Journal of Dentistry 90 (2019) 103207

Contents lists available at ScienceDirect

Journal of Dentistry

journal homepage: www.elsevier.com/locate/jdent

Combination of baicalein and ethanol-wet-bonding improves dentin bonding durability

Luyao Yi[a], Jian Yu[a], Lin Han[b], Tingting Li[c], Hongye Yang[a,*], Cui Huang[a,*]

[a] The State Key Laboratory Breeding Base of Basic Science of Stomatology (Hubei-MOST) & Key Laboratory for Oral Biomedical Ministry of Education, School & Hospital of Stomatology, Wuhan University, Wuhan, China
[b] CR&WISCO General Hospital, Wuhan, China
[c] Lanzhou Hospital of Stomatology, Lanzhou, China

ARTICLE INFO

Keywords:
Baicalein
Dentin
Adhesive
Ethanol-wet bonding
Antibacterial

ABSTRACT

Objectives: This study aimed to investigate the potential of baicalein combined with ethanol-wet bonding (EWB) in improving dentin bonding durability.
Methods: Sixty caries-free human third molars were randomly allocated into four groups and pretreated with solutions after sectioning and polishing. The pretreatments were prepared via dissolving baicalein in ethanol at concentrations of 0, 0.01%, 0.05% and 0.1% (w/v). Microtensile bond strength (MTBS) test, failure mode analysis and interfacial nanoleakage evaluation were conducted immediately or after thermocycling or 1 month of collagenase aging. In situ zymography, contact angle, antibacterial activity and bioactivity were comprehensively assessed.
Results: Results demonstrated that the three experimental groups exhibited higher MTBS and lower nanoleakage expression regardless of aging. MMP activity within hybrid layer and *Streptococcus. mutans* biofilm formation were inhibited in the experimental groups in a dose-dependent manner. Baicalein also reduced reactive oxygen species (ROS) expression in human dental pulp cells and resisted adhesive-induced cytotoxicity. Baicalein exhibited remarkable capabilities at concentrations higher than 0.05% (w/v).
Conclusion: Baicalein is a prospective candidate as bioactive dentin bonding agent. Combined with EWB, baicalein may form a functional bonding interface, thereby enhancing dentin bond strength and durability.
Significance: Joint efforts by baicalein and EWB provides a novel therapeutic strategy for obtaining ideal adhesive-dentin interface and prolonging the longevity of restorations.

1. Introduction

With the development of dental materials and minimally invasive dentistry based on prevention and preservation, adhesive restoration has become a common technology applied in dentistry [1,2]. Currently, the durability of dentin bonding remains an urgent clinical problem despite its immediate efficacy [3]. Weak dentin bonding causes the failure of various types of restoration, such as secondary caries, microleakage and restoration shedding [2]. It is considered that hydrolysis of bonding resin, enzymatic hydrolysis of collagen fibers by endogenous enzymes and secondary defects of bonding interface are three common causes of bonding failure [2,4,5].

Many targeted strategies are proposed to improve the durability of dentin bonding. The traditional wet-bonding theory by Kanca suggests that proper wetting can keep collagen fiber network of acid-etched dentin fluffy so that adhesive resin can maximally penetrate into collagen fiber network and form a hybrid layer [6]. Traditional dentin wet bonding refers to water wet bonding (WWB), the technical sensitivity of which is revealed as the humidity of water is uncontrollable. Therefore, ethanol wet-bonding (EWB) theory has been propounded; this theory proposes that gradient ethanol can replace water in the dentin matrix and promote hydrophobic resin penetration into collagen network, thereby producing a high-quality hydrophobic hybrid layer that can improve bonding strength and durability [7]. In contrast with WWB, EWB improves the bond strength of either hydrophobic or hydrophilic adhesives and reduces phase separation of interface [8,9].

Although traditional modified components such as chlorhexidine and glutaraldehyde, can effectively improve bonding stability, drug resistance and cytotoxicity cannot be underestimated [10–13].In recent years, biosafety and effectiveness of plant extracts have gradually been verified. Thus, plant extracts have been wildly used in clinical medicine and are research hotspot in dentistry, especially in dentin bonding

* Corresponding authors at: School & Hospital of Stomatology, Wuhan University, Wuhan, China.
E-mail addresses: yanghongye@whu.edu.cn (H. Yang), huangcui@whu.edu.cn (C. Huang).

https://doi.org/10.1016/j.jdent.2019.103207
Received 1 July 2019; Received in revised form 23 September 2019; Accepted 27 September 2019

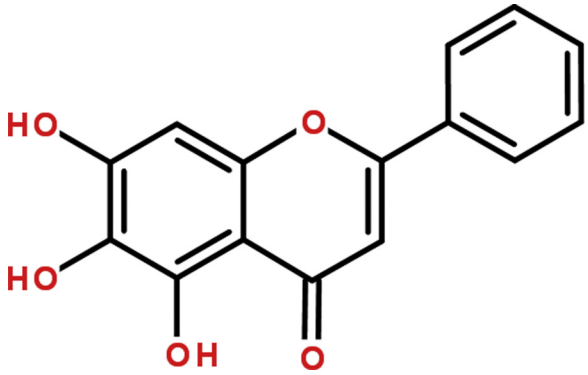

Fig. 1. Chemical structure of baicalein.

[14–16]. Flavonoids, such as proanthocyanidins, tea-polyphenols and quercetin, can prevent dentin collagen degradation and maintain dentin matrix stability, thereby enhancing dentin bonding strength [17–20]. Baicalein (5,6,7-trihydroxyflavone, $C_{15}H_{10}O_5$, molecular weight 270.24), a major flavonoid extract derived from *Scutellaria baicalensis Georgi* ("Huang-Qin" in Chinese), with excellent biocompatibility and multiple biological activities, such as anti-inflammatory, antibacterial, antioxidant, antitumor, antiviral and anti-fibrotic (Fig. 1) [21,22]. Its solubility in water is poor (16 μg/mL), but it can be improved in methanol, ethanol, acetone and other solvents, of which specific value is not yet clear. Besides, baicalein can be oxidized to red or dark green in an alkaline environment [23]. Studies have shown that baicalein can inhibit matrix metalloproteinase expression and biofilm formation, which is advantageous for improving dentin bonding durability, but few reports have been reported [24–26]. Additionally, it is well known that adhesives are toxic [27], while the detoxification effect of previous modified ingredients on adhesives has not been considered. Given that baicalein exhibits antioxidant properties that may be effective in protecting dental pulp cells from adhesive stimulation, we investigated the resistance of baicalein to adhesive-induced cytotoxicity.

To further explore the multi-functions of flavonoid extracts in dentin bonding, we dissolved baicalein in ethanol at appropriate concentrations as pretreatment and tested its potential to form functional bonding interface, including adhesion, antibacterial and biological properties.

The null hypotheses of this study are as follows:

(1) No differences occur in dentin bonding strength and interfacial nanoleakage expression between dentin pretreated with baicalein-containing ethanol and 100% ethanol even after thermocycling or collagenase aging is completed.
(2) No differences occur in MMP activity in hybrid layer between dentin pretreated with baicalein-containing ethanol and 100% ethanol.
(3) No differences occur in *S.mutans* biofilm formation between dentin pretreated with baicalein-containing ethanol and 100% ethanol.
(4) Baicalein pretreatment has no effect on adhesive- induced cytotoxicity.

2. Materials and methods

2.1. Fabrication of specimens and pretreatment agents

Sixty caries-free human third molars were collected with the informed consents of donors, and approval was obtained from the Human Research Ethics Committee, XX University. All tooth samples were cleaned immediately and stored in 0.1% thymol solution at 4 °C within 1 month prior to experimentation. The teeth were cut using a water-cooled low-speed diamond saw (Isomet; Buehler, Evanston, IL, USA) to remove crown and wet ground with 600-grit SiC sandpaper for 60 s to develop an enamel-free surface. The specimens were etched with 35% phosphoric acid for 15 s, then thoroughly rinsed with deionised water and dried using compressed air. Four primer solutions were randomly applied to specimens (n = 9 each group) with a microbrush for 60 s, which were prepared by dissolving baicalein in ethanol at concentrations of 0%, 0.01%, 0.05% and 0.1% (w/v).

Group 1: absolute ethanol (control group);
Group 2: 0.01% (w/v) baicalein-containing ethanol (0.01% group);
Group 3: 0.05% (w/v) baicalein-containing ethanol (0.05% group);
Group 4: 0.1% (w/v) baicalein-containing ethanol (0.1% group);

After lightly blotting with a filter paper, the ideal moist dentin surface was applied with Single Bond Universal (3 M ESPE, St. Paul, MN, USA) according to its instruction and irradiated for 15 s with a LED light-curing device (Bisco Inc., Schaumburg, IL, USA). Composite (Charisma, Kulzer, Germany) with a whole thickness of 4 mm was piled up in four increments and polymerized for 20 s each. All experimental procedures were conducted by a well-trained operator.

