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A prospective randomized clinical trial and an in vitro evaluation of the microtensile bond strength of a chlorhexidine-containing dentin bonding agent and a bulk fill composite material in primary teeth

Hülya Altıntop¹, Hasibe Elif Kuru² , Fahinur Ertuğrul³, Murat Türkün⁴ and Ebru Küçükylmaz^{1*}

Abstract

Objectives This study aimed to assess the microtensile bond strength of a chlorhexidine-containing dentin bonding agent compared with a standard adhesive, and to investigate its clinical success in Class II cavities in primary teeth, with a 12-month follow-up.

Materials and methods The study consisted of two parts: a prospective, single-blind, split-mouth randomized controlled clinical trial and an in vitro laboratory evaluation. Ethical approval were obtained for the both parts of the study. Forty pediatric patients (aged 5–9 years) with primary molars requiring Class II restorations were included in the clinical trial, where bulk-fill restorations bonded with a chlorhexidine-containing adhesive and a standard adhesive were evaluated based on FDI criteria at 3-month intervals for 12 months. The in vitro study examined the microtensile bond strength (μ TBS) of immediate and thermally aged specimens prepared with bulk-fill composite materials and adhesives with or without chlorhexidine. The data were analyzed using Mann Whitney U test for in-vivo, ANOVA test followed by Tukey's post hoc and Chi-square test for in-vitro study ($p=0.05$).

Results Clinically, both adhesives showed similar success rates (%100) across all FDI evaluation parameters after 12 months ($p > 0.05$). The chlorhexidine-containing adhesive demonstrated significantly higher microtensile bond strength than the standard adhesive in both immediate and aged samples ($p < 0.05$).

Conclusions The chlorhexidine-containing adhesive showed promising clinical success and improved bond strength compared to the standard adhesive. Longer follow-ups are needed to confirm its long-term durability. Incorporating chlorhexidine simplifies restorative procedures without compromising performance.

Clinical Relevance Chlorhexidine-containing adhesives may improve bond durability and procedural efficiency in pediatric dentistry, offering a practical and effective alternative for restoring primary molars.

*Correspondence:

Ebru Küçükylmaz
ebrukucukylmaz@hotmail.com; ebru.kucukylmaz@ikcu.edu.tr

Full list of author information is available at the end of the article



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Trial registration Invivo part of the study was registered in a public trial registry, www.clinicaltrials.gov (#NCT06257108). Registration Date 2nd. May 2024 (Retrospectively registered).

Keywords Chlorhexidine, Dentin bonding, Microtensile bond strength, Primary molars, Class II restoration, Clinical study

Introduction

In pediatric dentistry, the occurrence of carious lesions in primary teeth is a common feature in daily clinical practice [1]. The restoration of carious primary teeth involves a range of materials. Especially after the ban on dental amalgam in Europe in 2024, its production and export have been completely discontinued as of January 1, 2025, following the decision of the European Commission [2]. This has further emphasized the need for alternative restorative materials. Among these alternatives, the use of composite resins is highlighted by the AAPD due to the strong level of evidence available in the literature [3] and it is also noteworthy that they are the most commonly preferred material group for Class II restorations [4]. Despite the satisfactory properties exhibited by this material group, many failures are documented, primarily due to secondary caries [5–10]. A ten-year retrospective study on the survival of direct class II restorations revealed a shorter lifespan for restorations placed in children, particularly those at a higher risk of caries, emphasizing the critical need for providing the marginal integrity and effective cavity seal [7].

Residual bacteria and bacterial leakage from dentin-restoration interface are the primary causes of secondary caries. Other factors that can weaken the bond between the tooth and composite include polymerization shrinkage of composite resins, temperature changes in the oral cavity, chewing forces, and chemical attacks by acids and enzymes [11]. These factors can lead to marginal discoloration, microleakage, secondary caries, or pulpal inflammation [12].

Resin bonding agents play a dual role in attaching the composite to the tooth structure while providing a protective layer. However, the presence of bacteria on cavity surfaces poses a threat to the integrity of bonding agents, potentially compromising the bonding interface. The resin composite surface is susceptible to biofilm growth, directly impacting the longevity of tooth restoration. Lack of antibacterial activity of the resin materials makes it more susceptible to plaque and biofilm formation [13]. Hence, combining restorative materials with antibacterial properties is critical to obtain a resistant and durable composite-tooth interface [14].

Currently, different antibacterial agents have been incorporated into dental adhesives; such as quaternary ammonium methacrylate MDPB (12 methacryloyloxy-dodecylpyridinium bromide) [15], nisin peptide [16], dimethylaminododecyl methacrylate (DMADDM)

[17], glutaraldehyde [18], silver nanoparticles [19] and chlorhexidine [20]. These integrations hold promise for offering an innovative approach to addressing this persistent clinical challenge [21].

Chlorhexidine (CHX) is a cationic-bisguanide commonly used antibacterial material in dentistry. It can prevent bacterial growth and biofilm formation, induce bacterial cell death, and inhibit matrix metalloproteinases (MMP) enzymes [22]. MMPs are responsible for their proteolytic effect, degrading the collagen structure in the interface between dentin and restoration. By inhibiting them, CHX helps preserve the integrity of the dentin-restoration interface, leading to better preservation of the hybrid layer [20]. CHX is mostly carried out as a pre-treatment agent is applied on the etched dentin surface by rubbing, to maintain contact with the dentin surface. Also, there are some innovations in the dental market where CHX is added to the bonding system (primer and bonding agent) to reduce the number of steps and time of the operation. In a systematic review and meta-analysis, dentin bonding agents with CHX in %0.2 and above concentrations were found effective in terms of bond strength [23], however, there is a knowledge gap about micro-tensile bond strength of chlorhexidine-containing bond agents and well-designed clinical studies are needed to support the evidence.

In pediatric patients, restorative treatments are a challenge. Age, behavior management, and isolation problems are the main factors for success. Patient cooperation, relatively short chair time, and less technique-sensitive materials increase the chances of success [6]. Composite-based materials produced for bulk use have a light transmittance of up to 4 mm, allowing for one thick layer application without the need for layering. This benefit reduces contamination risk and shortens working time [24]. Additionally, bulk-fill composites exhibit less polymerization shrinkage and stress compared to conventional resin composites [25]. There is a lack of clinical studies evaluating the success of bulk-filling composite materials in primary teeth for pedodontic purposes [26]. The literature does not provide enough information on this material group. Therefore, this two-step study aims to assess the microtensile bond strength of a chlorhexidine-containing dentin bonding agent compared with standard adhesive and investigate its clinical success in Class II cavities, with a 12-month follow-up. Our null hypotheses are;

- i) There is no difference between the clinical evaluation results of deciduous teeth restored with dentin bonding agents containing and not containing chlorhexidine.
- ii) There is no difference between the microtensile bond strength values of teeth restored with dentin bonding agents containing and not containing chlorhexidine.

Materials and methods

The study consisted of two parts: (i) a prospective, single-center, single-blinded randomized split-mouth clinical trial and (ii) an in-vitro assessment of microtensile bond strength of bulk-fill composite using adhesives with or without chlorhexidine.

