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Effect of expiration date of universal adhesives on the alterations of micro tensile bond strength of composite resin to dentin

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ABSTRACT

Introduction: One of the factors affecting the success of bonded restoration is the use of appropriate adhesives and attention to their maintenance time. The aim of this study was to investigate the effect of three time periods related to the expiration date of two universal adhesives on the bond strength of resin composite to dentin.

Materials & Methods: In this in-vitro study, 30 intact third human molars were selected. The roots of the teeth were cut and the crown part was mounted in acrylic resin in such a way that the enamel of the buccal surface was clearly visible. Using abrasive disks, the enamel of the buccal surface of the teeth was abraded to create a flat dentin area with dimensions of 25 mm^2. The samples were randomly divided into 2 groups based on adhesive type (All Bond (Bisco, Fchaumburg,IL, USA) G-Permio and each group was divided into 3 subgroups based on expiration date. After the bonding process and fabrication of composite samples, microtensile bond strength (TBS) was measured at a speed of 1 mm/min. The data were analyzed using the Kruskal-Wallis test and post hoc Tukey's test. $P<0.05$ was considered as significant level.

Results: Significant differences were found among samples with different expiration dates in both All-Bond Universal ($p=0.0001$) and G-Premio ($p=0.0001$) groups in terms of microTBS (μTBS). In both adhesive groups, a significant difference was found between 2 months after expiration with expiration time and 2 months before expiration, but there was no significant difference between expiration time and 2 months before expiration.

Conclusion: The end of the expiration date has a reducing effect on the μTBS of universal adhesives investigated in this study. However, the amount of this effect varies depending on the type of adhesive.

Keywords: Dental Bonding,Tensile Strength, Composite Resins

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Introduction

Today, the use of restorative materials bonded to the teeth with adhesive techniques is very common. [1] Since the success of adhesive restorations depends on their adhesion, achieving stable adhesion is very important. [2]

Dental materials are stored for a long time on the shelf or in the refrigerator, and during this time, the constituents of the materials should not be separated or evaporated, reacted or degraded. [3] Otherwise, the resulting chemical changes may lead to a possible loss of adhesive bonding ability. [4]The formulations of adhesives are created to have maximum stability against early and accidental polymerization during storage before clinical use. In addition, their packaging is designed to be resistant to degradation by oxygen, humidity, light and heat. [2] Failure to form a suitable bond may be due to not only clinical process error, but also excessive adhesive retention time. [5] Accordingly, manufacturers always consider expiration dates for materials. [6] According to the American National Standards Institute (ANSI), the shelf life of a material is the time (from the date of manufacture) during which the material retains the physical and mechanical properties necessary to perform a specific purpose. [7]Most products have a minimum shelf life of two or three years from the date of manufacture. Of course, this does not necessarily mean that the material will deteriorate after the expiration date, but rather it does mean that by the time the expiration date is set, the manufacturer guarantees that the use of the material is safe and that its performance is as expected. [8] It is not yet clear to what extent the problems caused by the storage of the material beyond the shelf life increase or to what extent its properties are affected. [9]To evaluate the physical and chemical performance of materials, some criteria including mechanical, optical and surface properties as well as biocompatibility factors are proposed to determine and calculate the acceptable levels of stability. [3, 5, 7]These criteria are specifically used to assess the stability of medical materials. Although it may be a good start to set criteria for evaluating the stability of dental materials, other variables should also be considered, especially, because the conditions of transportation and storage of materials before preparation for clinical use are not always ideal. [6] These conditions, which may have a negative effect on the final quality and properties of the material, have rarely been studied in dental biomaterial literature. Therefore, it is clinically important to evaluate the effect of shelf life on the ability of adhesive systems. [10]The micro-tensile bond strength (μTBS) test was first introduced in 1994. This test is used as one of the most standard tests to evaluate bond strength in laboratories. [11] With advances in restorative dentistry, providing universal adhesives to reduce the process is expanding. These types of adhesives can be used as total-etch and self-etch as well as have bonding ability to enamel, dentin and a variety of restorative materials.