2.2. Microtensile bond strength (MTBS) test

Prior to MTBS test, specimens were soaked in deionized water and stored at 37 °C for 24 h, then cut parallel to the longitudinal axis to obtain 0.9 mm dental slabs. Nine slabs were randomly set aside for each group for nanoleakage evaluation (n = 6) and in situ zymography (n = 3), and the remaining slabs were further sectioned vertically to produce 0.9 mm × 0.9 mm beams. After peripherally situated unqualified beams were eliminated, almost 12 eligible beams were acquired per tooth. Four of them (n = 30 per group) were immediately subjected to MTBS test, and 4 (n = 30 per group) were tested after thermocycling. The remaining four were tested after 1 month of collagenase ageing. The thermocycled beams underwent 10,000 cycles in a temperature cycling chamber (HUAN-S, Wuhan, China), with beams remaining at 5 °C and 55 °C for 15 s considered to have underwent one cycle.

For collagenase-aged groups, *Clostridium histolyticum* collagenase (Sigma–Aldrich, St. Louis, MO, USA) was dissolved with artificial saliva to obtain 0.1 mg/ml aging solution. Beams were then immersed in the collagenase solution, which was renewed every 3 days, and stored in the dark conditions at 37 °C.

In an MTBS test, the prepared beams were attached to microtensile tester (Bisco Inc, Schaumburg, IL, USA) by using cyanoacrylate adhesive (Zapit; Dental Ventures of America, Corona, CA, USA). Load in tension was applied at 1 mm/min cross-head speed. Finally, the corresponding MTBS values (MPa) were computed by dividing the maximum load force by the dimension of beam gauged by vernier caliper.

2.3. Failure mode analysis

Field-emission scanning electron microscopy (FESEM) (Sigma, Zeiss, Germany) was used to observe the dentin surface of fractured specimens after MTBS test. Failure mode was classified as follows:(A) adhesive failure; (CD) cohesive failure in dentin; (CC) cohesive failure incomposite; or (M) mixed failure [28].

2.4. Interfacial nanoleakage evaluation

The slabs (n = 2 per subgroup) from immediate, thermocycled and collagenase aged groups were used to prepare the specimens for nanoleakage evaluation [29]. The slabs were painted with a thick layer of nail polish, except the area of 1 mm from the adhesive-dentin interface, and dipped into a 50 wt% (w/v) ammoniacal $AgNO_3$ solution (pH = 9.5) for a period of 24 h under light-proof condition. The slabs were then thoroughly rinsed with deionized water, immersed in developer solution and exposed to fluorescent light for 8 h. Each slab was sequentially wet-polished with 600-, 1000-, 1500-, 2000-grit SiC sandpapers and 0.5 μm diamond paste, and then subjected to ultrasonic cleaning. The prepared specimens (n = 20 per subgroup) were

observed via FESEM in a backscattered electron imaging mode. Ten fields-of-view along the adhesive-dentin interface were randomly captured. Image J (NIH, Frederick, MD, USA) was used to quantitatively calculate the percentage distribution of silver settlingsalong the interface. Nanoleakage expression was recorded by two inspectors as below: 0, no nanoleakage; 1, < 25% nanoleakage; 2, 25% ≤ 50% nanoleakage; 3, 50% ≤ 75% nanoleakage; and 4, > 75% nanoleakage [30]. Consistency between the results of observers was evaluated by Kappa test (K = 0.88).

2.5. In situ zymography

Fluorescence technique was used to compare the effects of 100% ethanol with 0.01, 0.05 and 0.1% (w/v) baicalein-containing ethanol on matrix metalloproteinase activity within hybrid layers. The remaining bonded slabs from the four groups (n = 3) were overlapped with one drop of quenched fluorescein-conjugated gelatin (E-12055, Molecular Probes, Eugene, OR, USA). These specimens were then put on a slide, overlaid with a coverslip and incubated in a light-proof wet box at 37 °C for 24 h. Confocal laser-scanning microscopy (CLSM) (FV1200, Olympus, Tokyo, Japan) was performed to image the quantity of green fluorescence (λex/λem = 494 nm/521 nm), which indicates MMP activity.

2.6. Surface contact angle test

Twenty-four 0.5 mm-thick dentin disks were produced, wet-polished and subjected to ultrasonic cleaning. The disks (n = 6 per group) were etched, rinsed and pretreated with pretreatment agents. Each dentin surface was dripped with one drop of deionized water and contact angle was measured using an OCA 20 contact angle system (Dataphysics, Germany) under ambient conditions.

2.7. Antibacterial activities

2.7.1. Bacterial culture and biofilm preparation

Fifteen dentin disks per group were used in antibacterial activity test, nine of which were subjected to MTT analysis, three for CLSM analysis and three for FESEM analysis. Prior to biofilm preparation, disks were etched with 35% phosphoric-acid, disinfected under UV-light for 2 h, pretreated with four experimental solutions (i.e. 0, 0.01%, 0.05% and 0.1% (w/v) baicalein-containing ethanol) and placed in a 24-well plate. Each well was added with the mixture of *S.mutans UA159* bacterial suspension and 1 mL of brain heart infusion (BHI) medium (BD, Sparks, MD, USA) containing 1% sucrose. The bacterial suspension was anaerobically cultured in BHI for 24 h and diluted to 10^8 CFU/ml in advance. The 24-well plates were placed in an anaerobic incubator at 37 °C for 24 h to form biofilm-coated specimens. Then *S.mutans* adhered to the surfaces of disks was washed away with sterile phosphate buffered saline (PBS).

2.7.2. Antibacterial evaluation by MTT assay

MTT assay was utilized to test the antibacterial effect of baicalein on *S. mutans* (n = 9). The specimens were transferred to a 24-well plate, and 1 mL of 0.5 mg/mL MTT solution was injected into each well and anaerobically cultivated at 37 °C for 4 h. After incubation, the MTT solution was pipetted out and an equal volume of dimethyl sulfoxide (DMSO) was added. The plate was then oscillated at a low speed in the dark for a period of 20 min and absorbance was recorded at 570 nm by a Power Wave plate reader (Bio-tek Instruments, Winooski, VT, USA). Five readings for each specimen were accomplished in each group. MTT assay was repeated three times.

2.7.3. Live/dead staining of biofilms

A live/dead bacterial viability kit (Molecular Probes, Invitrogen, USA) was used for biofilms staining (n = 3). The prepared specimens were stained for 15 min, lightly rinsed with PBS, and then analyzed by CLSM (Fluoview FV1200, Olympus, Tokyo, Japan) at 40 × magnification. Live bacteria stained with SYTO-9 emitted green fluorescence with an excitation of 488 nm, whereas dead bacteria stained with propidium iodide emitted red fluorescence with an excitation of 543 nm. For each specimen, 10 images were obtained by a consequent scanning along the z-stack from bottom to top at a Z-step of 2 μm. The 3D overlay image as well as quantitative data of biofilms including total biomass and distributions of live/dead bacteria were obtained by Imaris (Imaris 7.2.3, Bitplane, Zürich, Switzerland).

2.7.4. Antibacterial evaluation by FESEM

FESEM analysis was employed in detecting the quantity and morphology of *S. mutans* biofilm (n = 3). Biofilm-coated specimens were immobilized with 2.5% glutaraldehyde, and dehydrated with gradient ethanol (i.e. 30%, 50%, 70%, 80% and 90% for 20 min, 100% for 20 min twice). Before visualization by FESEM (Sigma, Zeiss, Germany), the disks were desiccated and sprayed with gold. For each specimen, three areas were randomly chosen for imaging.

2.8. Bioactivity evaluation

2.8.1. Cell culture and adhesive extracts preparation

Human dental pulp cells (hDPCs) seeded in 96-well plates (5×10^3 cells per well) were cultured in α-modified minimal essential medium (α-MEM; Hyclone, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT, USA) and 1% penicillin/streptomycin (Amresco LLC, Solon, OH, USA). These cells were incubated at 37 °C under the condition of 5% CO_2 for 24 h to ensure the adherence of the hDPCs to the well surface. The cells were then exposed to adhesive extracts in four adhesive groups. The extract was prepared as follows. Five drops adhesive was dripped into a 10 mL sterile vial and shaken gently to ensure that the liquid tile covered the bottom of the vial. The adhesive was light cured for 15 s to simulate the clinical procedures before 5 mL of medium was added. The extract medium was incubated under the same condition of cells for 24 h and filtered through a 0.22 μm syringe filter. Four kinds of medium containing baicalein at the concentrations of 0%, 0.01%, 0.05% and 0.1% (w/v) were produced as follows and applied to subsequent experiments. Different quantities of baicalein were incorporated into ethanol and then diluted with α-MEM at a ratio of 1:99.