The study protocol was approved by Izmir Katip Celebi University Faculty of Dentistry Ethical Committee on Human Research for both the in-vivo (76/2015) and in-vitro (42–44/2015) parts of the study. In-vivo part of the study was registered as a randomized, controlled, double-blind, and split-mouth trial in a public trial registry, www.clinicaltrials.gov (#NCT06257108). Written informed consent was obtained from all patients prior to tooth extraction, explicitly allowing the use of their extracted teeth for in-vitro research purposes.

In-vivo study

This study aims to compare the clinical effectiveness of CHX-containing bonding material with non-antibacterial bonding agents in Class II cavities of primary molars. A flowchart of the clinical study is shown in Fig. 1. The manuscript was written following the CONSORT (Consolidated Standards of Reporting Trials) guidelines. An informed consent including the details of the possible risks, discomforts, and benefits of the procedure was obtained from the parents of each patient.

Sample size and selection

The required sample size for this study was a minimum of 80 teeth (40 teeth for each group) to detect a significant difference, considering a Type I error of 0.05, a power of 98%, and an effect size (Cohen's h) of 0.476 [27]. The study was conducted on 40 patients aged 5–9 years (15 girls, 25 boys) who attended the Pediatric Dentistry Department. None of the patients included in the study was medically compromised.

Eligibility criteria

Preclinical oral examinations were completed before initiating the clinical stages of the study. The following criteria were considered in the selection of patients: (1) presence of at least one interproximal carious lesion not exceeding half of the dentin structure (D1 level-ICDAS 3 and 4) [28] both in the right and left sides of the mouth, (2) absence of bruxism or parafunctional habits (3)

acceptable cooperation level (scoring 3 or 4 on the Frankl scale), (4) patient and parent's willingness to attend follow-up appointments throughout the study.

Tooth selection

Teeth meeting the following criteria were included in the study: (1) the presence of pulp vitality with no clinical and/or radiological signs requiring endodontic treatment, (2) no previous treatment on the selected teeth, (3) radiographically, caries should not extend to one-third of the pulp, indicating only the need for a Class II restoration, (4) the absence of pathological root resorption, (5) the presence of adjacent teeth mesial and distal to the selected tooth and an opposing tooth in the oral cavity.

Randomization and blinding

Different restorative materials were applied to groups using a split-mouth design. 80 primary molars were randomly divided into two treatment groups with 40 teeth each: (I) standard adhesive applied group (control group), (II) CHX-added adhesive applied group (experimental group). Simple randomization was employed for selecting the restoration groups for the teeth. The choice of restorative material for each tooth was determined by a coin flip. Similarly, a coin flip decided which restoration material would be applied to which half of the jaw. The main researcher (unblinded to the study) flipped the coin immediately before the clinical procedure. All the clinical procedures were performed by one single unblinded, experienced, and trained with more than 4 years of experience in pediatric dentistry (H.A). Clinical success was assessed by two observers (M.M. and E.K.) who were blinded to the restoration procedure.

Clinical procedure

Periapical radiographs were taken during the examination session to confirm the eligibility of the selected teeth. Intraoral photographs of the teeth and restorations were captured using an intraoral camera (CS 1200, Carestream Health, Toronto, Canada) before cavity preparation and after the restoration completion.

The vitality of the teeth was tested by a cold thermal test (Chloroethyl; Wehr, Baden, Germany) and an electrical pulp test (Digitest; Parkell, Edgewood, NY, USA). The tooth was defined as vital if one of the methods provoked a response within normal limits for the tooth compared with neighboring teeth. Following topical anesthesia application using Vemcaine (Vem, Ankara, Turkey), the procedures commenced with local anesthesia using Fullcaine ampules (Onfarma, Samsun, Turkey). After isolating the relevant tooth with a rubber dam, carious lesions on the teeth were removed using diamond burs and No. 5 tungsten carbide burs. Rubber dam isolation was utilized during cavity preparation and restoration. After caries

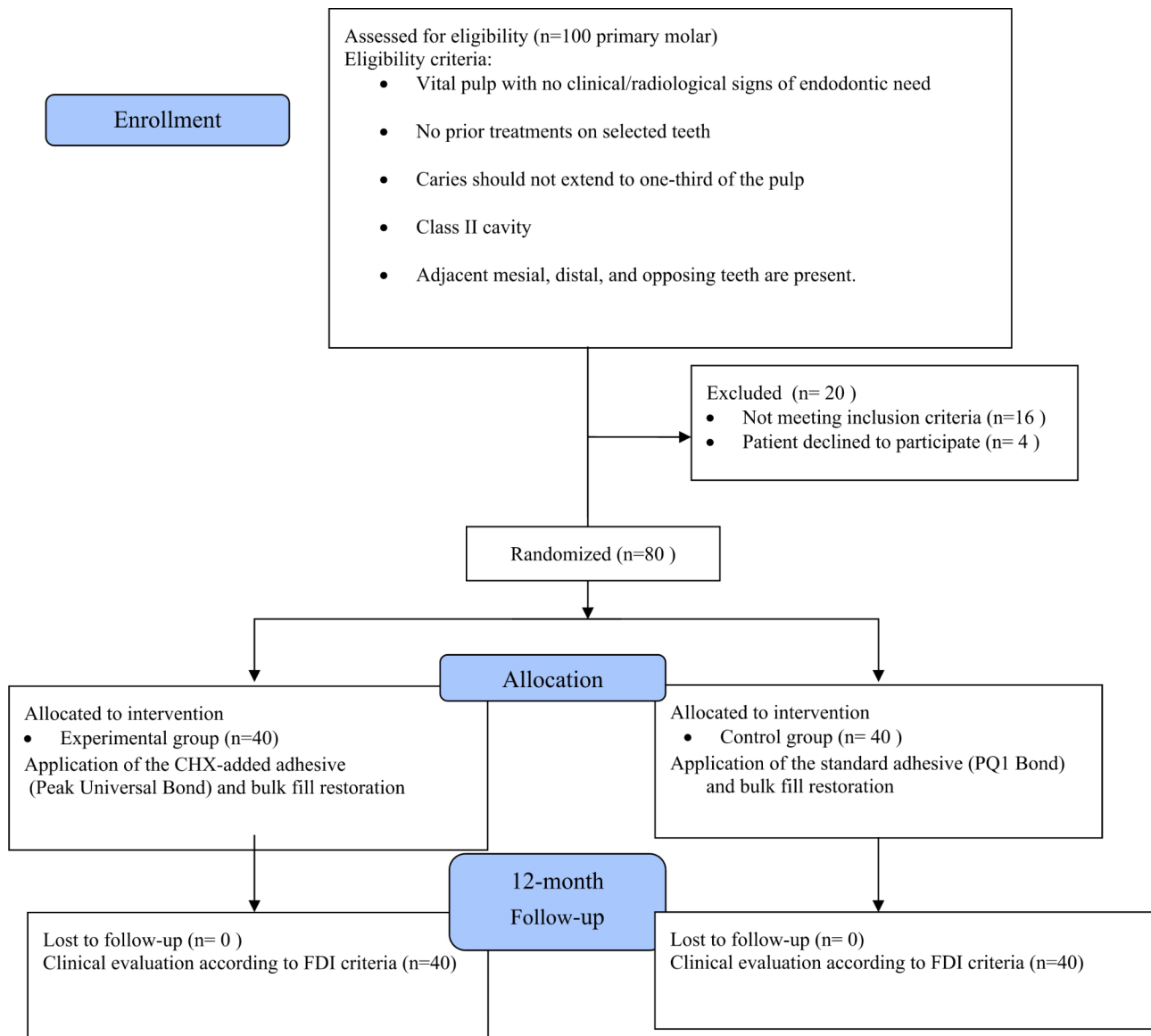


Fig. 1 Consort flow diagram of the clinical study

removal, the restoration process began, establishing suitable contact using the automatrix system (Kerr Corporation, Orange, USA) and an interdental wedge (Kerr Corporation, Orange, USA).

