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Original Article

Scanning electron microscope and shear bond strength analysis of Biofix and Orthocem two-step fluoridated orthodontic adhesives on human enamel

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Abstract

Introduction: The bonding process in orthodontic treatment is very important. This study aimed to evaluate the shear bond strength (SBS) and bond failure sites of stainless steel brackets bonded with two new two-step adhesives (Biofix (BF) and Orthocem (OC)) and a three-step adhesive (Transbond XT (TXT)).

Material & Methods: In this in vitro study, 66 extracted human premolars were collected and randomly divided into three groups (n=22). The brackets were bonded to each tooth with a) TXT, b) BF, and c) OC adhesives according to manufacturers' instructions. The SBS values of the brackets were measured 24 hours after thermocycling. Adhesive remnant index (ARI), enamel detachment index (EDI) and bond failure locations on bracket surfaces were qualitatively and quantitatively assessed using stereomicroscopic, scanning electron microscope (SEM) and energy dispersive x-ray (EDX) analyses. The data were analyzed using SPSS 22 software and ANOVA test. The significance level was defined at $P<0.05$.

Results: The means and standard deviations of SBS values for TXT, BF and OC adhesives were 22.49±4.58, 17.82±6.43 and 16.20±4.46 MPa, respectively. The SBS in the TXT group was significantly different from the two other groups, but the difference was not significant between the two other groups. Moreover, ARI and EDI were not significantly different between the three groups. The SBS values of BF ($P<0.001$) and OC ($P<0.001$) were not significantly different.

Conclusion: The adhesive SBS in the BF and OC groups was in the determined ranges to bond the orthodontic brackets. Therefore, these two adhesives can be used as a proper alternative for conventional bonding methods.

Keywords: Dentistry, Orthodontics, Scanning electron microscopy

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آنالیز میکروسکوپ الکترونی روبشی و بررسی استحکام باند برشی اتصال دو نوع ادهزیو ارتودنسی دو مرحله ای دارای فلوراید (Biofix and Orthocem) به مینای دندان انسان

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چکیده

مقدمه: پروسه ی باندینگ و افزایش سرعت آن در درمان ارتودنسی از اهمیت زیادی برخوردار است. هدف از این مطالعه، بررسی استحکام باند برشی و نواحی شکست باند در براکت های استیل ضد زنگ چسبانده شده توسط ادهزیوهای دو مرحله ای Orthocem (OC) و Biofix (BF) و ادهزیو سه مرحله ای Transbond XT (TXT) می باشد.

مواد و روش ها: در این مطالعه تجربی آزمایشگاهی، ۶۶ دندان پرمولر کشیده شده ی انسانی، بصورت تصادفی به ۳ گروه مساوی تقسیم شدند: در گروه اول براکت ها با TXT، در گروه دوم با BF و در گروه سوم با ادهزیو OC بر روی دندانها، طبق دستور کارخانه سازنده، باند شدند. ۲۴ ساعت بعد از ترموسایکلینگ، استحکام باند برشی براکت ها اندازه گیری شد. میزان رزین باقی مانده در سطح دندان ها (ARI) ، میزان تخریب مینا (EDI) و محل شکست باند به دو روش کمی و کیفی توسط استریومیکروسکوپ، اسکن میکروسکوپ الکترونی و آنالیز EDX ارزیابی شد. داده ها توسط نرم افزار SPSS 22 و تست ANOVA ارزیابی شد سطح معنی داری کمتر از ۰/۰۵ در نظر گرفته شده است.

یافته ها: میانگین استحکام باند برشی گروه اول: ۴/۵۸ ±۲۲/۴۹، گروه دوم: ۶/۴۳ ± ۱۷/۸۲و گروه سوم: ۴/۴۶ ± ۱۶/۲۰ می باشد. استحکام باند برشی گروه اول با سایر گروه ها اختلاف معنی داری داشت، ولی در دو گروه دیگر، این اختلاف معنی دار نبود($P<0.001$). همچنین، EDI و ARI در سه گروه اختلاف معنی داری را نشان نداد($P<0.001$).

