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Does double layer application of universal adhesives affect the bond strength on coronal and root dentin?

Ayşenur Bulut[1], Cansu Dağdelen Ahisha[1] and Sinem Akgül[1*]

Abstract

Background The aim of this study was to evaluate the effect of single- and double-layer application of two different universal adhesive systems on the bond strength of coronal and root dentin.

Methods Human dentin surfaces of 80 extracted maxillary incisors were used in this study. Coronal and root dentin surfaces were exposed, and standardized smear layers were created by polishing with silicon carbide abrasive paper for 30 s. The specimens were randomly divided into eight groups ($n = 10$). Gluma Bond Universal and Single Bond Universal adhesives were applied in single- and double-layer modes to both coronal and root dentin according to the manufacturers' instructions. A nanohybrid resin composite (G-ænial A'Chord, GC Corporation, Tokyo, Japan) was then applied. After 24 h of storage, the specimens were subjected to thermocycling, followed by microshear bond strength testing at a crosshead speed of 1 mm/min.

Results The microshear bond strength of Gluma Bond Universal to both coronal and root dentin was statistically significantly higher than that of Single Bond Universal. Double-layer application resulted in significantly higher bond strength values compared with single-layer application for both adhesive systems and dentin types. In the double-layer application groups, the bond strength of coronal dentin was statistically significantly higher than that of root dentin for both adhesive systems ($p = 0.001$; $p < 0.05$).

Conclusion Within the limitations of this in vitro study, double-layer application of universal adhesives increased bond strength, with higher values observed in coronal dentin than in root dentin. This application strategy may be considered a simple clinical approach to enhance the bonding effectiveness of universal adhesive systems.

Keywords Adhesive layer, Bond strength, Dentin, Universal adhesive

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Introduction

The simplification of adhesive systems and the reduction of technical sensitivity have become very popular in dentistry recently [1]. Simplification of adhesive systems has been achieved by introducing hydrophilic monomers and increasing the solvent content of the adhesive formulation to make the adhesive compatible with the wet dentin substrate [2]. This increased hydrophilicity leads to hydrolytic degradation, delayed formation of highly cross-linked polymers and phase separation. As a result, the interface may degrade over time [1]. Reductions in bond strength, often indicated in studies in the literature [3, 4], are caused by hydrolysis of collagen fibrils leading to destruction of the hybrid layer. Bond strength is influenced by the extent of resin infiltration into the exposed collagen fibrils. Ideally, the adhesive monomers should completely infiltrate the interfibrillar spaces [5].

The infiltration of adhesives into dentin and the thickness of the adhesive layer are directly related to the viscosity and chemical properties, However, the way it is applied also has a significant impact [6]. To improve the infiltration of monomers into the dentin surface, applications such as multilayer application, improved solvent evaporation, extended curing time, addition of a hydrophobic resin layer to strengthen the adhesive layer have been proposed [7, 8].

Several studies [6, 9] have shown that active application of the adhesive can accelerate the evaporation of the solvent, resulting in greater impregnation of the monomer into the smear layer. Active application of the adhesive may transport the acidic monomers to the underlying dentin, causing a more aggressive demineralisation and allowing better absorption of the monomers and a higher concentration of comonomers, which may improve the quality of the hybrid layer. To improve the infiltration of monomers into the dentin surface, applications such as multi-layer application, improved solvent evaporation, extended curing time, addition of a hydrophobic resin layer to strengthen the adhesive layer have been proposed [9]. In addition [10, 11], studies have shown that dual application of the adhesive improves interface quality by increasing resin saturation in the collagen network. However, several studies [12, 13] have reported that dual application may be ineffective for clinical applications.

Universal adhesives, while similar in appearance to one-step self-etch adhesives, have distinct differences in composition and clinical application as they are compatible with both total-etch and self-etch techniques [14, 15]. Numerous studies [14–16] have demonstrated that universal adhesives provide enamel and dentin bond strength comparable to or superior to previous generation one-step self-etch adhesives. Although universal adhesives are widely used in clinical practice, they do not fully overcome the disadvantages associated with one-step self-etch adhesives [17]. A certain amount of water and solvent is still required in universal adhesives, which can lead to similar interfacial phenomena and potentially compromise their long-term durability [2]. As with one-step self-etch adhesives, there is currently no clear consensus in the literature on the efficacy of applying two layers of universal adhesives [18]. Therefore, the aim of this study is to investigate the effect of single and double layer application of two different universal adhesives on bond strength to coronal and root dentin. The null hypotheses tested in this study were that (1) There is no difference between the bond strengths of two different universal adhesive systems to dentin (2) There is no difference between the bond strengths of the application methods of the adhesive systems to dentin (3) There is no difference between the bond strengths of the type of dentin to which the adhesive systems are applied.