Bonding procedures were performed according to the manufacturer's instructions. For both groups, 37% orthophosphoric acid (Ultra-Etch, Ultradent Products, South Jordan, USA) was applied prior to adhesive application to optimize resin-enamel bonding. PQ1 Bond, an etch-and-rinse adhesive, was applied according to the manufacturer's instructions [29]. Peak Universal Bond, a universal adhesive, was also used in the etch-and-rinse mode following phosphoric acid pre-etching [30]. For the experimental group, the prepared cavity was wetted with Peak Universal Bond, which contains 0.2% chlorhexidine

(Ultradent Products, South Jordan, USA). For the control group, PQ1 Bond, which does not contain any CHX compounds (Ultradent, South Jordan, USA), was applied using an applicator for 10 s, followed by applying slight air pressure for 10 s to disperse the solvent. After polymerization for 10 s using an LED light device (Valo, Ultradent Products, South Jordan, USA), Tetric N Ceram Bulk Fill Composite (Ivoclar Vivadent, Lichtenstein) was applied in horizontal layers of up to 4 mm, following the manufacturer's instructions, and polymerized for an additional 10 s. After completing the restorations, yellow-striped composite finishing burs (JINME, Guangdong, China) were used to contour the restorations. Subsequently, the restorations were polished using polishing discs (Shofu, Kyoto, Japan) with water cooling (Fig. 2).

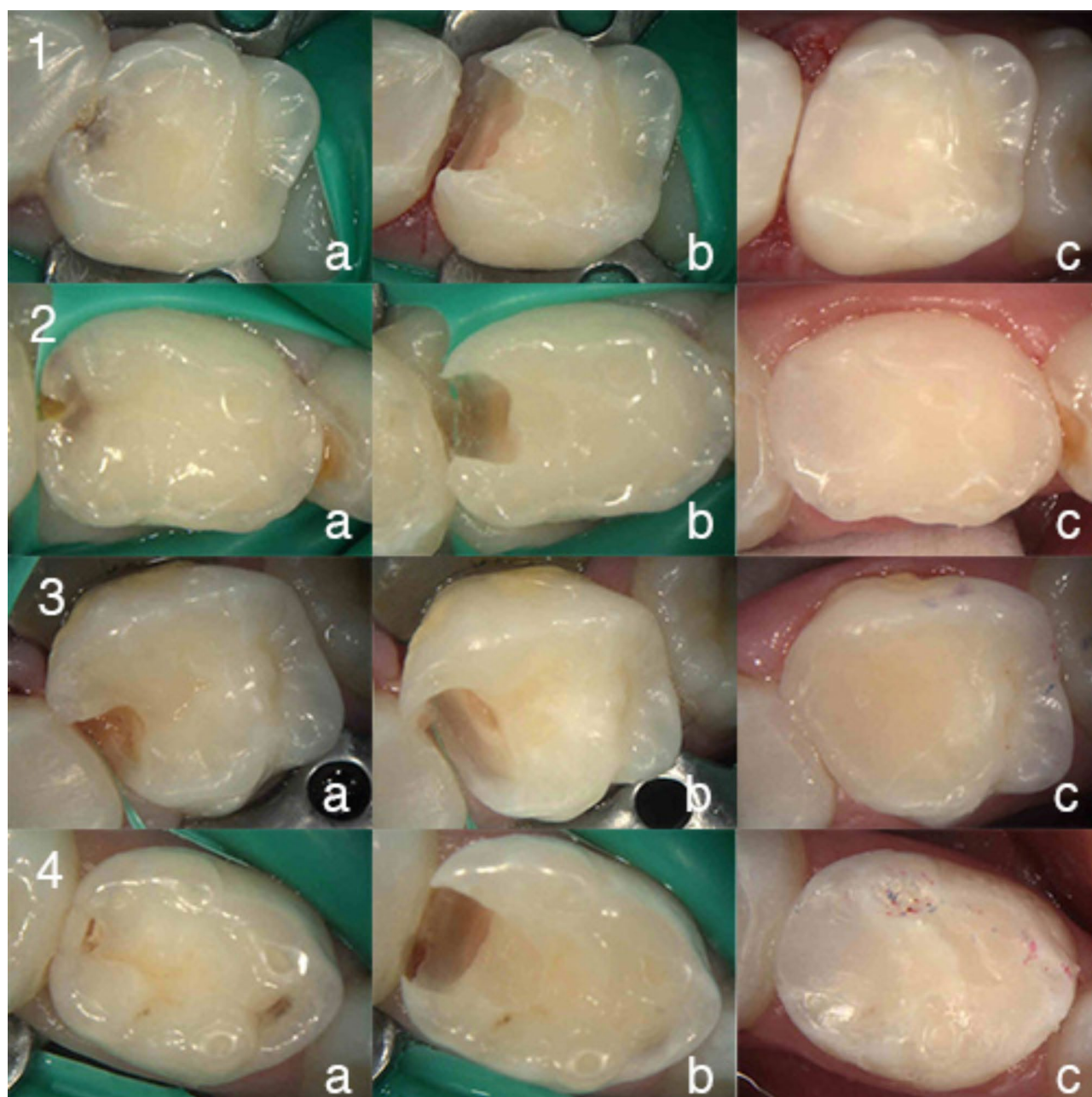


Fig. 2 Dental photograph example of the clinical. Clinical procedure (from left to right: **a.** initial photo, **b.** photos after caries excavation and the **c.** final restoration, from top to bottom; (1) right maxillary second primary molar, (2) right mandibular first primary molar, (3) left maxillary second primary molar, (4) left mandibular first primary molar)

Evaluation criteria

Two observers (M.M. and E.K.) trained and tested for the scoring system before the control sessions started. The scores for 15 teeth, not included in the study, were recorded at three different time intervals before the start of the control sessions. These scores were repeated, and intra-observer consistency was tested using the Kappa test ($\kappa = 0.80$). After completion of the restorations, two experienced observers (E.K and M.M) blinded to the applied material, scored the relevant tooth according to

the World Dental Federation (FDI) criteria [31] which included the categories of assessment for esthetic evaluation, functional assessment, biological responses to restorative materials. Examinations were performed using a mirror and probe under illumination, with teeth dried using air/water spray.

