

In vitro evaluation of the caries-preventive effect of bioactive resin composites for orthodontic clear aligner attachments

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Aim: The increased risk of caries during orthodontic treatment is a significant prevention challenge. This in vitro study aimed to evaluate the caries-preventive potential of bioactive composites used in clear aligner attachments.

Methods: A bioactive composite group consisted of Activa Bioactive Restorative (Pulpdent, USA), while a traditional flowable composite group included G-Aenial Universal Flo (GC, Japan). A total of 24 sound extracted human teeth were cleaned using pumice, and all surfaces except for a 3 × 3 mm area on the buccal crown were coated with acid resistant varnish. Standard acid etching and bonding protocols were applied to the exposed area, after which clear aligner attachments were created using a mould. The specimens underwent pH cycling, consisting of immersion in a demineralising solution for 48 hours followed by immersion in a remineralising solution for 24 hours, repeated three times. Sections were obtained from the attachment areas, and demineralisation depths were measured using a digital microscope. Statistical analysis was performed using an independent samples t-test.

Results: The mean lesion depth for the bioactive composite (Activa Bioactive Restorative) was 120 ± 29 µm, while the traditional flowable composite (G-Aenial Universal Flo) exhibited a mean lesion depth of 150 ± 41 µm. According to the t-test results, significantly less demineralisation depth was observed in the bioactive composite group ($p < 0.05$).

Conclusion: Under in vitro conditions, bioactive composites exhibited lower demineralisation depths compared to traditional flowable composites. The findings suggest that bioactive composites may offer caries-preventive benefits when used in clear aligner attachments, although further studies are needed to confirm the effects.

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Introduction

Clear aligners have been considered less effective than traditional fixed appliances in facilitating certain types of tooth movements, such as buccolingual inclination (torque), interocclusal sagittal adjustments, overjet correction, closure of extraction spaces, the establishment of occlusal contacts, and dental arch expansion.¹ Despite these limitations, clear aligner therapy remains highly popular in

contemporary orthodontics, primarily driven by increasing patient demand for alternative, “invisible” treatment options.² Clear aligners are generally preferred for mild to moderate malocclusions, relapse cases, and patients reluctant to use fixed appliances.³ This approach relies on the sequential use of custom-made clear thermoplastic aligners to gradually move teeth into desired positions. Tooth movements are facilitated by two primary mechanisms being a shape-

moulding effect and the use of auxiliary features in the form of attachments and power ridges which enhance tooth movement predictability.⁴

Caries development is a significant unwelcome side effect of orthodontic treatment. Although less common than that associated with fixed brackets and wires, clear aligner therapy can also accelerate caries formation. The incidence of white spot lesions, considered the initial stage of caries, has been reported to range from 1.2% to 35% in previous studies.^{5–7} Bioactivity is an emerging concept in restorative dentistry and refers to the ability of certain materials to elicit specific biological responses and form bonds with dental tissues.⁸ Technological advancements have led to the development of bioactive resins which enable the use of bioactive materials in restorative applications. Several studies have demonstrated that restorations made with bioactive resin composites can promote the remineralisation of demineralised enamel and help prevent caries progression.^{9–11}

Based on current evidence, it is conceivable that using bioactive resin composites for clear aligner attachments could mitigate the increased risk of caries development during orthodontic treatment. Therefore, the null hypothesis of the present study is that bioactive composites used for clear aligner attachments offer no additional protection against caries compared to conventional flowable composites.

Methods

The study was approved by the Clinical Research Ethics Committee of Karamanoğlu Mehmetbey University Faculty of Medicine (Decision No: 05-16). Extracted adult human teeth were stored in a solution containing 10% formalin and 0.1% thymol for preservation. Teeth exhibiting caries, fluorosis, abrasion, or any other deformities on the buccal surface were excluded from the study. A total of 24 teeth, comprising eight molars, eight premolars, and eight incisors, were selected for analysis and randomly assigned to two groups identified as a bioactive composite group and a conventional flowable composite group. Each group included an equal distribution of tooth types ($n = 12$).