Methods

Specimens preparation

The adhesive systems and their compositions, as well as the composition of the resin composite used in the study, are presented in Tables 1 and 2, respectively. The flow chart of the study is given in Fig. 1.

This in vitro study was designed as a factorial experiment. The study factors were dentin type (coronal and root dentin), adhesive system (Gluma Bond Universal and Single Bond Universal), and adhesive application mode (single- and double-layer application). The response variable was microshear bond strength (MPa). All experimental procedures were performed by a single trained operator.

In the study, 80 healthy human maxillary incisors extracted for periodontal reasons were used. The study

Table 1 The adhesive systems used in the study

Materials (Manufacturer)	Type	pH	Composition	Application Protocol
Gluma Bond Universal (Heraeus Kulzer, Hanau, Germany)	Universal	1.6	MDP phosphate monomers, 4-META, dimethacrylate resins, acetone, fillers, initiators, silane	Self-etch mode; actively applied for 20 s, gently air-dried, light-cured for 10 s (single-layer); second layer applied and light-cured for 10 s (double-layer)
Single Bond Universal 3 M ESPE, St. Paul, MN, USA)	Universal	2.8	MDP phosphate monomers, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, fillers, ethanol, water, initiators, silane	Self-etch mode; actively applied for 20 s, gently air-dried, light-cured for 10 s (single-layer); second layer applied and light-cured for 10 s (double-layer)

MDP Metakriloloksidesil dihidrojen fosfat, *HEMA* Hidroksietil metakrilat, *CQ* Kanforokinon, *META* Metakriloksietil trimelliat anhidrid

Table 2 The resin composite used in the study

Materials (Manufacturer)	Type	Filler amount (wt%, vol%)	Filler composition/size
G'aenial A'chord (GC Corporation, Tokyo, Japan)	Nanohybrid Resin Composite	82 – 65	Silica and strontium glass fillers; nano- and micro-sized particles (approximately 16–200 nm and up to 1 μm)

Bis-MEPP 2,2-Bis (4-metakriloksipolietoksifenil) propan

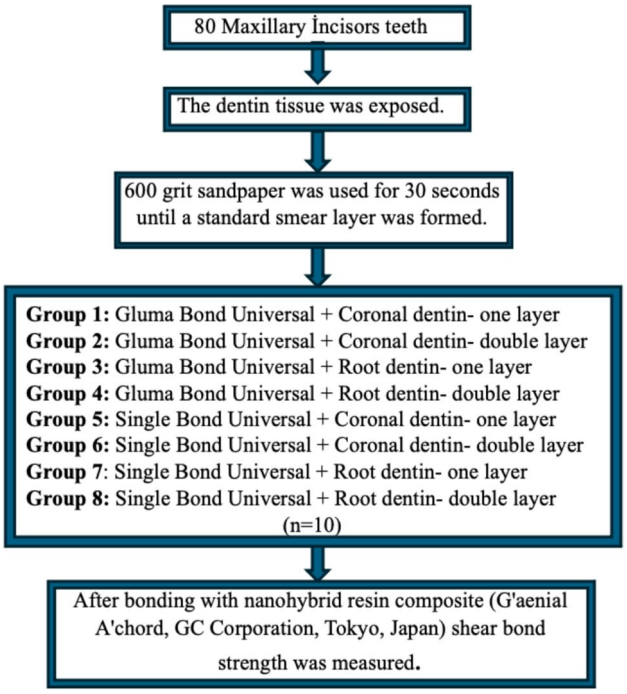


Fig. 1 Flow chart

protocol was reviewed and approved by the Gazi University Faculty of Dentistry Clinical Research Ethics Committee (Approval No: E-21071282-050.99-572988; Date: 27 January 2023). Extracted human teeth were collected as anonymous biological waste materials from routine dental extractions; therefore, individual informed consent was waived according to institutional policy. All procedures were conducted in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its subsequent amendments. After extraction, the teeth were cleaned of soft tissue remnants and stored in 0.1% thymol solution at 4 °C until use.

