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Effects of calcium hydroxide and Biodentine contamination on the bond strength of universal adhesives to deep dentin

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Abstract

Objective: This study aimed to evaluate the effects of calcium hydroxide (CH) and Biodentine contamination on the shear bond strength (SBS) of three universal adhesives to deep dentin.

Methods: Deep dentin surfaces were prepared from 90 extracted human third molars and randomly assigned to three dentin conditions: an uncontaminated control, calcium hydroxide (CH) contamination, or Biodentine contamination. Within each condition, specimens were allocated to Scotchbond Universal Plus, Gluma Bond Universal, or G-Premio BOND Universal (n = 10 per group), resulting in a 3 × 3 factorial design. All adhesives were applied in self-etch mode. Following composite build-up, specimens underwent 5,000 thermal cycles, after which SBS and failure modes were assessed. Data were analyzed using two-way analysis of variance (ANOVA) followed by the Bonferroni post-hoc test.

Results: Dentin condition and adhesive system significantly affected SBS (both $P < 0.001$), whereas their interaction was not significant ($P = 0.362$). Uncontaminated dentin exhibited significantly higher SBS than both the CH- and Biodentine-contaminated dentin groups (both $P < 0.001$), whereas no significant difference was detected between the two pulp-capping materials ($P = 0.483$). G-Premio BOND Universal demonstrated significantly higher SBS than Scotchbond Universal Plus ($P = 0.006$) and Gluma Bond Universal ($P < 0.001$). Adhesive failure was the predominant failure mode, and the distribution of failure modes did not differ significantly among groups ($P = 0.101$).

Conclusions: Calcium hydroxide and Biodentine contamination reduced SBS of universal adhesives to dentin. G-Premio BOND Universal demonstrated the highest overall SBS, irrespective of the dentin condition.

Keywords: Calcium Silicates, Calcium Hydroxide, Dentin-Bonding Agents, Dental Pulp Capping, Dentin, Shear Strength

Introduction

Calcium hydroxide (CH)-based liners and calcium silicate (CS)-based cements, including Biodentine, are widely used in vital pulp therapy (1, 2). Calcium hydroxide has antibacterial properties and can induce reparative dentin formation. However, its solubility, limited sealing ability, and long-term instability have contributed to the increasing use of calcium silicate-based materials, which offer more favorable biological and sealing properties (1, 3).

During the treatment of deep cavities, pulp-capping materials may come into contact with adjacent dentin. Although visible excess material should be removed before adhesive restoration, material particles or contact-related changes in the dentin surface may remain after bulk removal and potentially affect surface chemistry, resin infiltration, polymerization, or hybrid-layer formation (4, 5). A recent in vitro study found lower bond strength in dentin close to indirect pulp-capping materials, suggesting that these materials may affect bonding to adjacent dentin (6).

Bonding to deep dentin is less predictable than bonding to superficial dentin because of its greater dentinal tubule density, higher intrinsic moisture, and lower proportion of intertubular dentin, which may impair adhesive infiltration and interfacial stability (7, 8). Moreover, the bonding performance of universal adhesives may vary according to their acidity, application strategy, solvent composition, co-monomers, functional monomers such as 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP), and substrate characteristics (8).

There is limited evidence regarding the effect of CH or Biodentine contamination (i.e., contact with CH or Biodentine followed by removal of the visible material) on the bonding performance of universal adhesives. Furthermore, universal adhesives differ in acidity,

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solvent composition, functional monomers, and application protocols, and the bonding performance of these systems on dentin exposed to pulp-capping agents remains unpredictable. Therefore, the present study was designed to assess the shear bond strength (SBS) of three universal adhesives (Scotchbond Universal Plus, Gluma Bond Universal, and G-Premio BOND Universal) to deep dentin following CH or Biodentine contamination. It was hypothesized that SBS would not be significantly influenced by the dentin condition or the specific adhesive system.

Materials and methods

Study design and sample size calculation

This in vitro study was approved by the Health Sciences Research Ethics Committee of Hacettepe University (Approval No. SBA 25/271), and informed consent was obtained from all donors before tooth extraction.

The study employed a two-factor factorial design, with dentin condition and adhesive system as the independent variables. Dentin condition comprised three levels: uncontaminated control, CH contamination, and Biodentine contamination. The adhesive system comprised three levels: Scotchbond Universal Plus, Gluma Bond Universal, and G-Premio BOND Universal.

