Research Article

Enamel demineralization around orthodontic brackets bonded with new bioactive composite (in-vitro study)

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Abstract: background: This study aimed to evaluate the effect of bioactive composite (ACTIVA) on enamel demineralisation when used as an orthodontic adhesive, compared to other adhesives. Materials and methods: Human upper premolars (n=64) were randomly divided into two equal groups; the first group stored the bonded teeth in deionised water for 30 days at 37°C, and the second group exposed the bonded teeth to acidic media. Each group was further subdivided into four subgroups, with eight premolars in each subgroup, based on the type of adhesive used to bond metal brackets: non-fluoride-releasing adhesive (Transbond XT), fluoride-releasing adhesive (Light Bond), light-cured, resin-reinforced glass ionomer (GC Fuji ortho LC), and bioactive restorative composite (ACTIVA BioACTIVE-RESTORATIVE). Enamel demineralisation was assessed at baseline and after 30 days with a laser fluorescence device (DIAGNOdent™ Pen). Results: There were significant differences in fluorescence variation values (ΔFV) among all four tested adhesive systems in both water and acid groups after 30 days. Light Bond adhesive showed the highest fluorescence variation value, while glass ionomer showed the lowest, indicating less enamel demineralisation around the bracket. ACTIVA had less enamel demineralisation in acidic media. However, there was no significant difference in enamel demineralisation between water storage and acidic media groups. Conclusion: ACTIVA (RMGIC + composite) exhibited less enamel demineralisation than Light Bond; however, Fuji ortho LC showed the lowest enamel demineralisation.

Keywords: Enamel demineralization, fluoride-releasing adhesive, Laser fluorescence, bioactive material, ACTIVA.

Introduction

One of the most common drawbacks of orthodontic treatment with the fixed appliance is the development of white spot lesions (WSL), which demineralise enamel around the brackets [1]. These lesions can develop during a short period of about four weeks [2]. Fixed orthodontic appliances have a high risk of enamel demineralisation due to plaque accumulation around the attachments and an inadequate self-cleaning mechanism [3].

There are no specific guidelines for prevention of WSL during orthodontic treatment. However, orthodontists usually provide essential oral hygiene instructions before orthodontic treatment begins. Therefore, the patient has the most significant responsibility for preventing WSL [4]. In addition, one of the accepted methods to prevent WSL is the application of remineralisation agents (fluoride varnish or tooth mousse) that can restore minerals to the subsurface enamel and reduce WSL [5]. Furthermore, research has shown that using Resin-modified glass ionomer cement (RMGIC) as an orthodontic adhesive can reduce WSL. However, RMGIC has a significantly higher rate of bond failures than a resin-based composite bonding system [6]. The adhesive system type also significantly impacts the microleakage that can lead to the development of WSL [7].
An upgraded RMGIC, known as ACTIVA BioACTIVE-RESTORATIVE (ACTIVA), showed comparable flexural strength and flexural fatigue with flowable composites and more flexural strength and flexural

fatigue compared to RMGIC [8]. ACTIVA is a bioactive restorative material that stimulates the formation of hydroxyapatite layers and naturally remineralises the tooth-restoration interface by releasing calcium, phosphorus, and fluoride ions. Unlike composite resins, ACTIVA does not contain bisphenol A or its derivatives and is, therefore, more biocompatible [9]. In addition, research has shown that ACTIVA provides favourable adhesion properties and releases significant amounts of fluoride ions. Its biocompatibility is more excellent than traditional composite resin adhesive systems [10].
Therefore, this study assessed the enamel demineralisation around the brackets, bonded with ACTIVA, compared to other orthodontic adhesives, using the DIAGNOdent pen.

Materials and Methods

this study design comprehensively compared four distinct bracket bonding materials. Transbond XT (3M Unitek, Monrovia, Calif) with Transbond XT primer, Light Bond (Reliance Orthodontic Products, Itasca, Illinois, USA) with fluoride-releasing sealant resin, powder and liquid Fuji Ortho LC (GC Corporation, Tokyo, Japan) and ACTIVA restorative material (Pulpdent corporation, Watertown, USA) with assure bond (Figure 1). Stainless steel orthodontic brackets (Discovery® Smart, Dentaurum, for upper premolars) with bonding surfaces measuring 10.56 mm2 and a slot size of 0.022 inches.
Sixty-four permanent upper premolars with an intact buccal surface (no cracks, cavities, restorations, fluorosis, or chemical treatments) were collected. They were stored in a 0.1% (weight/volume) thymol solution for a week and then kept in deionised water until bonding [11].
Sixty-four teeth randomly divided into two groups, the water media and the acidic media groups, with 32 teeth each. The 32 teeth in each group were further divided into four groups of 8 teeth each, depending on the type of adhesive system used (Figure 1).

