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ORIGINAL RESEARCH

Effect of theaflavins pretreatment on bond strength and microleakage to deciduous dentin: an *in vitro* study

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Abstract

Background: Reliable bonding between resin composite restorations and deciduous dentin is clinically challenging. Theaflavins (TFs), black tea-derived natural polyphenols with collagen cross-linking, matrix metalloproteinase (MMP)-inhibiting and antibacterial activities, have not been studied as a deciduous dentin pretreatment agent. This study evaluated 2% (w/v) TFs for improving bond strength and marginal sealing, with 2% chlorhexidine (CHX) and distilled water (DW) as controls. **Methods**: Forty-eight caries-free primary molars were randomly assigned to micro-tensile bond strength (μTBS, 30 teeth) or microleakage (18 teeth) testing. Each group was subdivided into TFs, CHX, and DW subgroups, and these were further split into immediate/aged testing. After pretreatment and adhesive/resin application, μTBS measurement, fracture mode observation, interface morphology analysis, and International Organization for Standardization (ISO)-standard microleakage assessment were conducted; data were analyzed via SPSS 26.0 ($\alpha = 0.05$). **Results**: μTBS: In both immediate and aged groups, TFs group depicted the highest strength, followed by CHX and DW ($p < 0.05$). Fracture mode: After aging, the DW group showed predominantly type I interfacial failure, while the CHX and TFs groups displayed fracture mode III—a mode not observed in the DW group. Interface morphology: TFs group had the most and longest resin tags, while the thickest and most intact hybrid layer were observed in both immediate and aged groups. Microleakage: In both immediate and aged groups, the TFs group was lower than DW ($p < 0.05$) but showed no significant difference from CHX group ($p > 0.05$). TFs group had no significant change in microleakage after aging ($p > 0.05$). **Conclusions**: The 2% TFs pretreatment significantly improved bond strength and reduced microleakage in deciduous dentin, as compared with those with 2% CHX.

Keywords

Dentin bonding; Bond durability; Theaflavins (TFs); Microtensile bond strength (μTBS); Microleakage; Deciduous teeth; Chlorhexidine (CHX)

1. Introduction

Early childhood caries remains a prevalent oral disease worldwide, affecting over 621 million children [1–3]. It arises from an imbalance between demineralization and remineralization. It is driven by the interactions between tooth structure and microbial biofilms [4, 5]. Resin bonding restoration is a main treatment for early childhood caries because of its aesthetics and minimal invasiveness [6, 7]. However, the long-term stability of deciduous dentin bonding is affected by low mineralization, high organic content, and low dentinal tubule density, which make the hybrid layer more susceptible to degradation [8, 9]. Host-derived matrix metalloproteinases (MMPs) are activated in acidic environments to hydrolyze the exposed collagen fibers [10–13]. The residual microorganisms in dentinal tubules may cause secondary caries and microleakage, and lead to restoration discoloration, postoperative sensitivity, or failure [14–16].

Disinfectants like chlorhexidine (CHX) are used to inhibit microbial biofilms and enhance bonding durability [17–19]. A broad-spectrum MMPs inhibitor, CHX can also stabilize the bonding interface of primary and permanent teeth and maintains hybrid layer integrity by chelating zinc/calcium ions [20–23]. However, its usage in the clinic is limited because of cytotoxicity, tissue irritation, and tooth discoloration [24–28].

Theaflavins (TFs) are polyphenols extracted from black tea that possess antibacterial, anti-HIV (Human Immunodeficiency Virus), anticancer, and cholesterol-lowering activities with minimal toxicity toward host tissues [29–32]. Previous studies have shown that TFs exhibit inhibitory effects on *Streptococcus mutans*, anti-inflammatory functions, and antibacterial effects against periodontal diseases [33, 34]. TFs contain benzotropolone skeletons having adjacent carbonyls (capable of hydrogen bonding) and aromatic rings with phenolic hydroxyl and galloyl groups. These structural features are critical for collagen cross-linking and stabilization [35–37]. Additionally, TFs also inhibit MMPs [38–40]. Previous studies have demonstrated that TFs at 2% exhibited optimal cross-linking impact on dentin collagen [37]. It protected the collagen and enabled it to resist degradation by collagenase [40]. However, TFs' impact on deciduous dentin bonding remains unclear despite these beneficial characteristics. This study is designed to test the null hypothesis (H_0): 2% TFs pretreatment has no significant difference in microtensile bond strength (μTBS) or microleakage as compared to those of 2% CHX or distilled water (DW). DW is used as a blank control and 2% CHX as a positive control to test the above hypothesis. This study compares μTBS and microleakage of deciduous dentin after treatment with 2% TFs, 2% CHX, or DW. The results will provide a scientific basis for TFs' clinical use in primary tooth restoration.

2. Materials and methods

2.1 Ethical approval and tooth collection

The study herein was approved by the Ethics Committee of the Affiliated Stomatological Hospital of Nanchang University (No. 2024-005). The written informed consent was obtained for all the extracted teeth. Forty-eight non-carious and unrestored primary molars were selected for the study. Following visual and tactile examinations, soft tissues were cleaned, and calculus and surface pigments by hand scalers from the teeth and the teeth were stored in 4 °C physiological saline (refreshed daily) for usage within 1 month.

2.2 Reagents preparation

Based on a previous study by Liu *et al*. [36], 2% theaflavin was selected as the pretreatment agent for this study. A 2% TFs solution was prepared by dissolving 2 grams of TFs powder (Macklin, China) in 100 mL of dimethyl sulfoxide (DMSO, 67-68-5, Macklin, Shanghai, China). The solution was stored at room temperature. Commercial 2% chlorhexidine gluconate (CHX, 18472-51-0, Macklin, Shanghai, China) and distilled water (DW, CC-4039-06, Feituo Biotechnology, Nanchang, Jiangxi, China) were employed as the controls.

2.3 Micro-tensile bond strength (μTBS) experiment

2.3.1 Preparation of dentin bonding surface

Thirty primary molars were roots- and pulp-free and embedded in orthodontic self-curing resin (Nissin Dental Materials, China) with the occlusal surfaces 2 mm above the resin. Occlusal enamel was thoroughly and uniformly removed by using low speed cutting machine (Unicut 150, Maige, Suzhou, Jiangsu, China), which was guided by the visual judgment based on color under 5× stereomicroscope (SMZ800, Nikon, Tokyo, Japan) (enamel having white and translucent appearance, while dentin as pale yellow and opaque) and tactile judgment based on tissue hardness (enamel being the hardest tissue in human body). Afterward, the dentin surfaces were polished sequentially with 240-, 320-, and 600-mesh silicon carbide sandpapers for a total of 1 minute, followed by ultrasonic cleaning for 8 minutes [41]. The surfaces underwent etching by Scotchbond™ Universal Etchant (41263, 3M, Saint Paul, MI, USA) for 10 seconds, and were rinsed with pressurized air-water spray for 10 seconds, and then air-dried for 5 seconds. The route map of μTBS is shown in Fig. 1.

