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Effect of Baicalein on Matrix Metalloproteinases and Durability of Resin-Dentin Bonding

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Clinical Relevance

Baicalein used as a preconditioner in an etch-and-rinse adhesive system has potential value in clinical bonding procedures because it can effectively improve resin-dentin bonding durability and reduce interface nanoleakage *in vitro*.

SUMMARY

Objective: In an attempt to increase resin-dentin bonding quality, this study used baicalein as a preconditioner in an etch-and-rinse adhesive to evaluate its effect on matrix metalloproteinases (MMPs) and adhesive durability.

Methods: As a MMP inhibitor and potential collagen cross-linking agent, baicalein was used as a preconditioner in an etch-and-rinse adhesive system. The degree of conversion was evaluated by Fourier-transform infrared spectroscopy. EnzChek gelatinase/collagenase assay kits were then used to detect the MMP inhibitory effect of different concentrations of baicalein (0.1, 0.5, 2.5, and 5.0 μg/mL) on dentin powders. During *in vitro* bonding procedures, flat dentin surfaces on sound third molars were preconditioned with 2.5 μg/mL baicalein after being acid-etched; this step was followed by continuation of adhesive processes and build-up of resin composite. After resin-dentin stick preparation, bonding strength, failure mode, and interface nanoleakage were respectively evaluated via microtensile testing, stereomicroscopy, and field emission scanning electron microscopy either immediately or after storage in artificial saliva for three or six months. Data were analyzed by two-way analysis of variance and Tukey test (α=0.05).

Results: Baicalein at a concentration of 0-5.0 μg/mL did not influence the conversion of

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adhesives. However, it inhibited the activities of dentin-bond gelatinase and collagenase, especially at a concentration of 2.5 μg/mL, while effectively increasing microtensile bonding strength and decreasing nanoleakage *in vitro*, both immediately and after aging.

Conclusions: Baicalein used as preconditioner in an etch-and-rinse adhesive system has an anti-MMP function and effectively improves resin-dentin bonding durability *in vitro*, which has potential value in clinical bonding procedures.

INTRODUCTION

Although innovation in adhesive techniques has occurred since the "hybrid layer" was proposed,[1] the problem that exposed collagen fibrils deep inside the hybrid layer are vulnerable to hydrolysis or enzymolysis remains unsolved.[2-4] The discrepancy between the depth of acid-etching and resin infiltration in etch-and-rinse adhesive system leads to further catastrophic failure of the bonding interface, which significantly influences the durability of resin-dentin adhesive.

Studies[5-16] have found that the use of matrix metalloproteinase (MMP) inhibitors or cross-linkers is effective in protecting the dentin collagen matrix in the hybrid layer. MMP inhibitors such as chlorhexidine can terminate collagen degradation caused by host-derived endogenous MMPs,[5-7] including gelatinases (MMP-2 and MMP-9) and collagenases (MMP-1 and MMP-8).[2] Furthermore, cross-linking agents stabilize collagen fibrils and interfere with the identification and complexation of protease.[8] Some cross-linking agents, such as glutaraldehyde,[9,10] proanthocyanidins,[11-14] and genipin,[15,16] can markedly increase immediate or aging dentin bonding strength. However, because of cytotoxicity,[17,18] the use of chemical cross-linkers is restricted to laboratory experiments, while natural agents have a greater potential for clinical usage as a result of their biosafety and cost-effectiveness.

Baicalein (BAI) is one of the major flavonoids in *Scutellaria baicalensis*. Recently, an increasing number of studies have demonstrated that BAI can inhibit the activity and expression of MMP-2 and MMP-9 via PI3K/AKT,[19,20] p38-MAPK-NF-κB,[21,22] and MAPK-ERK1/2[23,24] signal pathways in various tumor cells. The effect of BAI on these anti-MMPs may offer more plausible reasons for its use in dentin adhesives. Moreover, BAI and proanthocyanidins are natural plant polyphenols that share a similar molecular structure with phenolichydroxyl functional groups,[25,26] suggesting they share comparable cross-linking properties. A previous study[27] showed that BAI could improve enzymatic resistance of the dentin collagen matrix better than could proanthocyanidins and quercetin at a concentration of 50 g/L. Therefore, we speculated that because of its potential MMP inhibition and cross-linking abilities, BAI is capable of stabilizing collagen fibrils and protecting the integrity of the hybrid layer in dentin bonding.