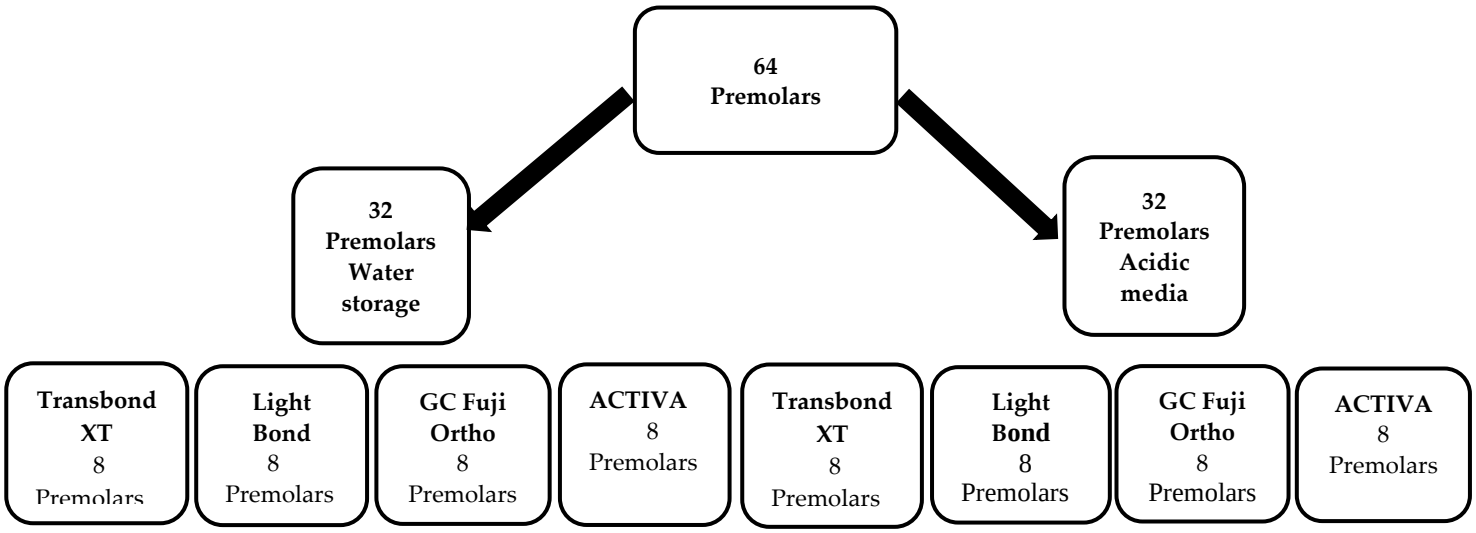

Figure 1: Sample distribution.

The buccal tooth surface polished for 10 seconds using non-fluoridated pumice. Then, the teeth were washed and dried for an additional 10 seconds [12]. We placed a piece of adhesive tape (7x7 mm) on the middle of the buccal tooth surfaces and painted the remaining tooth surfaces with nail varnish twice during three hours. After 24 hours, we removed the adhesive tape and cleaned any remaining adhesive with a cotton pellet soaked in alcohol. To ensure there is no remaining adhesive, inspect the tooth surface under a stereomicroscope (10 X) [13].

The buccal enamel surfaces of all 64 teeth were treated with 37% phosphoric acid for 30 seconds, followed by an air-water rinse. The primer was applied to the etched enamel surface and polymerised by an LED light curing unit. However, the teeth bonded with Fuji Ortho were not pretreated with a primer.

The bracket base was covered with adhesive paste or cement and positioned in the middle of the buccal tooth surface. To produce a consistent adhesive thickness, a weight of 200 gm was applied to each bracket for 10 seconds [12]. We removed the excess adhesive and then applied an LED light curing unit with a curing intensity of 1500 mw/cm² for 40 seconds (10 seconds on each side of the bracket) [14].

After bonding, the 64 teeth were stored in deionised water for 24 hours at 37°C. Afterward, we randomly divided the 64 teeth into two groups, one for water media and the other for acidic media, with 32 teeth in each group.