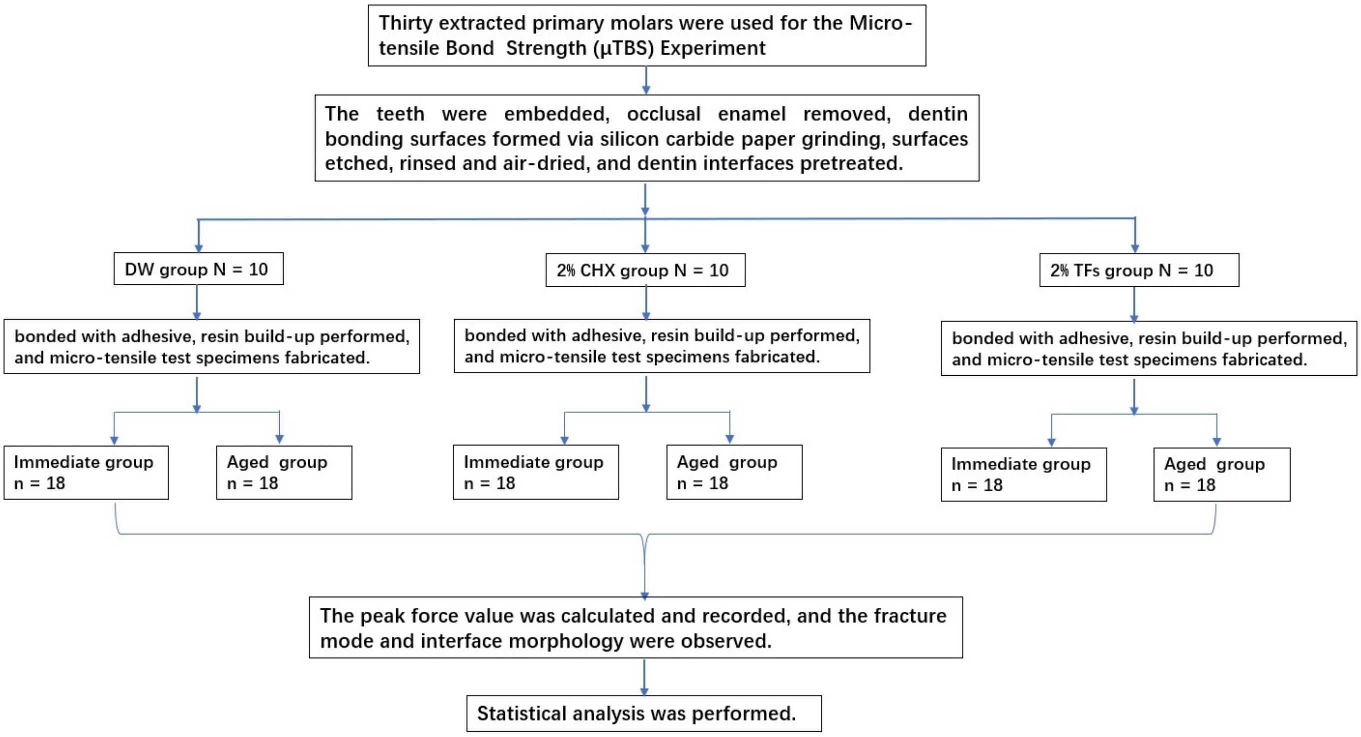


FIGURE 1. Experimental technical route map of μTBS. DW: distilled water; CHX: Chlorhexidine; TFs: Theaflavins.

2.3.2 Grouping and dentin surface pretreatment

The above prepared teeth were randomly divided into the TFs, CHX, and DW groups, 10 each, and then split into Immediate and Aged subgroups. Dentin surfaces were treated by corresponding reagents for 60 seconds [24, 36], followed by light blowing for 5 seconds.

2.3.3 Bonding and resin stacking

The following operations were performed according to the instructions of adhesive and light-cured resin: Single Bond Universal adhesive (3M, USA) was applied to the dentin surface and kept for 20 seconds [42], followed by air-blowing through three-way syringe for 5 seconds and light curing for 10 seconds.

Filtek™ Z350 XT universal nano-composite resin (3M, USA) was applied in incremental layers with each layer of maximum 2 mm thickness. All the layers were light-cured for 40 seconds. The total thickness of resin build-up was ~4 mm.

2.3.4 Preparation of micro-tensile specimens (n = 18)

Each bonded tooth was sectioned along tooth's long axis using hard tissue microtome (Unicut 150, Maige, Suzhou, Jiangsu, China) to prepare strips (1 × 1 × 10 mm) (Fig. 2A). Each tooth produced 2–4 specimens yielding a total of 18 specimens per group for the μTBS testing. All specimens were stored in 37 °C artificial saliva for 24 hours.

2.3.5 μTBS testing

Immediate group: Specimens were fixed to the test stage of micro-tensile tester (Bisco, Pittsburgh, PA, USA) by cyanoacrylate adhesive (Kisling, Switzerland) (Fig. 2B). The tester was operated at a crosshead speed of 1 mm/min until specimen fractured. The peak fracture load was recorded, and actual bonding area was measured by electronic vernier caliper. μTBS was calculated as fracture load/cross-sectional area (unit: MPa).

For the Aged group, specimens were placed in thermal cycling chamber for alternate water bath cycles: 30 seconds in a 55 (±0.5) °C hot water bath, followed by 30 seconds in cold at 5 (±0.5) °C, with a total of 1000 cycles. The aged specimens were then tested for μTBS using the same method as for the immediate group.

2.3.6 Observation of fracture modes

Fracture modes of specimens (before/after aging) were studied under 5× stereomicroscope (Nikon, Japan) and classified as follows (Fig. 3): Type I (Adhesive fracture): fractures at the bonding interface between deciduous dentin and resin with no resin residues on dentin surface; Type II (Cohesive fracture): fractures within the resin or deciduous dentin; Type III (Mixed fracture): combination of Type I and II with residual dentin or resin on the fracture surface [43].