The sample size was calculated using G*Power software (version 3.1.9.7; Heinrich Heine University Düsseldorf, Düsseldorf, Germany) for analysis of variance (ANOVA), assuming an effect size of 0.40, an alpha level of 0.05, and a statistical power of 80%. The calculation indicated that a minimum of 80 specimens was required. To ensure equal allocation across the nine experimental groups, 90 teeth were included, with 10 specimens assigned to each group. Specimens were randomized using a computer-generated sequence after preparation of the deep dentin surfaces.

Table 1 presents the commercial products used in this study and their application protocols.

Specimen preparation

Table 1. Commercial products and their application protocols used in this study

Commercial product	Material type	Principal components	Manufacturer	Application protocol
Scotchbond™ Universal Plus (SBUP)	Universal adhesive	10-MDP, HEMA, silane mixture, dimethacrylate resins, silica filler, Vitrebond copolymer, photoinitiators, ethanol, water	3M ESPE, St. Paul, MN, USA	Applied in self-etch mode by actively rubbing onto the dentin surface for 20 seconds, followed by gentle air drying for 5 seconds to evaporate the solvent and light curing for 10 seconds.
Gluma Bond Universal (GBU)	Universal adhesive	4-META, 10-MDP, methacrylate monomers, photoinitiator system, acetone, water	Kulzer GmbH, Hanau, Germany	Applied to the dentin surface and actively rubbed for 20 seconds. Excess solvent was removed by gentle air drying for 5 seconds, followed by light curing for 10 seconds.
G-Premio BOND Universal (GPBU)	Universal adhesive	10-MDP, 4-MET, MDTP, dimethacrylate monomers, silica, initiator, stabilizer, acetone, water	GC Corporation, Tokyo, Japan	Applied to the dentin surface and left undisturbed for 10 seconds. The surface was thoroughly air-dried for at least 5 seconds until the solvent had evaporated, followed by light curing for 10 seconds.
Life™ (CH)	Calcium hydroxide-based liner (two-paste)	Calcium hydroxide, zinc oxide, and glycol salicylate	Kerr, Orange, CA, USA	Equal amounts of the base and catalyst pastes were mixed for 10–15 seconds to obtain a homogeneous mixture. The material was applied as a 0.5–1.0-mm-thick layer onto deep dentin and left undisturbed for 2–3 minutes to allow chemical setting.
Biodentine™	Calcium silicate-based cement	Tricalcium silicate, calcium carbonate, zirconium oxide, and calcium chloride in aqueous solution	Septodont, Saint-Maur-des-Fossés, France	The capsule was activated and mixed in a high-speed amalgamator for 30 seconds. The mixed material was applied as a uniform layer approximately 1 mm thick and left undisturbed for 12 minutes to allow initial setting.
Tetric® N-Ceram Bulk Fill	Nanohybrid resin-based composite	Bis-GMA, Bis-EMA, UDMA, barium aluminum silicate glass fillers/Isofiller, Ivocerin	Ivoclar Vivadent, Schaan, Liechtenstein	Placed in a single 3-mm increment according to the manufacturer's recommended bulk-fill thickness and light-cured for 20 seconds.

Ninety freshly extracted, caries-free human third molars were screened under 2.5 × magnification to exclude specimens with cracks, structural defects, caries, restorations, or developmental abnormalities. Following extraction, the teeth were stored in 0.1% thymol solution at 4°C and tested within 3 months of extraction. The roots were removed using a water-cooled low-speed diamond saw, and the crowns were embedded in autopolymerizing acrylic resin.

Figure 1 presents a schematic overview of the procedures used in this study. Flat dentin surfaces were prepared by removing the occlusal enamel and underlying dentin with a diamond disc until the outline of the pulp chamber was visible, while maintaining an approximately 0.5-mm-thick dentin barrier. The surfaces were then sequentially polished with 280-, 400-, and 600-grit silicon carbide papers under running water to standardize the smear layer. Specimens with pulpal exposure were excluded. The specimens were then ultrasonically cleaned in distilled water for 10 minutes to remove debris and examined under a light microscope at 10 × magnification to confirm the absence of pulpal exposure. Specimens exhibiting pulpal exposure or visible defects were excluded and replaced before randomization.