The purpose of this study was to evaluate the influence of BAI on MMPs and resin-dentin adhesive. The null hypothesis tested was that BAI used as a preconditioner in an etch-and-rinse adhesive system does not stabilize resin-dentin interfaces or increase bonding durability.

METHODS AND MATERIALS

Degree of Conversion (DC) Measurement

A flow chart of this study is shown as Figure 1. The resin monomer conversion of the preconditioner-adhesive complex was determined using Fourier-transform infrared spectroscopy (FTIR), as previously reported,[28,29] with a slight modification. BAI (Sigma-Aldrich, St Louis, MO, USA) was dissolved in 1% dimethyl sulfoxide (DMSO) to achieve the required concentrations (0.1, 0.5, 2.5, and 5.0 μg/mL) as a preconditioner. Then 5 μL of preconditioner was smeared upon clean 2.0 × 2.0-cm^2 polypropylene membranes. After gentle drying, 5 μL of Adper™ Single Bond 2 (3M ESPE, St Paul, MN, USA) adhesive was applied, and another cleaned polypropylene membrane was covered (n=5). DMSO at 1% in distilled water was used as a solvent control group. Absorption spectra (before and 20 seconds, 60 seconds, five minutes, 10 minutes, and 30 minutes after curing) were then observed with a FTIR spectrometer (Nicolet 6700, Thermo Scientific, Waltham, MA, USA). Each specimen was cured for 20 seconds at 0.5-cm distance with a light-curing unit (Elipar™ S10 LED, 3M ESPE). The DC of unreacted double carbon bonds was calculated from the ratio of absorbance intensities of aliphatic C=C (peak at 1638.6 cm^{-1}) and aromatic C=C (peak at 1609.4 cm^{-1}) before and after curing using the following formula:

$$DC(\%) = \left(1 - \frac{Abs(C=C)_{After}/Abs(C\cdots C)_{After}}{Abs(C=C)_{Before}/Abs(C\cdots C)_{Before}}\right) \times 100\%.$$

Tooth Collection

Caries-free human third molars of patients aged 18-39 years were collected with informed consent under

