providing water stability and a stress-relaxing effect. [45] Also the adhesive solution was applied in two layers as recommended by the manufacturer.

Okuda et al., [46] reported that the first layer (called priming layer) doubled the early bond strength of this adhesive, while the second applied adhesive layer showed higher extent of polymerization and less water permeability. Such properties may affect the bond strength.

After six month of water storage, in the current study, found that Scotchbond MP (three-step total-etch adhesives) recorded the highest significant value of silver nitrate percentage followed by Futurabond DC one-step self-etch) while the lowest silver nitrate percentage value was recorded in Clearfil SE bond two-step self-etch adhesives.

The result of current study of Nanoleakage was agreed with results of **Reis et al.,** [47,48] who found that the amount of silver nitrate is increased after storage in water due to degradation of resin-dentin interface.

In the current study it was found that there was non-significant inverse correlation between shear bond strength values and nanoleakage of silver nitrate penetration, and this result was agreed with **Guzman-Armostrong** [49]**, Okuda** [46]**, Pereira** [50] that showed there is no correlation between nanoleakage and bond strength and regarding the type of adhesives used and time of immersion have significant effect, which also was agreed with the present study.

CONCLUSION

Under the limitations of this in-vitro study, the results suggest that:

1- No adhesives was able to totally prevent water-induced nanoleakage of resin dentin interfaces

2- Self etch adhesive system (Clearfil SE bond) is long standing durable adhesive to nanoleakage than total etch adhesive system.

3- There is no significant correlation between the shear bond strength and nanoleakage of three adhesive systems used.

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