نتیجه گیری: استحکام باند کامپوزیت در دو گروه OC و BF در محدوده ی تعیین شده جهت باند براکت های ارتودنسی بوده و میتوان این دو ادهزیو را به عنوان جایگزینی مناسب برای روش های باند معمول مورد استفاده قرار داد.

واژگان کلیدی: دندانپزشکی، ارتودنسی، میکروسکوپ الکترونی روبشی

Introduction

Orthodontic brackets should endure the masticatory loads, deliver optimal orthodontic force, and simply be removed at the end of the treatment with minimal or no damage to the tooth surface.[1,2] Many factors, including the duration and concentration of the etchant, adhesive material and general features of brackets such as clinician's expertise and design, influence the mechanical adhesion of orthodontic brackets.[3] Bracket bonding failure is challenging for the practitioner, affects the appliance efficiency, imposes economic impacts on the practice and gives rise to potential and significant delays in the treatment process.[4,5] Some underlying causes of bond failure are the type of adhesive used[6] and the bracket base size.[7] The traditional bonding system in orthodontics is a three-step mechanism,[8] leading to longer procedures. The two-step system combines steps two and three in one step, reducing the chair time for patients. Release of fluoride from these systems could potentially reduce the frequently occurring demineralized white spot lesions (DWSLs) adjacent to the bracket in orthodontic patients. Fixed orthodontic appliances are a challenge for oral hygiene and provide greater surface area for the adhesion of plaque; the irregular shapes of the

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appliances also limit the self-cleaning ability of saliva, lips, tongue and cheeks, eventually increasing the risk of incipient caries on tooth surfaces that are not usually prone to caries attack. A recent study showed that orthodontic patients had a significantly higher incidence of DWSLs than a control group of participants who did not undergo orthodontic treatment. The fluoride ions are capable of precipitating within the enamel prisms, promoting the re-mineralization of the tooth surface.[9] Moreover, it seems that the incidence of enamel color changes associated with orthodontic bonding can be reduced by eliminating step two of the 3-step mechanism of bonding. The enamel color alterations might be caused by the irreversible penetration of resin tags into the enamel structure.[10]

Accordingly, this study aimed to measure and compare the shear bond strength (SBS) and adhesive remnant index (ARI) score of two new fluoridated orthodontic adhesives [Biofix (BF) and Orthocem (OC)] and compare them with Transbond XT (TXT). The study's null hypothesis stated that there was no significant difference in the SBS values and debonded locations between the different groups.

Materials & Methods

This study was approved by the Ethics Committee of Babol University of Medical Sciences, Babol, Iran (under the code mubabol.rec.1392.19). Sixty-six intact maxillary premolar teeth extracted for orthodontic reasons were selected. Previously restored teeth or teeth with enamel defects or cracks (observed at ×10 magnification) were excluded. The teeth were disinfected with 0.05% thymol solution to prevent bacterial growth[11] and then stored in normal saline solution at room temperature. The teeth were randomly assigned to three groups (n=22). After 15 seconds of polishing with non-fluoridated and oil-free pumice, using a rubber cup and a low-speed handpiece, the buccal surface of each tooth was rinsed and dried by air. Stainless steel maxillary premolar brackets (Standard Edgewise 0.22-Dentarum, Pforzheim, Germany) were bonded to the teeth with a different adhesive in each group according to the manufacturer's instructions. The average surface of the orthodontic bracket base was 11.85 mm^2. The same operator bonded all the brackets. The bonding adhesives were light-cured with an LED light-curing unit (Valo, Ultradent, South Jordan, UT, USA) using 1000 mW/cm^2 power confirmed by a radiometer.