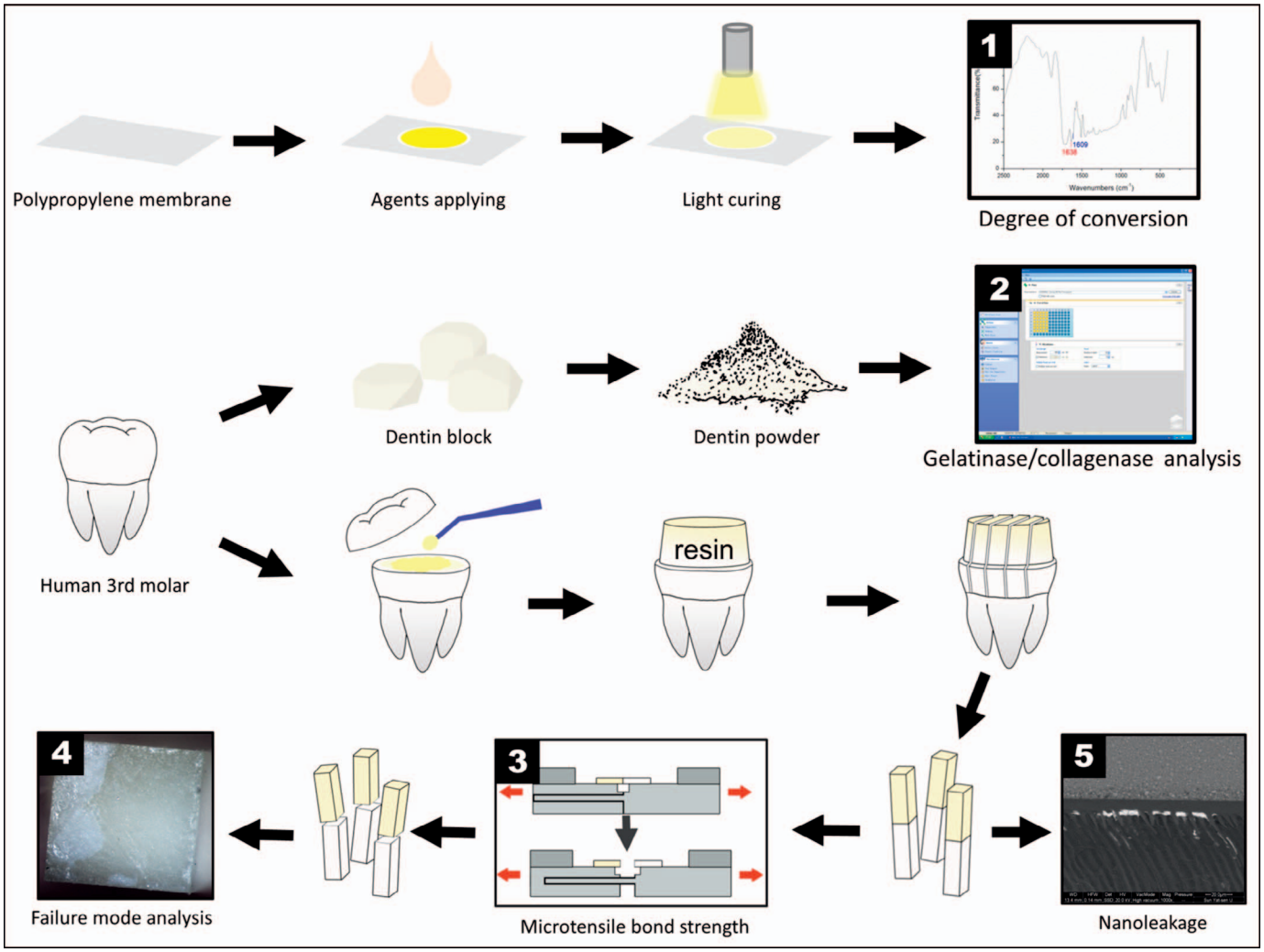

Figure 1. *Flow chart of this study. (1) Degree of conversion measurement obtained using FTIR. (2) Gelatinase and collagenase analysis obtained using EnzChek assay kits. (3) Bonding strength evaluation using microtensile tester. (4) Failure mode analysis using stereomicroscope. (5) Nanoleakage evaluation by FE-SEM.*

a protocol approved by the relevant ethics committee (approval code ERC-2014-23). Teeth were cleaned and stored in 0.5% chloramine-T solution at 4°C and were used within two weeks.

Gelatinolytic and Collagenolytic Activity Evaluation

The gelatinolytic and collagenolytic activities of dentin were measured by means of EnzChek gelatinase/collagenase assay kits (E-12055, Molecular Probes, Eugene, OR, USA), basically as previously reported.[30] Following this step, teeth were sectioned at the level of the cemento-enamel junction and enamel and pulp tissues were removed. After verifying that only the dentin remained using a stereomicroscope (M205A, Leica, Solms, Germany), the dentin blocks were dehydrated in anhydrous acetone at 4°C, then frozen in liquid nitrogen; we then continued triturating and sieving through multiple screens to obtain a fine powder smaller than 80 μm in size. The dentin powders were then demineralized with 35% phosphoric acid for 15 seconds and rinsed with distilled water.