Water media group: 32 bonded teeth were kept in deionised water at 37°C inside sealed containers in the incubator for 30 days. Water refreshments was performed daily to prevent the cumulative effects [15,16].

Acidic media group: A 500 ml batch of the acidic solution (pH=2.5) was made by gradually adding 1.5 ml of HCl [1M] to distilled water. To simulate the wet oral environment, we immersed the 32 bonded teeth in the acidic solution for five minutes, three times per day, for 30 days. Two-hour breaks were employed between acidic exposures. The teeth were kept in deionised water at 37°C for the rest of the day. We routinely replaced each storage media daily, and the teeth were washed and air-dried before and after each session [16].

Laser fluorescence measurements

Enamel demineralisation around the bracket was assessed in both groups using the DIAGNOdentTM Pen 2190 (KaVo, Biberach, Germany) and a sapphire fissure probe. The DIAGNOdent penis is a laser fluorescence instrument that emits red light with a wavelength of 655 nm and gathers the fluorescence from the carious tissue. This fluorescence is quantified on a scale from 0 to 99, with larger values often found in more advanced carious lesions [17]. The DIAGNOdent pen is used for the early detection of caries and is superior to the visual assessment technique in terms of sensitivity and specificity [18].

The DIAGNOdentTM pen according calibrated to the manufacturer's instructions. Before inspecting each of the bonded teeth, the calibration was rechecked [19]. The initial measurements were taken at the time of bracket bonding to establish a baseline fluorescence value for each bonded tooth, known as FV1. Laser fluorescence measurements were collected on the buccal surfaces, three times each at 1 mm from the brackets' mesial, distal, gingival, and occlusal margins. The mean value was then calculated [20].

To collect the fluorescence from all directions, we used the DIAGNOdentTM Pen, positioning its tip. Each site was air-dried for 5 seconds while holding it perpendicular to the tooth surface [19].

The final DIAGNOdent pen readings (FV2) were taken in different storage media after 30 days for each bonded tooth. We used the difference between the initial fluorescence value (FV1) and the final fluorescence value (FV2) to calculate the fluorescence variation (ΔFV).

Statistical analysis

SPSS Version 29 used to analyze the obtained data. Data in all groups were tested for normality by using Shapiro-Wilk. We used the Kruskal-Wallis test to assess the mean differences in fluorescence variation (ΔFV) among the groups within water storage (data were not normally distributed). Simultaneously, we employed a one-way analysis of variance (ANOVA) to assess the mean differences in fluorescence variation (ΔFV) among the groups within acidic media (data were normally distributed). We considered $p < 0.05$ as statistically significant for all tests.

Results

To assess intra-examiner reliability, the same examiner repeated the laser fluorescence measurements for 12 bonded teeth after 10-day intervals. In addition, a second examiner repeated the laser fluorescence measurements for the same 12 bonded teeth to assess inter-examiner reliability. The intraclass correlation coefficient (ICC) was used to determine intra- and inter-examiner reliability and showed excellent agreement, as shown in Table 1.

Table 1: Intra-examiner and inter-examiner reliability of laser fluorescence measurements, assessed by ICC for 12 bonded teeth.

Reliability	N	ICC	95% CI
Inter-examiner	12	0.932	0.766-0.982
Intra-examiner	12	0.923	0.741-0.977

Shapiro-Wilks test used to determine the data distribution's normality and assign the appropriate statistical tests. The findings showed significant differences between water storage groups, leading to data that did not follow a normal distribution ($P<0.05$), whereas there were no significant differences between acidic media groups, resulting in data that were normally distributed ($P>0.05$); as shown in Table 2.

Table 2: Testing the normality of distribution by Shapiro-Wilk test for the laser fluorescence measurement in different adhesive groups.

Media	Groups	Shapiro-Wilk test		
		Statistic	df	p-value
Water	**Transbond XT**	0.868	8	0.143
	Light Bond	0.803	8	**0.031**
	GC Fuji Ortho	0.783	8	**0.019**
	ACTIVA	0.812	8	**0.038**
Acid	**Transbond XT**	0.868	8	0.143
	Light Bond	0.940	8	0.607
	GC Fuji Ortho	0.872	8	0.158
	ACTIVA	0.967	8	0.876

The mean differences in fluorescence variation (ΔFV) within both groups (water and acidic media) were compared. The results in Table 3 and Table 4 revealed highly significant differences in both groups.

Table 3: Kruskal Wallis Test (Pairwise test) comparison of the laser fluorescence test in water media groups.