Sample preparation method: Group 1 (TXT) (Unitek/3M, St Paul, Minn, USA): The buccal surface of each tooth was etched with 37% phosphoric acid gel (Ultra-Etch, Ultradent, South Jordan, USA) for 30 seconds, rinsed for 20 seconds and finally dried using moisture-free air for 20 seconds until the enamel achieved a white and frosty appearance. The bonding agent (Sealant, Transbond XT Primer) and TXT adhesive was applied to the bracket base, with the bracket positioned 4 mm below the cusp tip, approximately on the middle of the buccal surface of the tooth, using a 300-gr force[12] for 10 seconds with a tension and compression gauge (Dentarum-Germany). This force was previously defined with Correx Gauge (Dentarum-Germany) after which the excess bonding resin was removed using a sharp scaler. Subsequently, the adhesive on the bracket base was light-cured for 10 seconds from the mesial and 10 seconds from the distal aspects based on the manufacturer's instructions.

Group 2 (BF) (**Biodinamica, Ibipora, PR, Brazil**): Etching, rinsing and drying were carried out similar to the group 1. After detecting the frosty appearance, without the priming agent, BF composite was applied, and the bonding protocol was followed according to the manufacturer's instructions.

Group 3 (OC) (**FGM- Joinville, Santa Catarina, Brazil**): The same procedure was used to prepare this sample as well, except that the force applied (450 gr) to the adhesive caused the adhesive to extrude from the borders of the bracket because OC had higher consistency compared to two other adhesives.

All the three samples were thermocycled (Nemo Industrial, Mashhad, Iran) in water for 400 cycles; each cycle consisted of three phases of hot water bath for 30 seconds, cold water bath for 30 seconds and a dwell time of 20 seconds.[13,14] The bracketed teeth were immersed in distilled water in sealed containers and kept at room temperature, allowing adequate water absorption and equilibrium. The teeth were then mounted in molds. The internal surface of each mold was coated with vaseline, and the teeth were fixed using 19×25-inch rectangular stainless steel wire and O-rings (Ortho–Technology, USA). Each tooth was positioned at the center of the mold, and the rectangular wire was fixed to the mold using sticky wax so that the teeth remained fixed when the acrylic resin was applied. Auto-polymerizing acrylic resin was applied, and the

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teeth were embedded in acrylic resin to the level of their cementoenamel junction. After polymerization of acrylic resin, the teeth in acrylic blocks were separated from the mold. The brackets' SBS values were measured by a universal testing machine (Zwick/ Roell, Germany) at a crosshead speed of 0.5 mm/min. The testing machine was prepared using a chisel-edged plunger. The edge of the plunger was positioned at the enamel–composite interface.[15] The peak force levels, automatically recorded by the testing machine, were converted into stress per unit area (MPa) by dividing the force (N) by the mean unit area of the bracket base (11.85 mm^2). ANOVA was used to compare SBS between the groups at a significance level of $P<0.05$.

Residual adhesive: After debonding, all the teeth and brackets were examined at ×10 magnification under a stereomicroscope (Nikon Instrument INC, USA). The remnants of the adhesive material were evaluated using ARI and scored by assessing the resin material-to-enamel surface ratio.[16] ARI was used to determine the sites of the bond failure between the enamel, the adhesive and bracket base.

Furthermore, Razi Metallurgy Research Institute dismantled the remaining enamel and brackets to determine the bond quality, using SEM (VEGA\TESCAN) and EDX analyses. Ten brackets in each group (30 brackets in total) were randomly selected for SEM and EDX analyses. The first set of images obtained was perpendicular to the bracket base, at a magnification of ×35 (Fig. 1). Data were reviewed, and the amount of ARI on the brackets was determined by the following rating system,[16] and bond failure sites were visualized under an SEM:

Grade 1: No composite remaining on the bracket base
Grade 2: <10% of composite remaining on bracket base
Grade 3: >10% and less than 90% of composite remaining on the base
Grade 4: >90% of composite remaining on the base
Grade 5: All the composite remaining on the bracket