Grouping and dentin conditions

The prepared specimens were assigned to one of three dentin condition groups (n = 30), as follows:

- *Uncontaminated control*: No pulp-capping material was applied to specimens in the control group.
- *CH contamination*: A calcium hydroxide liner (Life™; Kerr, Orange, CA, USA) was mixed according to the manufacturer's instructions and applied as a 0.5–1.0-mm-thick layer to the prepared deep dentin surface. The material was allowed to remain undisturbed for 2–3 minutes. The visible bulk of the material was then carefully removed using a hand excavator without applying pressure to the dentin surface, followed by gentle wiping of the entire

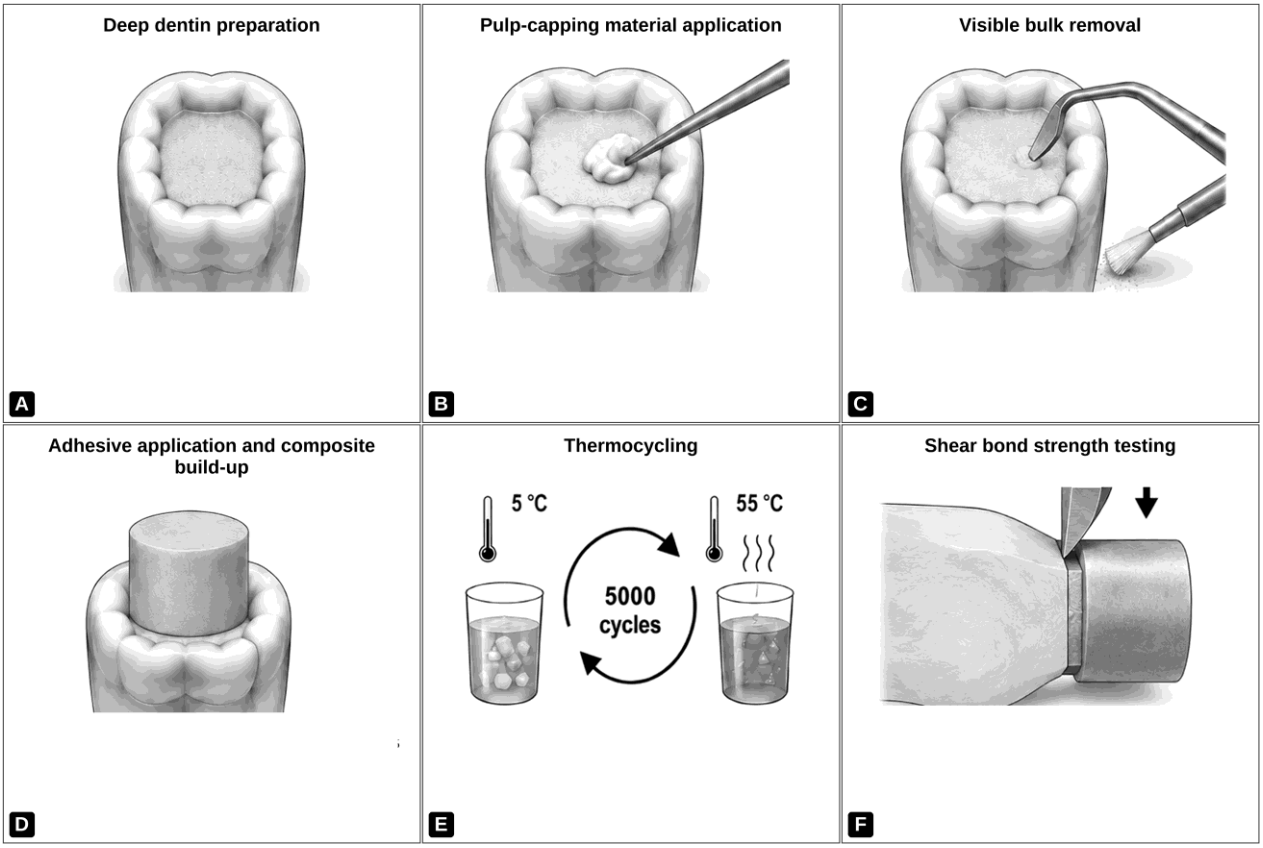

Figure 1. Schematic representation of specimen preparation and shear bond strength (SBS) testing. (A) Standardized deep-dentin preparation with approximately 0.5 mm of residual dentin between the prepared surface and the pulp chamber; (B) application of calcium hydroxide or Biodentine as a uniform 0.5–1.0-mm-thick layer; (C) removal of the visible material with a hand excavator, followed by microbrush cleaning; (D) application of a self-etch adhesive and fabrication of a composite cylinder measuring 2.5 mm in diameter and 3 mm in height; (E) thermocycling for 5,000 cycles between 5°C and 55°C, with a dwell time of 30 seconds and a transfer time of 10 seconds; and (F) measurement of shear bond strength using a chisel-shaped blade at a crosshead speed of 1 mm/min.

contacted surface with a microbrush to remove any remaining visible material.