The root surface of the extracted incisors to be tested was removed from the enamel-dentin junction, and the enamel tissue on the buccal surfaces was removed with the help of a hard tissue cutting device (Brillant 210 Precision Cutting Device, Ata Quality Assured, England) in a way that exposed the dentin tissue. The buccal surfaces of the teeth were placed in autopolymerized acrylic resin surrounded by a polyvinylchloride (PVC) cylinder, and the exposed dentin surfaces of the teeth were sanded with a sanding and polishing device (Mecapol P230, Presi, Türkiye) to create a standard smear layer using 600 grit sandpaper for 30 s. The samples were randomly divided into eight groups (n: 10).

Adhesive application protocol

In this study, eight experimental groups were established based on the type of dentin substrate (coronal or root dentin), the adhesive system used (Gluma Bond Universal or Single Bond Universal) and the number of adhesive layers applied (one or two layers). In groups 1 and 2, Gluma Bond Universal adhesive (Heraeus Kulzer, Hanau, Germany) was applied to coronal dentin.

In Group 1, a single layer of adhesive was actively applied to the dentin surface for 20 s, gently air-dried, and then light cured for 10 s using an LED light-curing unit (GC D-Light Pro, GC Corporation, Tokyo, Japan) operating in standard mode with a light intensity of 1400 mW/cm². In Group 2, the adhesive was applied in two layers: after the first application (20 s) and gentle air drying, a second layer was actively applied for a further 20 s and then light cured for 10 s. Groups 3 and 4 followed the same protocol as groups 1 and 2, but the adhesive was applied to the root dentin. Groups 5 and 6 used Single Bond Universal (3 M ESPE, St. Paul, MN, USA) on coronal dentin. In Group 5, a single layer was applied for 20 s, gently air dried and light cured for 10 s. In Group 6, two layers were applied, similar to the protocol used in Group 2. Groups 7 and 8 received the same Single Bond Universal protocol as Groups 5 and 6, but on root dentin.

After application of the adhesive system, a transparent tube (Tygon, Norton Performance Plastic Co., Akron, OH, USA) with an inner diameter of 0.8 mm and a height of 1 mm was placed, and a nanohybrid resin restorative material (G'aenial A'chord, GC Corporation, Tokyo, Japan) was applied. The resin composite was then light cured for 20 s using the same LED light-curing unit (GC D-Light Pro, GC Corporation, Tokyo, Japan) operating in standard mode with a light intensity of 1400 mW/cm². After light curing, the Tygon tube was carefully removed using a scalpel blade, leaving a standardized resin composite cylinder bonded to the dentin surface.

Measurement of shear bond strength

All samples were immersed in distilled water in an incubator at 37 °C for 24 h. Then, the thermal cycling test ranging from 5 to 55 C^0 was repeated 10,000 times with an immersion time of 10 s in each bath and an inter-bath

exchange time of 5 s [19]. The shear bond strength of the samples was measured using a Universal testing machine (Shimadzu IG-IS; Kyoto, Japan). The load was applied to the interface at a cross-head speed of 1 mm/min until failure and the stress-strain curve was analyzed with the machine's software program. The force causing the fracture was recorded in Newtons (N). This value was then divided by the sample surface area to convert the mean shear bond strength values to Megapascals (MPa). Failures were classified as adhesive, cohesive, or mixed and were examined by a single observer using a stereomicroscope (SV11, Zeiss, Oberkochen, Germany) at 50× magnification. Cohesive failures included fractures occurring within dentin or resin composite.

Statistical analysis

IBM SPSS Statistics 22 was used for statistical analysis in the evaluation of the results obtained in the study. The suitability of the parameters for normal data was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests, and it was found that the parameters were suitable for normal distribution. Homogeneity of variances was evaluated using Levene's test. A three-way ANOVA test was used to evaluate the effects of dentin, adhesive application method, and adhesive system on bond strength. Significance was assessed at the $p < 0.05$ level.

Results

The microshear bond strength values are presented in Table 3. Three-way ANOVA revealed that the adhesive system, application mode, and dentin location had statistically significant effects on microshear bond strength ($p < 0.05$) (Table 4).