The bracket base surface coated by gold was transferred to the machine, and x-ray beams were irradiated in a vacuum environment. The reflected electrons were collected by the optical photon detectors and converted into a visible image. Thus, the entire surface of the bracket was again photographed and recorded by the device. EDX analysis recorded the emitted energy from the bracket surface elements and determined the atomic weight of the elements. Iron, silicon, phosphorus and calcium (Fe, Si, P and Ca) indicated brackets, resin and tooth enamel, respectively.[17] The P, Ca, Si and Fe values were calculated based on weight percentages (Fig. 2).

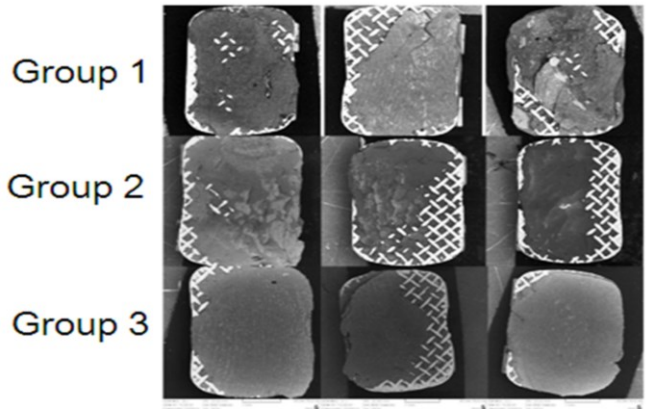

Fig. 1. SEM of debonded brackets.

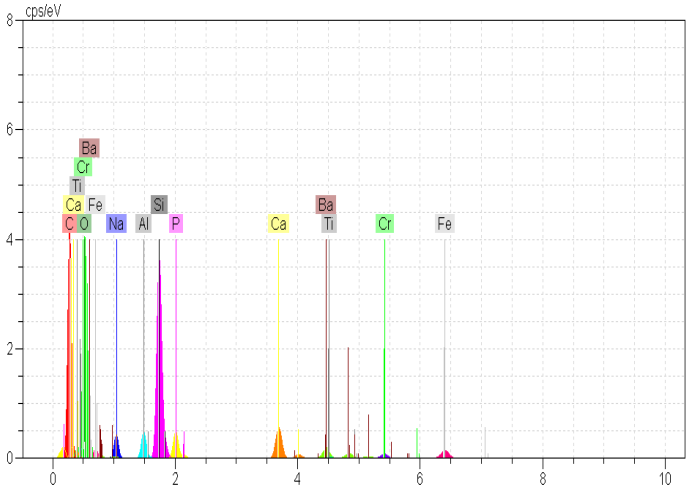

Fig. 2 A sample EDX spectrum of a bracket base composed of P, Ca, Si and Fe.

Statistical analysis: Statistical analysis was performed by SPSS 22 (SPSS Inc., Chicago, IL, USA). The means and standard deviations were used to analyze the quantitative data, and the numbers and frequency percentages were used for qualitative analysis of data. ANOVA was used to compare the quantitative data between the three groups. Chi-squared test was applied to compare the qualitative data between the groups. Statistical significance was defined at $P<0.05$.

Results

Quantitative results are presented in Table 1. The use of three different adhesives yielded the following results.