After drying, the demineralized dentin powders were equally assigned to seven groups, as follows: preconditioned with BAI (0.1, 0.5, 2.5, and 5.0 μg/mL BAI), 2% chlorhexidine, 1% DMSO, or distilled water for 120 seconds, respectively. Adper™ Single Bond 2 was applied for one minute, and the reaction was stopped by acetone; this step was followed by rinsing and dehydration. Eighty milligrams of dentin powders and 20 μL of 100 μg/mL gelatin/collagen substrates were added to 96-well plates and incubated at 37°C (n=5). Fluorescent cleavage products were

recorded under a microplate reader (Infinite 200 PRO, Tecan, Männedorf, Switzerland) with ~495-nm absorption maxima and ~515-mm fluorescence emission maxima at one hour, 24 hours, 48 hours, and 72 hours. The relative fluorescence unit (RFU) of each well was corrected by subtracting the background fluorescence value of the non-enzyme control. Tests were repeated twice and the average taken.

Microtensile Bond Strength (μTBS) Evaluation

The occlusal enamel and superficial dentin of teeth were removed perpendicular to the long axis of each tooth. After verification using a stereomicroscope, a standardized smear layer was created on the exposed middle coronal dentin with 600-grit silicon carbide paper. The teeth were then equally and randomly assigned to four treatment groups: exposed dentin surfaces were preconditioned with 2.5 μg/mL BAI, 5% glutaraldehyde, 1% DMSO, and distilled water for 120 seconds for each group after etching with 35% phosphoric acid for 15 seconds. Adper™ Single Bond 2 adhesive was applied and cured and then the coronal height was reconstructed with 4-5-mm thick layers of resin composite (Filtek Z250, 3M ESPE). After storing in distilled water at 37°C for 24 hours, teeth were sectioned perpendicular to the long axis of the adhesive surfaces with cutting equipment (Accotom-50, Struers, Rodovre, Denmark) to create 0.9 mm × 0.9 mm × 8 mm resin-dentin sticks as nontrimming specimens for the microtensile test.[31] Each tooth was cut into 12 sticks. These sticks were then randomly divided into three subgroups; one group was tested immediately and the others were stored in artificial saliva at 37°C for three and six months as aging groups. The artificial saliva was changed weekly.

The adhesive surface area (S) of each resin-dentin stick was calculated with the help of a digital caliper (with an accuracy of 0.01 mm) and then microtensile testing was performed at a loading speed of 1 mm/min (T-6102K, BISCO, Schaumburg, IL, USA). The maximum loading force (F) was recorded and the μTBS was calculated using the following formula: $\mu TBS\ (MPa) = F/S$.

Failure Mode Analysis

After microtensile testing, all specimens were collected and observed with a stereomicroscope at 100× magnification. Failure modes were classified as follows:[32,33] 1) adhesive failure: failure occurred at the adhesive agent or in the layers between the adhesive agent and the dentin or resin; 2) cohesive failure: failure occurred at the inner parts of the dentin or resin; and 3) mixed failure: mixture of adhesive and cohesive failures.

Interfacial Nanoleakage Testing

Three sticks of each tooth remained for interfacial nanoleakage evaluation,[34] including the immediate group and the three-month and six-month storage groups. Ammoniacal silver nitrate was prepared according to a protocol previously described.[35] The sticks were coated with two layers of nail varnish applied up to 1 mm thick on the bonded interfaces and placed in the 50 mass% ammoniacal $AgNO_3$ in darkness for 24 hours. They were then rinsed thoroughly in distilled water three times and immersed in photodeveloping solution for eight hours under a fluorescent light. All of the sticks were wet-polished with silicon carbide paper to remove the nail varnish and were then polished down with 800-, 1500-, 3000-, and 5000-grit silicon carbide paper. The sticks were then carbon coated and analyzed with a field emission scanning electron microscope (FE-SEM; Quanta 400F, FEI, Hillsboro, OR, USA) operated in the backscattered electron mode. Three discontinuous fields of vision at the central area of each sample's hybrid layer were photographed. The percentage of the total hybrid layer length with nanoleakage was calculated with the Image J program (ImageJ 1.48v, National Institutes of Health, Bethesda, MD, USA).