- *Biodentine contamination*: Biodentine™ (Septodont, Saint-Maur-des-Fossés, France) was prepared according to the manufacturer's instructions. The capsule was activated and mixed for 30 seconds, and the material was applied as a uniform layer approximately 1 mm thick to the prepared deep dentin surface. The material was allowed to remain undisturbed for 12 minutes. The visible bulk was then carefully removed using a hand excavator without pressure on the dentin surface, followed by gentle wiping of the entire contacted surface with a microbrush.

To minimize operator-dependent variability, the same operator performed all contamination procedures using the same standardized two-step application and removal sequence.

Adhesive application

Within each dentin condition, specimens were assigned to one of three universal adhesive systems (n = 10). All adhesives were applied in the self-etch mode according to the manufacturers' instructions. The study groups were as follows:

- *Scotchbond Universal Plus:* Scotchbond Universal Plus (3M ESPE, St. Paul, MN, USA) was actively rubbed onto the dentin surface for 20 seconds, followed by gentle air-drying for 5 seconds and light-curing for 10 seconds.
- *Gluma Bond Universal:* Gluma Bond Universal (Kulzer GmbH, Hanau, Germany) was actively rubbed onto the dentin surface for 20 seconds, followed by gentle air-drying for 5 seconds and light-curing for 10 seconds.
- *G-Premio BOND Universal:* G-Premio BOND Universal (GC Corporation, Tokyo, Japan) was applied to the dentin surface and allowed to remain undisturbed for 10 seconds, followed by thorough air-drying for at least 5 seconds and light-curing for 10 seconds.

All adhesives were light-cured using an Elipar™ LED light-curing unit (3M, St. Paul, MN, USA) at an irradiance of 1200 mW/cm². The irradiance was verified with a dental radiometer before specimen preparation.

Composite build-up

A cylindrical plastic mold with an internal diameter of 2.5 mm and a height of 3 mm was positioned on the bonded dentin surface. A bulk fill composite resin (Tetric N-Ceram Bulk Fill; Ivoclar Vivadent AG, Schaan, Liechtenstein) was placed into the mold as a single 3-mm increment and light-cured for 20 seconds using the same LED light-curing unit. After polymerization, the mold was carefully removed.

Thermocycling

Following composite build-up, all specimens were subjected to 5,000 thermal cycles between 5°C and 55°C using a thermocycling device (MTE-101; MOD Dental, Esetron Smart Robotechnologies, Ankara, Türkiye). Each cycle consisted of a 30-second dwell time in each water bath and a 10-second transfer time between baths. Following thermocycling, the specimens were subjected to shear bond strength (SBS) testing.

Shear bond strength measurement

SBS was determined using a universal testing machine (LR5K; Lloyd Instruments, Fareham, Hampshire, UK). Each specimen was secured in a custom-made holding device with the bonded dentin surface positioned parallel to the direction of the applied shear load. A chisel-shaped blade was positioned as close as possible to the adhesive interface at the base of the resin composite cylinder without contacting the dentin surface. The load was applied parallel to the bonded interface at a crosshead speed of 1 mm/min until failure occurred. The maximum load at failure was recorded in Newtons (N). Shear bond strength (SBS) was calculated in megapascals (MPa) by dividing the failure load by the bonded area. For a cylindrical mold with an internal diameter of 2.5 mm, the bonded area was calculated as 4.91 mm² using the formula πr^2 (r = 1.25 mm).

Failure mode analysis

The fractured surfaces were examined at 20 × magnification using an optical microscope and classified as adhesive, cohesive, or mixed failure according to previously described criteria (9, 10).

Adhesive failure: Adhesive failure was defined as failure in which at least 80% of the fractured surface occurred at the adhesive–dentin interface, with no substantial cohesive involvement of either the dentin or the resin composite (9, 10).