2.3.7 SEM analysis of the interface

Representative specimens were sectioned along the bonding interface, etched by Scotchbond™ Universal Etchant (3M, USA) for 10 seconds, and rinsed for 10 seconds. After dehydration with ethanol, drying, and gold sputtering, the number/length of resin tags and hybrid layer thickness were analyzed by scanning electron microscopy (SEM; Sigma560, Zeiss, Oberkochen, BW, Germany) (500×/1000× magnification).

2.4 Microleakage experiment

2.4.1 Preparation of dentin bonding surface

The roots and coronal pulp were removed for the remaining 18 deciduous molars, and pulp chambers were sealed with flowable resin (3M, USA). The teeth were then embedded in self-curing resin to attain regular square models. A 4 × 2 × 1 mm occlusal Class I cavity was fabricated on tooth's occlusal surface using a BR-S45 bur (MANI, Japan) and measured with a calibrated periodontal probe (DEVEMED, Germany). The cavity floor was polished sequentially using 180-, 240-, 320-, and 600-mesh silicon carbide sandpapers (for a total of 1 minute), followed by the ultrasonic cleaning of 8 minutes to obtain dentin bonding surface. The route map of microleakage evaluation is shown in Fig. 4.

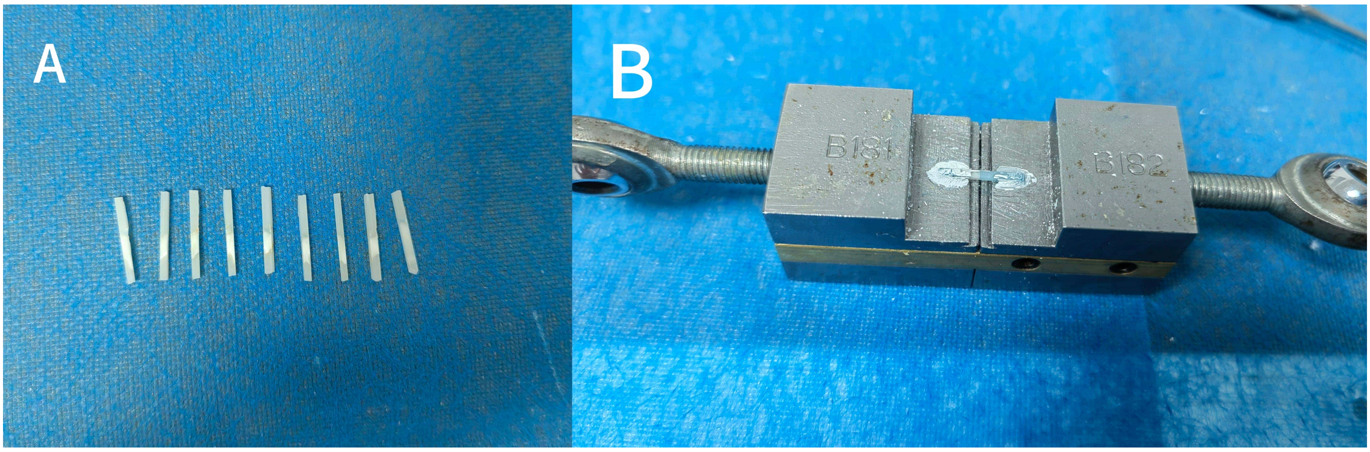


FIGURE 2. Micro-tensile test specimens and instruments. (A) Dentin specimen prepared; (B) Microtensile specimen fixed on tester.

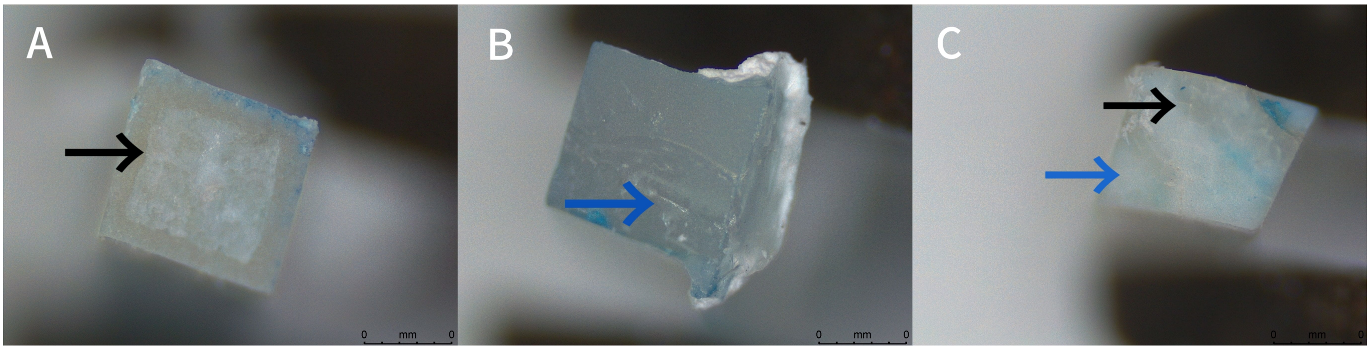


FIGURE 3. Images (A), (B) and (C) show Type I (Adhesive), Type II (cohesion) and Type III (mixed) fractures, respectively. The black arrows indicate bonding interface fracture; and the blue arrows indicate intra-resin fracture.