2.8.2. CCK8 assay

After the old medium was pipetted out, 100 μL of medium containing a series of baicalein concentrations was added into the wells of the experimental groups, and 100 mL of fresh medium was added into the wells of the control groups (i.e. blank control and adhesive pretreatment only). Subsequently, 10 μL adhesive medium was exposed to cells after 1 h, except for the blank control. The cells were cultivated for 24 h and cultured with 110 μL of CCK8 (Dojindo, Tokyo, Japan) solution at 37 °C in the darkness condition for a period of 4 h. Absorbance was measured at 450 nm by using a microplate reader. Percent survivals were plotted relative to blank control cells to test the protective effect of baicalein against adhesive-induced cytotoxicity. CCK8 assay was implemented in sextuplicate.

2.8.3. ROS measurement

Intracellular generation of reactive oxygen species (ROS) was monitored using a human reactive oxygen species ELISA kit (Applygen Technologies, Beijing, China). HDPCs were seeded on the slides of cells (10^4 per slide) and cultured for 24 h. Then the cells were incubated with 10 μM 2,7-Dichlorofluorescin diacetate (DCFH-DA) under a light-proof condition and put in a 37 °C incubator for 30 min. Prior to observations, the samples were washed gently with PBS three times and placed into a cassette. ROS was determined by a fluorescence microscope (DP71, OLYMPUS, China) with a blue excitation filter. Five fields were

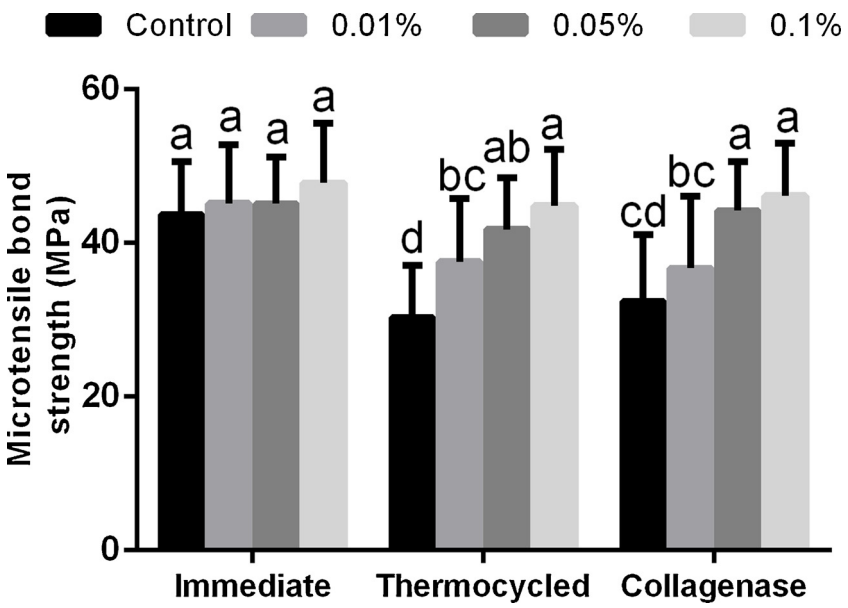

Fig. 2. Microtensile bond strengths of each group. Data are shown as means ± SD. Groups with the same superscripts are not significantly different ($P > 0.05$).

observed under the same conditions.

2.8.4. Statistical analysis

Two-way ANOVA and the post-hoc Tukey's test were used to interpret the MTBS test. Kruskal-Wallis test and Dunnett's post-hoc test were employed to determine the statistical differences in scores among the nanoleakage groups, whereas Cohen's kappa test was used to assess inter-examiner reliability. One-Way ANOVA and following post-hoc Tukey's test were used for analyzing Surface contact angle, live/dead bacteria distributions, MTT and CCK8 assay results. SPSS (IBM SPSS Statistics 22.0, Armonk, NY, USA) was applied for statistical analysis. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. MTBS

The MTBS results computed from the four groups are plotted in Fig. 2. No significant difference was observed among the four immediate groups ($p > 0.05$). After 10,000 thermocycling cycles, the bond strength of each group decreased at varying degrees, except for the 0.1% group. Compared with the experimental groups, the control group manifested the lowest bond strength ($p < 0.05$). In the collagenase aging groups, the bond strength of 0.05% and 0.01% groups was not affected ($p > 0.05$), whereas that of the other two groups was significantly reduced ($p < 0.05$). The Two-way ANOVA results indicated that dentin bonding strength was evidently affected by aging mode ($F = 18.881$, $p = 0.000$) and concentration of pretreatment agent ($F = 24.045$, $p = 0.000$). The strong interaction between aging mode and pretreatment agent ($F = 3.088$, $p = 0.006$) implied that the differences among groups were caused by the above two factors.

3.2. Failure mode analysis

Fig. 3 presented the frequency distribution of failure mode. Taking control and 0.1% group for instance, the dominant failure mode in the control group was cohesive failure in dentin regardless of aging. Although the rate of cohesive failure in dentin in the 0.1% group increased after thermocycling and one-month collagenase aging, it was lower than control. Representative FESEM images were shown in Fig. 4A–D.

3.3. Interfacial nanoleakage evaluation

Table 1 summarized the quantitative analysis data of nanoleakage expression. Kruskal-Wallis test revealed that irrespective of aging (i.e. thermocycling and collagenase aging), the specimens pretreated with baicalein-containing ethanol solution displayed lower nanoleakage expression ($p < 0.05$) in comparison with the control group. Baicalein down-regulated nanoleakage expression in a dose-dependentmanner, that is, nanoleakage expression gradually decreased as baicalein concentration increased. Nanoleakage expression in the aged groups exceeded that in the immediate groups. Fig. 5 illustrated the typical FESEM images of nanoleakage of the control and 0.05% groups. The controls showed white silver particles continuously deposited on the hybrid layer and even filled dentinal tubules (Fig. 5A, C and E). The 0.05% group demonstrated silver uptake intermittently distributed along the interface (Fig. 5B, D and F).

3.4. In situ zymography

Fig. 6 demonstrated the representative CLSM images of gelatin zymograms obtained from the control or baicalein-containing ethanol groups. Intense green fluorescence was widely observed within hybrid layer in the control group, indicating high gelatinolytic activity (Fig. 6A). With the incorporation of baicalein, the fluorescence was significantly decreased and presented an intermittent distribution. Especially in the 0.1% group, fluorescence was barely detected (Fig. 6D).

3.5. Surface contact angle test

Fig. 7 described the detection results of surface contact angle and corresponding views. The average contact angle of the control, 0.01%, 0.05% and 0.1% groups were 38.3° ± 8.0°, 53.5° ± 3.8°, 68.7° ± 6.5° and 85.0° ± 8.3°, respectively. The contact angles of the baicalein pretreated groups were higher than that of the control, which may be beneficial for the quality of hybrid layer.

3.6. Antibacterial activity evaluation

The result of MTT assay was illustrated in Fig. 8. Decreased metabolic activity of *S.mutans* was observed in the baicalein-containing ethanol pretreated groups ($P < 0.05$), and metabolic activity

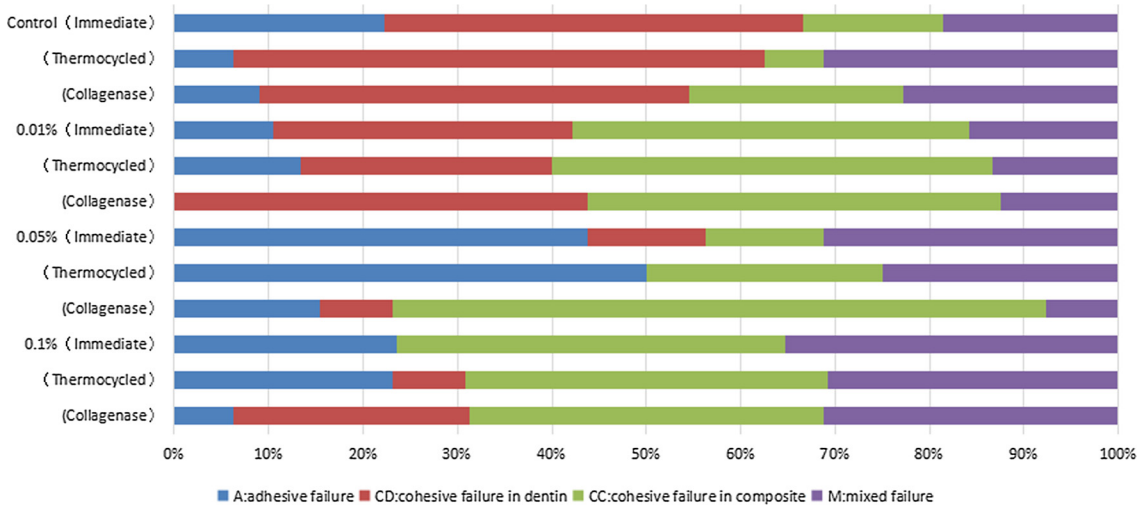

Fig. 3. Distribution of four failure modes following MTBS test.

decreased as the concentration of baicalein increased. Fig. 9 presented the representative Z-stacked confocal images of *S.mutans* biofilms growing on the specimen surface from each group. From the reconstructed 3D overlay images of 10 layers (Fig. 9A1–D1), the control group was mainly stained green, representing live bacteria, whereas the experimental group was mainly stained red, representing dead bacteria, especially in the 0.05% and 0.1% groups. The line plots (Fig. 9 A2–D2) summarized the corresponding relative distribution of live/dead bacteria. Although the dead biomass was high in the ethanol-pretreated control group, the ratio of live/dead biomass was significantly reduced after the addition of baicalein. In particular, the total biomass area in the 0.1% group was less than half that of the control group, suggesting the strong antibacterial properties of baicalein. Fig. 10 exhibited the typical FESEM images of *S.mutans* biofilm from the control and 0.01% groups. In the control group (Fig. 10A, B), thick and dense bacterial biofilm covering the entire dentin surface was observed. Upon pretreatment of dentin specimens with 0.01% baicalein-containing ethanol (Fig. 10C, D), few bacteria grew and were merely scattered on the dentin surface.