Cohesive failure: This type of failure was defined as failure occurring predominantly within dentin or resin composite, with at least 80% of the fractured area confined to one substrate.

Mixed failure: Mixed failure was defined as a combination of adhesive and cohesive components in which neither component accounted for at least 80% of the fractured area.

The distribution of failure modes was recorded for each experimental group.

Statistical analysis

Statistical analyses were performed using SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, NY, USA). The normality of the SBS data was assessed using the Shapiro–Wilk test, and the homogeneity of variances was assessed by Levene's test.

A two-way ANOVA was performed with dentin condition and adhesive system as fixed factors. The material condition × adhesive system interaction was evaluated, followed by assessment of the main effects. When significant main effects were detected, post hoc pairwise comparisons were performed using the Bonferroni test.

Failure-mode distributions were analyzed using the Fisher–Freeman–Halton exact test because of sparse expected frequencies. Statistical significance was set at $P < 0.05$.

Results

Shear bond strength

Table 2 presents the shear bond strength (SBS) values for the experimental groups. No pre-test failures occurred during specimen preparation or thermocycling, and all 90 specimens were included in the analysis.

Two-way analysis of variance (ANOVA) demonstrated significant main effects of dentin condition ($P < 0.001$) and adhesive system ($P < 0.001$) on SBS. The dentin condition × adhesive system interaction was not statistically significant ($P = 0.362$; Table 2). Therefore, the main effects were interpreted independently.

Regarding dentin condition, the uncontaminated control showed significantly higher overall SBS (11.15 ± 2.69 MPa) than both the CH contamination (6.98 ± 1.84 MPa, $P < 0.001$) and the Biodentine contamination (7.74 ± 1.65 MPa, $P < 0.001$) groups, whereas the CH and Biodentine contamination groups did not differ significantly ($P = 0.483$).

Regarding adhesive systems, G-Premio BOND Universal demonstrated significantly higher overall SBS (9.99± 2.61 MPa) than Scotchbond Universal Plus (8.27± 2.95 MPa, $P = 0.006$) and Gluma Bond Universal (7.62± 2.72MPa, $P < 0.001$). No significant difference was observed between Scotchbond Universal Plus and Gluma Bond Universal ($P = 0.667$).

Failure mode analysis

Figure 2 shows the distribution of failure modes across the experimental groups. Adhesive failure was observed in 88 of 90 specimens (97.8%) and was the predominant failure mode in the experimental groups. One cohesive failure and one mixed failure were observed, both in the uncontaminated control group bonded with G-Premio BOND Universal adhesive system. The distribution of failure modes did not differ significantly among the study groups ($P = 0.101$).

Discussion

The present study evaluated the effects of dentin contamination with pulp capping materials on shear bond strength of several adhesive systems to deep dentin. The experimental model was designed to evaluate the effect of prior contact with pulp-capping materials followed by visible bulk removal (i.e., dentin contamination) on bonding to deep dentin. This approach allowed standardized evaluation of the effect of pulp-capping material contact on adhesive bonding.

Both calcium hydroxide (CH) contamination and Biodentine contamination resulted in significantly lower SBS than the uncontaminated control group, while the two contaminated groups did not differ significantly. G-Premio BOND Universal demonstrated the highest overall SBS among the tested adhesives, irrespective of

Table 2. Mean and standard deviation of shear bond strength (MPa) for the study groups according to dentin condition and adhesive system

Dentin condition	Scotchbond Universal Plus	Gluma Bond Universal	G-Premio BOND Universal	Total
	Mean ± SD	Mean ± SD	Mean ± SD	
Uncontaminated control	10.88 ± 2.99	9.76 ± 2.96	12.82 ± 1.77	11.15 ± 2.69^{a}
CH contamination	7.00 ± 2.05	6.47 ± 2.22	7.48 ± 1.21	6.98 ± 1.84^{b}
Biodentine contamination	6.94 ± 1.88	6.62 ± 1.57	9.66 ± 1.16	7.74 ± 1.65^{b}
Total	8.27 ± 2.95^{A}	7.62 ± 2.72^{A}	9.99 ± 2.61^{B}	8.62 ± 2.71
The effect of dentin condition	P < 0.001			
The effect of adhesive system	P < 0.001			
The interaction of dentin condition × adhesive system	P = 0.362			

Different superscript lowercase letters within the same column indicate significant differences among dentin conditions ($P < 0.05$). Different superscript uppercase letters within the same row indicate significant differences among adhesive systems ($P < 0.05$).