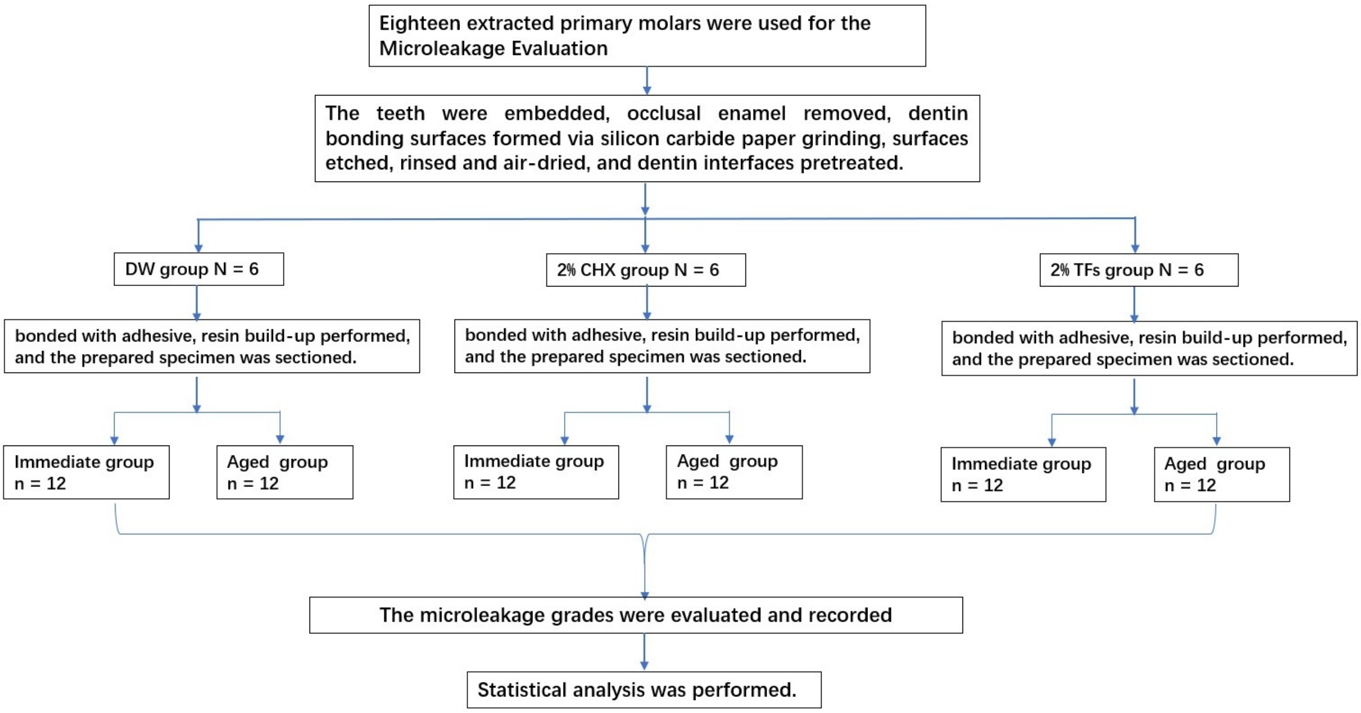


FIGURE 4. Experimental technical route map of microleakage evaluation. DW: distilled water; CHX: chlorhexidine; TFs: Theaflavins.

2.4.2 Grouping, pretreatment, bonding, and aging

Specimens were divided into 6 subgroups with 3 teeth in each (consistent with μTBS experiments): DW-Immediate, DW-Aged, CHX-Immediate, CHX-Aged, TFs-Immediate, TFs-Aged. Dentin pretreatment and resin bonding were accomplished by following the same protocol as in Section 2.3.2–2.3.3. Immediate subgroups were tested immediately, while aged undergone 1000 thermal cycles as indicated in Section 2.3.5.

2.4.3 Preparation of microleakage specimens (n = 12)

Teeth with completed bonding were coated by two layers of nail polish (Feituo Biotechnology, China) ~1 mm outside the preparation area. The teeth were then immersed for 24 hours in 0.5% toluidine blue aqueous solution (FZ-0014646, Feituo Biotechnology, Nanchang, Jiangxi, China). After removal, excess dye was cleaned off from the specimen surfaces. The teeth were sectioned into 1 mm-thick slices using hard tissue microtome. Each tooth produced 4 slices yielding 12 slices per group. All slices underwent microleakage evaluation.

2.4.4 Microleakage evaluation

A single tester observed and recorded the dye penetration at filling margins under a 5× stereomicroscope [44]. Grading followed the ISO standard (ISO/Technical Specification (TS) 11405:2015) with microleakage classified in 5 grades: Grade 0, No dye penetration; Grade 1, Minimal dye penetration but not exceeding the enamel-dentin junction; Grade 2, Dye penetration beyond enamel-dentin junction, however not exceeding 1/2 of the dentin thickness under filling; Grade 3: Dye penetration beyond 1/2 of dentin thickness but not reaching the cavity floor or reaching the floor without merging with dye on opposite side; Grade 4: Dye merging with dye on the opposite side [45] (Fig. 5).

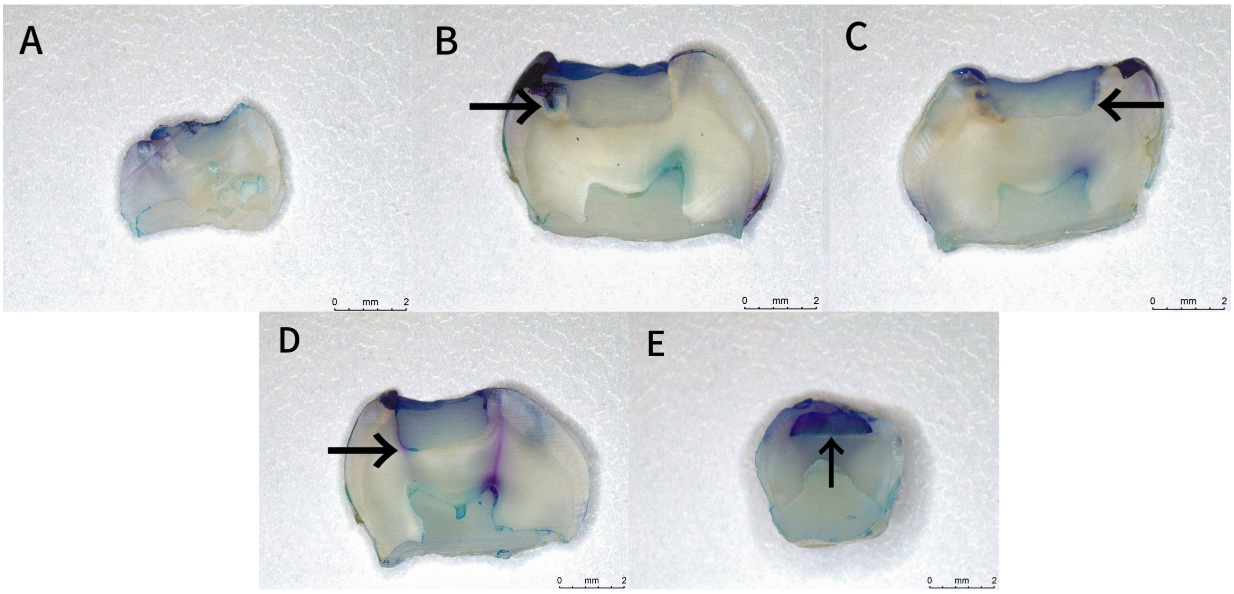


FIGURE 5. Microleakage grades (body microscope, 5×). (A) Grade 0; (B) Grade 1; (C) Grade 2; (D) Grade 3; (E) Grade 4. Black arrows indicate leakage depth.

