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Effect of Egg Shell and White Tea Dentin Pretreatment on Microtensile Bond Strength of Universal Adhesive System

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ABSTRACT

Purpose: This study was conducted to evaluate the effect of egg shell and white tea dentin pre-treatment on microtensile bond strength between resin composite and dentin surface. **Materials and Methods:** Forty-five extracted molar teeth were divided into three subgroups according to dentin pretreatment ; with no treatment (control), Egg shell, and white tea. Each group was subdivided for microtensile testing into three equal subgroups, either after 24 hours (T0), after 1 month (T1), and after 3 months (T2). The occlusal enamel was removed. And according to each group one of pretreatments was used. Resin composite blocks were fabricated on dentin after application of self etch adhesive. The teeth were longitudinally sectioned to get composite-dentin beams, two central beams were selected from 45 teeth in order to have 90 beams. Specimens were assessed after 2 storage periods in artificial saliva: either one month (T1) or three months (T2) storage period. Specimen were subjected to microtensile bond strength testing. **Results:** at T0, there was no statistically significant difference in mean μ TBS between different dentin pre-treatments, while at T1 and T2, white tea group showed significantly the highest μ TBS mean values, followed by egg shell group, then control group. **Conclusion:** Natural remineralizing agents could enhance microtensile bond strength of universal bonding system in self etch mode, and remineralizing potential of natural agents has stabilized by time.

INTRODUCTION

Dental caries is considered a disease affecting human teeth. It is a period of demineralization and remineralization which alternate

KEYWORDS

White tea, eggshell, dentin.

- Paper extracted from Doctor thesis titled “Effect of egg shell and white tea on dentin pretreatment on microtensile bond strength and their Performance in Managing Deep Caries”
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with each other through fluoride, calcium and phosphorous⁽¹⁾. Remineralization is a natural repair process of carious teeth, when demineralization is severe, natural remineralization is not enough to stop the caries process, therefore, remineralizing materials were developed to stop caries⁽²⁾.

Dentin remineralization increases in proportion to minerals concentration. Where the collagen fibrils in the dentin act as a scaffold for mineral crystallites promoting its remineralization⁽³⁾. Many natural remineralizing agents have been widely used in the dental field among these material is chicken egg shell powder and white tea. Chicken egg shell contains insoluble and soluble proteins and its outer crust is an uneatable left as a waste⁽⁴⁾.

The powder of chicken egg shell is composed of calcium carbonate 98.2%, phosphate 0.9% and magnesium 0.9%, so it is a source of mineral salts as calcium carbonate⁽⁵⁾, it is used in many therapeutic treatments as treatment of osteoporosis as it increases bone mineral density in senile osteoporotic patients when it was taken as an oral supplement for 12 months^(6,7).

Tea is the beverage which is consumed by more than two thirds of the world's population. Most of commercial tea comes from the dried leaf named *Camellia sinensis* which was native to China, but later on, it spreads to India and Japan, then to Europe and Russia then to the whole world for the last three decades. It has been observed that low risk of cancer, cardiovascular disease and osteoporosis in populations who drink tea. According to its method of processing; tea is classified into three different types white tea, green tea and black tea. Tea contains catechins as Epigallocatechin gallate (EGCG). It has antioxidant and therapeutic properties for many diseases such as cancer. White tea has catechins and it is made of young tea leaves or buds which is immediately steamed after their harvest to inactivate the action of polyphenols oxidase enzyme which inhibits the action of catechins.

White tea has an anti-collagenolytic action, which may play a role in stabilization of the

collagen and maintain the collagen in expanded form, so intrafibrillar spaces are kept open for remineralization⁽⁸⁾.

Microtensile bond strength measurement is used to evaluate the bond effectiveness of different adhesive system. It is done by applying load on the specimen, which consist of dentin and composite until fracture. Realizing the benefits of natural products, therefore this study aimed to determine the effect of egg shell and white tea dentin pretreatment on microtensile bond strength of universal adhesive system.