Media	Kruskal Wallis Test			Groups		p-value
	X2	df	p-value			
Water	13.53	3	0.004	Transbond XT	Light Bond	0.337
					GC Fuji Ortho	0.022
					ACTIVA	0.423
				Light Bond	GC Fuji Ortho	0.007
					ACTIVA	0.873
				GC Fuji Ortho	ACTIVA	0.012

Table 4: The Post-hoc Games-Howell test compares the laser fluorescence test in acidic media groups.

Media	Post hoc test	Groups		p-value
Acidic	Games-Howell	Transbond XT	Light Bond	0.521
			GC Fuji Ortho	0.021
			ACTIVA	0.308
		Light Bond	GC Fuji Ortho	0.000
			ACTIVA	0.003
		GC Fuji Ortho	ACTIVA	0.101

Mann-Whitney U test used to compare the data and assess how the ageing media (water media and acidic media) would impact the demineralisation of enamel while considering the four tested materials. According to the findings, the enamel demineralisation did not differ significantly between the water storage and acidic media groups, as indicated in Table 5.

Table 5: Using the Mann-Whitney test, compare the effects of the ageing medium on the enamel demineralisation related to the four tested materials.

Groups	Media	Descriptive statistics			Comparison	
		N	Median	Mean Rank	MWU test	p-value
Transbond XT	Water	8	2.875	7.19	21.5	0.268
	Acid	8	4.250	9.81		
Light Bond	Water	8	6.500	8.31	30.5	0.874
	Acid	8	6.000	8.69		
GC Fuji Ortho	Water	8	0.540	8.94	28.5	0.711
	Acid	8	0.875	8.06		
ACTIVA	Water	8	3.125	9.88	21	0.247
	Acid	8	2.250	7.13		

Discussion

The most common side effect of fixed orthodontic treatment is white spot lesions (WSL), with prevalence rates ranging from 2% to 96% [(1)]. The new bioactive restorative material (ACTIVA) is proposed to have the advantages of fluoride release as in glass ionomer cements and the superior physical properties of resin-based composites. ACTIVA restorative composite exhibited significantly less enamel demineralisation adjacent to the dental restorative materials than resin-based composite [(21)].

The current study aimed to assess the ACTIVA composite's effectiveness in preventing demineralisation of enamel around metal brackets, compared to other composites, including Transbond XT, Light Bond and GC Fuji Ortho. We used water and acidic media for 30 days.

The acidic media protocol was used in this study to mimic the oral environment. It is based on the idea that a person wearing fixed orthodontic appliances consumes acidic liquids (pH 2.5) for five minutes, three times daily. The water storage of bonded teeth was observed for 30 days at 37°C to rule out any potential effects of prolonged water storage on the enamel demineralisation, which occurs in conjunction with the acidic attack [(16)]. To our knowledge, only a few studies have assessed the effect of long-term water storage on fluoride-releasing adhesives. Our results revealed statistically significant differences between the evaluated adhesive systems in both groups. The laser fluorescence test revealed that the Light Bond group

exhibited the highest mean of fluorescence variation (ΔFV), indicating more enamel demineralisation around the bracket. The GC Fuji Ortho group exhibited the lowest mean of ΔFV, which can be attributed to the longer-lasting release of Fluoride by the Fuji Ortho LC [22]. On the other hand, we found that enamel demineralisation adjacent to ACTIVA in water media was more than the Fuji Ortho group, which may be due to less fluoride release from ACTIVA composite compared to Fuji Ortho [23]. In acidic media, enamel demineralisation adjacent to ACTIVA was also more than Fuji Ortho, but the difference was not statistically significant.

Although the difference was not statistically significant, enamel demineralisation adjacent to ACTIVA composite was less than Transbond XT composite, as previously reported by Saunders et al. study [24]. However, in that study, they used a microscope to assess demineralisation and the teeth were immersed in an artificial caries solution for only three days. In addition, we observed less enamel demineralisation adjacent to the bracket bonded with Fuji Ortho compared to Transbond XT. This finding is consistent with previous studies and is explained by Fuji Ortho's ability to release Fluoride when the pH drops [25-28].

Moreover, we observed less enamel demineralisation adjacent to the ACTIVA composite than Light Bond, and the difference was significant. This can be explained by the fluoride release from ACTIVA [24]. To date, no previous study has compared ACTIVA with Light Bond.