2.5 Statistical analysis

The μTBS data were expressed as mean ± standard deviation ($\bar{x} \pm s$). A normal distribution was confirmed by the Shapiro-Wilk test ($p > 0.05$). One-way analysis of variance (ANOVA) and Least Significant Difference (LSD) *post-hoc* test were employed to compare the differences between groups. The bond strength before and after aging was compared by Student *t*-test to determine statistical significance. Non-parametric rank sum test was used to analyze the microleakage data. The significance level was set at $\alpha = 0.05$, and $p < 0.05$ was considered as statistically significant. The data were analyzed using SPSS 26.0 (IBM Corporation, Armonk, NY, USA) program.

2.6 Quality control of experiments

This study implemented standardized quality control measures to reduce the impact of operational errors on data stability. All specimens were prepared by the same operator to ensure consistency. The adhesive application time and light-curing parameters were calibrated in bonding process to avoid human-induced operational variations. The loading speed for mechanical testing was automatically controlled by the instrument.

3. Results

Micro-tensile Bond Strength (μTBS): Immediate group: TFs group had the highest μTBS (32.26 ± 1.68 MPa), which was significantly higher than the CHX (25.90 ± 1.60 MPa) and DW (24.13 ± 1.21 MPa) groups ($p < 0.05$ for both) (Table 1). The μTBS of all groups after aging was decreased with the TFs group remaining at the highest (27.61 ± 2.09 MPa, $p < 0.05$), compared with the other two groups (Table 2).

TABLE 1. Bond strength of the immediate group (n = 18, $\bar{x} \pm s$).

Group	Bond Strength (MPa)	*p* value compared with Group A
DW	24.13 ± 1.21	-
CHX	25.90 ± 1.60	<0.001
TFs	32.26 ± 1.68	<0.001

DW: distilled water; CHX: chlorhexidine; TFs: Theaflavins.

TABLE 2. Bond strength of the aged group (n = 18, $\bar{x} \pm s$).

Group	Bond Strength (MPa)	*p* value compared with Group A
DW	16.88 ± 1.26	-
CHX	23.74 ± 1.67	<0.001
TFs	27.61 ± 2.09	<0.001

DW: distilled water; CHX: chlorhexidine; TFs: Theaflavins.

Statistical analysis of data before and after simulated aging revealed statistically significant differences for all three groups (Table 3). These suggest that simulated aging had varying degrees of impact on the bonding performance of the different groups. Nevertheless, the aging impact on CHX and TFs groups was less than that on DW group. It indicates that the dentin pretreatments using CHX or TFs incurred stability and durability to deciduous dentin bonding.

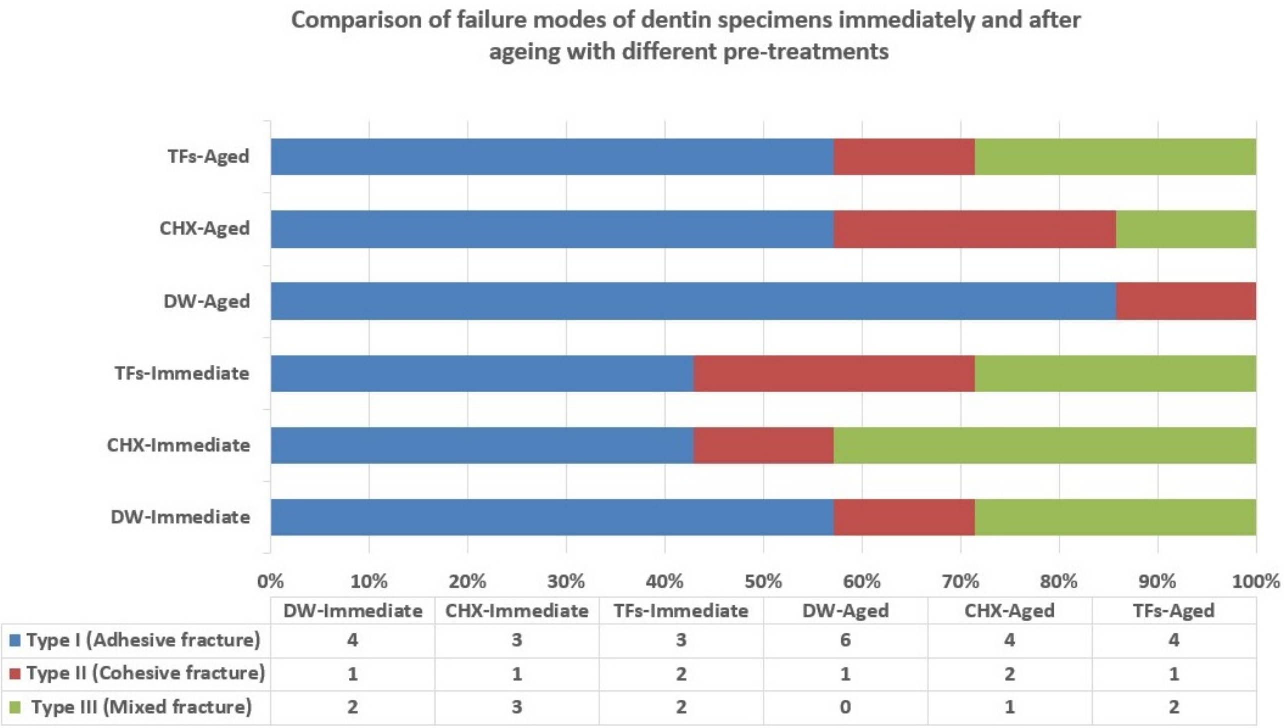
Fracture Mode: As shown in Fig. 6, all immediate groups displayed predominantly Type I and Type III failures. Among the aged groups, the DW group predominantly exhibited fracture mode I, while the CHX group and the TFs group, in addition to fracture modes I and II, also displayed fracture mode III—a mode not observed in the DW group—with a higher proportion of mixed/cohesive failures (linked to stronger bonding).

SEM analysis: Among the Immediate groups (Fig. 7), only a small number of resin tags were observed in the DW group with

TABLE 3. Comparison of bond strengths between immediate and aged groups with different pretreatments (n = 18, $\bar{x} \pm s$).

Immediate Group		Aged Group		t	p
Group	Bond Strength (MPa)	Group	Bond Strength (MPa)		
DW	24.13 ± 1.21	DW	16.88 ± 1.26	17.603	<0.001
CHX	25.90 ± 1.60	CHX	23.74 ± 1.67	3.959	<0.001
TFs	32.26 ± 1.68	TFs	27.61 ± 2.09	7.348	<0.001

DW: distilled water; CHX: chlorhexidine; TFs: Theaflavins.