Gluma Bond Universal demonstrated significantly higher microshear bond strength values than Single Bond Universal when applied to both coronal and root dentin, regardless of whether a single- or double-layer application was used ($p < 0.05$).

For both adhesive systems, double-layer application resulted in significantly higher microshear bond strength values compared with single-layer application on both coronal and root dentin ($p < 0.05$). In addition, coronal dentin exhibited significantly higher bond strength values than root dentin in both single- and double-layer applications for both adhesive systems ($p < 0.05$).

The distribution of failure modes observed after the microshear bond strength test is presented in Fig. 2. Adhesive failures were predominantly observed in specimens subjected to single-layer adhesive application on both coronal and root dentin. In contrast, mixed failures were more frequently observed in double-layer applications. Cohesive failures in dentin and resin composite were the least frequently observed failure modes across all groups.

Discussion

In the oral cavity, restorations are constantly exposed to a complex, moist and aggressive environment due to thermal changes, biofilm and mastication. Rapid interfacial deterioration of restorations and even the failure of the restoration not only represent a major economic burden in terms of healthcare expenditure but can also be detrimental to tooth survival if secondary caries due to interfacial deterioration further weakens the tooth structure [20]. Adhesive technology is therefore an important factor in achieving strong restorations with a long clinical life [21].

In this study, a statistically significant difference in shear bond strength was observed between the two different universal adhesive systems tested. The first null hypothesis is therefore rejected. Bonding of universal adhesives to dentin is achieved by chemical bonding of the functional monomer in the adhesive to dentin [22]. The chemical bonding capacity of adhesives is basic, as it depends on the functional monomer it contains. 10-MDP is considered the most effective acidic functional monomer because it demineralises dentin, ionically binds to

Table 3 Evaluation of groups in terms of microshear bond strength

Dentin	Adhesive application	Gluma Bond Universal	Single Bond Universal	
		Mean ± SD	Mean ± SD	*p*
Coronal	Single Layer	23.09 ± 0.57^{Aa}	19.35 ± 0.52^{Ba}	0.001*
	Double Layer	24.59 ± 0.54^{Ab}	20.87 ± 0.55^{Bb}	0.001*
	p	0.001*	0.001*	
Root	Single Layer	15.70 ± 0.63^{Ac}	14.89 ± 0.74^{Bc}	0.017*
	Double Layer	18.32 ± 0.77^{Ad}	16.28 ± 0.73^{Bd}	0.001*
	p	0.001*	0.001*	
Coronal-Root	Single Layer p	0.001*	0.001*	
	Double Layer p	0.001*	0.001*	

Different uppercase letters indicate statistically significant differences between adhesive systems within the same column (same dentin type and application mode)

Different lowercase letters indicate statistically significant differences between application modes or dentin locations within the same row ($p < 0.05$)

Three-way ANOVA Test *$p < 0.05$

Table 4 Distribution of fracture types of specimens subjected to micro shear bond strength test (Type I (Adhesive), type II (Cohesive), type III (Mixed))

Groups	Procedures Applied to Specimens	Failure Type	Number of Failures
Group 1	Gluma Bond Universal + Coronal dentin + Single layer	Tip I	7
		Tip II	0
		Tip III	3
Group 2	Gluma Bond Universal + Coronal dentin + Double layer	Tip I	3
		Tip II	1
		Tip III	6
Group 3	Gluma Bond Universal + Root dentin + Single layer	Tip I	5
		Tip II	2
		Tip III	3
Group 4	Gluma Bond Universal + Root dentin + Double layer	Tip I	3
		Tip II	2
		Tip III	5
Group 5	Single Bond Universal + Coronal dentin + Single layer	Tip I	6
		Tip II	1
		Tip III	3
Group 6	Single Bond Universal + Coronal dentin + Double layer	Tip I	2
		Tip II	2
		Tip III	6
Group 7	Single Bond Universal + Root dentin + Single layer	Tip I	6
		Tip II	1
		Tip III	3
Group8	Single Bond Universal + Root dentin + Double layer	Tip I	4
		Tip II	1
		Tip III	5