The scores represented successful restorations (Score 1), minor defects (Score 2), acceptable defects (Score 3), restorations requiring repair (Score 4), and restorations requiring replacement (Score 5). The treatments were

evaluated using the same method for 12 months with 3-month intervals by the same examiners.

In-vitro study

The study aimed to compare the microtensile bond strength values of dentin bonding systems with and without chlorhexidine, for both immediate bonding and aged specimens.

Sample size

The sample size for the microtensile test in this study was estimated using data from a similar study [32]. The parameters for the calculation were as follows: effect size (d)=0.86, statistical significance level α =0.05, and statistical power $(1-\beta)$ =0.80. Based on these parameters, it was determined that a minimum of 23 beams per group were required to achieve sufficient statistical power.

Tooth Preparation

A total of 24 freshly extracted, caries-free, and unrestored permanent human molars were selected. Samples were stored in distilled water at +4 °C for a maximum of 3 months. Following the cleaning of the teeth and their preservation, the tooth roots were cut 2 mm below the cemento-gingival junction, and all of the occlusal enamel was removed using a water-cooled, low-speed diamond saw (Isomet 1000, Buehler, Lake Bluff, IL) to expose a superficial dentin surface. Enamel removal was verified using a stereomicroscope (Olympus SZ61, Olympus Optical Co, Tokyo, Japan). Subsequently, dentin surfaces were ground with 400, 600, and 800 grit silicon carbide papers for 60 s underwater to establish a standard smear layer [33].

The teeth were then randomly categorized into experimental and control groups. A bonding agent containing chlorhexidine (Peak Universal Bond, Ultradent, South Jordan, USA) was used for the experimental group, and a non-antibacterial bonding agent (PQ1, Ultradent, South Jordan, USA) was used for the control group. Prior to bonding, all dentin surfaces were etched with 37% phosphoric acid for 20 s, rinsed with water, and gently air-dried to maintain a moist bonding environment. Peak Universal Bond, a universal adhesive, was applied to the experimental group using an applicator for 10 s, ensuring complete surface coverage, followed by gentle air dispersion for 10 s to evaporate the solvent. PQ1 Bond, an etch-and-rinse adhesive was applied in a similar manner to the control group. Both adhesives were then polymerized using an LED light (Valo, Ultradent Products, South Jordan, USA) for 10 s. Tetric N Ceram Bulk Fill Composite (Ivoclar Vivadent, Lichtenstein) was applied in horizontal layers of up to 4 mm and polymerized for an additional 10 s with an LED light. After completion of the restorations, the control and experimental groups were

again randomly divided into two subgroups (immediate bonding samples and thermally aged samples) ($n=6$ teeth) [33]. Finished restorations were then incubated in distilled water at 37 °C for 24 h. In the group of thermally aged specimens, the samples underwent 5000 cycles of thermal aging (equivalent to 6 months of in vivo function) [34] using a thermal cycling device (Nova Co., Konya, Turkey) with temperatures ranging from 5 °C to 55 °C. The teeth were held for 30 s at each temperature and 5 s between temperature changes.

Microtensile bond strength test

The specimens were stored in distilled water at 37 °C for 24 h, then a low-speed diamond saw was used to section the teeth under continuous water cooling. Two cuts were made in a mesiodistal direction along the long axis of the teeth with a 1 mm thick diamond disc and then the center restorative part of the tooth was sectioned buccolingual with 1 mm width. Each specimen was examined under 40× magnification using a stereomicroscope to detect interfacial defects. Inappropriate specimens were excluded. Only central, nontrimmed beams from each tooth were selected for the μ TBS test. The study was conducted with a total of 140 specimens (35 beams of each group). The bonding surface areas were calculated by measuring the dimensions of the rods using a digital micrometer (Mitutoyo, Japan). Samples were subjected to bond strength evaluation using a universal testing machine (Shimadzu, Model AGS-X 5kN, Shimadzu Corporation, Kyoto, Japan). Each beam was attached to a custom-made jig using cyanoacrylate glue and a tensile load was applied at a cross-head speed of 1 mm/min and with a maximum 5000 N force until the beam fractured. The amount of load required for fracture recorded in newtons was converted to megapascals (Mpa) by using the formula: $S=L/A$, where S is the bond strength in megapascals (MPa), L =test load (N), A =adhesive area (mm^2) [35]. If any debonding occurred during specimen sectioning or mounting, the μ TBS was recorded as 0 MPa and these samples were not included in the statistical analyses. To assess the mode of failure the fractured specimens were examined with a stereomicroscope. Photomicrographs were taken at 40X magnification, and the failure mode (cohesive, adhesive, or mixed failure) was identified for each specimen.

Statistical analysis

In the in-vivo part of the study, the assumption of normal distribution was assessed using the Shapiro-Wilk test. When normality was confirmed, a two-way ANOVA test was performed to examine the interaction effects between independent groups. For categorical variables, Fisher's Exact test was applied in cases where the sample size assumption (expected count >5) was not met.

To analyze relationships between dependent categorical variables, the Marginal Homogeneity test was used. All analyses were performed using IBM SPSS Statistics version 27. A significance level of $\alpha = 0.05$ was adopted for all tests.

For the in-vitro part, in order to compare the microtensile bond strength values according to fracture type and experimental groups, a two-way ANOVA test was conducted. This test allowed the evaluation of both main effects (fracture type and adhesive group) and their interaction. Following the ANOVA, pairwise comparisons within groups were performed using the Bonferroni post hoc test to identify specific group differences. A significance level of $p < 0.05$ was considered statistically significant in all tests.

Results

Clinical success

A total of 40 pediatric patients (15 girls and 25 boys) aged between 5 and 9 years (mean age: 7.3 ± 1 years) were included in the study. In total, 80 primary molars were evaluated, with 32 teeth from the maxillary arch and 48 from the mandibular arch. Table 1 shows the number of teeth in the study, categorized by location.

The restored teeth were evaluated every three months for one year using the FDI criteria. The distribution of scores by study groups is presented in the Table 2, and Fisher’s Exact tests were used to analyze the relationships between them. The analyses revealed no statistically significant relationship between the study groups and the scores ($p > 0.05$). The distribution of scores across the groups was homogeneous.

To evaluate changes in scores over time within each study group, Marginal Homogeneity tests were conducted. A statistically significant difference was observed between the 6th and 9th month evaluations ($p = 0.034$). However, no significant differences were found between the 3rd and 6th months or between the 9th and 12th months ($p = 1.000$ and $p = 0.157$, respectively) (Table 3).

Microtensile bond strength evaluation

The total number of beams was 144. Bonding failure was observed in 2 beams in the group. These beams were excluded from the study. In addition, 2 beams were excluded due to manipulation error. As a result, the total number of beams included in the study was 140. Estimated mean microtensile bond strengths of the aged and immediate samples are shown in Table 4; Fig. 3.

The distribution of microtensile bond strength (MPa) measurements according to fracture types and study groups was presented, and a two-way ANOVA test was used for the comparisons. The analysis showed no statistically significant difference between the fracture types regardless of the study groups ($p = 0.505$), whereas a statistically significant difference was observed between the study groups regardless of the fracture types ($p < 0.001$). The interaction effect between the group and fracture type was not statistically significant ($p = 0.085$). Representative fracture patterns are presented in stereomicroscope images (Fig. 4).