	DW-Immediate	CHX-Immediate	TFs-Immediate	DW-Aged	CHX-Aged	TFs-Aged
🟦 Type I (Adhesive fracture)	4	3	3	6	4	4
🟥 Type II (Cohesive fracture)	1	1	2	1	2	1
🟩 Type III (Mixed fracture)	2	3	2	0	1	2

FIGURE 6. Comparison of failure modes of dentin specimens tested immediately and after ageing. DW: distilled water; CHX: chlorhexidine; TFs: Theaflavins.

short lengths and no cross-linking, while moderate increases were observed with the CHX group. In contrast, the TFs group had significantly more resin tags at dentin bonding interface along with the marked increase in penetration length, as well as the thickness and extent of hybrid layer.

Following aging (Fig. 8), destruction of the hybrid layer was indicated in the DW group at dentin bonding interface with visible gaps between the dentin and resin. The CHX group had increased number of resin tags at the dentin bonding interface, while a small number of micro-gaps was also present. The TFs group had significant increases in the number of resin tags at dentin bonding interface with intact hybrid layer but with no visible gaps.

These results demonstrated that pretreatments with TFs solution could improve the stability of bonding interface, reduce hybrid layer destruction, and enhance dentin bonding durability. Taken all together, these results rejected the null hypothesis (H_0) to demonstrate that pretreatments by 2% TFs could significantly improve the immediate and post-aging bond strength of deciduous dentin.

Microleakage Evaluation: Microleakage severity from the highest to lowest in immediate groups was ranked as follows: DW > CHX > TFs (Table 4). Inter-group comparisons indicated that the microleakage grade of the TFs group was significantly lower compared with the DW group ($p < 0.05$), while no statistically significant differences existed between the CHX and DW groups, or between the TFs and CHX groups ($p > 0.05$). All groups after thermal cycling aging depicted increased microleakage, although the order of severity was unchanged (DW > CHX > TFs). The microleakage grade of TFs group after aging was significantly lower compared with that of the DW group ($p < 0.05$), and no statistically significant differences existed between the CHX and DW groups, or between the TFs and CHX groups ($p > 0.05$). Intra-group comparisons suggest that the microleakage severity of the DW group was more significant after aging than the immediate group ($p < 0.05$), whereas the CHX and TFs groups had no significant changes before and after aging ($p > 0.05$). Again, these results rejected the null hypothesis (H_0) and suggest that pretreatments with 2% TFs can reduce the microleakage and maintain the stability of marginal sealing after aging.

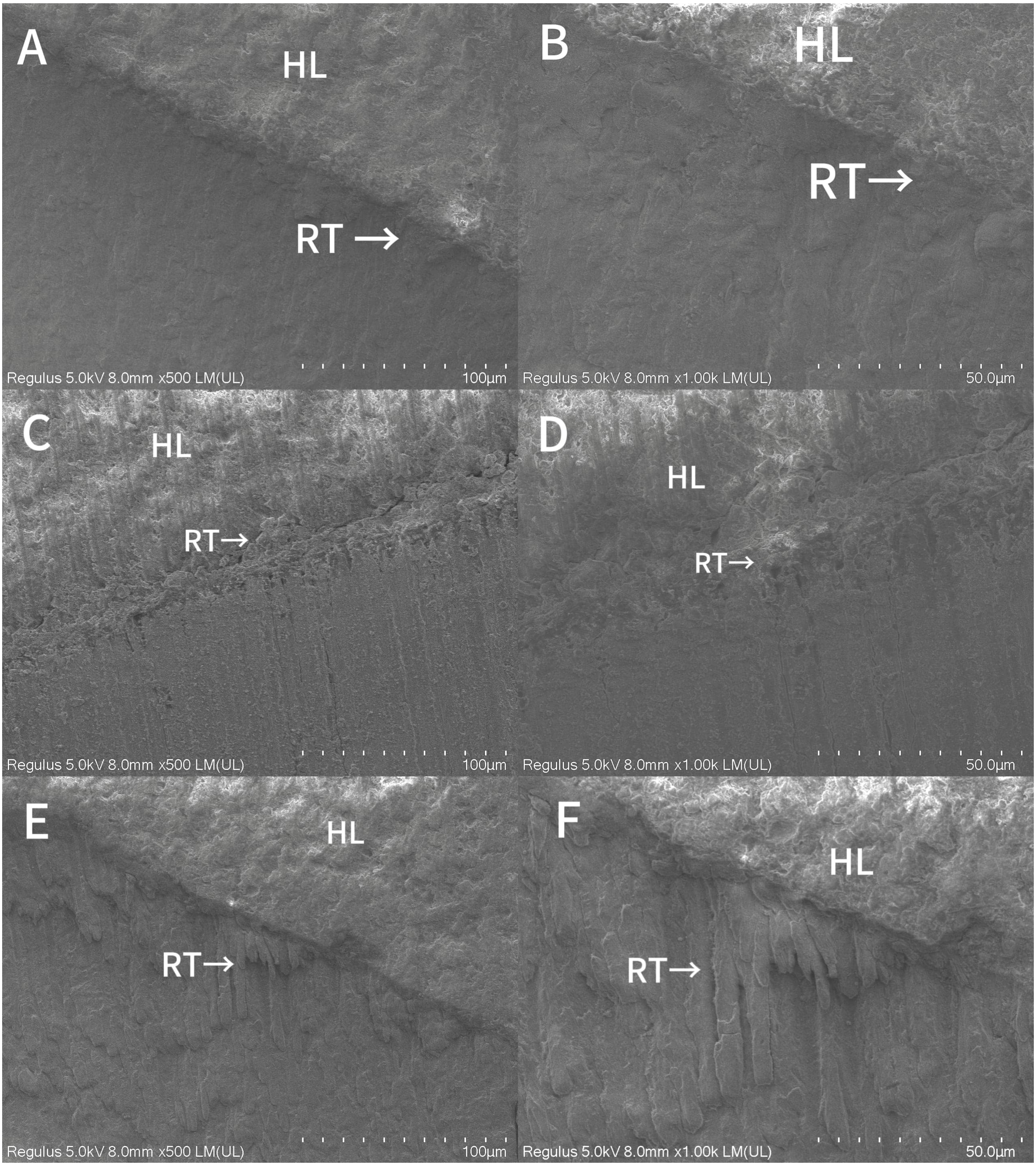


FIGURE 7. The bonding interface morphology of the immediate groups pretreated with various solutions under SEM. (A) Distilled water pretreatment group (500×); (B) Distilled water pretreatment group (1000×); (C) CHX pretreatment group (500×); (D) CHX pretreatment group (1000×); (E) TFs pretreatment group (500×); (F) TFs pretreatment group (1000×). HL: Hybrid layer; RT: Resin tag.