Statistical Analysis

In the bonding evaluation, the tooth was considered the statistical unit. The μTBS (MPa) and interfacial nanoleakage (%) from the same experimental unit were averaged for statistical purposes at each storage time. After observing normality and homogeneity of variances, data were subjected to a two-way analysis of variance, and multiple comparisons were performed with the Tukey test at $\alpha = 0.05$ using SPSS 22.0 (IBM Corp, Chicago, IL, USA).

RESULTS

Degree of Conversion

The DC results are shown in Figure 2. DC increased dramatically in all groups initially, but the rate of increase decreased and tended to stabilize with time. No significant differences were found among different pretreatments groups at different time points ($p>0.05$).

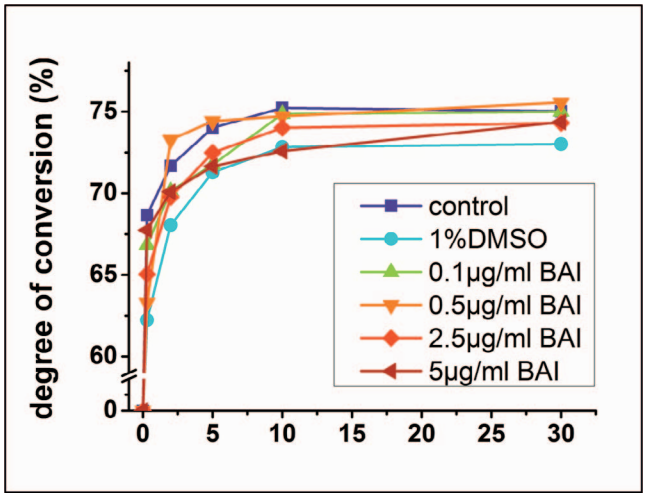

Figure 2. *Degrees of conversion of different groups had no statistical differences at different time points (p>0.05).*

Gelatinolytic/Collagenolytic Activity

Results of the EnzChek assay kit for gelatinolytic activities are shown in Table 1. The 2.5 μg/mL BAI and 2% chlorhexidine groups had significantly lower RFU values than other groups at all time points ($p<0.05$), but there were no significant differences between them ($p>0.05$). However, the 5.0 μg/mL and 0.1 μg/mL BAI groups showed no significant differences with the control group after being incubated for ≥48 hours ($p>0.05$). The 1% DMSO group and control group had the highest RFU values at all time points, with no significant difference between them ($p>0.05$). Similarly, collagenolytic activities increased in all groups over time (Table 2). The 2.5 μg/mL and 0.5 μg/mL BAI and 2% chlorhexidine groups showed an equally strong inhibitory effect on collagenase ($p<0.05$), while the 5.0 μg/mL and 0.1 μg/mL BAI groups had an attenuating effect, but the effect was still better than that associated with the control group ($p<0.05$). However, in contrast to gelatinase testing, significant differences between the 1% DMSO and control groups in collagenase testing were recorded at 24 hours and 48 hours ($p<0.05$).

Microtensile Bonding Strength

The results for μTBS are shown in Table 3 (premature failures were not included in the statistical analysis). In both the immediate and aging groups, the μTBS values of both the 5% glutaraldehyde and 2.5 μg/mL BAI groups were significantly higher than that of the control group ($p<0.05$). Although the 2.5 μg/mL BAI group had slightly lower values than did the 5% glutaraldehyde group, there were no significant differences between groups ($p>0.05$). The μTBS values of the 1% DMSO group were significantly lower than those of the 2.5 μg/mL BAI and 5% glutaraldehyde groups ($p<0.05$) but higher than that of the control group ($p<0.05$), with statistical differences.