MATERIAL AND METHODS

This in vitro study was approved by the Ethical Committee of Faculty of Dental Medicine for Girls, Al Azhar -University (REC-OP-21-02)

Teeth selection and preparation

Forty five extracted human molar teeth were selected anonymously for this study. All collected teeth were extracted for therapeutic reasons from patients of age group (20-40), which were free of cracks, caries and with no hypoplastic defects. The occlusal enamel was removed in all collected teeth in a direction perpendicular to its long axis in order to expose flat dentin surface with a round bur (Komet, Germany) mounted in a low-speed driller (8000 rpm) (Everase, china), an indentation of 1 mm was made and guided by a rubber stopper glued to the shaft of round bur then the final depth was reached by regrinding of the surface with a grinding milling machine using #180-grit silicon carbide papers under water coolant papers (Gamberini s.r.l, Via Della Bastia, Caslecchio Di Reno, Italy), then the teeth were embedded in self-curing acrylic resin (Acrostone Dental Factor, England) surrounded by two half split teflon mould which was removed after polymerization of the acrylic resin. At the end of the study teeth were disposed in a medical waste container.

Preparation of Egg shell solution

Twelve chicken eggs were cleaned with distilled water and kept in hot boiling water for 10 mins at 100°C. The eggshells were removed and crushed to small particles with sterile mortar and pestle. The tiny crushed particles were left in a muffle furnace (Neycraft Model JFF 2000) at 1200°C to be sure that resulting powder is pathogen free, after that the dried powder was milled by using ball mill machine (planetary –ball-mill-pm -400) for 10 hours ,speed 350 rpm .The average particle size was 35:65 nm.

One gram of chicken egg shell powder was measured by electronic balance (Electronic balance, OHAUS , USA), then it was dissolved in 20ml of 4% acetic acid and the clear fluid at the top was moved to another beaker and finally the pH of solution was 11.4 checked by a pH meter (Mettler Toledo)⁽⁹⁾.

Preparation of white tea solution

Fuding White tea was manufactured in Fujian Guanglin Fu tea industry company, 10 gm of tea powder was measured by electronic balance ,then 100 ml of boiled distilled water were poured , stirring for 1 minute, then filtered using No(1) filter paper (Whatman, united kingdom) ⁽³⁾.

Dentin surface pretreatment

According to each group, one of the pretreatment was used: No pretreatment (A) , Egg shell solution was scrubbed for 60 seconds with bond brush, then rinsed for 30 seconds with distilled water(B), white tea solution was scrubbed for 60 seconds with bond brush then rinsed for 30 seconds with distilled water (C). All surfaces were gently dried with no desiccation ⁽¹⁰⁾, then Scotchbond™ Universal adhesive bond (3M ESPE, St. Paul, MN,USA) was applied using disposable brush and was rubbed continuously for 20 seconds, then gentle air drying was done for five seconds until the bond no longer moves , then it was light cured for 20s following manufacturer instructions by a LED light-curing

unit of 470 wavelength (Elipar S10, 3M, ESPE). The light intensity was 1000 mW/cm² placed perpendicular and at a standardized distance by one mm glass slide ⁽¹¹⁾.

Preparation of Resin Composite Blocks

A specially constructed flat two halves split Teflon ring mould (6x6mm in diameter and 4mm high) was used for the fabrication of resin composite blocks on the dentin substrate. An external metal ring was used surrounding the two halves of the teflon to keep the mould assembled. Cubical composite blocks were prepared inside the centre of Teflon mould using a visible light activated nano hybrid restorative resin composite (Filtek™ Z250 XT Universal restorative 3MESPE, St. Paul, MN, USA,). The Teflon ring was placed on the surface of dentin treated with self-etch adhesives, then the resin composite was incrementally packed using titanium-coated instrument (Cosmdent, Inc., Chicago, Illinois). Two increments, each of 2 mm in thickness was light cured with 470 wavelength (Elipar 3M, ESPE) for 20 seconds . The light intensity was 1000 mW/cm² which was periodically checked every 10 specimens using a radiometer (Demeteron 100, Kerr, Midelton, USA). Resin Composite blocks were further light cured for 20 seconds at each side after their removal from the split Teflon mould.