Table 1.Means and standard deviations of quantitative indicators (SBS, EDX and ARI) in different groups

Group Indicator	SBS (N/mm^2)	EDX			ARI	
		Fe	Si	Ca + P	(stereomicroscope)	(SEM)
TXT	22.49±4.58[a]	6.35±1.93[a]	11.80±0.68[a]	5.94±2.08[a]	3.5±0.51[a]	3.4±0.52[a]
BF	17.82±6.43[b]	2.65±0.99[a]	7.28±0.67[a]	9.20±2.64[a]	3.76±0.54[a]	3.4±0.52[a]
OC	16.20±4.46[b]	4.61±1.35[a]	17.13±1.69[b]	10.16±3.93[a]	3.64±0.66[a]	3.4±0.52[a]

Shear bond strength: Different symbols (a and b) in each column indicate significant differences between the two groups at P<0.05. The numbers in the table are means ± SD. Test for analysis: ANOVA. The mean SBS values of the study groups are shown in Table 1. Statistical analysis of the shear bond strength values of the three groups revealed a significant difference (P<0.001) in the bonding strength of the TXT, due to its higher mean bond strength, compared to the other two groups. Furthermore, ANOVA showed that the SBS values of BF (P<0.001) and OC (P<0.001) were not significantly different. The destruction of enamel (EDX*):* ANOVA indicated that the amounts of Fe, Ca and P after deboning were significantly different (P=0.03 and P=0.01, respectively), with a statistically significant difference in the amount of Si (P<0.001). The element means and standard deviations in different groups are presented in Table 1. The maximum amounts of Si, Ca and P were observed in the OC group, while the highest amount of iron was seen in the TXT group.

Qualitative findings: The results for the qualitative variables as a whole are presented in Table 1.

A) ARI* (stereomicroscope/the amount of adhesive remaining on the enamel surface*): The amount of adhesive remaining on the tooth surface in different groups based on the Bishara-built ranking is illustrated in Tables 3 and 4. Both observers reported that the highest amount of composite remaining on the tooth was related to the TXT, while the lowest one was reported in BF. However, based on ANOVA, the remaining adhesive value showed no statistically significant difference between the units (P=0.327).

B) ARI* (SEM/the amount of adhesive remaining on the enamel surface*): In Table 2, based on SEM images, the adhesive remaining on the bracket surface in different groups based on Bishara built ranking revealed the amount of resin remaining on the bracket at different levels with no significant difference between the images.

Table 2. Frequency distribution percentages of the adhesive remaining on teeth in different groups (ARI by stereomicroscope (SM) and SEM)

Group Grade	100% (score 1)		>90% (score 2)		10-90% (score 3)		<10% (score 4)		0 (score 5)	
	SM	SEM	SM	SEM	SM	SEM	SM	SEM	SM	SEM
TXT	0	0	0	0	0	11 (50%)	13 (60%)	11 (50%)	9 (40%)	0
BF	0	0	0	0	0	7 (31.6%)	13 (60%)	13 (63.2%)	9 (40%)	2 (5.3%)
OC	0	0	0	0	0	10 (45.5%)	13 (60%)	10 (45.5%)	9 (40%)	2 (9.1%)

Test for analysis: Chi-square test

Discussion

The results of the present study can be summarized in two sections: a) the SBS and b) the remaining resin, enamel damage and fracture location. Shear bond strength: The current study showed that the SBS of brackets bonded with TXT adhesive was significantly higher than that of BF and OC, while the difference in bond strength between BF and OC was not significant. The results varied in bond strength, ranging from 3.5 to 27.8 MPa, indicating the lack of a standard method for testing the bond strength. Since testing conditions can influence the bond strength, an attempt was made to simulate the oral environment with high precision and use specific test methods to increase the accuracy as much as possible. In 1975, in a meta-analysis, Reynolds[18] suggested that the minimum bond strength of orthodontic treatments in vitro was 6−8 MPa. In this study, the results indicated that all the three composites, including BF, OC and TXT, had bond strength beyond the minimum requirements listed; thus, they might be

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applicable in the clinical setting. In the present study, the bond strength of the three composites was higher than the minimum recommended (6−8 MPa) by Reynolds for clinical use, consistent with the results of studies by Minaei Basharik et al,[5] Arici et al,[6] D'Attilio et al[8] and Uysal et al,[19] who reported that the values were >20 MPa. Nevertheless, the differences in the results could be explained by multiple settings and factors in studies.