Prior to the attachment bonding procedure, the crowns of the teeth were meticulously cleaned using pumice and water until no visible debris remained. The buccal surfaces were then etched with 37%

phosphoric acid gel for 30 seconds, followed by thorough rinsing with water and complete drying using an air syringe. A universal adhesive, G-Premio Bond (GC International AG, Luzerne, Switzerland), was applied in accordance with the manufacturer's instructions and polymerised using a LED curing unit (Valo Cordless, Ultradent, 1000 mW/cm²) for 20 seconds. Activa Bioactive Restorative (Pulpdent) was applied to the bioactive composite group, while G-Aenial Universal Flo (GC International AG, Luzerne, Switzerland) was used for the conventional flowable composite group. Detailed information about the materials examined in the present study are found in Table I. Both materials were placed into moulds prepared from clear aligner sheets to achieve a standardised thickness and diameter of 2 mm and were then polymerised for 20 seconds. Any excess composite material around the attachment site was carefully removed using a scalpel. To standardise the exposure area, the teeth were coated with an acid-resistant varnish, leaving a 3 x 3 mm area on the buccal crown surface and the attachments exposed.

The demineralisation solution was prepared by dissolving 2.2 mM calcium chloride (CaCl₂), 2.2 mM sodium phosphate (NaH₂PO₄), and 0.05 M acetic acid (CH₃COOH) in distilled water. The pH of the solution was adjusted to 4.4 using an appropriate amount of potassium hydroxide (KOH), in accordance with protocols commonly preferred by previous research.^{15–17}

An artificial saliva solution with a pH of 6.8 was prepared following the formulation described by Gal et al.¹⁸ to apply and serve during the remineralisation phase. The solution contained 125.6 mg/L sodium chloride (NaCl), 963.9 mg/L potassium chloride (KCl), 227.8 mg/L calcium chloride hydrate (CaCl₂·H₂O), 178 mg/L ammonium chloride (NH₄Cl), 189.2 mg/L potassium thiocyanate (KSCN), 336.5 mg/L sodium sulphate (Na₂SO₄), 200 mg/L urea (CH₄N₂O), 630.8 mg/L sodium bicarbonate (NaHCO₃), and 654.5 mg/L potassium dihydrogen phosphate (KH₂PO₄), dissolved in distilled water.

The teeth from each group were initially placed in sealed containers filled with 120 mL of the demineralisation solution (10 mL per tooth) for 48 hours. Subsequently, they were transferred to separate containers with the same volume of artificial saliva for 24 hours. The demineralisation-remineralisation

Table 1. Information about the adhesive and composites used in the study

Type	Material and Manufacturer	Composition
Bioactive composite	Activa Bioactive Restorative <i>Pulpdent, Watertown, MA, USA</i>	Blend of diurethane and other methacrylates with modified polyacrylic acid (44.6%), amorphous silica (6.7%), and sodium fluoride (0.75%), reactive glass particles, shock absorbing ionic resin component containing acidic monomers with antimicrobial properties. Filler load: 56% by weight ⁽¹²⁾
Conventional flowable composite	G Aenial Universal Flo <i>GC, Tokyo, Japan</i>	Urethane dimethacrylate (UDMA), bisphenol A ethoxymethacrylate (bis-MEPP), triethyleneglycol dimethacrylate (TEGDMA), silicon dioxide, strontium glass, pigment, photoinitiator Filler load: 69% by weight ⁽¹³⁾
Universal adhesive	G-Premio Bond <i>GC, Tokyo, Japan</i>	10-MDP, 4-META, 10-methacryloyloxydecyl dihydrogen thiophosphate, methacrylate acid ester, distilled water, acetone, photo-initiators, silica fine powder ⁽¹⁴⁾

cycle was repeated three times to simulate the oral pH cycle. The remineralisation period was intentionally kept shorter to promote the formation of artificial carious lesions. During each solution change, the teeth were rinsed thoroughly with distilled water and upon completion of the cycles, the teeth were stored in containers filled with distilled water until the sectioning procedure.