According to studies on the μTBS of universal adhesives to bond to teeth, regardless of the type of the used adhesives (self-etch or total-etch), these adhesives will improve the bond strength of the tooth. However, challenges associated with previous generations of adhesives including being bio-protective have not been overcome yet. [12]Over time and after the expiration date, universal adhesives are expected to exhibit unsuitable physical and chemical properties. These unsuitable properties affect the bond strength, leakage and materials' degrees of conversion (DC). [13]In 2019, Cuevas-Suarez et al. evaluated the μTBS to dental dentin, DC and nanoleakage expression of 8 dental adhesives based on their expiration date and concluded that longer storage time and storing conditions affect the performance of universal adhesive systems. [6]In 2020, Mazzitelli et al. studied on the shelf life of universal adhesives and investigated the μTBS and endogenous enzymatic activity of a universal adhesive system. They have concluded that the use of universal adhesive beyond the expiration date due to higher endogenous enzymatic activity after the end of the shelf life leads to reduced bonding performance and

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shortened durability of the restorations. [14] Accordingly, the aim of the present study was to investigate the effect of the expiration date of universal adhesives on the μTBS of resin composite to dentin

Materials & Methods

This study was evaluated after obtaining the ethical approval from Babol University of Medical Sciences (IR.MUBABOL.REC.1400.099). According to the previous study and based on the information of similar articles [6, 7], 30 sound third human molars without any caries and defects were selected and after cleaning the soft tissue residues from their surfaces, they were kept in 0.5% Chloramine T (Merck, Darmstadt, Germany) for seven days.[6] The sample size was calculated using the following formula:

$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 (S_1^2 + S_2^2)}{(\mu_1 - \mu_2)^2}$$

Then, the teeth were removed from the disinfectant solution and washed. The teeth were kept in distilled water at 4 ° C until use. To prepare the specimens, the roots of the teeth were cut and the crown was placed in acrylic resin so that the enamel of the buccal surface was completely exposed.Using abrasive disks (model no. 11-1280-250, Buhler Ltd., Lake Bluff, IL, USA), the enamel of the buccal surface of the teeth was abraded to create a flat dentin area with dimensions of 25 mm^2. The surface of the exposed dentin was abraded with 600-grit abrasive paper (Norton: Saint-Gobain Abrasivos Ltda) for 30 seconds under water to create a smear layer.Then, the samples were randomly divided into 2 groups (n=15) based on the type of adhesive, and each group based on time (2 months before expiration date, expiration date and 2 months after expiration date) was categorized into 3 subgroups (n=5):

(The bottles of both adhesives used in this study were first opened 2 months before expiration date).

First group: G-Premio adhesives, 2 months before expiration date: To prepare the specimens of this group, after applying G-Premio Bond (GC,Tokyo,Japan) on the prepared surface with a microbrush, we waited for 10 seconds, and then they were gently air-dried for 5 seconds. Next, the photoactivation was performed with LED (Valo, Kenr, USA) for 10 seconds with 800 mW/cm^2 of energy intensity. After preparing each 5 specimens, the intensity of the device was checked using a radiometer. After bonding, the resin composite (Filtek Z250, 3M ESPE, St. Paul, MN, USA) was placed in three 2-mm layers on the specimens, and each layer separately was photoactivated and polymerized for 30 seconds. The specimens were then immersed in 37 ºC distilled water for 24 hours and kept in an incubator.

Second group: G-Premio adhesives, at the expiration date: The preparation process of specimens was the same as that in the first group.

Third group: G-Premio adhesives, 2 months after expiration date: The preparation process of specimens was the same as that in the first group.

Fourth group: All-Bond Universal adhesives, 2 months before expiration date: To prepare the samples of this group, after application of All-Bond Universal (Bisco, FchaumburgIL, USA) with microbrush, it was placed on the surface of the specimens through scrubbing for 10-15 seconds. After that, the second layer was put on the surface of the specimens in the same way and air-dried for 5 seconds and photoactivated with LED (Valo, Kenr, USA) for 10 seconds. After all 5 samples, the intensity of the device was checked using a radiometer. Next, the

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composites were placed on the specimens as in the first group, and the prepared specimens were immersed in distilled water for 24 hours and kept in an incubator. Other steps were performed as described in the first group.

Fifth group: All-Bond Universal adhesives, at the expiration date: The preparation process of specimens was the same as that in the fourth group.

Sixth group: All-Bond Universal adhesives, at the expiration date, 2 months after expiration date: The preparation process of specimens was the same as that in the fourth group. At all steps, the specimens were kept in an aqueous medium during experiments.