Uysal et al[19] reported bond strengths of 25.5 MPa for metal brackets bonded by TXT. Like the current study, the brackets with larger size (3M, 12 mm^2) were used, the excess composite was removed with an explorer, and thermocycling was not carried out in their study. In a study by Minaei Basharik et al,[5] the SBS for brackets with TXT was 25.26 MPa. The laboratory conditions, selection of tooth samples, the bracket size and experimental procedures were the same in their study and in our studies, but the specimen mounting method in acrylic resin was different in these studies.

Arici et al[6] applied metal brackets with 11.9-mm cross-section. In their study, the SBS in the control group (No-Mix Adhesive/Leone) without thermocycling was reported at 22.9 MPa; at 200 rpm thermocycling, it was reported at 21.6 MPa; and at 2000 thermocycling, it was reported at 18.8 MPa. Furthermore, D'Attilio et al[8] used human premolars. Their methods were the same as those of the present study; they applied SBS for TXT and metal bracket, reporting a value of 23.47 MPa. Uysal et al[19]used larger brackets (3M, 12 mm^2) and mounted the samples similar to the present study and the bracket SBS for TXT was at 25 MPa in their study. The SBS was 16.56 MPa in the study of Arash et al[20] (lower than that of the present study) although their methods and models were similar to those in the current study; this difference might be due to the use of smaller brackets (lower base area: Dentaurum, 9.93 mm^2) in their study.

Van Noort et al[21] and Unterbrink et al[22] reported that the ultimate bond strength depends on the bonding surface of the bracket and its development, which could justify the higher values reported in the present study. The brackets applied in the present study had a cross-sectional area of 11.8 mm^2 (American Orthodontic), while the brackets used in the reviewed studies had different surface areas.

Furthermore, in other studies, such as the one carried out by Ravadgar et al,[14] the composite extruding around the bracket was removed with a scaler and then polished by a diamond bur after light-curing. However, in the present study, the excess composite was removed only with an explorer to prevent possible damage to the bond, which could increase the bonded surface. Moreover, higher bond strength values in this study might have resulted from differences in administrating the laboratory procedures, such as lower thermal cycles, different teeth that were selected and variations in the amounts of force exerted by the laboratory tools.

Literature review shows that although OC and BF have been marketed for years, no complete study has been undertaken to assess their bond strength accurately. The only study conducted to assess the bond strength of these two composites was carried out by Scribante et al,[7] who reported the bond strength of OC at 13.78 MPa and TXT at 17.67 MPa. This study indicated a statistically significant difference between the two; however, the difference between the bond strengths evaluated in their study and the present study could be justified by variations in laboratory procedures and use of bovine teeth in their study.

Remaining resin, enamel damage and fracture location:

Stereomicroscopic images of enamel and SEM images of brackets were scored to evaluate the images quantitatively in the present study. The results of this evaluation indicated no significant differences in the amounts of enamel damage between the three experimental groups, while these results represented lower values by using a stereomicroscope compared to an SEM. However, the results of both images in our study indicated no significant differences in the severity of enamel damage between the three types of adhesives.

Therefore, it can be pointed out that the higher the residual adhesive remaining on the tooth surface, the higher the bond strength would be due to the stronger bond formed between the enamel and the adhesive material.[21] This can be attributed to either a direct connection between a higher TXT bond strength and the surface (higher SBS) compared to other groups or the amount of resin remaining on the enamel in this group. In the ARI under the stereomicroscope, both observers reported no color difference between the enamel and composite due to the similarity between the two, even at a magnification of ×20, and it was observed that this test had no necessary precision to detect the location of fractures. Thus, the results differed from those yielded by electron microscopy. Therefore, it can be claimed that this method is not efficient and convenient for the

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evaluation of the bond fracture location, and further studies are needed to evaluate the efficacy of these methods. However, for quantification in this study, we used EDX analysis and the remaining elements' weight percentages on bracket bases.