According to Bonferroni post-hoc tests, statistically significant differences were found between the Aged Peak and Immediate Peak groups compared to the Aged PQ1 and Immediate PQ1 groups ($p < 0.05$). The MPa values in the Aged Peak and Immediate Peak groups were higher than those in the Aged PQ1 and Immediate PQ1 groups.

Discussion

In this study, clinical success and microtensile bond strength of two bonding agents: Peak Universal Bond, which contains chlorhexidine (CHX), and Peak PQ1, which does not, were compared. The results of the study showed no significant difference in clinical success between the two bonding agents when used with bulk-fill composite restorations in primary molars over 12-month period. Therefore, the null hypotheses for the in vivo study were accepted. However, the in vitro part of the study revealed that Peak Universal Bond’s bond strength was significantly higher than that of PQ1 Bond in both immediate and aged samples, leading to the rejection of the null hypothesis for the in vitro study.

In alignment with the global efforts to reduce mercury emissions outlined in the Minamata Convention, the European Commission enacted a 2024 regulation that prohibits the use, manufacture, and export of dental amalgam effective January 1, 2025, with special emphasis on its prohibition in deciduous teeth, children under 15, and pregnant or breastfeeding women [36]. This international regulatory shift has prompted a global move toward alternative materials. Resin-based composites have emerged as the most commonly used alternative, as also emphasized by the AAPD due to the strong evidence base supporting their use. Nevertheless, studies comparing amalgam and composite restorations in children have reported higher failure rates for resin-based materials, particularly in large, multi-surface cavities, primarily due to secondary caries and technique sensitivity [37]. Thus, while composite resins are now the predominant choice, their clinical success depends heavily on proper technique, isolation, and caries risk assessment. Material selection should therefore balance regulatory mandates,

Table 1 Included teeth groups categorized by the location (n)

Teeth groups (n = 80)	First molar (n)	Secondary molar (n)
Maxilla (n)	13	19
Mandibula (nn)	28	20

Table 2 Distribution of scores by study groups and relationships among them

FDI Criteria	Time	Score	Peak Universal			PQ1			Test statistic	P
			n	%	%G.	n	%	%G.		
Surface Lustre	3-month	Score 1	38	50,0	95,0	38	50,0	95,0	-	1,000
		Score 2	2	50,0	5,0	2	50,0	5,0		
	6-month	Score 1	38	50,0	95,0	38	50,0	95,0	-	1,000
		Score 2	2	50,0	5,0	2	50,0	5,0		
	9-month	Score 1	38	50,0	95,0	38	50,0	95,0	-	1,000
		Score 2	2	50,0	5,0	2	50,0	5,0		
Marginal and Surface Staining	12-month	Score 1	38	50,0	95,0	38	50,0	95,0	-	1,000
		Score 2	2	50,0	5,0	2	50,0	5,0		
	3-month	Score 1	39	50,0	97,5	39	50,0	100,0	-	1,000
		Score 2	1	100,0	2,5	0	0,0	0,0		
	6-month	Score 1	39	50,0	97,5	39	50,0	100,0	-	1,000
		Score 2	1	100,0	2,5	0	0,0	0,0		
	9-month	Score 1	38	50,0	95,0	38	50,0	97,4	-	1,000
		Score 2	2	66,7	5,0	1	33,3	2,6		
	12-month	Score 1	38	50,0	95,0	38	50,0	97,4	-	1,000
		Score 2	2	66,7	5,0	1	33,3	2,6		
Color Match	3-month	Score 1	39	49,4	97,5	40	50,6	100,0	-	1,000
		Score 3	1	100,0	2,5	0	0,0	0,0		
	6-month	Score 1	39	49,4	97,5	40	50,6	100,0	-	1,000
		Score 3	1	100,0	2,5	0	0,0	0,0		
	9-month	Score 1	39	49,4	97,5	40	50,6	100,0	-	1,000
		Score 3	1	100,0	2,5	0	0,0	0,0		
	12-month	Score 1	39	49,4	97,5	40	50,6	100,0	-	1,000
		Score 3	1	100,0	2,5	0	0,0	0,0		
	3-month	Score 1	37	49,3	92,5	38	50,7	95,0	-	1,000
		Score 2	3	60,0	7,5	2	40,0	5,0		
Aesthetic-Anatomic Form	6-month	Score 1	37	49,3	92,5	38	50,7	95,0	-	1,000
		Score 2	3	60,0	7,5	2	40,0	5,0		
	9-month	Score 1	36	48,6	90,0	38	51,4	95,0	-	0,675
		Score 2	4	66,7	10,0	2	33,3	5,0		
	12-month	Score 1	36	48,6	90,0	38	51,4	95,0	-	0,675
		Score 2	4	66,7	10,0	2	33,3	5,0		
Material Fracture and Retention	3-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
	6-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
	9-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
	12-month	Score 1	39	49,4	97,5	40	50,6	100,0	-	1,000
Marginal Adaptation		Score 2	1	100,0	2,5	0	0,0	0,0		
	3-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
	6-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
	9-month	Score 1	39	49,4	97,5	40	50,6	100,0	-	1,000
		Score 2	1	100,0	2,5	0	0,0	0,0		
	12-month	Score 1	39	50,0	97,5	39	50,0	97,5	-	1,000
Occlusal Contour and Wear		Score 2	1	50,0	2,5	1	50,0	2,5		
	3-month	Score 1	39	49,4	97,5	40	50,6	100,0	-	1,000
		Score 2	1	100,0	2,5	0	0,0	0,0		
	6-month	Score 1	39	49,4	97,5	40	50,6	100,0	-	1,000
		Score 2	1	100,0	2,5	0	0,0	0,0		
	9-month	Score 1	39	49,4	97,5	40	50,6	100,0	-	1,000
		Score 2	1	100,0	2,5	0	0,0	0,0		
	12-month	Score 1	39	49,4	97,5	40	50,6	100,0	-	1,000
		Score 2	1	100,0	2,5	0	0,0	0,0		

Table 2 (continued)