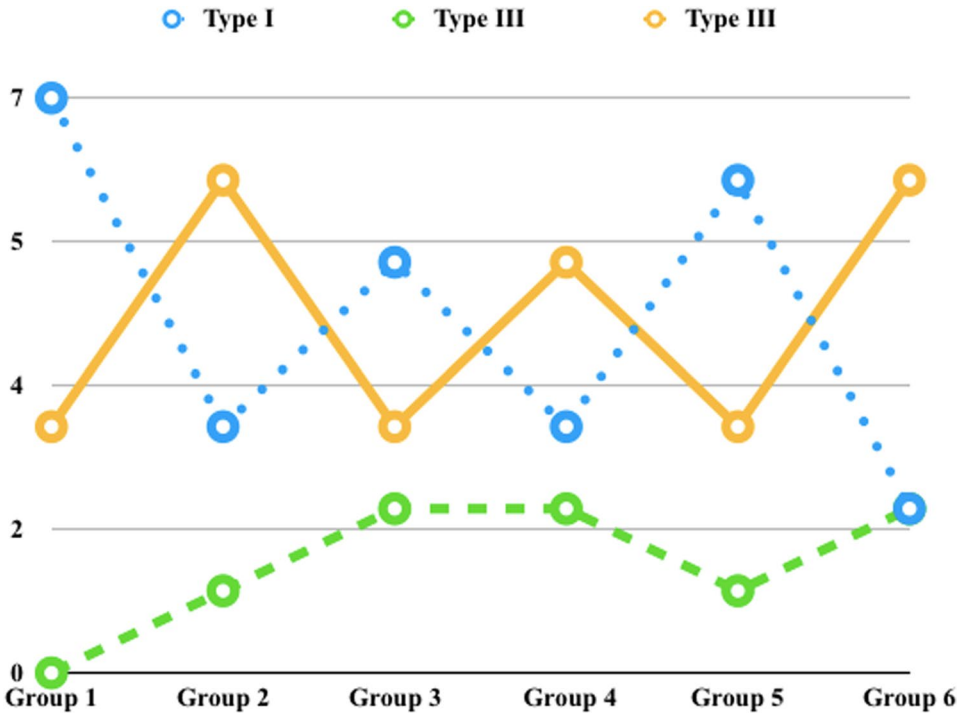


Fig. 2 Distribution of fracture types observed in specimens subjected to the micro-shear bond strength test. Type I = Adhesive failure, Type II = Cohesive failure (in dentin or resin composite), Type III = Mixed failure. Results are shown for each adhesive system, dentin location (coronal/root), and application mode (single/double layer)

calcium in hydroxyapatite and forms stable nano-layered calcium salts [15]. This type of chemical bonding does not increase the immediate bond strength, but it has been reported in the literature that it can improve the long-term stability of adhesive interfaces [16, 23]. Therefore, many current adhesive systems contain 10-MDP. Single Bond Universal and Gluma Bond Universal contain 10-MDP as the acidic functional monomer [24, 25]. Gluma Bond Universal contains the acidic monomer 4-META with two carboxyl groups in addition to 10-MDP. It also has a moderately strong acidity (pH = 1.6). Single Bond Universal also contains a copolymer of polyalkenoic acid, which can also chemically bond to hydroxyapatite. However, it has been reported that this polyalkenoic acid copolymer competes with the 10-MDP functional monomer for calcium binding sites in hydroxyapatite and may also inhibit monomer polymerisation due to its high molecular weight [26, 27].

Gluma consists of HEMA and glutaraldehyde [28]. HEMA provides hydrophilic properties, facilitates penetration of the adhesive into dentin tubules, and leads to the formation of a high-quality hybrid layer. The glutaraldehyde component reacts with albumin present in dentinal tubules, precipitating as protein. These protein precipitates contribute to the desensitizing effect and establish a strong and stable bond with phosphate groups in adhesive cement [28]. Sailer et al. [29] demonstrated a significant improvement in SBS values following the application of a desensitizer containing glutaraldehyde/HEMA prior to luting with self-adhesive cement. Similarly, Stawarczyk et al. [30] and Kulunk et al. [31] reported that Gluma significantly increased the SBS of self-adhesive resin cement to dentin. In the present study, the use of Gluma Bond Universal, which contains both 10-MDP and glutaraldehyde/HEMA, may have enhanced chemical bonding to hydroxyapatite while promoting a stable hybrid layer, contributing to the higher bond strength values observed. These mechanistic effects highlight how the specific composition of Gluma can influence adhesive performance.