The results confirmed that the weight percentages of Fe, SI, P and Ca on the bracket surfaces were significantly different; however, Ca and P levels suggested enamel damage in all the samples, and the records demonstrated variations between different groups; it should be noted that this damage was not visible through direct observation.

Furthermore, EDX analysis revealed more enamel destruction in the OC group compared to the other groups. Although the OC group had the lowest bond strength among the three groups, this difference could not represent the potential relationship between the bond strength and enamel destruction. The ARI scores for remaining resin levels and Fe percentages obtained through EDX analysis revealed a potential relationship between the two. However, these did not conform to the ARI results; i.e., the ARI scores revealed most resin remaining on the tooth surface for the TXT, while EDX analysis demonstrated a greater Fe percentage as well. It seems that the fractures occurred in the bracket−adhesive interface or within the brackets.

However, the Si, Ca and P percentages in the OC group were higher compared to the other groups, suggesting fractures within the adhesive or between the adhesive and enamel. Nevertheless, it should be noted that ARI has various shortcomings that cannot be measured, but they affect the patient experience, such as adhesive thickness. Nonetheless, they are taken into account in the EDX analysis. Accordingly, the analysis through the use of two methods cannot be compared in different groups and conditions. By using the collective data and measuring the presence of phosphorus, calcium, silicon and iron in the samples, it was inferred that the failure of the bond occurred in all the three groups, and the exact location could not be determined. The failure could occur in bracket bonding, bracket and the resin bonding, within resin, between the tooth and resin and on the enamel surface of the teeth.

The results of the present study revealed the highest amounts of Fe in group 1, but the highest amounts of Si and Ca + P were recorded in group 3; additionally, the lowest amounts of Si and Fe were recorded in BF. These data demonstrated that the fractures in the TXT group occurred within the bracket or between the bracket and adhesive, while in the two other groups, it represented fractures at enamel−adhesive interface.

Moreover, it has been reported that the maximum bond strength should not exceed the enamel's cohesive strength (about 14 MPa)[23] to prevent the risk of damage to the enamel during the bracket debonding. With this in mind, it appears the damage to enamel was minimal in the present study, similar to the reviewed studies. The results showed that the bond strength was higher in the in vitro experiments compared to the in vivo experiments since intraoral conditions, such as humidity, temperature changes and other variables in the oral cavity, weakened the bond strength. Moreover, the force exerted by the machine is only a shearing force, while in clinical settings, it is a combination of torsional, tensile and shearing forces. On the other hand, the teeth are stored in water in vitro; therefore, they are more fragile. Hence, the fractures at the enamel−adhesive interface and enamel damage occur at a higher rate in vitro than in the clinical setting.[22] It seems that the risk of damage to enamel during debonding in clinical treatments is less than that in vitro. Thus, standardizing and achieving a precise criterion to evaluate the bond strength of the new adhesives requires more definitive studies.

Conclusion

In this study, the adhesive SBS in the BF and OC groups was suitable for orthodontic bracket bonding, indicating that these bonding agents and techniques can be a proper alternative for the conventional bonding method to facilitate the bonding process and decrease DWSLs. Based on the results of the present study and comparisons made with other studies, it appears that the enhancement in the bonding surface area via an increase in bracket base cross-section results in an increase in the bond strength.

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Authors' Contributions

The study was designed by Fatereh Samadi and Manouchehr Rahmati Kamel. Fatereh Samadi and Manouchehr Rahmati Kamel defined the conceptual content of the research. The study data were collected by Fatereh Samadi .Statistical analysis and interpretation of data were accomplished by Valiollah Arash and Soraya Khafri. The manuscript was prepared by Fatereh Samadi and Soraya Khafri and revised by Fatereh Samadi. Faezeh Abolghasemzadeh contributed to the design and implementation of the research. Study supervision was performed by Manouchehr Rahmati Kamel.

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