FDI Criteria	Time	Score	Peak Universal			PQ1			Test statistic	P
			n	%	%G.	n	%	%G.		
Approximal Anatomic Form	3-month	Score 1	38	50,7	95,0	37	49,3	92,5	-	1,000
		Score 2	2	40,0	5,0	3	60,0	7,5		
	6-month	Score 1	38	50,7	95,0	37	49,3	92,5	-	1,000
		Score 2	2	40,0	5,0	3	60,0	7,5		
	9-month	Score 1	37	50,0	92,5	37	50,0	92,5	-	1,000
		Score 2	2	40,0	5,0	3	60,0	7,5		
		Score 3	1	100,0	2,5	0	0,0	0,0		
	12-month	Score 1	37	50,0	92,5	37	50,0	92,5	-	1,000
		Score 2	2	40,0	5,0	3	60,0	7,5		
		Score 3	1	100,0	2,5	0	0,0	0,0		
Patient Satisfaction	3-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
	6-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
	9-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
	12-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
Post-operative (hyper) Sensitivity and Tooth Vitality	3-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
	6-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
	9-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
	12-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
Recurrence of Caries	3-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
	6-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
	9-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
	12-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
Tooth Integrity	3-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
	6-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
	9-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
	12-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
Adjacent Mucosa	3-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
	6-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
	9-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
	12-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
Oral and General Health	3-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
	6-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
	9-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
	12-month	Score 1	40	50,0	100,0	40	50,0	100,0	-	-
Total	3-month	Score 1	550	49,9	98,2	552	50,1	98,7	1,204	0,715
		Score 2	9	56,3	1,6	7	43,8	1,3		
		Score 3	1	100,0	0,2	0	0,0	0,0		
	6-month	Score 1	550	49,9	98,2	552	50,1	98,7	1,204	0,715
		Score 2	9	56,3	1,6	7	43,8	1,3		
		Score 3	1	100,0	0,2	0	0,0	0,0		
	9-month	Score 1	546	49,8	97,5	551	50,2	98,6	2,441	0,250
		Score 2	12	60,0	2,1	8	40,0	1,4		
		Score 3	2	100,0	0,4	0	0,0	0,0		
	12-month	Score 1	545	49,8	97,3	550	50,2	98,4	2,371	0,271
		Score 2	13	59,1	2,3	9	40,9	1,6		

%. Row percentage and %G.: Column percentage for groups

evidence-based performance, and patient-specific clinical factors.

The main reason for restoration failure in primary teeth is secondary caries and bonding failures such as retention loss of adhesive restorations and marginal defects

[38, 39]. These failures are often caused by water sorption [40] and degradation of the collagen fibrils in the hybrid layers after activation of MMPs [41]. Although chlorhexidine has proven MMP-inhibiting activity, its recommended protocol involves applying 2% CHX digluconate

Table 3 Distribution of scores over time and relationships within study groups

FDI Criteria	Peak Universal			PQ1		
	3–6 month	6–9 month	9–12 month	3–6 month	6–9 month	9–12 month
	<i>p</i>	<i>p</i>	<i>p</i>	<i>p</i>	<i>p</i>	<i>p</i>
Surface Lustre	1,000	1,000	1,000	1,000	1,000	1,000
Marginal and Surface Staining	1,000	0,317	1,000	-	0,317	1,000
Color Match	1,000	1,000	1,000	-	-	-
Aesthetic/Anatomic Form	1,000	0,317	1,000	1,000	1,000	1,000
Material Fracture and Retention	-	-	0,317	-	-	-
Marginal Adaptation	-	0,317	1,000	-	-	0,317
Occlusal Contour and Wear	1,000	1,000	1,000	-	-	-
Approximal Anatomic Form	1,000	0,317	1,000	1,000	1,000	1,000
Patient Satisfaction	-	-	-	-	-	-
Post-operative (hyper) Sensitivity and Tooth Vitality	-	-	-	-	-	-
Recurrence of Caries	-	-	-	-	-	-
Tooth Integrity	-	-	-	-	-	-
Adjacent Mucosa	-	-	-	-	-	-
Oral and General Health	-	-	-	-	-	-
Total	1,000	0,059	0,317	1,000	0,317	0,317

*1–3 scores are clinically acceptable, while scores 4 and 5 are considered clinically unsuccessful

Table 4 Distribution and comparison of MPa measurements according to fracture type and study groups

	Adhesive	Cohesive	Mixed	Total
	Mean ± SD (Median)	Mean ± SD (Median)	Mean ± SD (Median)	Mean ± SD (Median)
Aged Peak	25,88 ± 3,13(27,04)	22,78 ± 3,95(23,44)	22,84 ± 5,7(25,41)	23,78 ± 4,64(25,3)
Immediate Peak	20,87 ± 2,08(20,7)	24,86 ± 2,95(24,1)	23,97 ± 4,24(24,3)	23,82 ± 3,62(23,75)
Aged PQ1	17,86 ± 3,75(19)	18,88 ± 4,00(19,36)	18,10 ± 3,34(17,97)	18,42 ± 3,70(19)
Immediate PQ1	17,73 ± 1,46(17,81)	19,97 ± 3,56(19,77)	20,26 ± 3,74(20,15)	19,74 ± 3,46(19,35)
Total	21,16 ± 4,60(19,7)	21,57 ± 4,33(22,33)	21,49 ± 4,75(20,86)	21,44 ± 4,54(21,22)
Source of Variation	Sum of Squares Type III	Mean Square	F	P
Fracture Type	20,084	10,042	0,686	0,505
Group	732,209	244,070	16,677	< 0,001*
Fracture Type * Group	167,031	27,838	1,902	0,085

*Significance level: **p* < 0,05

on acid-etched dentin before adhesive application. While the use of 2% CHX for 60 s as a non-rinse primer on etched dentin has been shown to be beneficial [42, 43], this procedure adds an extra step to the bonding protocol, which can be impractical in pediatric dentistry due to children's limited attention spans. Furthermore, the incorporation of CHX into the bonding system ensures the delivery of an optimal concentration and reduces practitioner-to-practitioner variability. Consequently, the present study compared the clinical success and bond strength of Peak Universal Bond (with CHX) and Peak P-Q1 Bond (without CHX).

For the in vivo part of the study, FDI criteria were used for the clinical evaluation of restorations, reported as practical, relevant, and standardized assessment tools [44]. These criteria consist of three main sections: esthetics, functional, and biological properties, each with several items. Post-operative sensitivity, secondary caries, tooth vitality, material fracture, retention, and marginal adaptation indicate the quality of bonding success, while

other criteria provide insights into restoration success [31]. In this study, both bonding agents showed high clinical success according to these criteria, with no significant difference found clinically between Peak P-Q1 and Peak Universal Bond. Recent meta-analysis showed that 0.1% and 0.2% CHX increased long-term bond strength stability, and provided statistical evidence for the clinical inclusion of CHX in the bonding system, corroborating our results [23]. This suggests that the CHX-included Peak Universal Bond can be used as a bonding material without requiring an extra CHX application step.