Beam Preparation:

Isomet 4000, (Buehler Lt, IL, USA) was used for sectioning of teeth. For the longitudinal sectioning, a specially designed gripping attachment was used to keep acrylic blocks with mounted teeth firm in place during sectioning to assure the perpendicular relation between the cutting disc and the occlusal surface. Serial sectioning was done in buccolingual direction than horizontal final cut at cemento enamel junction to get beams of (0.9 mm x 0.9 mm) in area , two central beams were selected from each tooth to have a total of 90 beams. 30 beams per group, 10 beams in each final class (n=10). Each beam was composed of dentin and composite with adhesive

at the interface. In order to facilitate identification of beam location; whether peripheral or central, the surfaces of composite restorations were painted with permanent ink so that the end of central beams would have a different color from peripheral ones. Each beam was stored in artificial saliva in a sealed plastic cone labelled according to subgroup and tooth of origin.

Microtensile Bond Strength Measurement:

The assessment was done using microtensile bond strength testing, and the durability was examined after three storage periods (1day, 1month and 3months). Moreover, bond failure modes were analyzed using stereomicroscope.

Statistical analysis

Statistical analysis was done using IBM SPSS Statistics Version 2.0 for Windows. Data was presented as mean, standard deviation (SD) and percentage. For checking data normality Kolmogorov-Smirnov and Shapiro-Wilk tests were conducted . The significance level was set at $P \leq 0.05$. Two-Way ANOVA was performed for comparing effect of study variables and their interaction on microtensile bond strength. One-Way ANOVA followed by Tukey’s post-hoc test were used for comparing effect of different dentin pre-treatments on microtensile bond strength at each evaluation time. ANOVA repeated measures was conducted for comparing effect of evaluation time on microtensile bond strength within each dentin pre-treatment group.

RESULTS

Microtensile bond strength

Effect of dentin pre-treatment on microtensile bond strength at each evaluation time:

Results of the effect of microtensile bond strength test at each evaluation time using One-Way ANOVA then Tukey’s post-hoc test (Table1 and Fig.1) showed that there was no evidence of statistically significant

difference in mean μ TBS between different dentin pre-treatments at T0 ($P=0.814$). While at T1, there was statistical significant difference where white tea group yielded the significantly higher μ TBS mean values ($P<0.001$) compared to egg shell and control groups, which were having similar rank statistically. At T2, there was statistical significant difference where white tea group yielded the significantly higher μ TBS mean values ($P<0.001$) compared to egg shell and control groups, followed by egg shell group, then control group which showed the significantly lowest μ TBS values ($P<0.001$).

Table (1): Mean±SD and P-value for the effect of dentin pre-treatment on microtensile bond strength at each evaluation time.

	T0	T1	T2
Control	13.2±1.8 ^a	14.8±2.4 ^b	14.0±2.4 ^c
Egg shell	13.9±3.9 ^a	17.9±2.4 ^b	19.2±3.6 ^b
White tea	12.9±3.1 ^a	26.9±2.9 ^a	36.4±2.1 ^a
P-value	0.814NS	<0.001*	<0.001*

*: significant at $P \leq 0.05$; NS: non-significant at $P > 0.05$

Means with different superscript lowercase letters in each column are statistically significantly different at $P \leq 0.05$

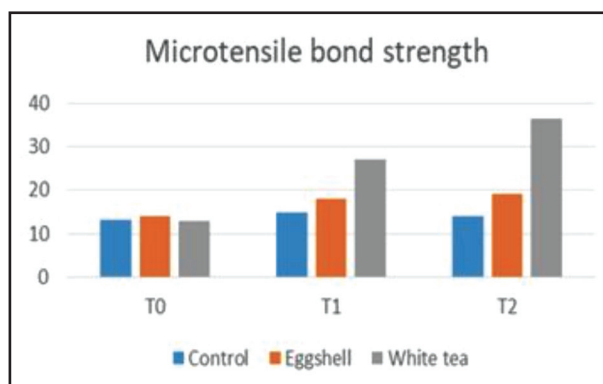


Figure (1): Bar chart showing microtensile bond strength at each evaluation time after different dentin pre-treatments