Table 1
Percentage distribution of nanoleakage scores in each group for immediate, after 10,000 thermocycling cycles and one-month collagenase aging.

Groups	Time	Score percentage (%)					Statistical difference
		0	1	2	3	4	
Control	Immediate	0	8	27	41	24	bc
	Thermocycled	0	0	0	22	78	a
	Collagenase	0	0	14	34	52	a
0.01%	Immediate	0	0	61	31	8	bcd
	Thermocycled	0	0	3	43	54	a
	Collagenase	0	0	10	50	40	ab
0.05%	Immediate	7	32	54	7	0	e
	Thermocycled	0	15	22	52	11	bcd
	Collagenase	13	32	23	23	9	de
0.10%	Immediate	13	60	27	0	0	f
	Thermocycled	0	21	29	43	7	cd
	Collagenase	0	17	57	26	0	de

The Kruskal–Wallis test with Dunnett's post hoc test. Groups with the same letters are not statistically different ($P > 0.05$).

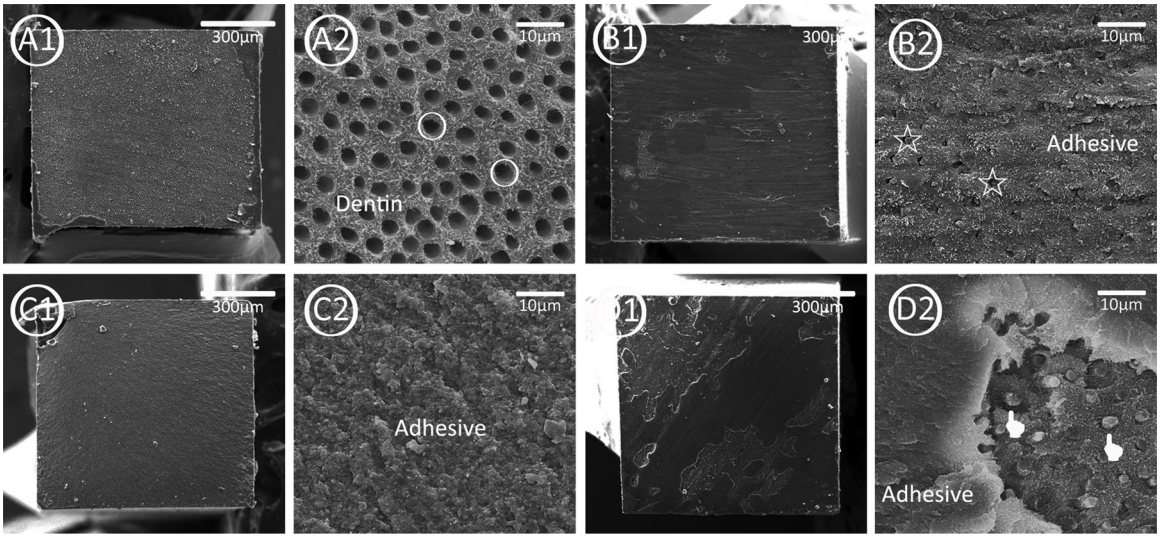

Fig. 4. Representative FESEM images of fractured dentine surface following MTBS test. Low-magnification (150×) images presented in (A1-D1) and high-magnification (3000×) images presented in (A2-D2), respectively, indicate the general condition and detail of fractured surfaces. (A1,A2) Cohesive failure in dentin, from thermocycling-aged control group; (B1,B2) Cohesive failure in composite, from collagenase-aged 0.01% group; (C1,C2) Adhesive failure, from immediate 0.05% group; (D1,D2) Mixed failure, from immediate 0.1% group. Circles: open dentin tubules; Pentagrams: sealed dentin tubules; Pointers: resin tags.

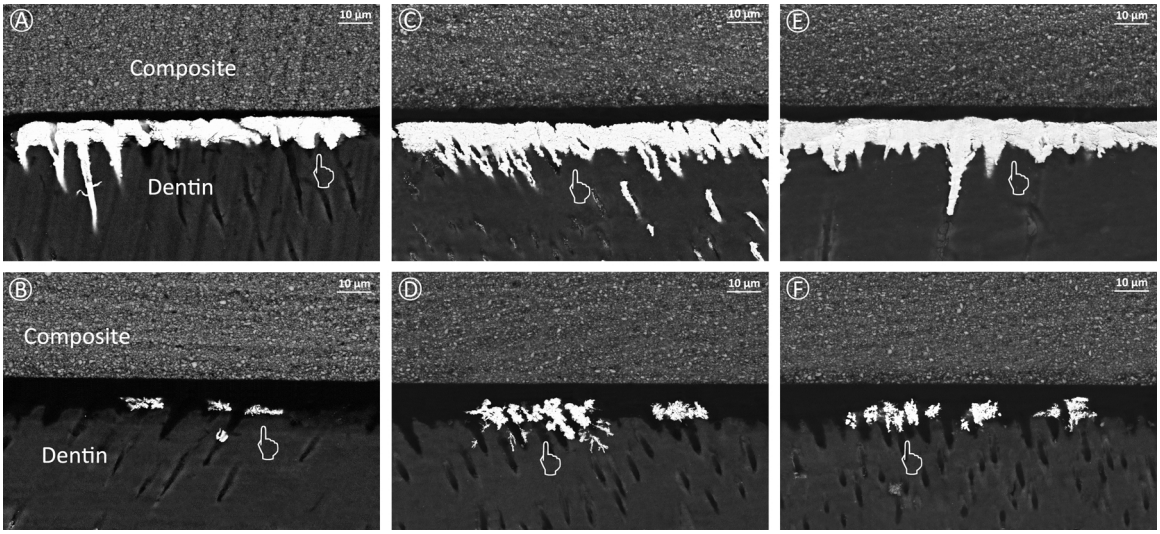

Fig. 5. Representative FESEM images (1000×) of nanoleakage expression from control and 0.05% group for immediate, after 10,000 thermocycling cycles and one-month collagenase aging. (A) immediate control group; (B) immediate 0.05% group; (C) thermocycling-aged control group; (D) thermocycling-aged 0.05% group; (E) collagenase-aged control group; (F) collagenase-aged 0.05% group. Pointer: silver deposits.

3.7. Bioactivity evaluation

Baicalein is a plant extract with low cytotoxicity. Here we examined the protective effects of baicalein against adhesive-induced cytotoxicity. The statistical results of CCK8 were demonstrated in Fig. 11A. Compared with the blank group, the cell viability of hDPCs exposed to dentin adhesive extracts was decreased ($p < 0.05$). Cytotoxicity was enhanced upon the addition of ethanol into the medium ($p < 0.05$). After baicalein was added, adhesive-induced cytotoxicity was effectively reduced, and cell viability in the 0.01% group was equal to that in the blank group. The cell viability of the 0.05% and 0.1% groups even significantly exceeded the blank group ($p < 0.05$), indicating that baicalein potentially promoted cell proliferation. Intracellular ROS was detected by fluorescence microscopy with DCFH-DA, representative pictures of which were shown in Fig. 11B–E. The adhesive and control groups showed stronger green fluorescence than the blank group, indicating the increase in ROS production. The hDPCs incubated with baicalein-containing medium represented by the 0.05% group, only showed weak fluorescence, which was consistent with CCK8 results.

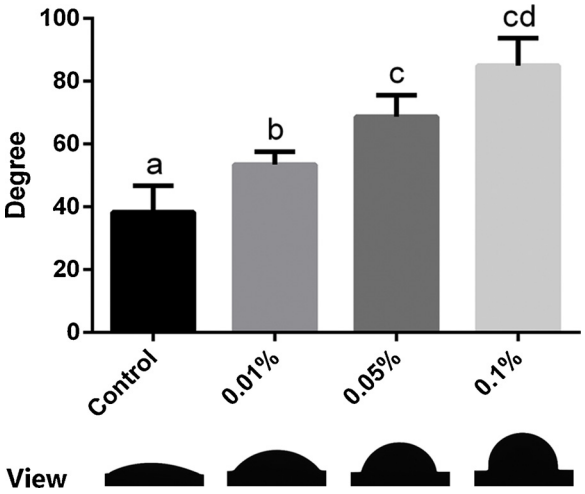

Fig. 7. The degree of surface contact angle and corresponding views of each group. Groups with the same superscripts are not significantly different ($P > 0.05$).