The teeth were mounted on a slow speed diamond cutter device, and horizontal sections of 150 µm thick were obtained from the attachment area. Images of the sections were acquired at 4x magnification using a digital microscope (Optikam B9, Optika, Italy). Measurements were performed using ImageJ (NIH, USA) software. Although demineralisation depths were relatively uniform in most samples, some specimens showed shallower lesions adjacent to the attachment, with increasing depth noted at greater distances. To minimise potential marginal influences, all measurements were standardised at a distance of 500 µm from the attachment. This distance was chosen because microscopic leakage between the mould and the tooth during attachment fabrication, along with localised ion release from the composite material, may directly affect lesion depth at the attachment margins.

Preliminary statistical analyses, including Levene's test for homogeneity of variances and the Shapiro-

Wilk test for normality, were conducted to determine the suitability of the parametric tests. The data demonstrated normal distribution and homogeneity of variances across groups ($p > 0.05$) and so differences between the composite groups were evaluated using an independent samples t-test. A p-value of less than 0.05 was considered statistically significant.

Results

At the end of the pH cycling process, the visual examination of the tooth sections under the digital microscope revealed areas of opacity resembling white spot lesions on the outer enamel surface between the composite attachment and the acid-resistant varnish. Although the demineralisation depths were relatively uniform in most samples, some specimens exhibited shallower lesions near the attachment area, while increasing depth was observed as the distance from the attachment increased. Representative images from both groups are presented in Figure 1.

In the conventional flowable composite group (G-Aenial Universal Flo), the mean depth of the artificially induced carious lesions was 150.46 ± 40.89 µm ($n = 12$). In contrast, the bioactive composite group (Activa Bioactive Restorative) demonstrated a significantly lower mean lesion depth of 119.60 ± 29.39 µm ($n = 12$). The distribution of

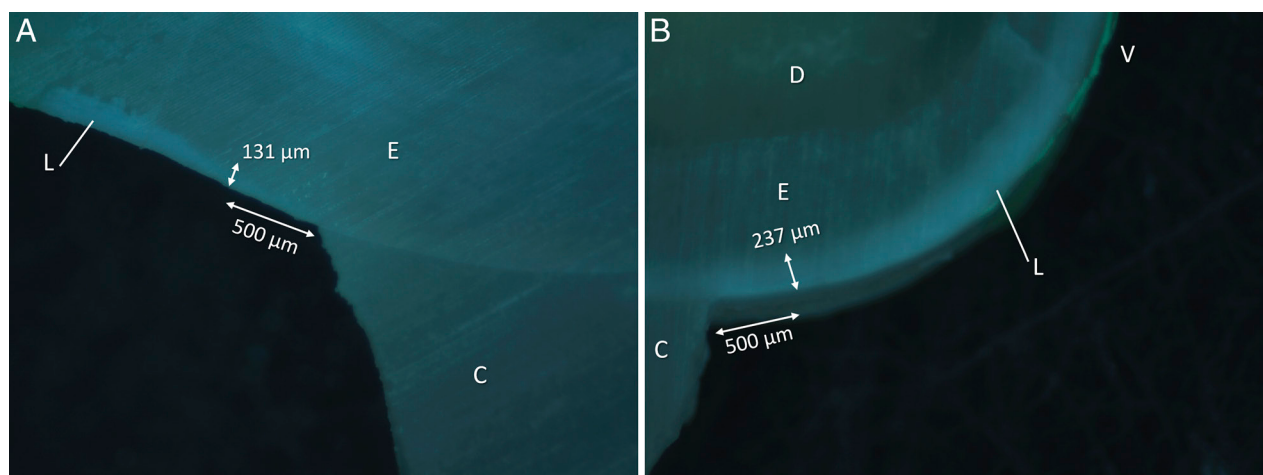


Figure 1. Representative images obtained from sections of the attachment area using a digital microscope. (A) Activa Bioactive Restorative, (B) G-Aenial Universal Flo. In the images, C represents the composite attachment, D represents the dentine, E represents the enamel, L represents the artificial lesion, and V represents the acid-resistant varnish.

lesion depths for both groups is illustrated in the box plot shown in Figure 2.