For thermocycling, the specimens were floated in a cold water bath at 5±2 ºC and a hot water bath at 55±2 ºC. The immersion time of the specimens in each chamber was 30 seconds, and the total time of one cycle was 90 seconds. This work was repeated 1500 times. Next, the specimens were cut at intervals of one millimeter using a disk (Microremet, remet, Bologna,Italy) to obtain dentin-resin rods with an approximate cross-sectional area of 1 mm^2. The size of the cross-sectional area of the specimens was confirmed with a caliper (Mitutoyo IP67 Resolution: 0.01mm). The rods were fixed separately to the universal testing machine platform using cyanoacrylate adhesive and subjected to tensile stress at a speed of one millimeter per minute until failure occurred. The stress at which the failure occurred was recorded in Newton as the failure load and was divided by the cross-sectional area of the specimens (in square millimeters). The value obtained from μTBS was in megapascal (MPa). Failure specimens were examined under a photomicroscope at ×40 magnification, and the types of failure were determined as adhesive failure, cohesive failure in dentin, cohesive failure or mixing failure in resin composite. Finally, the data were entered into SPSS 26 and analyzed using the Kruskal-Wallis test and post hoc Tukey's test. A value of $p<0.05$ was statistically considered significant. The general composition of materials used in this study is provided in Table1.

Table 1. Test materials name, manufacturers and compositions

Materials	Composition	Maufacture	Lot number*
All-Bond Universal	10-MDP, 2-HEMA, Bis-GMA, ethanol, waters, photoinitiator	Bisco, SchaumburgIL, USA	1900001237
G-permio Bond Universal	10-MDP, 4-MET, MTDP, methacrylicacid ester, Silica, acetone, water, photoinitiator	GC cord, Tokyo, Japan	1906112
Composite Z250	Matrix: Bis-GMA, UDMA, BisEMA Filler : zirconium, silica	3M ESPE, St. Paul, MN, USA	NC33470

*According to the manufactures information. 10-MDP : 10-methacryloyloxydecyl dihydrogen phosphate, 2-HEMA: 2-hydroxyethyl methacrylate, Bis-GMA: Bisphenol-A-glycidyl dimethacrylate, 4-MET: 4-methacryloxyethyl trimellitic acid, MDTP: methacryloyloxydecyl dihydrogen thiophosphate, UDMA : Urethane dimethacrylate , BisEMA: Bisphenol A ethoxylate dimethacrylate

* Identification number assigned to a particular quantity or lot of material from a single manufacturer.

Results

In the current study, the μTBS of two adhesives -All-Bond Universal and G-Premio to dentin were evaluated using 30 healthy third human molars over three time periods: 2 months before expiration date, expiration date and 2 months after expiration date. The mean and standard deviation of the μTBS of adhesives to dentin in terms of MPa were investigated. In the present study, the value of $p<0.05$ was statistically considered significant. Table 2.

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presents a comparison of the subgroups and the mean±standard deviation of microtensile bond strength in all subgroups.

Table 2. Descriptive statistics of the groups and comparison of μTBS values

Adhesive	Time (Subgroup)	Sample size N(%)	Mean±SD (Mpa)
	2 months before expiration date	5 (16.6%)	18.61±4.13
	Expiration date	5 (16.6%)	16.95±3.35
	2 months after expiration date	5 (16.6%)	13.66±2.29
	2 months before expiration date	5 (16.6%)	11.13±2.34
	Expiration date	5 (16.6%)	10.81±2.63
	2 months after expiration date	5 (16.6%)	3.65±1.78

A significant difference was found between samples with different expiration dates in both All-Bond (p=0.0001) and G-Premio (p=0.0001) groups in terms of μTBS. In a more detailed study, in the All-Bond group, there was a significant difference between 2 months after expiration date with expiration date (p=0.01) and 2 months before expiration date (p=0.0001), but this difference was not significant between the expiration date and 2 months before expiration date (p=0.28). In addition, in the G-Premio group, there was a statistically significant difference between 2 months after expiration date with expiration date (p=0.0001) and 2 months before expiration date (p=0.0001) in terms of μTBS, but no significant difference was found between the expiration date and 2 months before expiration date (p=0.9). Moreover, in all three adhesive expiration dates, the μTBS of All-Bond was significantly higher than that of G-Premio (p= 0.001 in all three cases). A simultaneous study of the effect of adhesive type and expiration date showed that the interaction of these two factors had a significant effect on μTBS (p=0.012). Furthermore, figure 1 illustrates the mean μTBS of the samples by each group. Results of the failure modes determined by optical microscopic evaluation are shown in Table 3.