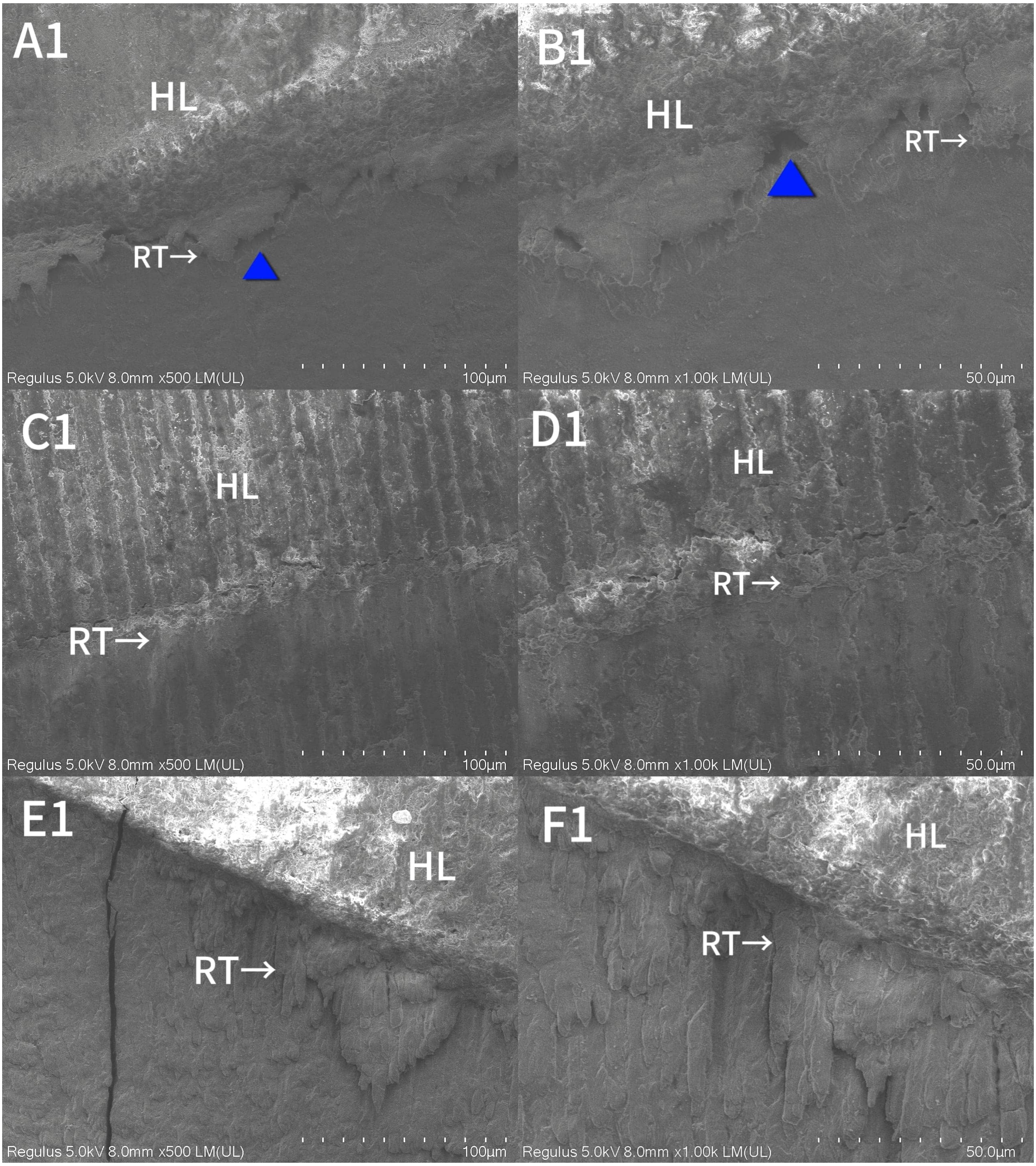


FIGURE 8. The bonding interface morphology of the aged groups pretreated with various solutions under SEM. (A1) Distilled water pretreatment group (500×); (B1) Distilled water pretreatment group (1000×); (C1) CHX pretreatment group (500×); (D1) CHX pretreatment group (1000×); (E1) TFs pretreatment group (500×); (F1) TFs pretreatment group (1000×). HL: Hybrid layer; RT: Resin tag. Blue triangular icons indicate cracks.

TABLE 4. Comparison of microleakage before and after aging with different pretreatment agents (n, %).

Group	n	Microleakage Grade					z	p
		0	1	2	3	4		
DW-Immediate	12	0 (0.00)	2 (16.67)	5 (41.67)	4 (33.33)	1 (8.33)	−2.041	0.041
DW-Aged	12	0 (0.00)	1 (8.33)	2 (16.67)	3 (25.00)	6 (50.00)		
CHX-Immediate	12	1 (8.33)	2 (16.67)	5 (41.67)	3 (25.00)	1 (8.33)	−1.477	0.140
CHX-Aged	12	0 (0.00)	1 (8.33)	4 (33.33)	4 (33.33)	3 (25.00)		
TFs-Immediate	12	1 (8.33)	5 (41.67)	5 (41.67)	1 (8.33)	0 (0.00)	−1.856	0.063
TFs-Aged	12	1 (8.33)	2 (16.67)	3 (25.00)	4 (33.33)	2 (16.67)		

$p < 0.05$ indicates significant differences. DW: distilled water; CHX: chlorhexidine; TFs: Theaflavins.

4. Discussion

The results presented here have shown that TFs at 2% as a pretreatment agent for deciduous dentin enhanced the immediate bond strength, long-term stability, and marginal sealing of resin-dentin bonds. The TFs has displayed superior performance compared with that of commonly used 2% CHX solution. These results reject the null hypothesis (H_0) and strongly suggest that 2% TFs pretreatment is significantly more effective than 2% CHX in improving the bond strength of deciduous dentin and reducing microleakage.

Deciduous dentin compromises the long-term stability of resin bonding because of its low mineralization, high organic content, unique dentinal tubule structure, and high endogenous matrix metalloproteinases (MMPs) activity [46]. Strategies for improving the bonding durability include the inhibition of MMPs activity to protect collagen or using cross-linking agents to strengthen the collagen network. Chemical inhibitors, such as CHX, can inhibit MMPs, but they have limitations, including cytotoxicity [25–28]. Some natural cross-linking agents, such as proanthocyanidins (PA), also cause discoloration [47]. However, TFs being the natural polyphenol extract of black tea depict dual biological activities, *i.e.*, collagen cross-linking and MMPs inhibition, along with biosafety [48–50]. It is considered a promising novel approach in addressing the challenges of deciduous dentin bonding.