Effect of evaluation time on microtensile bond strength within each dentin pre-treatment group:

Results of the effect of microtensile bond strength test within each dentin pretreatment using One-Way ANOVA then Tukey’s post-hoc test measures (Table 2 and Fig.2) showed that there was a statistically significant difference in mean μ TBS between different evaluation times within each pre-treatment group (P=0.045 in control group, P=0.024 in egg shell group and P<0.001 in white tea group). In control group, μ TBS mean values at T1 were significantly higher than T0; while μ TBS values at T2 differ significantly from those at T0 and T1. In egg shell group, μ TBS mean values at T0 were significantly lower than those at T1 and T2, which were having same rank statistically. In white tea group, μ TBS mean values were significantly the highest at T2, followed by T1, then T0 which showed significantly lowest μ TBS values.

Table (2): Mean±SD and P-value for the effect of evaluation time on microtensile bond strength within each dentin pre-treatment group.

	Control	Eggshell	White tea
T0	13.2±1.8 ^b	13.9±3.9 ^b	12.9±3.1 ^c
T1	14.8±2.4 ^a	17.9±2.4 ^a	26.9±2.9 ^b
T2	14.0±2.4 ^{ab}	19.2±3.6 ^a	36.4±2.1 ^a
P-value	0.045*	0.024*	<0.001*

*: significant at P≤0.05

Means with different superscript lowercase letters in each column are statistically significantly different at P≤0.05.

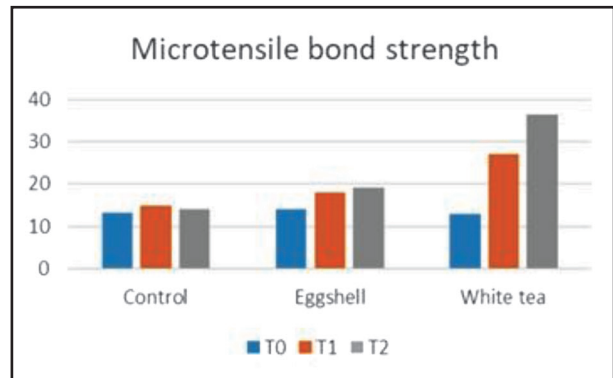


Figure (2): Bar chart showing microtensile bond strength within each dentin pre-treatment group at different evaluation times.

Fractographic Analysis:

Failure modes were:

Type I: Adhesive failure, the fracture site within the adhesive.

Type II: Cohesive failure in dentin, the fracture site within dentin.

Type III: Cohesive failure in composite, the fracture site within composite.

Type IV: Mixed failure, the fracture site started from the adhesive to the resin composite or dentin (Adhesive + Cohesive in dentin) or (Adhesive + Cohesive in composite)⁽¹²⁾.

Effect of all variables on the mode of failure of all groups:

In all groups adhesive failures type I followed by mixed failure type IV were the most common modes of failure, followed by cohesive in dentin then cohesive in composite as shown in Table (3), the greatest percent of failures type I adhesive failure (70%) was noted in control group (G1) at 3 months, whereas the least percentage (30%) of adhesive was noted in egg shell group (G2) at 3 months, regarding mixed failure Type IV, the greatest percent (50%) was noted in egg shell

group(G2) at baseline, whereas the least percentage (0%) was in egg shell group (G2) at 1 months, regarding cohesive in dentin type II, the greatest percent (30%) was noted in white tea group (G3) at 1 month., regarding cohesive in composite type III, the greatest percent (30%) was noted in egg shell group (G2) at 3 month .