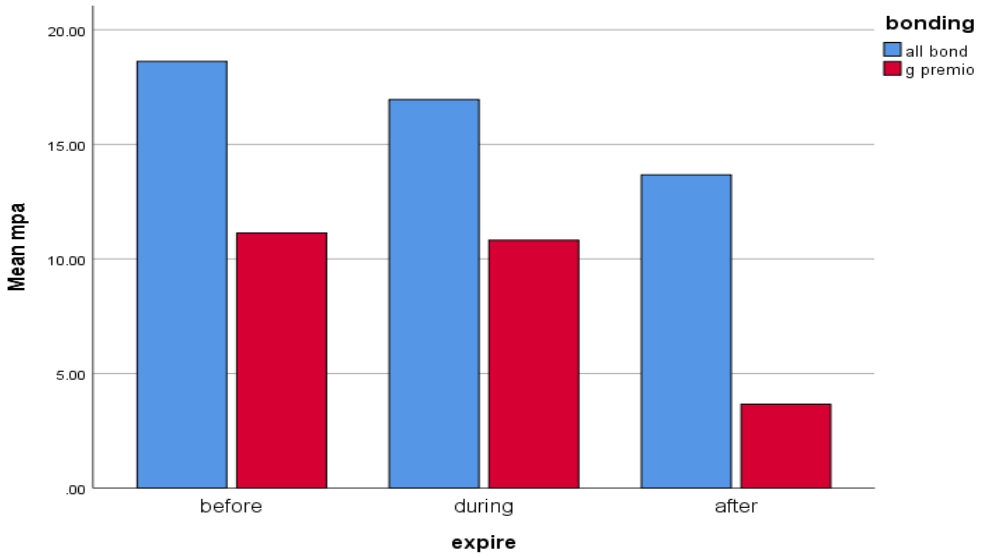

Figure 1. Mean microtensile bond strength of the studied samples by each group

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Table 3. Distribution of failure modes as observed by optical microscopy at magnification ×40

	Time (subgroups)	Adhesive	Cohesive in dentin	Cohesive in composite	Mix
	2 months before the expiration date 20 N(%)	16(80%)	0	4(20%)	0
	Expiration date 20 N(%)	16(80%)	0	4(20%)	0
	2 months after the expiration date 20 N(%)	14(70%)	0	6(30%)	0
	2 months before the expiration date 20 N(%)	17(85%)	0	3(15%)	0
	Expiration date 20 N(%)	16(80%)	0	4(20%)	0
	2 months after the expiration date 20 N(%)	18(90%)	0	2(10%)	0

Discussion

The findings of the present study showed that the μTBS of All-Bond was significantly higher than that of G-Premio in all three adhesive expiration dates (2 months before expiration date, expiration date and 2 months after expiration date). According to the results, a significant difference was found between samples with different expiration dates in both All-Bond and G-Premio groups in terms of μTBS. In both All-Bond and G-Premio groups, this difference was significant between 2 months after expiration date with expiration date and 2 months before the expiration date but was not significant between the expiration date and 2 months before expiration date. Simultaneous study of the effect of adhesive type and expiration date indicated that the interaction of these two factors had a significant effect on μTBS. The results of the Papadogiannis et al. in 2019 are in line with those of the current study. The All-Bond adhesive is a self-etch with a very mild pH (pH=3.1) and contains 2-hydroxyethylmethacrylate (HEMA) with ethanol/water solvents, while the G-Premio universal adhesive is a 2-HEMA-free adhesive with low pH.[15]