TFs are poorly soluble in water but readily soluble in organic solvents like DMSO, alcohols, and ethyl acetate. Previous research reveals that DMSO does not reduce short- or long-term bond strength between resin and dentin [51, 52]. In this study, TFs are thus dissolved in DMSO to prepare 2% TFs solution for deciduous dentin bonding experiments.

The bond strength of deciduous dentin in this study is evaluated by comparing the micro-tensile bond strength (μTBS) immediately and after aging. The μTBS is regarded as the gold standard for evaluating resin-dentin bonding performance because of operation simplicity, reproducibility, and realistic simulation capability to test the clinical bonding effects [53].

The common accelerated aging methods for simulating the aging process in oral environment include sodium hypochlorite solution immersion, water storage, pH cycling, and thermal cycling [54]. Immersion for 1 hour in 10% sodium hypochlorite solution can replicate the aging effects caused by 10,000 thermal cycles or 6 months of water storage [55]. Despite hypochlorite solution immersion being efficient and time saving, its oxidizing properties may denature collagen, which in turn interfere with the evaluation of true aging behavior of bonding interface. Moreover, water storage is time-consuming, while the mechanism of pH cycling affecting bonding interface remains controversial and lacks standardization. Thermal cycling was thus selected as the artificial aging method in this study. The literature also indicates that 1000 thermal cycles can simulate the 6-month physiological aging of primary teeth in oral cavity [51]. This method reflects the stress effects of temperature changes on bonding interface and aligns with the actual clinical environment.

Results in this study show that μTBS values of TFs-pretreated group in both the immediate and aged groups are significantly higher than those of the DW (blank control) and CHX groups ($p < 0.05$). SEM analyses provided further support for these results. More and longer resin tags were observed at the bonding interface after TFs pretreatment. The hybrid layer was thicker after aging, and its structure in TFs group remains intact with no obvious gaps, whereas the blank group has hybrid layer destruction and interfacial gaps. Fracture mode analysis further confirms the TFs advantages. TFs group exhibits a higher proportion of mixed and cohesive failures, *i.e.*, modes associated with stronger bonding forces. These results have demonstrated that TFs pretreatment enhances immediate bonding strength and resists enzymatic and hydrolytic damage during aging, probably by stabilizing the collagen matrix of hybrid layer. Consequently, superior long-term bonding stability is attained. The underlying mechanism may involve the cross-links formation of phenolic hydroxyl and galloyl groups in TFs molecular structure with dentin collagen. This enhances the mechanical strength and enzyme resistance of collagen and inhibits MMPs activity.

In this study, it is observed that the adhesive interface exhibits excellent bonding strength and stability after pretreatment with TFs. In addition to the above-mentioned mechanisms, certain interactions between TFs and adhesive components may also exist. TFs, as the polyphenolic compounds, are rich in phenolic hydroxyl and galloyl groups. Their phenolic hydroxyl groups can form hydrogen bonds with carboxyl groups of the adhesive, which enhance the intermolecular forces at interface. This may positively affect and improve the bonding strength. Techniques such as Fourier transform infrared spectroscopy (FTIR), Raman imaging, or mass spectrometry can be applied in the future to further explore the specific interaction mechanisms between TFs and adhesive monomers.

Marginal microleakage leads to secondary caries around restorations, postoperative sensitivity, and restoration failure [55–57]. A 0.5% toluidine blue solution is used as a marker in microleakage studies [58]. Therefore, it was used in this study where the depth of blue dye penetration at restoration margin indicates the degree of microleakage at dentin bonding interface. Results show that the TFs pretreatment reduces the degree of microleakage in immediate and aged groups $p < 0.05$ *vs.* the DW group. The CHX group shows slight improvement in reducing microleakage compared with DW group however, although the difference was not statistically significant ($p >$ 0.05). These suggest that CHX stabilizes the existing bonding interface by inhibiting MMPs, but shows limited improvement in the initial sealing of interface and resists leakage during aging. Likely, the anti-microleakage efficiency of TFs can be attributed to two factors: (1) The collagen cross-linking induced by TFs maintains the structural integrity of hybrid layer, reduces collagen collapse, and forms microvoids; (2) TFs penetrate deeply into demineralized dentin because of their low molecular weights and good fluidity for sufficient cross-linking.

This experiment verified that the bonding effect of TFs solution pretreatment on primary tooth dentin is superior to that of CHX and DW. However, this experiment used DMSO as the solvent for TFs. Whether the application of DMSO in this experiment has a beneficial effect on dentin bonding needs to be verified. To confirm this view, the experiment should add a DMSO control group to compare with the TFs solution, and check if there is a statistical difference between the two groups. This will help verify whether it is tea flavonoids themselves that improve the dentin bonding performance and durability, or the synergistic effect with DMSO. Secondly, it is necessary to further determine the cytotoxicity of the TFs solution on odontoblasts and dental pulp cells, as well as the stability of the solution.

In conclusion, this study verifies that TFs solution can improve the bonding performance and stability of deciduous dentin as observed through series of experiments, including μTBS testing of deciduous dentin specimens, fracture mode analysis, SEM analysis of dentin bonding interface, and comparison of microleakage degrees. These results have demonstrated that TFs is promising as a pretreatment agent in deciduous dentin bonding and restoration, and laid a solid foundation for further studies on its potential in clinical applications.

5. Conclusions

2% theaflavins pretreatment significantly enhances immediate bond strength and maintains post-aging bonding stability in deciduous dentin. Theaflavins show comparable microleakage control to 2% chlorhexidine. This study provides strong evidence that theaflavins from black tea are promising as a natural dentin pretreatment agent.

ABBREVIATIONS

TFs, theaflavins; CHX, chlorhexidine; μTBS, micro-tensile bond strength; SEM, scanning electron microscope; MMPs, matrix metalloproteinases; DW, distilled water; ANOVA, analysis of variance; H_0, hypothesis; DMSO, dimethyl sulfoxide; PA, proanthocyanidins; FTIR, Fourier transform infrared spectroscopy; ISO, International Organization for Standardization; TS, Technical Specification; LSD, least significant difference.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

LCL and YH—designed the research study and performed the research. BNH—drafted the manuscript. LCL, BNH, YH, JJJX, XYT, FL, XC and LPZ—participated in the discussion. All authors contributed to editorial modifications, read, and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ethics Committee of Stomatological Hospital Affiliated to Nanchang University (Approval No.: 2024-005). Informed consent was obtained from all participants and their legal guardians for their donation of dental samples. The experimental procedures adhered to the principles of the Declaration of Helsinki.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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