Table (3) Different modes of failure in all groups:

Failure Groups	Adhesive Failure	Cohesive in dentin	Cohesive in Composite	Mixed Failure
T0	6(60%)	0(0%)	0(0%)	4(40%)
T1	6(60%)	2(20%)	0(0%)	2(20%)
T2	7(70%)	0(0%)	1(10%)	2(20%)
T0	5(50%)	0(0%)	0(0%)	5(50%)
T1	6(60%)	2(20%)	2(20%)	0(0%)
T2	3(30%)	0(0%)	3(30%)	4(40%)
T0	6(60%)	0(0%)	0(0%)	4(40%)
T1	4(40%)	3(30%)	0(0%)	3(30%)
T2	6(60%)	0(0%)	1(10%)	3(30%)

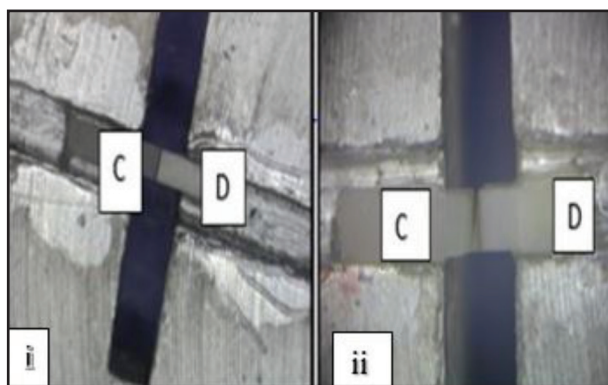


Figure (3): Adhesive failure (i), Mixed failure (ii), C: composite, D: dentin

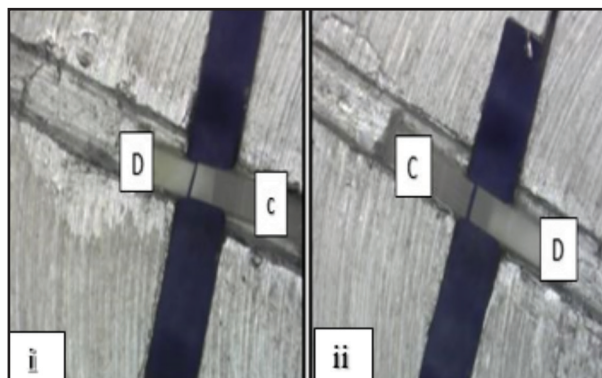


Figure (4): Cohesive failure in dentin (i), Cohesive failure In composite (ii), C: composite , D: dentin

DISCUSSION

Dental caries results from loss of balance between oral microbiota. Any increase in eating sugar or decrease in salivary flow results in a decrease in pH leading to change in the proportions of bacterial species which leads to predominance of species with an acid-producing or acid-tolerating nature causing dental caries. The cavitation of teeth depends on balance between demineralization caused by bacterial biofilm, and remineralization which depends on fluoride, calcium and phosphate⁽¹³⁾.

Remineralization of dentin is a challenge as it does not intend to repair dentin only, but also improving the dentin bond stability and treat hypersensitivity. Dentin forms the biggest portion of hard tooth structure and consists of tubules. The tubules are lined internally by mineralized and non-collagenous intratubular dentin and surrounded externally with intertubular dentin. Dentin demineralization after acidic challenges results in peritubular dentin dissolution, which is detected by the enlargement of the tubule lumen, while the shape of intertubular dentin is maintained. There are different strategies for remineralization of demineralized dentin. The traditional ion-based strategy which depends on the deposition of phosphate and calcium ions on the demineralized dentin reducing the solubility of hydroxyapatite.

With this strategy, remineralization will be absent in areas where there are no seed crystallites, so this classical ion-based crystallization strategy is not capable for remineralizing demineralized dentin ⁽¹⁴⁾.