In addition, the All-Bond universal adhesive containing bulk bisphenol-A-glycidyl dimethacrylate (Bis-GMA) monomer provides hydrophobic aliphatic dimethacrylates for resilience, stiffness and strength in the polymer network as well as efficient cross-linking with the hydrophobic resin restoratives.They have found that dimethacrylate monomers do not polymerize completely because of steric hindrance and C=C bonds remain in the polymer network, but adding low molecular weight monomethacrylates such as 2-HEMA may result in rapid copolymerization with the residual C=C groups of the bulky monomers. [15] In their study, it was revealed that G-Premio compared to All-Bond had a lower DC, maybe related to the presence of multiple acidic monomers (methacryloyloxydecyl dihydrogen thiophosphate (MTDP), 10-methacryloyloxydecyl dihydrogen phosphate (10-MPD) and 4-methacryloxyethyl trimellitic acid (4-MET)) and residual solvents as well as the absence of the non-acidic 2-HEMA monomer. [15] Solvent type is an important and influential factor affecting the durability and clinical success of adhesives. In dentistry, ethanol, water and acetone are the most common solvents. G-Premio and All-Bond adhesives contain acetone and ethanol as solvents, respectively. Several studies have stated that the bond strength of washing and etching systems highly depends on the type of solvent. [16] The results of Chowdhury et al. study in 2021 are similar to those of the present study. G-Premio universal adhesive consists of a high volatile

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acetone solvent as well as in a short time of adhesive application, the acetone evaporates quickly and water remains. Incomplete polymerization due to the high residual water and heterogeneity of the adhesive layer caused by lack of HEMA leads to a weak premature and interface failure of the bond. [17] In addition, Dilsad et al. (2018) have explained that repeated opening of bonding bottles causes the solvent to evaporate faster, leading to lower bond strength in the acetone-based adhesive system. Further, thinner layers are created in acetone-based adhesives through higher vapor pressure of acetone compared to ethanol and water; therefore, the resulting adhesive layer is more susceptible to degradation. [16] The results of the ongoing study are the same as those of Sugimura et al. in 2019. They have pointed out that the concentration of water is much lower (3 volume%) in the All-Bond universal adhesive than in the G-Premio adhesive (25 volume%), resulting in a hydrophobic adhesive-tooth interface, resistant to hydrolytic degradation . [18]

It is important to note that excess water in the adhesive leads to the formation of poor quality bond layers due to incomplete polymerization. On the other hand, the accumulation of water in the adhesive due to solvent evaporation causes weaker chemical interaction and demineralization. [18] On the other hand, Choi et al. in 2017 investigated the effect of dentin moisture on the bond strength of universal adhesives and came to the opposite conclusion of the present study. They have described that because G-Premio adhesive has a higher concentration of water, it opens collapsed collagen networks caused by excessive drying of the dentin, facilitating the penetration of the resin. [19] In contrast to the present study, Khamverdi et al. investigated the 10-MDP and HEMA monomer and concluded that the 10-MDP phosphate monomer in universal adhesives could justify their ability to etch and bond to different surfaces.When universal adhesives are used in self-etch mode, they protect the demineralized dentin moisture and prevent collagen fibrils collapse. In self-etching mode, the etched surface is not rinsed. Thus, the calcium and phosphate ions resulting from the dissolution of hydroxyapatite crystals form a chemical bond with the 10-MDP monomer, while in the All-Bond adhesive, the HEMA monomer competes with 10-MDP monomer to bond to the hydroxyapatite crystals and reduces the formation of 10-MDP-calcium salts in the resin-dentin interface. HEMA monomer due to the chemical composition of monomer the acrylate weakens the mechanical properties of the polymerized bond, which has an adverse effect on the hydraulic degradation of the adhesive layer, leading to the separation of the resin-dentin interface. HEMA can prevent the 10-MDP monomer nanolayering mechanism and reduce the bond strength of the universal adhesive. [20]

Since the present research was an in vitro study, ethical considerations had no effect on the research process. One of the limitations of this study was to find sound extracted teeth that were free from caries and cracks, and to find universal adhesives with the desired expiration date. It is suggested that further research should be conducted considering effective properties such as the degree of conversion and hardness of the adhesive layer, as well as the effects of factors such as saliva, masticatory forces, thermal changes and clinical conditions of the oral environment.

Conclusion

These results clearly demonstrated that the mechanical properties of the universal adhesives especially micro tensile bond strength decrease after the end of shelf life. However, the amount of this effect varies depending on the type of adhesive.

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Conflicts of Interest

There was no conflict of interest.

Authors' Contribution

The study was designed by Fariba Ezoji. The study data were collected by Golara Azarpira. Analysis and interpretation of data, drafting of the manuscript, and critical revision of the manuscript for important intellectual content were performed by Faraneh Mokhtarpour and Fariba Ezoji. Study supervision was conducted by Fariba Ezoji and Ghazaleh Ahmadizenouz.

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