In pediatric patients, restorative materials such as amalgam, glass ionomer, resin-modified glass ionomer, compomer, and resin composite are commonly used, with compomer frequently chosen for primary tooth restorations due to its fluoride-releasing properties [45, 46]. However, in this study, bulk-fill resin composite was selected as the restorative material to objectively evaluate the effectiveness of the chlorhexidine-containing dentin bonding agent and prevent antibacterial fluoride

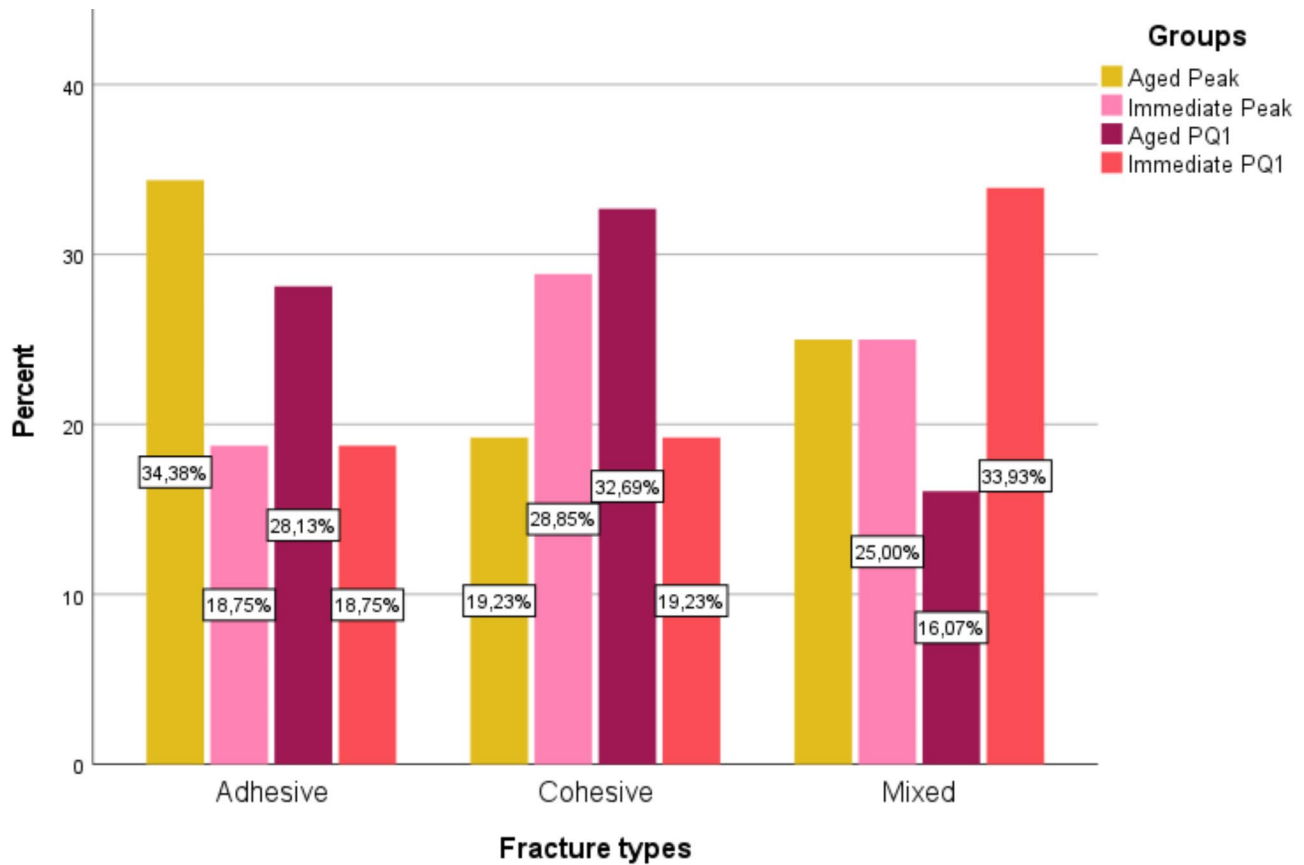


Fig. 3 Graphical presentation of the comparison of the microtensile strength of samples, classified into the fracture types

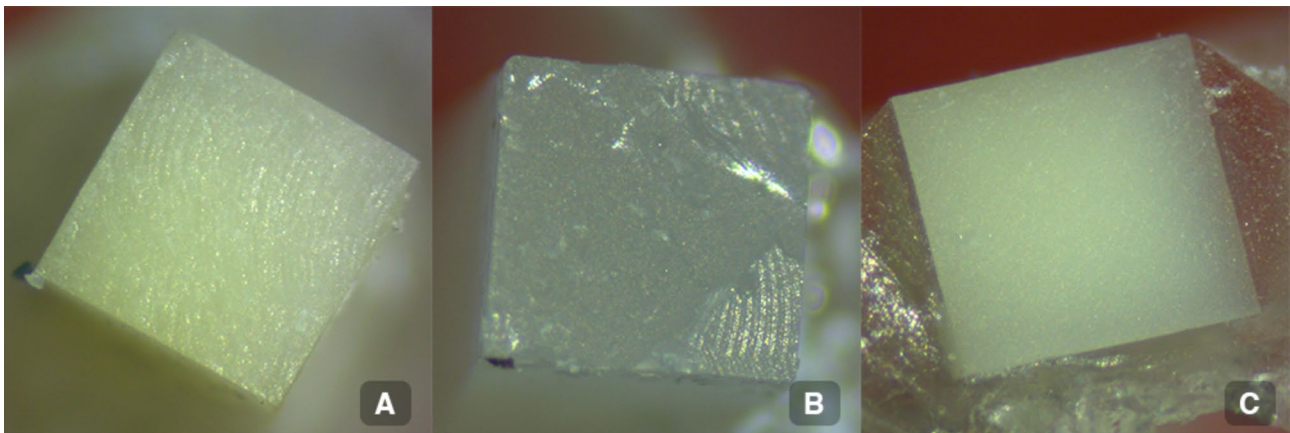


Fig. 4 Representative fracture patterns in stereomicroscope images. (A: adhesive, B: mixed and C: cohesive fracture)

from compomer resin from affecting the study results. Conventional composites require incremental application of no more than 2 mm to minimize polymerization shrinkage, which increases working time and demands precision. In contrast, bulk-fill composites enable larger increments of 4–5 mm, reducing contamination risk and working time while exhibiting lower polymerization shrinkage and stress [47]. Given the ease of use, time efficiency, and limited clinical research on bulk-fill

composites in primary teeth, this material was chosen for the study. Our results demonstrated the clinical success of bulk-fill composites in Class II restorations of primary molars over a 12-month follow-up. Similar to our results, several studies have shown no significant clinical differences between conventional and bulk-fill composites in primary and permanent molars [48, 49]. Therefore, bulk-fill composites can be considered a reliable restorative

material for pediatric patients, offering reduced chairside time and efficient handling.

In the *in vitro* part of the study, Peak Universal Bond exhibited significantly higher microtensile bond strength than PQ1 Bond in both immediate and aged measurements. This can be attributed to the presence of 0.2% chlorhexidine in Peak Universal Bond, providing better resistance to polymer degradation and MMP-induced collagen degradation during aging. Additionally, structural differences between the two bonding agents, such as PQ1 Bond's higher viscosity, may reduce resin penetration through dentin tubules and affect bond quality. The literature reveals limited studies on the bond strength of Peak Universal Bond. Sabatini found no difference in bond strength and MMP inhibitor effectiveness between adding chlorhexidine to bonding agents and its use as a cavity disinfectant [50]. Muñoz et al. reported no statistically significant differences in the immediate bond strength of Peak Universal Bond compared to control groups for both etch-and-rinse and self-etch applications, concluding that chlorhexidine did not negatively affect bond strength [51]. These findings support that Peak Universal Bond does not negatively impact immediate bond values. On the other hand, neither PQ1 Bond nor Peak Universal Bond showed a statistically significant difference in microtensile bond strength between their immediate measurements and those taken after thermal aging. Carillho et al. reported that using 2% chlorhexidine as a cavity disinfectant led to less degradation of the bonding agent after aging, due to inhibition of MMP activation and prevention of collagen degradation [52]. While Carillho et al. used 2% chlorhexidine, the Peak Universal Bond in this study contained only 0.2% chlorhexidine. The lack of a significant difference in bond strength between immediate and aged measurements of Peak Universal Bond may be due to the stable yet lower concentration of chlorhexidine in its formulation. Sabatini similarly found no significant difference in the immediate and 6-month aged shear bond strengths of Peak Universal Bond [50], supporting the argument that chlorhexidine reduces hybrid layer degradation.