Failure Mode Analysis

The results of the failure mode analysis are shown in Figure 3. Mixed and adhesive failures were the most common in each group. With the aging processing, adhesive failures increased, especially in the control and 1% DMSO groups, with growth rates of 27% and 17%, respectively. However, the increase was not significant in the 5% glutaraldehyde and 2.5 μg/mL BAI groups, with mixed failure still being the predominant failure mode after six months of aging.

Nanoleakage

The nanoleakage results are shown in Table 4, and representative images under FE-SEM are presented in Figure 4. The nanoleakage percentage of the 2.5 μg/mL BAI group was significantly lower than that in the control group, especially before aging ($p<0.05$). The 5% glutaraldehyde group acted as a positive control group and had a low nanoleakage percentage, and there was no significant difference between the result before and after aging ($p>0.05$). Although the 1% DMSO group had a relatively lower nanoleakage result than the control group ($p<0.05$), it was still higher than that in the 2.5 μg/mL BAI group ($p<0.05$).

Table 1: *Relative Fluorescence Intensity (Means and Standard Deviations) of Dentin-bond Gelatinase*[a]

	Control	2% CHX	1% DMSO	0.1 μg/mL BAI	0.5 μg/mL BAI	2.5 μg/mL BAI	5.0 μg/mL BAI
1h	27.94 ± 6.33 A	3.29 ± 2.60 B	22.16 ± 1.71 AC	26.16 ± 2.32 A	17.18 ± 4.09 CD	2.75 ± 2.89 B	14.89 ± 1.34 D
24h	54.69 ± 6.53 A	14.26 ± 2.42 B	53.55 ± 3.61 A	38.37 ± 4.99 CD	28.23 ± 5.64 C	15.62 ± 6.29 B	44.64 ± 4.88 AD
48h	59.11 ± 6.12 A	23.71 ± 2.90 B	58.30 ± 3.43 A	54.09 ± 8.63 A	37.87 ± 4.37 C	25.75 ± 4.23 B	48.86 ± 5.00 A
72h	71.96 ± 10.40 A	39.93 ± 3.87 BC	71.96 ± 7.80 A	64.88 ± 7.50 AD	51.39 ± 6.09 CD	33.05 ± 3.58 B	60.85 ± 5.48 AD

Abbreviations: BAI, baicalein; CHX, chlorhexidine; DMSO, dimethyl sulfoxide.
[a] *Different uppercase letters indicate significant differences between treatment groups at the same time point (p<0.05). Control = distilled water.*

Table 2: *Relative Fluorescence Intensity (Means and Standard Deviations) of Dentin-bond Collagenase*[a]

	Control	2% CHX	1% DMSO	0.1 μg/mL BAI	0.5 μg/mL BAI	2.5 μg/mL BAI	5.0 μg/mL BAI
1h	31.42 ± 9.01 A	1.60 ± 1.49 B	22.79 ± 7.52 AC	20.61 ± 3.59 CD	3.55 ± 2.21 B	2.95 ± 2.52 B	10.81 ± 2.10 BD
24h	49.64 ± 6.09 A	13.00 ± 5.48 B	37.75 ± 5.39 C	36.56 ± 4.34 C	23.01 ± 4.08 BD	18.59 ± 5.74 B	30.24 ± 4.90 CD
48h	59.95 ± 3.38 A	24.48 ± 5.87 B	48.24 ± 7.54 C	46.30 ± 4.05 CD	29.94 ± 1.25 BE	27.31 ± 4.84 BE	37.12 ± 5.40 DE
72h	69.17 ± 4.65 A	36.59 ± 4.40 B	61.41 ± 6.00 AC	54.85 ± 5.38 C	40.72 ± 3.55 B	35.45 ± 3.69 B	51.57 ± 7.41 C

Abbreviations: BAI, baicalein; CHX, chlorhexidine; DMSO, dimethyl sulfoxide.
[a] *Different uppercase letters indicate significant differences between treatment groups at the same time point ($p<0.05$). Control = distilled water.*