Doğan et al. [32] investigated the repair bond strength of the resin composite after three different surface treatments using Tokuyama Universal Bond, Prime Bond Universal, Gluma Bond Universal and Clearfil SE Bond and reported that silane + Gluma Bond Universal showed the highest bond strength. In the present study, we also obtained a higher bond strength value with Gluma Bond Universal, which can be attributed to the moderately strong acidity of the adhesive and the presence of the 10-MDP functional monomer in the adhesive.

A double-layer application of a universal adhesive can be carried out in two ways: with or without the first layer being exposed to light. If the first layer is not exposed to light, it has been found that the unpolymerised acidic monomers of the second layer can increase the corrosiveness of the adhesive and increase the thickness of the adhesive layer to some extent [33, 34]. In other words, after the first layer of adhesive has been applied and the solvent has been removed and polymerised, the monomer concentration increases with the application of the second layer, thus improving resin infiltration and the quality of the hybrid layer [35, 36]. The hybrid layer is formed by the infiltration of hydrophilic functional monomers into demineralised dentin. This layer plays an important role in bonding dentin to adhesive resin [37]. It has been reported that when universal adhesives are used in self-etch mode, a thin and less distinct hybrid or transitional layer is formed due to limited collagen exposure, and a clearly defined hybrid layer is often not observed under SEM evaluation. In contrast, the etch-and-rinse technique results in a thicker and more clearly identifiable hybrid layer [15].

Applying two layers of universal adhesive in succession can result in a thicker, higher-quality hybrid layer, which could improve bond strength [38]. Applying a double adhesive layer results in a thicker adhesive layer. This layer may have improved mechanical properties, which could help to reduce polymerisation stresses and provide better stress distribution during testing [35, 39]. However, removing the solvent from a thicker adhesive layer may be more difficult [40]. After adhesive application, the solvent must be thoroughly evaporated, as any remaining solvent in the adhesive layer may weaken the adhesive interface and reduce bond strength [41]. This potential problem following double-layer application can be addressed by evaporating the solvent and light-curing the first layer before applying the second layer [42]. However, it has been reported that resin infiltration may not increase when the second layer is applied over a polymerised first layer [43]. An excessively thick adhesive layer may also compromise marginal integrity in the long term [41]. Furthermore, there is no consensus on the optimal adhesive layer thickness for dental adhesives. It has also been reported that applying two layers increases the hardness of the adhesive layer and the resin-dentin interface, thus improving the bond strength of universal adhesives [12, 15]. Furthermore, applying two layers can help to compensate for potential application errors, resulting in a more homogeneous adhesive layer [15]. From a clinical perspective, a single-layer application may be sufficient for superficial dentin where adhesive infiltration is less challenging. In contrast, double-layer application could be particularly advantageous for deep or sclerotic dentin, where wider tubules, higher moisture content, or increased mineralization may reduce monomer penetration. In these cases, applying a second adhesive layer can enhance hybrid layer quality and improve bond strength, providing a more reliable adhesive interface in challenging clinical scenarios.

In line with previous findings, this study considers the observed improvement in bond strength of universal adhesives to be due to the increased quality of the hybrid layer, as well as the increased thickness and quality of the adhesive layer resulting from the double-layer application. In light of these findings, we also rejected our second null hypothesis.

It has been reported that the formation of the smear layer affects the bond strength of adhesives by altering its thickness and quality [44, 45]. In clinical practice, the smear layer is formed through preparation using diamond and tungsten carbide burs. Studies have shown that the smear layer formed using diamond and tungsten carbide burs is denser, thicker, and rougher than that produced using 600-grit silicon carbide (SiC) abrasive papers [46].

In the early phases of adhesive research, 600-grit SiC papers were the most frequently used abrasive for generating a standardized smear layer on dentin surfaces in laboratory studies and are still commonly employed by manufacturers prior to the introduction of new adhesive systems [47]. However, because the smear layer created by regular-grit diamond burs differs substantially from that produced by 600-grit SiC, several authors have recommended the use of coarser abrasives, such as 180-grit or 120-grit SiC, to better approximate clinical conditions [13].