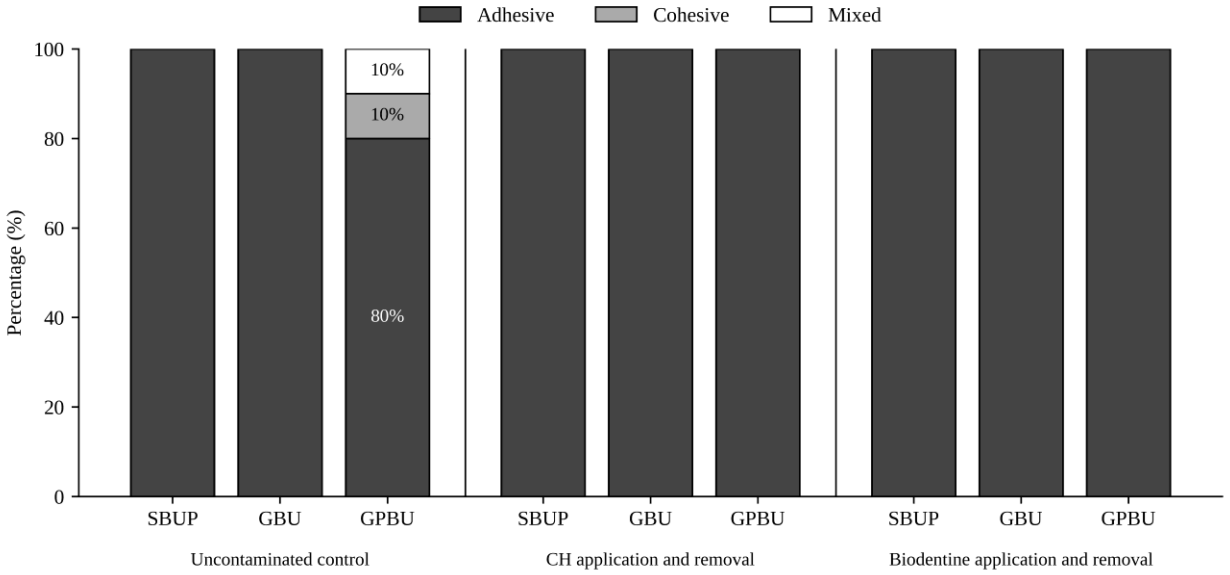

Figure 2. Distribution of failure modes among the experimental groups. Stacked bars show the percentages of adhesive, cohesive, and mixed failures according to pulp-capping material condition and adhesive system. CH: calcium hydroxide; SBUP: Scotchbond Universal Plus; GBU: Gluma Bond Universal; GPBU: G-Premio BOND Universal.

the dentin condition. No significant difference in bond strength was observed between Scotchbond Universal Plus and Gluma Bond Universal.

Bonding to deep dentin is challenging because of its greater dentinal tubule density, higher intrinsic moisture, and lower proportion of intertubular dentin. These factors may reduce micromechanical retention and complicate solvent evaporation and monomer infiltration (11-13), and therefore compromise adhesive bonding to deep dentin. In the present study, the dentin specimens were prepared in close proximity to the pulp chamber to simulate the highly permeable and moisture-rich substrate encountered in deep cavities.

Neither pulp-capping material was intentionally retained on the dentin surface. The visible bulk was mechanically removed with a hand excavator, and the surface was gently wiped with a dry microbrush to remove loose remnants of the material. This approach allowed removal of the visible bulk while avoiding excessive cleaning that could eliminate clinically relevant microscopic remnants. Thus, the lower SBS observed in the CH and Biodentine groups may be related to residual microscopic material or changes in the dentin surface caused by prior material contact, which may affect subsequent adhesive bonding (6, 14).

The CH contamination resulted in lower SBS than the uncontaminated control. Life is a salicylate-based, two-paste calcium hydroxide liner containing calcium hydroxide, zinc oxide, and glycol salicylate; therefore, the observed reduction in SBS cannot be attributed solely to calcium hydroxide or its alkalinity. Residual calcium-containing particles, remnants of the pulp-capping material, or contact-induced changes in the dentin surface could potentially alter wetting, monomer penetration, or interaction with mineralized dentin. Ballal et al. (14) reported that alkaline biomaterial remnants increased dentin alkalinity and reduced the bond strength of resin-based adhesives.