An independent samples t-test revealed a statistically significant difference in lesion depth between the two groups ($p < 0.05$), indicating that the bioactive composite resulted in less demineralisation compared to the conventional flowable composite. Detailed descriptive statistics and comparative analysis are provided in Table II.

Discussion

The present study investigated whether caries development could be reduced by using newly developed bioactive composites instead of conventional flowable composites for bonding attachments for clear aligner orthodontic therapy. The null hypothesis, stating that bioactive composites would not provide caries-preventive benefits compared to conventional flowable composites, was rejected. The results demonstrated that the depths of artificially induced carious lesions were significantly less in teeth with applied attachments made from bioactive composites compared to those with attachments made from conventional flowable composites after undergoing demineralisation-remineralisation cycles.

There is a continuous cycle of demineralisation in the oral environment by which inorganic components are dissolved from the dental hard tissues and subsequently redeposited during a

remineralisation process. When this balance shifts toward demineralisation, notably during orthodontic treatment, carious lesion formation may occur. During the initial stages of demineralisation, enamel maintains its external morphology. A progressive loss of inorganic content leads to increased lesion depth and eventually, the surface may fracture under mechanical stress. Once this point is reached, even

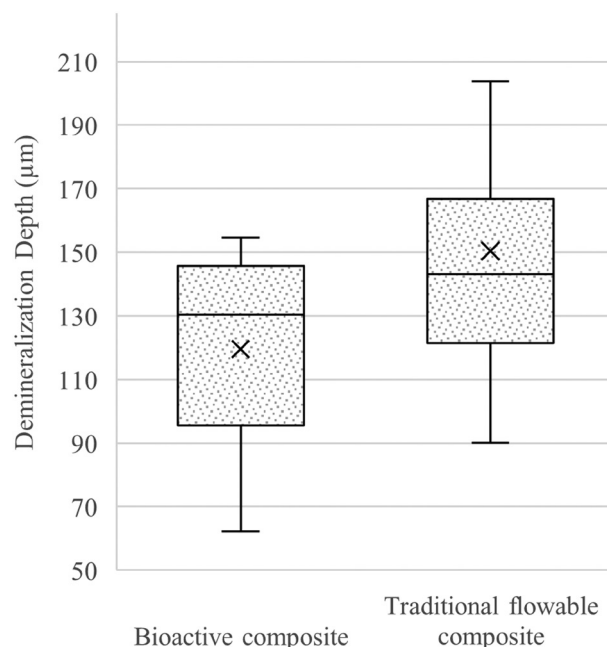


Figure 2. Box and whisker plot showing the demineralisation depth of the artificial lesions in both groups.

Table II. Independent sample t test results

Material	n	Mean	Std. Dev.	t	df	p
Conventional flowable composite	12	150,46	40,89	2,123	22	0,045
Bioactive composite	12	119,60	29,39			

It has been determined that the demineralization depth in enamel after pH cycles differs between clear aligner attachments made with bioactive composite and conventional flowable composite. The demineralization depth is lower in bioactive composites.

if the balance shifts back toward remineralisation, the carious process cannot be easily halted, and restorative intervention is typically required.¹⁹ Therefore, interventions that can moderate caries risk during orthodontic treatment are of critical importance.

In contemporary orthodontic practice, clear aligner attachments may be fabricated using a variety of composite types, including packable, flowable, bulk-fill, or specialised attachment resin materials.²⁰ The bioactive composite used in the present study, Activa Bioactive Restorative, is available in a flowable form. Therefore, a conventional flowable composite, G-Aenial Universal Flo, was selected for the control group. Notably, a prior clinical study demonstrated that flowable composites significantly reduce attachment preparation time compared to packable composites, without compromising the one-year survival rate of the attachments.²¹ Activa Bioactive Restorative is frequently preferred in bioactivity research due to its unique properties. It has been noted that these materials do not contain Bis-GMA or its derivatives, which have traditionally been considered the main component of dental composites. Their ability to release calcium, phosphate, and fluoride ions, along with initial claims by the manufacturer that they could be used without the application of an adhesive, led some researchers to describe them as resin-modified glass ionomer cements. However, subsequent studies have indicated that restorations performed without an adhesive exhibit a high failure rate, leading to current recommendations that they be used in conjunction with an adhesive system.^{22,23} Additionally, the composites have demonstrated antibacterial effects against *Streptococcus mutans*, which is considered a primary factor in the development of dental caries.²⁴