Another strategy for dentin remineralization is Biomimetic remineralization which is another approach by stabilizing dentin biomimetic analogs of non-collagenous proteins and providing intrafibrillar mineralization of collagen. It is thought that non-collagenous proteins, specific Matrix metalloproteinase (MMPs) and enzymes secreted by odontoblasts help in dentin mineralization. They lead to Ca/P nucleation then apatite crystallization. It is bottom up strategy of remineralization which does not depend on crystallites, it works on bringing back the mechanical properties of dentin thus increasing the longevity of resin- dentin bond. Considering the recent trend in treatment of diseases which encourages the use of natural products rather than conventional medicine. Several herbal or other natural compounds have been used as remineralizing agents. Depending on its specific component, they could affect mineral precipitation and saturation, have an antimicrobial action, or stabilize collagen that act as a scaffold for mineral deposition ^(15,16).

Egg shell powder (ESP) mainly consist of 94% calcium phosphate, 4% organic matter, 1% magnesium carbonate and low concentration of strontium, fluoride, manganese, zinc and copper ions. Researchers have found that ESP can be used as a bone graft substitute as it is biocompatible, no disease transfer risks, availability and also it can be prepared with minimal cost. The minerals of ESP when come in contact with carious lesions; they pass through the superficial layer obstructing the surface porosities. ESP solution can be used as a remineralizing agent in preventive dentistry as an alternative to fluoride ⁽¹⁷⁾.

White tea originates from the plant *Camelia sinensis*. Its origin is in China mostly from Fujian, nowadays it is cultivated all over the world. The white tea has a specific technique of harvesting and it

is covered with a layer of silvered hair characterizing its colour, its leaves are naturally dried in the air to keep its benefits. It has the least amount of caffeine and less processed compared to green and black tea. The most important ingredient in white tea is polyphenols, which are natural antioxidants. It contains flavanols or catechins, such as epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechingallate (ECG), and epicatechin (EC). It also contains alanine, and amino acids. White tea has beneficial effects in treatment of several diseases such as neurodegenerative and cardiovascular diseases, diabetes, obesity ^(18,19).

The pH of universal adhesives used as self-etch is 2.2 to 3.2. Self-etch universal adhesives have mild or very mild etching effect, the universal adhesives have bond strength reported to be high and by applying it with active rubbing motions, fresh monomers are released which enhance adhesive infiltration ^(20,21).

Bond strength testing is easy, fast and the most common method for measuring the success of adhesive systems ⁽²²⁾. Microtensile bond strength has been widely used more than any bond strength test. Microtensile bond strength testing is used to evaluate the interfacial bond strengths on specimen below 1mm² permitting multiple specimens to be prepared from each tooth. The specimen's geometry has a big role in microtensile bond strength testing; so that the non-trimming method was used to obtain beam sticks which was claimed to be easy in preparation with fewer defects that might affect bond strength results ⁽²³⁾.

Artificial saliva with pH adjusted to 7.2 used as a storage medium. It is considered an important factor that affects the bond durability. Period of immersion in artificial saliva and the frequency of its change is very important to prevent any degradation process so artificial saliva was refreshed every day and the beams were washed with distilled water ⁽²⁴⁾. Therefore, this study was conducted to determine the effect of egg shell and white tea on microtensile bond strength of universal adhesive system.

Regarding the effect of different dentin pretreatment on microtensile bond strength at each evaluation time, results showed that there was no significant difference in mean μ TBS between different dentin pre-treatments at T0. While at T1, there was statistical significant difference between different pretreatment groups where white tea group yielded the significantly highest μ TBS mean values compared to eggshell and control groups. At T2, there was statistical significant difference among different pretreatment groups where white tea group showed the highest μ TBS mean values, followed by egg shell group, then control group which showed the significantly lowest μ TBS values .