4. Discussion

The multi-effects of baicalein on dentin bonding were evaluated by combining baicalein and EWB in the present study. Our results demonstrated that baicalein-containing ethanol solution as a pretreatment agent resisted resin-dentin interface ageing caused by collagenase and thermocycling, reduced interfacial nanoleakage expression, inhibited MMP activity in hybrid layer, suppressed the formation of *S.mutans* biofilm, and prevented adhesive-induced cytotoxicity. These findings indicated that baicalein is one of the most prospective candidates for dental modified materials. Hence, the null hypotheses were rejected.

EWB has been proven to improve bond strength of either hydrophobic or hydrophilic adhesives and reduce the phase separation of interface relative to WWB [8], because it could replace water in the

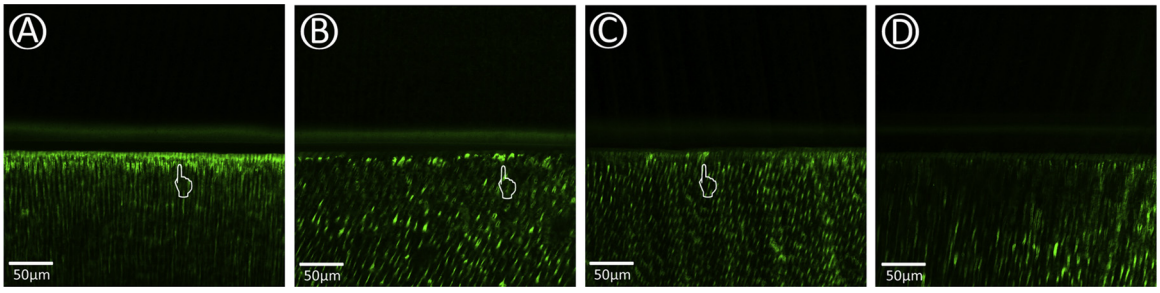

Fig. 6. Representative CLSM images of in situ zymography marked with fluorescein-quenched gelatin. (A) control group; (B) 0.01% group; (C) 0.05% group; (D) 0.1% group. Pointer: MMP activity indicator.

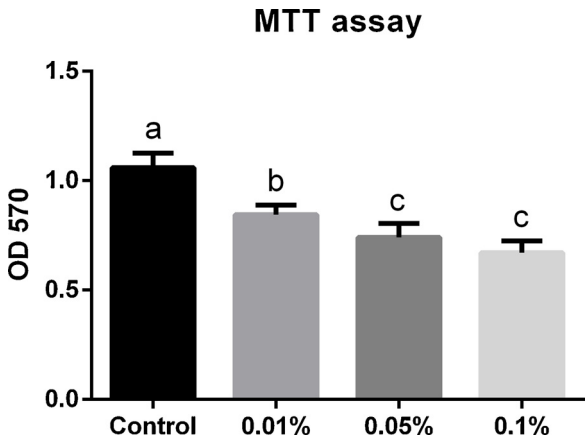

Fig. 8. The average OD570 values after *S. mutans* incubating on the dentin surfaces for 24h. Groups with the same superscripts are not significantly different ($P > 0.05$).

dentin matrix and promote hydrophobic resin penetration into collagen network, thereby producing a high-quality hydrophobic hybrid layer that can improve bonding strength and durability [7,9]. However, as it turned out, the bond strength was unfortunately reduced by a large margin after thermocycling or collagenase aging in our experiment, indicating that EWB does not necessarily improve bonding durability.

The polymerization shrinkage of dental composite materials is the factor that cannot be ignored in the failure of dental restoration. Generally, temperature changes in oral conditions affect the mechanical properties of dental restorative materials, thus, it is necessary to assess the sensitivity of restorative material to temperature changes [31]. In the present study, MTBS results demonstrated that the values of baicalein containing groups significantly lower than that of the control group, especially the 0.1% group, in which MTBS was not reduced after thermocycling. These results suggested that the combination of baicalein and EWB may resist interface degradation caused by intraoral temperature change and improve bonding durability.

Hybrid layer, a structure of demineralized dentin collagen fibers wrapped by resin matrix, plays a crucial role in successfully dentin bonding in both etch-and-rinse and self-etch adhesive system [32]. However, despite recent tremendous advances in dental materials, hybrid layer remains imperfect and is degraded over time. Exposed mineral-free collagen fibers surrounded by water occur at the bottom of hybrid layer, which lack the protection of resin [2]. Hydrolysis of adhesive resin and enzymatic hydrolysis of collagen fiber are two major degradation modes of hybrid layer. In addition to bacterial-derived enzymes, endogenous enzymes, mainly MMPs and cysteine cathepsins, can also degrade dentin collagen [2,5]. MMPs are endogenous proteases dependent on Zn^{2+} and Ca^{2+}, and MMP-2, MMP-9 are widely present in dentin. EWB protects hybrid layer by removing free water, exhibiting excellent bonding durability [2,33]. Flavonoids, such as baicalein, are natural polyphenolic compounds that coordinate with metal ions and interplay with peptidases, whose catalytic activity depends on the metal cations. Baicalein has been identified and widely used as natural inhibitors of MMPs [24,34–36]. To date, it was found that flavonoids such as proanthocyanidins, tea-polyphenols, quercetin and naringin could inhibit dentin collagen degradation and maintain dentin matrix stability [17–20]. In the present study, the group treated with baicalein-containing ethanol solutions possessed less bond strength loss than the control group even after collagenase aging. In particular, the bond strengths of the 0.05% and 0.1% groups were identical to those of the immediate groups.

Nanoleakage which was first described by Sano et al. in 1994 is a phenomenon in which porosity can be observed at the bottom of the hybrid layer [37]. Nanoleakage develops as follows. Acid-etching leads to the inhomogeneous demineralization of dentin surface. The deeper the dentin, the lower is the demineralization degree. Ultimately, there is a layer of dentin that is not etched at all. Continuing the bonding process on this basis, the resin cannot penetrate and fill into this deep area. The size of these unfilled gaps is approximately10-50 nm [37,38]. The interrelation between nanoleakage expression and bond strength has not yet been determined. F.R. Tay found that nanoleakage negatively affected dentin bonding strength corroborating our results [39]. Here we reported that the amount of nanoleakage expression was concordant with MTBS. As baicalein concentration was increased, silver particles deposited on the hybrid layer considerably decreased, though the amount deposited in the aging group was more than that in the immediate group. Nevertheless, M. Hashimoto and Paul G. F. Ding found that nanoleakage expression was not necessarily associated with MTBS [40,41]. Therefore, the relationship between nanoleakage expression and bond strength should be further studied.

As mentioned above, MMPs, especially MMP-2 and MMP-9, are important factors causing the enzymatic hydrolysis of dentin collagen fiber [2]. Endogenous MMPs are originally bound to mineralized dentin collagen, and exposed after acid etching. Its activity can be activated by subsequent acid monomers [42]. In the current study, *in situ*

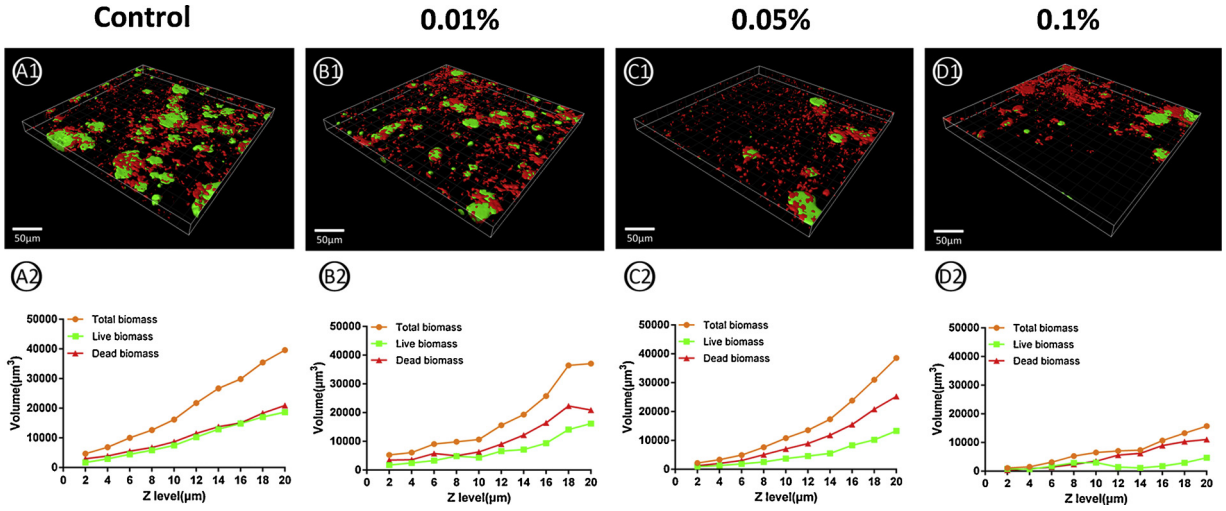

Fig. 9. Live/dead staining of *S.mutans* bioflms. Representative reconstructed CLSM 3D overlay images of *S.mutans* biofilms presented in (A1-D1) and corresponding relative distribution of total, live, and dead bacteria along the z-stack at a z-step of 2 μm presented in (A2-D2). (A1,A2) control group; (B1,B2) 0.01% group; (C1,C2) 0.05% group; (D1,D2) 0.1% group.