Despite these differences, the use of 600-grit SiC remains a widely accepted and standardized method in in vitro bond strength studies, allowing for reproducible smear layer formation and facilitating meaningful comparisons with previous studies. Therefore, in the present study, dentin surfaces were prepared using 600-grit SiC abrasive. The acidic monomers in universal adhesives can penetrate the smear layer and dissolve the hydroxyapatite structure to varying degrees depending on the level of acidity. Active application of acidic primers through prolonged rubbing aims to remove the smear layer, creating a better micromechanical and chemical bond in the dentin [44]. This process provides more effective acidification and interaction between the acidic monomers and the dental tissues. It spreads the acidic products into the hybrid layer, facilitates the evaporation of solvents, and causes a higher percentage of monomers to be incorporated into the smear layer. Spreading the acidic monomers into the deeper layers of dentin provides more aggressive demineralisation and better interaction with the smear layer and underlying dentin tissue [13, 48]. Therefore, in our study, we actively applied universal adhesives to the dentin surface, which we believe also supports bond strength.

One of the most important factors affecting the adhesion strength of the adhesive system to dentin is its complex tissue structure and the variety of its chemical components. As the depth from the dentin surface increases, the chemical composition of the dentin changes: the collagen content decreases; the number and diameter of the tubules increase; the water content of the dentin increases; and the permeability of the dentin increases. These changes result in a difference in bond strength [49]. Dentinal tubules are similar in both crown and root dentin, from the cement to the pulp. However, differences between dentin near the cement and dentin near the pulp are more pronounced in the crown than in the root. Since tubule density and peritubular dentin thickness are both greater in the crown than in the root, the proportion of tubular area is lower in root dentin, with less peritubular dentin present [49]. Alnaftachi et al. [50] evaluated the bond strength of four universal adhesives to root and crown dentin, reporting that all adhesives exhibited higher bond strength values with crown dentin than root dentin. This is because root dentin has fewer dentin tubules, and its permeability is much lower than that of crown dentin. Consequently, the infiltration capacity of the hydrophilic resin in root dentin is reduced, resulting in lower bond strength. In our study, a higher bonding value was observed in crown dentin, which is consistent with previous research. Therefore, the third null hypothesis was rejected.

In order to avoid waiting a long time to determine the durability of the material, there are various methods available to age the samples in vitro. In the oral environment, restorations are exposed to chemical, physical and mechanical processes, so there are mechanical, biological, chemical and physical ageing factors to consider. This study aimed to determine bond strength, and given that universal adhesives' biggest deficiency is their tendency to physical deterioration, thermal cycling was chosen as the ageing process [51].

The fracture types of the groups in our study were examined and a relationship was found between bonding strength and fracture type. More adhesive fractures were observed in the first group, where the universal adhesive agent was applied in a single layer, while mixed-type fractures were more common in the second group, where it was applied in two layers. Adhesive fractures are preferable to cohesive fractures, which cause fractures in the material itself. However, with high bonding strength, more cohesive fractures are observed. The higher incidence of mixed fractures in the group where the agent was applied in two layers can be explained by the stronger bond to the dentin in this group [52, 53].

This is an in vitro study that does not replicate intraoral conditions and may produce results that differ from those observed in a clinical setting. Factors present in the oral cavity, such as saliva, enzymes, food and beverages at different pH levels and temperatures, may affect resin composites. Further in vivo studies are required to evaluate the effect of universal adhesives on the bond strength and longevity of coronal and root dentin.

Conclusion

In this in vitro study, Gluma Bond Universal adhesive demonstrated higher dentin bond strength than Single Bond Universal adhesive, and a double-layer application further enhanced bonding performance. These results suggest that both the adhesive type and application protocol significantly influence bond efficacy, particularly on deep or sclerotic dentin. Mechanistically, the improved performance of Gluma Bond Universal may be attributed to its functional monomer composition, which enhances chemical interaction with dentin. Clinically, tailoring adhesive selection and application technique to the dentin characteristics could optimize restoration longevity and reduce the risk of bond failure. However, these in vitro findings need to be validated in well-designed clinical studies to inform evidence-based adhesive selection and protocol standardization in routine restorative practice.

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Authors' contributions
All authors contributed to each stage of the study to the same degree. All authors read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study protocol has been reviewed and approved by the [Gazi University Faculty of Dentistry Clinical Research Ethics Committee] (Approval No: [E-21071282-050.99-572988], Date: [27 January 2023]). Extracted human teeth were collected as anonymous biological waste materials from routine dental extractions; therefore, individual informed consent was waived according to institutional policy. All procedures were conducted in accordance with the ethical standards of the institutional and/or national research committee and the 1964 Declaration of Helsinki and its subsequent amendments.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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