The dentin pretreatment using white tea group yielded the highest significant μ TBS mean values compared to eggshell and control groups, this might be due to the presence of catechins, such as epicatechin(EC),epigallocatechin(EGC), epicatechingallate(EGCG) and epigallocatechin gallate (EGCG). ECG and EGCG are considered as MMP inhibitor. EGCG binds to collagen by hydrogen bonding and hydrophobic interaction, preventing action of collagenase at the treated site. In this way it could stabilize the collagen and maintain it in an expanded state which influence the bond strength of the restoration and their durability maintaining higher values of bond strength. Moreover ,white tea is considered as antioxidant which prevents the action of oxidants that might be released into dentin through aging which prevent the harmful effect of free radicals thus preserving its structural integrity (25-27).These results are in accordance with other studies (28,29)which found that catechins found in large amounts in white tea increased the bond strength. On the contrary other study (30) found that application of tea showed no significant difference in immediate bond strength values but it was considerable stable in the long-term.

Egg shell dentine pretreatment group showed significantly higher μ TBS mean values than the control group , as the egg shell solution had a pH of 11.7 which increases the activity of hydroxyl

ions ,so the water remained in dentinal tubules and collagen fibrils facilitates the movement of calcium ion, moreover,10-MDP (10-methacryloxydecyl dihydrogen phosphate) in universal adhesives bonds to calcium ions chemically in dentinal tubules and collagen fibrils forming a thicker hybrid layer(31).

The egg solution should be maintained alkaline as the low PH has greater concentration of H+ ions which combines with the available anions and thus reducing the ions required for bonding (32). These results are in accordance with other study (33). On the other side ,other study found that egg shell solution showed no remineralizing effect(34).

Regarding the effect of evaluation time on microtensile bond strength within each dentin pretreatment group, results revealed that there was a statistically significant difference in mean μ TBS between different evaluation times within each pre-treatment group. In control group, μ TBS mean values at T2 differ significantly from those at T0 and T1. In egg shell group, μ TBS mean values at T0 were significantly lower than those at T1 and T2. In white tea group, μ TBS mean values were significantly the highest at T2, followed by T1, then T0 which showed significantly the lowest μ TBS values. It may be due to action of self-etch adhesives which do not totally expose the dentin collagen matrix, and it maintains more remaining hydroxyapatite crystal in the hybrid layers which decrease the activation of dentin MMPs . The calcium ions which are released from the matrix during self-etch form insoluble calcium salts with functional monomers like10-MDP (10-methacryloyloxydecyl dihydrogen phosphate). The deposition of MDP-Ca salts at the adhesive interface might increase its mechanical strength. As it self-assemble into nanolayers, protecting the hybrid layer against hydrolytic degradation(35).

Moreover, the microtensile bond strength values were higher after storage in artificial saliva in all groups, because artificial saliva contains many minerals like Ca++, PO4--, Na+ and urea. During storage, deposition of these minerals on the surface

resulted in the formation of film probably composed of calcium which prevents hydrolytic degradation of the collagen fibrils which results in an increase in the bond strength⁽³⁶⁾. On the contrary, it was found that the microtensile bond strength values decreased after storage which may be due to storage in water leading to the degradation of resin -dentin bond strength by time⁽³⁷⁾.

Regarding the mode of failure; the greatest percent of failure was type I adhesive failure which was noted in control group(G1) at 3 months, whereas the least percentage of adhesive failure was noted in egg shell group (G2) at 3 months. Regarding cohesive failure in dentin type II, the greatest percent was noted in white tea group (G3) at 1 month. Regarding cohesive failure in composite type III, the greatest percent was noted in egg shell group (G2) at 3 months. Regarding mixed failure Type IV, the greatest percent was noted in egg shell group(G2) at baseline, whereas the least percentage was in egg shell group(G2) at 1 month.

The adhesive failure was the most common failure followed by mixed failure in composite, which is in accordance with other study⁽³⁸⁾ as the majority of failures were adhesive which may be due to the nature of specimen and that the microtensile testing provide stress distribution at the interface so more adhesive failures and less cohesive failures^(39,40).

CONCLUSION

Under the limitation of this study, it was concluded that: natural remineralizing agents could enhance microtensile bond strength of universal bonding system used in self etch mode and remineralizing potential of natural agents has stabilized by time.

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RECOMMENDATIONS

Further clinical studies should be done.

CONFLICT OF INTEREST

There is no conflict of interest.

FUNDING

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