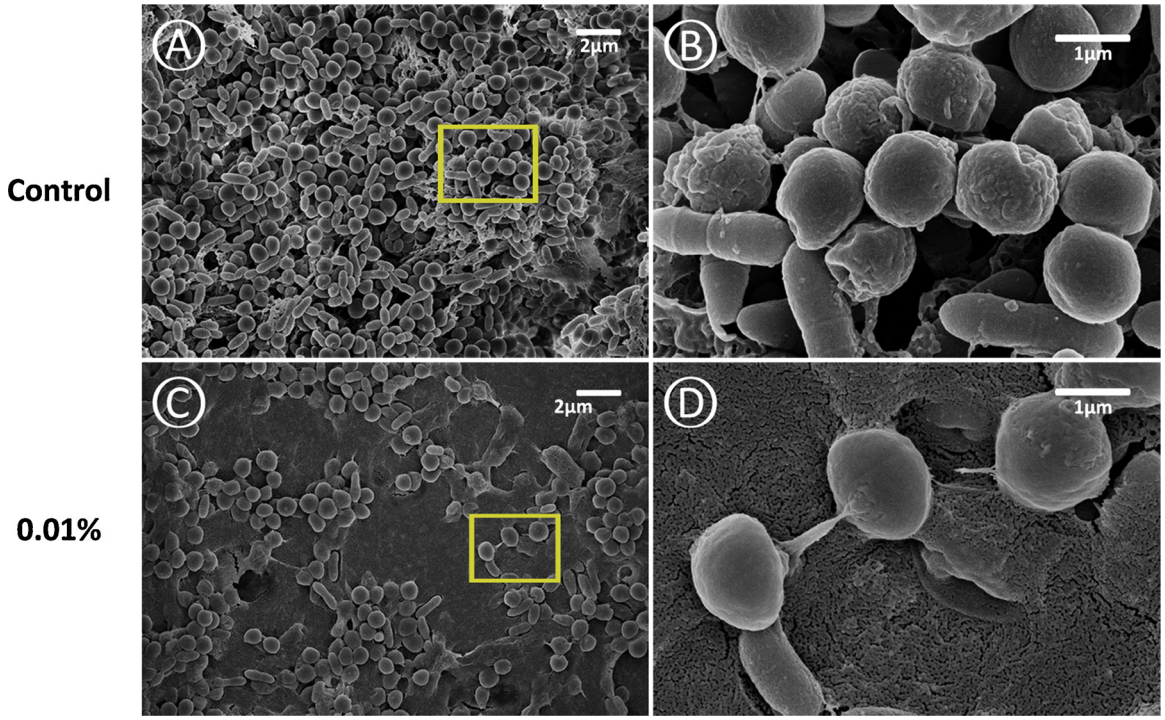

Fig. 10. Representative FESEM images of the *S.mutans* biofilm cultured on the dentin surface from the control (A,B) and 0.01% group (C,D). The high-magnification images of (A,C) are shown in (B,D).

zymography was used to compare the effects between 100% ethanol and baicalein-containing ethanol on matrix metalloproteinase activity within hybrid layers. This technique allows relative proteolytic activity within hybrid layer to be directly located. Intense green fluorescence was widely observed within hybrid layer in the control group, indicating high MMP activity. With the incorporation of baicalein, the fluorescence was reduced, presenting intermittent distribution. Especially in the 0.1% group, green fluorescence was barely detected. This finding may be attributed to the fact that baicalein is an inhibitor of matrix metalloproteinase, interfering with MMP-2 and MMP-9 expression levels and down-regulating their gelatinolytic activities [24,34]. The mechanism for the inhibition of MMPs by baicalein is speculated as follows. Firstly, baicalein can competitively bind to the active center of MMPs by chelation of metals such as Zn^{2+}. Secondly, baicalein may crosslink with MMPs and alter the three-dimensional structure of them, thereby resulting in their inactivation. Thirdly, baicalein may change or cover the recognition site of MMPs by crosslinking with collagen, interfering with the binding process of ligands and receptors, thus protecting collagen from degradation [43].

Dentin is a moist matrix whose hydrophilicity may cause the easy degradation of bonding interface over time. Bonding with hydrophilic adhesives and resins can increase water absorption and reduce mechanical properties and bonding durability, while the use of hydrophobic materials can prevent these problems [44,45]. Previous study has shown that surface contact angle of exposed collagen fibers was changed by using bio-modifier as primer, and hydrophilicity reduction of dentin surface may improve bonding quality by promoting the volatilization of water and organic solvents [7]. As shown in the results of

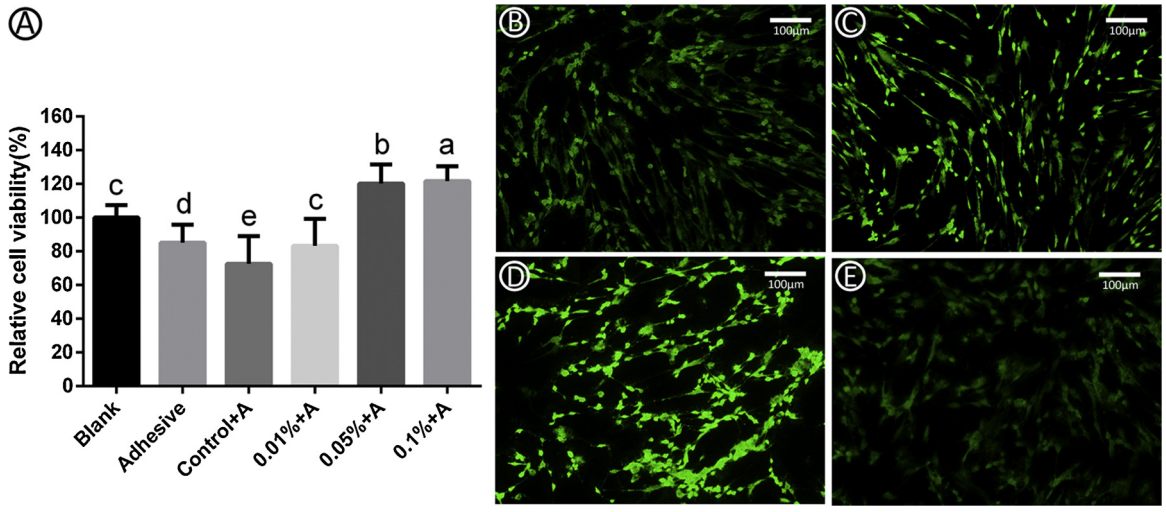

Fig. 11. Bioactivity evaluation. (A) Relative cell viability of hDPCs after incubation with α-MEM, adhesive extracts, and a series of concentrations of baicalein-containing medium (0, 0.01%, 0.05%, 0.1%) doped with adhesive extracts. (B–E) Representative fluorescence microscope images of intracellular ROS from the blank (B), adhesive (C), control (D), 0.05% (E) group.

the current study, as the concentration of baicalein was increased, the contact angle of acid-etched dentin surface increased, which might reduce the swelling of dentin matrix, thereby decreasing hydrolysis possibility and protecting dentin collagen from degradation [46].

Secondary caries, a carious lesion occurring at the restoration margins, is the main cause of restoration failure [4]. It begins in dental plaque (bacterial biofilm) and acts as a crucial factor for the development of oral diseases [47]. *S.mutans* is an early colonizer of dental plaque and the major cariogenic bacterium of dental caries [48].Therefore, inhibiting the growth of bacterial biofilm on restorations is of great significance to reduce the risk of secondary caries and prolong the life of restoration. Baicalein is one of the most commonly used herbal medicines in the treatment of bacterial and viral infections. It shows remarkable antimicrobial effects on *Escherichia coli, Staphylococcus, Pseudomonas aeruginosa* and *S.mutans* [25,26,49–51]. According to MTT results, metabolic activity of *S.mutans* in the 0.01% group was lower than control group. The CLSM results revealed that the live/dead biomass of the three experimental groups decreased in a dose-dependent manner relative to control group. In particular, the total biomass area of the 0.1% group was even less than half that of the control group. The high dead biomass of the control group was likely caused by ethanol, a powerful fungicide that can coagulate proteins in bacteria. The results of FESEM also demonstrated the prominent anti-*S.mutans* activity of the baicalein-containing ethanol solution, corroborating the above two results. The antimicrobial mechanism of baicalein against *S.mutans* may include affecting cell membrane permeability, inhibiting nucleic acid or protein synthesis, blocking ATP synthesis and inhibiting bacterial metabolism and DNA to poisomerase, thereby inhibiting microbe attachment and biofilm formation [49,50,52].