The Biodentine contamination also resulted in significantly lower SBS than the uncontaminated control. Biodentine is a tricalcium silicate-based cement. During hydration, it forms calcium silicate hydrate and calcium hydroxide and releases calcium and hydroxyl ions. Longer-term investigations of Biodentine–dentin interfaces have described tag-like structures, mineral infiltration zones, and calcium- and phosphate-containing interfacial layers (15, 16). However, these studies used maturation periods substantially longer than the 12-minute initial setting period employed in the present study; therefore, the formation of a mature mineral infiltration zone cannot be assumed under the current protocol. The present findings propose that early hydration products, residual particles, or other contact-induced surface alterations may have persisted after bulk removal, potentially producing a heterogeneous dentin surface and affecting the interaction of the self-etch adhesives with deep dentin.

The CH and Biodentine contamination groups did not differ significantly in SBS. Both materials may have produced a less favorable bonding substrate through residual particulate material or contact-related substrate modification, although the underlying

physicochemical processes are unlikely to be the same (14-16). Because residual material and surface chemistry were not directly evaluated in this study, the relative contributions of these mechanisms to the reduction in SBS remain unclear.

In the present study, CH was allowed to remain undisturbed on deep dentin for 2–3 minutes, whereas Biodentine was left undisturbed for 12 minutes. The different contact periods used for CH and Biodentine in this study were based on their respective setting characteristics. Life is a calcium hydroxide pulp-capping material with a reported working time of approximately 2–3 minutes, whereas Biodentine has an initial setting time of approximately 9–12 minutes (17, 18).

The three universal adhesives differed in their composition and application protocols. G-Premio BOND Universal, Scotchbond Universal Plus, and Gluma Bond Universal all contained 10-MDP, but differed in their solvent systems, functional monomers, and other components. Scotchbond Universal Plus is a mildly acidic adhesive (pH approximately 2.7), whereas Gluma Bond Universal and G-Premio BOND Universal are more acidic, with pH values of approximately 1.6–1.8 and 1.5, respectively. The compositional and procedural differences between these adhesives may have contributed to the observed variation in bonding performance.

In this study, G-Premio BOND Universal demonstrated the highest overall SBS among the adhesive systems. The higher bond strength of G-Premio BOND Universal may be due to the combined effects of its formulation and application protocol. Previous studies have similarly indicated that the bonding performance of universal adhesives is influenced by adhesive composition, solvent characteristics, monomer distribution, and polymerization behavior (19, 20). However, because these parameters and the resin–dentin interfacial morphology were not directly evaluated in the present study, the specific mechanisms underlying the differences in SBS among adhesive systems remain uncertain and warrant further investigation.

Several limitations of the present study should be acknowledged. Only the self-etch application mode was investigated. Aging was limited to 5,000 thermocycles and did not include prolonged water storage, artificial saliva exposure, or mechanical fatigue. Residual material, surface chemistry, and resin–dentin interfacial morphology were not directly characterized. Only one CH liner and one calcium silicate-based cement were evaluated, limiting generalization to other pulp-capping materials. In addition, the substrate-contact model did not reproduce clinical variables such as cavity geometry, moisture control, protective bases or liners, and other operative factors that may influence adhesive performance. Future studies should incorporate surface and interfacial characterization, longer-term aging protocols, and alternative adhesive strategies to further assess how the bond strengths of different adhesives are influenced by pulp-capping materials.

Conclusions

Within the limitations of this in vitro study, dentin contamination with calcium hydroxide or Biodentine caused a significant reduction in shear bond strength of universal adhesives compared with the uncontaminated dentin condition. Among the adhesives tested, G-Premio BOND Universal showed the highest overall SBS, irrespective of the dentin condition.

Acknowledgements

None.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

U.K.V. and E.U.Ö. contributed to the conceptualization and methodology of the study. U.K.V., B.E.K., and E.U.Ö. contributed to the study design and investigation. U.K.V. and C.B. contributed to data curation, formal analysis, and interpretation of the results. U.K.V. and E.U.Ö. contributed to manuscript preparation. All authors contributed to the critical revision of the manuscript. E.U.Ö. supervised the study. All authors read and approved the final manuscript.

Ethical considerations

This in vitro study was approved by the Health Sciences Research Ethics Committee of Hacettepe University (Approval No: SBA 25/271).

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