In the present study, carious lesions were artificially induced, and the experimental environment did not contain *Streptococcus mutans*. However, the fluoride

release from the bioactive composite, attributed to its sodium fluoride content, may have contributed to the reduced lesion depth. Fluoride, in addition to inhibiting the biological activity of *S. mutans*, can inhibit demineralisation, accelerate remineralisation, and enhance the resistance of remineralised enamel to subsequent acid challenges through the formation of fluorapatite.²⁵ Additionally, bioactive composites release calcium and phosphate ions, which can promote the formation of an hydroxyapatite-like mineral phase.²⁶ These synergistic effects likely explain the superior performance of bioactive composites observed in the present study.

Jaiswal et al. placed Class V restorations on primary teeth using the bioactive composite Activa Bioactive Restorative, the glass ionomer GC Gold Label (GC International AG, Luzerne, Switzerland) and the conventional composite Z350XT (3M ESPE, St Paul, MN, USA), subjecting them to an acid cycle for 7 days. A demineralisation depth observed at the margins of the restorations was lower in the Activa Bioactive Restorative group.²⁷ In an additional in vitro study, Ibrahim et al. evaluated dentine microhardness after a 7-day pH cycling protocol of restorations placed using the bioactive composites Beautiful Kids SA (Shofu, Japan) and Predicta Bioactive Bulk-Fill (Parkell, NY, USA), as well as the conventional flowable composite G-Aenial Universal Flo, which was also used in the present study. It was noted that restorations made with bioactive composites exhibited less demineralisation compared to those placed with the conventional flowable composite.²⁸ While the present findings are consistent with previous in vitro studies showing reduced demineralisation following the use of bioactive composites, a meta-analysis of clinical studies conducted by Carvalho et al. reported that bioactive composites do not provide additional benefits in preventing secondary caries.²⁹ This discrepancy may be attributed to the complex dynamics of the oral environment, including

factors related to salivary flow, biofilm formation, and patient-specific variables, which are difficult to replicate in vitro.

The potential caries-preventive properties of bioactive composites have also attracted the attention of orthodontists, who have investigated caries development around brackets when bonded using Activa Bioactive Restorative instead of the standard Transbond XT. Ali et al. subjected dental samples to pH cycling for 30 days and evaluated subsequent demineralisation using laser fluorescence. No statistically significant difference was observed between the groups.³⁰ However, using the same composites and a shorter 3-day pH cycling protocol, Saunders et al. reported significantly lower demineralisation around brackets in the Activa Bioactive Restorative group compared to the Transbond XT group.³¹

In the present study, a lower demineralisation depth was observed in the bioactive composite group used for clear aligner attachments. Although most samples showed a uniform demineralisation depth, a shallower demineralisation region was seen near the attachment in some samples, particularly in those with bioactive composites (Figure 1). This may be due to the higher concentrations of calcium, phosphate and fluoride ions released from the bioactive composites in regions close to the attachment. In the present study, the samples were statically kept in the solution, which may have affected ion distribution and be considered a limitation of the study. Using a bench shaker during the incubation period could enhance homogenisation, thereby better simulating the dynamic conditions of the oral environment. Additionally, the results of the present study should be interpreted with caution, due to other inherent limitations of in vitro conditions. Future in vitro studies may incorporate additional methods such as micro-computed tomography or white spot lesion surface area analysis or, alternatively, focus on long-term clinical trials to validate the caries-preventive potential of bioactive composites under real-life orthodontic conditions.

Conclusion

Bioactive composites exhibited lower surrounding demineralisation depths compared to conventional flowable composites. Considering the findings and potential advantages identified in the present

study, bioactive composites may offer caries preventive benefits and be a promising alternative to conventional bonding materials for bonding clear aligner attachments.

Conflict of interest

The authors declare that there is no conflict of interest.

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