It has been suggested that Light Bond released relatively little Fluoride over time in a steadily decreasing manner after exposing them to various ageing media [29]. In addition, that study found that Fuji Ortho LC has detectable fluoride penetration, which is beneficial in reducing enamel demineralisation, whereas this property cannot be recognised for Light Bond [29]. This may be related to the effect of the primer layer in Light Bond, which prevents Fluoride from penetrating the enamel surface [22]. These results were consistent with the results of the present study. However, these findings are inconsistent with the results of the Wilson and Donly study [30], which observed no significant differences between Fuji Ortho LC and Light Bond in reducing enamel demineralisation adjacent to brackets. This difference may be related to the shorter immersion period of bonded teeth in the demineralisation solution in their research (5 days). We found no significant difference in the enamel demineralisation adjacent to the bracket bonded by Transbond XT and Light Bond, and these findings were consistent with Pascho's et al. study [31].

Since this study is in vitro, it cannot accurately mimic the complex dynamic biological system of the oral environment, which significantly impacts both demineralisation and remineralisation, including factors such as the presence of plaque, saliva, and pH variations. Additionally, chemical agents, rather than bacteria and their acids, promoted demineralisation in this study. Furthermore, the number of teeth was 8 in each group, and the study period was only 30 days.

Conclusion

Demineralisation of enamel adjacent to the bracket bonded with ACTIVA composite was less than Transbond XT and Light Bond. However, the ACTIVA composite showed more demineralisation compared to Fuji Ortho LC.

Conflict of interest

The authors have no conflicts of interest to declare.

Author contributions

NA, LM; study conception and design. NA; data collection and Methodology. AH; statistical analysis. NA, LM interpretation of results. NT; original draft manuscript preparation, Writing -review & editing. All authors reviewed the results and approved the final version of the manuscript to be published.

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Ethical approval

The study was approved by the College of Dentistry/University of Baghdad's local ethics commission. project number, 596422, Ref. number:596

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نزع المعادن من المينا حول أقواس تقويم الأسنان المرتبطة بمركب نشط بيولوجيا جديد (دراسة في المختبر)

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المستخلص:

الملخص: واحدة من العيوب الأكثر شيوعا لعلاج تقويم الأسنان مع الجهاز الثابت هو تطور آفات البقع البيضاء (WSLs)، التي تطورت خلال فترة قصيرة من حوالي أربعة أسابيع. تهدف هذه الدراسة إلى تقييم تأثير المركب النشط بيولوجيا (ACTIVA)على نزع المعادن من المينا عند استخدامه كمادة لاصقة

لتقويم الأسنان، مقارنة بالمواد اللاصقة الأخرى. المواد والطرق: 64 ضواحك علوية بشرية قسمت عشوائيا إلى مجموعتين متساويتين. قامت المجموعة الأولى بتخزين الأسنان المستعبدة في ماء منزوع الأيونات لمدة 30 يوما عند 37 درجة مئوية، وعرضت المجموعة الثانية الأسنان المستعبدة لوسائط حمضية. قمنا بتقسيم كل مجموعة إلى أربع مجموعات فرعية، مع ثمانية ضواحك في كل مجموعة فرعية، بناء على نوع المادة اللاصقة المستخدمة لربط الأقواس المعدنية التقويمية: مادة لاصقة لا تطلق الفلورايد(Transbond XT) ، لاصقة تطلق الفلورايد(Light Bond) ، (GC Fuji ortho LC)، ومركب نشط بيولوجيا .(ACTIVA BioACTIVE-RESTORATIVE) تم تقييم نزع المعادن من المينا في بداية عملية اللصق وبعد 30 يوما باستخدام جهاز الليزر (DIAGNOdent™ Pen). النتائج: كانت هناك اختلافات ذات دلالة إحصائية كبيرة في قيم التباين الفلوري (ΔFV) بين جميع أنظمة اللصق الأربعة التي تم اختبارها في كل من مجموعات الماء والحمض بعد 30 يوما. أظهر لاصق Light Bond أعلى قيمة تباين، بينما أظهر Fuji Ortho LC أدنى قيمة، مما يشير إلى انخفاض نزع المعادن من المينا. كان لدى ACTIVA نسبة أقل من إزالة المعادن من المينا في الوسائط الحمضية. ومع ذلك، لم يكن هناك فرق كبير في نزع المعادن من المينا بين مجموعات الماء والوسائط الحمضية.الخلاصة: أظهر مركب ACTIVA BioACTIVE-RESTORATIVE نزع معادن المينا أقل من Light Bondومع ذلك، أظهر Fuji Ortho LC أدنى نزع معادن للمينا.