Baicalein can also alleviate cytotoxicity caused by other drugs [53,54]. Polymerized monomers in adhesives and resins such as hydroxyethyl methacrylate (HEMA) and triethylene glycol dimethacrylate (TEGDMA) can diffuse into dental pulp through dentinal tubules and do damage to dental pulp cells [27]. Therefore, this present study focused on whether baicalein affects adhesive-induced cytotoxicity. The results showed that the addition of baicalein could effectively reduce the cytotoxicity caused by the adhesive, and high baicalein concentration might stimulate the proliferation of hDPCs, which needs follow-up research. Kang, K. A. et al. found that baicalein can protect cells by inhibiting oxidative stress-induced DNA damage, lipid peroxidation and protein oxidation by scavenging ROS in cells [55]. In our study, as baicalein concentration was increased, green fluorescence representing the ROS in hDPCs decreased. We speculate that baicalein may resist the adhesive induced-cytotoxicity by inhibiting ROS formation, but this assumption must be verified.

Our study validated that the combination of EWB strategy and baicalein could efficiently enhance the quality and durability of dentin bonding by simultaneously inhibiting the hydrolysis of hybrid layer and enzymatic hydrolysis of collagen fiber. Baicalein could also inhibit the formation of *S. mutans* biofilm, thereby preventing restoration failure caused by secondary caries. Moreover, baicalein exhibited high biological safety and bioactivity, with lower toxicity and drug-resistance compared with modifiers such as CHX and glutaraldehyde. Baicalein could resist adhesive-induced cytotoxicity and reduces the damage to hDPCs. However, attention should be paid to several limitations. First, baicalein with low solubility is easy to precipitate. A suitable concentration must be found prior to clinical use. Second, although baicalein possesses antibacterial, anti-enzymatic and other biological effects, its ability to promote remineralization requires further confirmation before it can be defined as an ideal bioactive dental material.

5. Conclusion

The present study suggested that baicalein is a prospective candidate for dentin bonding materials with bioactivity, which combined with EWB inhibits hydrolysis of hybrid layer, enzymatic hydrolysis of dentin collagen and biofilm formation of *S.mutans*, and resists the adhesive-induced cytotoxicity. The proposed strategy may provide a novel therapeutic strategy for durable adhesive restoration.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgments

This work was financially supported by National Natural Science Foundation of China (81701012), Youth Clinical Research Fund of Chinese Stomatological Association (CSA-B2018-01), Natural Science Foundation of Hubei Province (2017CFB798), and Lanzhou Innovation and Entrepreneurship Project (2017-RC-13).

References

[1] C.A. Murdoch-Kinch, M.E. McLean, Minimally invasive dentistry, J. Am. Dent. Assoc. 134 (1) (2003) 87–95.
[2] L. Breschi, T. Maravic, S.R. Cunha, A. Comba, M. Cadenaro, L. Tjaderhane, D.H. Pashley, F.R. Tay, A. Mazzoni, Dentin bonding systems: from dentin collagen structure to bond preservation and clinical applications, Dent. Mater. 34 (1) (2018) 78–96.
[3] B. Lorenzo, M. Annalisa, R. Alessandra, C. Milena, D.L. Roberto, D.S.D. Elettra, Dental adhesion review: aging and stability of the bonded interface, Dent. Mater. 24 (1) (2008) 90–101.
[4] M. Bernardo, H. Luis, M.D. Martin, B.G. Leroux, T.A. DeRouen, Survival and reason for failure of amalgam versus composite posterior restorations placed in a randomized clinical trial, J. Am. Dent. Assoc. 138 (6) (2007) 775–783.
[5] C.A. Stewart, Y. Finer, Biostable, antidegradative and antimicrobial restorative systems based on host-biomaterials and microbial interactions, Dent. Mater. 35 (1) (2019) 36–52.
[6] J.R. Kanca, Improving bond strength through acid etching of dentin and bonding to wet dentin surfaces, J. Am. Dent. Assoc. 123 (9) (1992) 35–43.
[7] D.H. Pashley, F.R. Tay, R.M. Carvalho, F.A. Rueggeberg, K.A. Agee, M. Carrilho, A. Donnelly, F. Garcia-Godoy, From dry bonding to water-wet bonding to ethanol-wet bonding. A review of the interactions between dentin matrix and solvated resins using a macromodel of the hybrid layer, Am. J. Dent. 20 (1) (2007) 7–20.
[8] F.T. Sadek, D.H. Pashley, Y. Nishitani, M.R. Carrilho, A. Donnelly, M. Ferrari, F.R. Tay, Application of hydrophobic resin adhesives to acid-etched dentin with an alternative wet bonding technique, J. Biomed. Mater. Res. A. 84 (1) (2008) 19–29.
[9] F.R. Tay, D.H. Pashley, R.R. Kapur, M.R. Carrilho, Y.B. Hur, L.V. Garrett, K.C. Tay, Bonding BisGMA to dentin–a proof of concept for hydrophobic dentin bonding, J. Dent. Res. 86 (11) (2007) 1034–1039.
[10] A. Al-Ammar, J.L. Drummond, A.K. Bedran-Russo, The use of collagen cross-linking agents to enhance dentin bond strength, J. Biomed. Mater. Res. B. Appl. Biomater. 91 (1) (2009) 419–424.
[11] F.C. Lessa, I. Nogueira, C. Huck, J. Hebling, C.A. Costa, Transdentinal cytotoxic effects of different concentrations of chlorhexidine gel applied on acid-conditioned dentin substrate, J. Biomed. Mater. Res. B. Appl. Biomater. 92 (1) (2010) 40–47.
[12] H. Schweikl, G. Schmalz, Glutaraldehyde-containing dentin bonding agents are mutagens in mammalian cells in vitro, J. Biomed. Mater. Res. 36 (3) (1997) 284–288.
[13] R. Verma, U.P. Singh, S.P. Tyagi, R. Nagpal, N. Manuja, Long-term bonding effectiveness of simplified etch-and-rinse adhesives to dentin after different surface pretreatments, J. Conserv. Dent. 16 (4) (2013) 367–370.
[14] M. Ondua, E.M. Njoya, M.A. Abdalla, L.J. McGaw, Anti-inflammatory and antioxidant properties of leaf extracts of eleven South African medicinal plants used traditionally to treat inflammation, J. Ethnopharmacol. 234 (2019) 27–35.
[15] S. Akyuz, N.O. Chousein, O. Sacan, R. Yanardag, S. Kalayci, A. Yarat, F. Sahin, Antibacterial and photodynamic effects of some plant extracts for cavity disinfection, Photodiagn. Photodyn. Ther. 26 (2019) 48–52.
[16] L.I. Xiang-yang, S. Bao-an, Progress in the development and application of plant-based antiviral agents, J. Integr. Agric. 16 (12) (2017) 2772–2783.
[17] D.J. Epasinghe, C.K. Yiu, M.F. Burrow, F.R. Tay, N.M. King, Effect of proanthocyanidin incorporation into dental adhesive resin on resin-dentine bond strength, J. Dent. 40 (3) (2012) 173–180.
[18] L. Kang, H. Yang, H. Yan, Y. Sun, X. Chen, J. Guo, J. Yue, H. Cui, Quercetin as a simple but versatile primer in dentin bonding, RSC Adv. 7 (58) (2017) 36392–36402.
[19] H. Yang, J. Guo, D. Deng, Z. Chen, C. Huang, Effect of adjunctive application of epigallocatechin-3-gallate and ethanol-wet bonding on adhesive-dentin bonds, J. Dent. 44 (2016) 44–49.
[20] H. Yang, K. Li, H. Yan, S. Liu, Y. Wang, C. Huang, High-performance therapeutic quercetin-doped adhesive for adhesive-dentin interfaces, Sci. Rep. 7 (1) (2017) 8189.
[21] M. Li-Weber, New therapeutic aspects of flavones: the anticancer properties of Scutellaria and its main active constituents Wogonin, Baicalein and Baicalin, Cancer