Adhesive systems play a key role in the clinical success of restorations by ensuring long-lasting bonding between restorative materials and dental hard tissues, particularly by reducing microleakage, postoperative sensitivity, and secondary caries [53]. In pediatric dentistry, the need for simplified techniques with reliable bond strength has led to growing interest in self-etch systems due to their shorter application time and reduced technique sensitivity [54]. However, studies comparing self-etch and etch-and-rinse systems in primary teeth have reported varying results. Soares et al. found that an etch-and-rinse system (Prime&Bond XP) provided significantly higher bond strength to primary enamel compared to self-etch

systems *in vitro*, though self-etch adhesives also showed acceptable adhesion levels [53]. In clinical settings, both Soares and Lenzi reported similar clinical performance between self-etch and etch-and-rinse systems in primary molar Class II restorations after 12–18 months [55]. Nevertheless, Donmez et al. observed superior marginal adaptation and reduced marginal staining with etch-and-rinse adhesives over a 3-year follow-up [56]. In our study, we used an etch-and-rinse adhesive and a universal adhesive system, both applied with 37% phosphoric acid pretreatment to optimize bonding, particularly to enamel. Clinically, both adhesives demonstrated comparable success over 12 months. However, in the *in vitro* microtensile bond strength analysis, statistically higher bond strength values were obtained in the aged and immediate universal adhesive groups compared to the PQ1 groups. These findings support the literature suggesting that phosphoric acid etching prior to universal adhesive application enhances bonding effectiveness in pediatric restorative treatments.

In this study, the most frequently observed fracture type in the microtensile bond strength test was mixed fractures, followed by cohesive and adhesive fractures. The classification of fracture types in the specimens provides insights into the distribution of stress in the restorative material, tooth, and resin-dentin interface according to the applied test method [50, 57]. An important factor influencing bond strength is the application technique. Considering that both bonding agents in this study were applied using the etch-and-rinse technique, high bond strength was expected. acid etching increases surface roughness, removes the smear layer, and opens dentin tubules, facilitating effective bonding [51, 58, 59]. Muñoz et al. reported that mixed fractures were the most common in their study evaluating the bond strength of a chlorhexidine-containing dentin bonding agent (Peak Universal Bond), followed by adhesive and cohesive fractures in all groups [51]. Another study using a two-step etch-and-rinse adhesive system investigated the effect of applying 2% chlorhexidine between the two steps on bond strength, revealing that mixed fractures were the most common, followed by adhesive and cohesive fractures [52]. The fracture type findings of this study's microtensile bond strength test showed a similar distribution of fracture types for Peak Universal Bond and PQ1 Bond, can be attributed to the etch-and-rinse application method.

This current study was a prospective, split-mouth, randomized controlled trial, which is recommended for clinical trials to obtain robust results. The 100% recall rate achieved in this study is a notable strength, as patient dropout is a common challenge in clinical research. The successful follow-up of all participants ensured complete data collection and minimized the risk of bias associated

with loss to follow-up. This high recall rate can be attributed to effective patient communication, parental cooperation, and the relatively short follow-up period of 12 months. Also, as a future strength, although bulk-fill composites prove their success on permanent teeth [60], there is limited knowledge in the clinical success of bulk-fill composites in primary teeth. However, several limitations exist: because of the split-mouth design of the study, operator blinding could not be achieved. But to overcome this issue, the assessor blinding was achieved. Another limitation is that a 12-month follow-up can be considered a limited time for evaluating the restoration's clinical success. However, long-term follow-up is challenging for primary teeth due to physiologic exfoliation. Also, in the in vitro part of the study, a micro-tensile bond strength test was applied to the aged samples prepared to resemble the long-term performance of the agents. Another limitation is that the bonding materials investigated were applied to different teeth of the same participant during one session. While this could affect the study results, this approach was chosen to ensure the same intraoral condition for the objective comparison of the agents; different environmental and subject-specific factors might have influenced the clinical success of the restoration in different participants, making fully equal conditions impossible. Suggestions for future studies would be to increase the sample size and evaluate clinical success with long-term follow-up. Also, in vivo investigation of the antibacterial activity of CHX-included bonding material is recommended for better understanding of the performance.

Conclusion

In conclusion, within the limitations of this study, the bonding agent with added chlorhexidine (CHX) showed high clinical success similar to the standard adhesive in primary molars over a 12-month follow-up period. Moreover, the CHX-added adhesive demonstrated significantly higher microtensile bond strength compared to the adhesive without CHX. These results indicate that CHX-added adhesives can be effectively used in primary molars due to their strong bonding ability, which is achieved by inhibiting matrix metalloproteinases (MMPs). Additionally, the inclusion of CHX in the bonding agent eliminates the need for a separate application step, thereby reducing chair time during the restoration procedure, which may particularly be beneficial in pediatric dentistry. Further research with longer follow-up periods is necessary to confirm these findings.

Abbreviations

CHX	Chlorhexidine
AAPD	American Academy of Pediatric Dentistry

Acknowledgements

The authors would like to thank Dr. Merve MEŞE for their contributions.

Author contributions

H.A: Conceptualization, Methodology, Investigation, Data curation, Formal analysis; H.E.K: Data curation, Visualization, Writing- Original Draft, Writing - Review & Editing; F.E: Methodology, Supervision, Writing - Review & Editing; M.T: Methodology, Supervision, Writing - Review & Editing; E.K.: Conceptualization, Methodology, Supervision, Writing- Original Draft, Writing - Review & Editing. All authors read and approved the final manuscript.

Funding

No financial funding was obtained for this study.

Data availability

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

The study protocol was approved by Izmir Katip Celebi University Faculty of Dentistry Ethical Committee on Human Research for both the in-vivo (76/2015) and in-vitro (42–44/2015) parts of the study. The procedures used in this study adhere to the tenets of the Declaration of Helsinki. An informed consent including the details of the possible risks, discomforts, and benefits of the procedure was obtained from the parents of each patient.

Consent for publication

Not Applicable.

Competing interests

The authors declare no competing interests.

Author details

¹Faculty of Dentistry, Department of Pediatric Dentistry, Izmir Katip Çelebi University, Izmir, Turkey

²Faculty of Dentistry, Department of Pediatric Dentistry, Uşak University, Uşak, Turkey

³Faculty of Dentistry, Department of Pediatric Dentistry, Ege University, Izmir, Turkey

⁴Faculty of Dentistry, Department of Restorative Dentistry, Ege University, Izmir, Turkey

Received: 9 February 2025 / Accepted: 4 April 2025

Published online: 13 April 2025

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