Treat. Rev. 35 (1) (2009) 57–68.
[22] C.C. Lin, D.E. Shieh, The anti-inflammatory activity of Scutellaria rivularis extracts and its active components, baicalin, baicalein and wogonin, Am. J. Chin. Med. 24 (1) (1996) 31–36.
[23] J. Xing, X. Chen, Y. Sun, Y. Luan, D. Zhong, Interaction of baicalin and baicalein with antibiotics in the gastrointestinal tract, J. Pharm. Pharmacol. 57 (6) (2005) 743–750.
[24] N. Chandrashekar, A. Selvamani, R. Subramanian, A. Pandi, D. Thiruvengadam, Baicalein inhibits pulmonary carcinogenesis-associated inflammation and interferes with COX-2, MMP-2 and MMP-9 expressions in-vivo, Toxicol. Appl. Pharmacol. 261 (1) (2012) 10–21.
[25] C. Duan, S. Matsumura, N. Kariya, M. Nishimura, T. Shimono, In vitro antibacterial activities of Scutellaria baicalensis Georgi against cariogenic bacterial, Pediatr. Dent. J. 17 (1) (2007) 58–64.
[26] E.J. Jang, S.M. Cha, S.M. Choi, J.D. Cha, Combination effects of baicalein with antibiotics against oral pathogens, Arch. Oral Biol. 59 (11) (2014) 1233–1241.
[27] I.P. Caldas, G.G. Alves, I.B. Barbosa, P. Scelza, F. de Noronha, M.Z. Scelza, In vitro cytotoxicity of dental adhesives: a systematic review, Dent. Mater. 35 (2) (2019) 195–205.
[28] A. Cova, L. Breschi, F. Nato, A.J. Ruggeri, M. Carrilho, L. Tjaderhane, C. Prati, R. Di Lenarda, F.R. Tay, D.H. Pashley, A. Mazzoni, Effect of UVA-activated riboflavin on dentin bonding, J. Dent. Res. 90 (12) (2011) 1439–1445.
[29] N. Hiraishi, C.K. Yiu, N.M. King, F.R. Tay, Effect of 2% chlorhexidine on dentin microtensile bond strengths and nanoleakage of luting cements, J. Dent. 37 (6) (2009) 440–448.
[30] V.P. Saboia, F. Nato, A. Mazzoni, G. Orsini, A. Putignano, M. Giannini, L. Breschi, Adhesion of a two-step etch-and-rinse adhesive on collagen-depleted dentin, J. Adhes. Dent. 10 (6) (2008) 419–422.
[31] M.R. Ayatollahi, M.Y. Yahya, A. Karimzadeh, M. Nikkhooyifar, A. Ayob, Effects of temperature change and beverage on mechanical and tribological properties of dental restorative composites, Mater. Sci. Eng. C. Mater. Biol. Appl. 54 (2015) 69–75.
[32] N. Nakabayashi, K. Kojima, E. Masuhara, The promotion of adhesion by the infiltration of monomers into tooth substrates, J. Biomed. Mater. Res. 16 (3) (1982) 265–273.
[33] L. Tjaderhane, F.D. Nascimento, L. Breschi, A. Mazzoni, I.L. Tersariol, S. Geraldeli, A. Tezvergil-Mutluay, M. Carrilho, R.M. Carvalho, F.R. Tay, D.H. Pashley, Strategies to prevent hydrolytic degradation of the hybrid layer-A review, Dent. Mater. 29 (10) (2013) 999–1011.
[34] Y.W. Chiu, T.H. Lin, W.S. Huang, C.Y. Teng, Y.S. Liou, W.H. Kuo, W.L. Lin, H.I. Huang, J.N. Tung, C.Y. Huang, J.Y. Liu, W.H. Wang, J.M. Hwang, H.C. Kuo, Baicalein inhibits the migration and invasive properties of human hepatoma cells, Toxicol. Appl. Pharmacol. 255 (3) (2011) 316–326.
[35] L. Crasci, L. Basile, A. Panico, C. Puglia, F.P. Bonina, P.M. Basile, L. Rizza, S. Guccione, Correlating in vitro target-oriented screening and docking: inhibition of matrix metalloproteinases activities by flavonoids, Planta Med. 83 (11) (2017) 901–911.
[36] L. Wang, Y. Ling, Y. Chen, C.L. Li, F. Feng, Q.D. You, N. Lu, Q.L. Guo, Flavonoid baicalein suppresses adhesion, migration and invasion of MDA-MB-231 human breast cancer cells, Cancer Lett. 297 (1) (2010) 42–48.
[37] H. Sano, T. Shono, T. Takatsu, H. Hosoda, Microporous dentin zone beneath resin-impregnated layer, Oper. Dent. 19 (2) (1994) 59–64.
[38] B. Van Meerbeek, Y. Yoshida, P. Lambrechts, G. Vanherle, E.S. Duke, J.D. Eick, S.J. Robinson, A TEM study of two water-based adhesive systems bonded to dry and wet dentin, J. Dent. Res. 77 (1) (1998) 50–59.
[39] M. Ekambaram, C.K. Yiu, J.P. Matinlinna, N.M. King, F.R. Tay, Adjunctive application of chlorhexidine and ethanol-wet bonding on durability of bonds to sound and caries-affected dentine, J. Dent. 42 (6) (2014) 709–719.
[40] M. Hashimoto, J. De Munck, S. Ito, H. Sano, M. Kaga, H. Oguchi, B. Van Meerbeek, D.H. Pashley, In vitro effect of nanoleakage expression on resin-dentin bond strengths analyzed by microtensile bond test, SEM/EDX and TEM, Biomaterials 25 (25) (2004) 5565–5574.
[41] P.G. Ding, D. Wolff, T. Pioch, H.J. Staehle, B. Dannewitz, Relationship between microtensile bond strength and nanoleakage at the composite-dentin interface, Dent. Mater. 25 (1) (2009) 135–141.
[42] A. Mazzoni, F.D. Nascimento, M. Carrilho, I. Tersariol, V. Papa, L. Tjaderhane, R. Di Lenarda, F.R. Tay, D.H. Pashley, L. Breschi, MMP activity in the hybrid layer detected with in situ zymography, J. Dent. Res. 91 (5) (2012) 467–472.
[43] J. Li, B. Chen, N. Hong, S. Wu, Y. Li, Effect of baicalein on matrix metalloproteinases and durability of resin-dentin bonding, Oper. Dent. 43 (4) (2018) 426–436.
[44] J.C.F. Almeida, M.F. De Goes, M.R.O. Carrilho, Durability of bonded-dentin treated with hydrophilic versus hydrophobic primers, Dent. Mater. 26 (2) (2010) e137.
[45] A.A. Leme, C.M. Vidal, L.S. Hassan, A.K. Bedran-Russo, Potential role of surface wettability on the long-term stability of dentin bonds after surface biomodification, J. Biomech. 48 (10) (2015) 2067–2071.
[46] C.S. Castellan, P.N. Pereira, R.H. Grande, A.K. Bedran-Russo, Mechanical characterization of proanthocyanidin-dentin matrix interaction, Dent. Mater. 26 (10) (2010) 968–973.
[47] R.H. Selwitz, A.I. Ismail, N.B. Pitts, Dental caries, Lancet 369 (9555) (2007) 51–59.
[48] H.J. Busscher, M. Rinastiti, W. Siswomihardjo, H.C.V.D. Mei, Biofilm formation on dental restorative and implant materials, J. Dent. Res. 89 (7) (2010) 657–665.
[49] N. Chinnam, P.K. Dadi, S.A. Sabri, M. Ahmad, M.A. Kabir, Z. Ahmad, Dietary bioflavonoids inhibit Escherichia coli ATP synthase in a differential manner, Int. J. Biol. Macromol. 46 (5) (2010) 478–486.
[50] B.Y. Yun, L. Zhou, K.P. Xie, Y.J. Wang, M.J. Xie, Antibacterial activity and mechanism of baicalein, Yao Xue Xue Bao 47 (12) (2012) 1587.
[51] Z. Zeng, L. Qian, L. Cao, H. Tan, Y. Huang, X. Xue, Y. Shen, S. Zhou, Virtual screening for novel quorum sensing inhibitors to eradicate biofilm formation of Pseudomonas aeruginosa, Appl. Microbiol. Biotechnol. 79 (1) (2008) 119–126.
[52] Y. Xie, W. Yang, F. Tang, X. Chen, L. Ren, Antibacterial activities of flavonoids: structure-activity relationship and mechanism, Curr. Med. Chem. 22 (1) (2015) 132–149.
[53] B.D. Sahu, J.M. Kumar, M. Kuncha, R.M. Borkar, R. Srinivas, R. Sistla, Baicalein alleviates doxorubicin-induced cardiotoxicity via suppression of myocardial oxidative stress and apoptosis in mice, Life Sci. 144 (2016) 8–18.
[54] X. Zhang, Y. Yang, L. Du, W. Zhang, G. Du, Baicalein exerts anti-neuroinflammatory effects to protect against rotenone-induced brain injury in rats, Int. Immunopharmacol. 50 (2017) 38–47.
[55] K.A. Kang, R. Zhang, M.J. Piao, S. Chae, H.S. Kim, J.H. Park, K.S. Jung, J.W. Hyun, Baicalein inhibits oxidative stress-induced cellular damage via antioxidant effects, Toxicol. Ind. Health 28 (5) (2012) 412–421.