DISCUSSION

This study evaluated the influence of BAI on MMPs and a resin-dentin adhesive. Acting as the connecting media between the dentin and adhesive resin, the hybrid layer plays an important role in resin-dentin adhesives. The complete infiltration and curing of the adhesive agent in the demineralized dentin matrix are extremely important for quality hybrid layer formation. However, the prerequisite for use of BAI as a MMP inhibitor and cross-linking agent in the bonding procedure to improve adhesive strength is that it does not alter the inherent curing characteristic of the adhesive. In this study, the results from FTIR showed that BAI had no significant influence on the curing behavior of adhesive within a concentration range of 0-5.0 μg/mL, indicating that it could be used as a preconditioner in follow-up experiments.

The present results of EnzChek gelatinase/collagenase assay kits showed that BAI has a direct and remarkable inhibitory effect on the total activity of dentin-bond gelatinase/collagenase, especially at concentrations of 0.5 μg/mL and 2.5 μg/mL. Previous studies[36,37] have determined that the C6 and C7 hydroxyl groups of BAI have powerful metal-chelate characteristics and can chelate transition metals such as Zn^{2+}. In consideration of MMPs as a family of Zn^{2+}/Ca^{2+}–dependent enzymes, the competitive reaction of metal ions might be the main inhibitory mechanism of BAI on MMPs. Moreover, cross-linked collagen has a higher resistance to collagenase digestion,[38-41] indicating that there might be a strong relationship between cross-linking and MMP inhibition. Prompted by the bonding mode of BAI and human serum albumin,[42] BAI can act as a potential cross-linker since its hydroxyl groups can form hydrogen bonds with the amide carbonyl of proteins. Furthermore, it has been reported that cross-linkers can cross-link not only proteins but also proteases. This may directly interfere with the molecular mobility of proteases or inactivate C-terminal telopeptidases, maintaining telopeptides' ability and sterically blocking collagenase binding to the critical peptide bond.[43,44] Therefore, we speculated that the MMP inhibitory mechanism of BAI in endogenous dentin-bond MMPs might be as follows. First, BAI could compete with the active center of the enzyme through a metal-chelating effect, grabbing metal irons such as Zn^{2+} to suppress the activity of MMPs. Second, BAI might cross-link and alter the three-dimensional structure or molecular mobility of MMPs, resulting in the loss of the collagen enzymolysis ability of the latter. Third, BAI might cross-link with dentin collagen fibers through hydrogen bonds, changing or covering the recognition sites of MMPs in collagen to interfere with enzymatic coordination and complexation, thereby protecting noncoated collagen from degradation.

However, the inhibitory effect of BAI on gelatinase/collagenase was not a simple positive correlation, and a concentration limitation of BAI still existed. The increasing concentration of BAI might result in a competition between BAI molecules themselves for the bonding sites of protease, leading to the attenuation of the inhibitory effect. Another reason might be that the high concentration of

Table 3: *Microtensile Bond Strengths (MPa, Means and Standard Deviations) of Different Treatment Groups at Different Aging Time Points*[a]

	Control	1% DMSO	5% GD	2.5 μg/mL BAI
Immediate	41.89 ± 5.18 Aa	49.32 ± 5.03 Ba	58.86 ± 4.29 Ca	58.32 ± 3.95 Ca
3 mo	34.46 ± 6.22 Ab	43.59 ± 5.8 2Bb	56.10 ± 5.8 9Ca	53.07 ± 3.97 Cb
6 mo	26.82 ± 5.30 Ac	40.73 ± 4.91 Bb	51.86 ± 6.42 Cb	52.43 ± 5.43 Cb

Abbreviations: BAI, baicalein; DMSO, dimethyl sulfoxide; GD, glutaraldehyde.
[a] *Different uppercase letters indicate significant differences between different treatment groups. Different lowercase letters indicate statistically significant differences between different aging time points ($p<0.05$). Control = distilled water.*

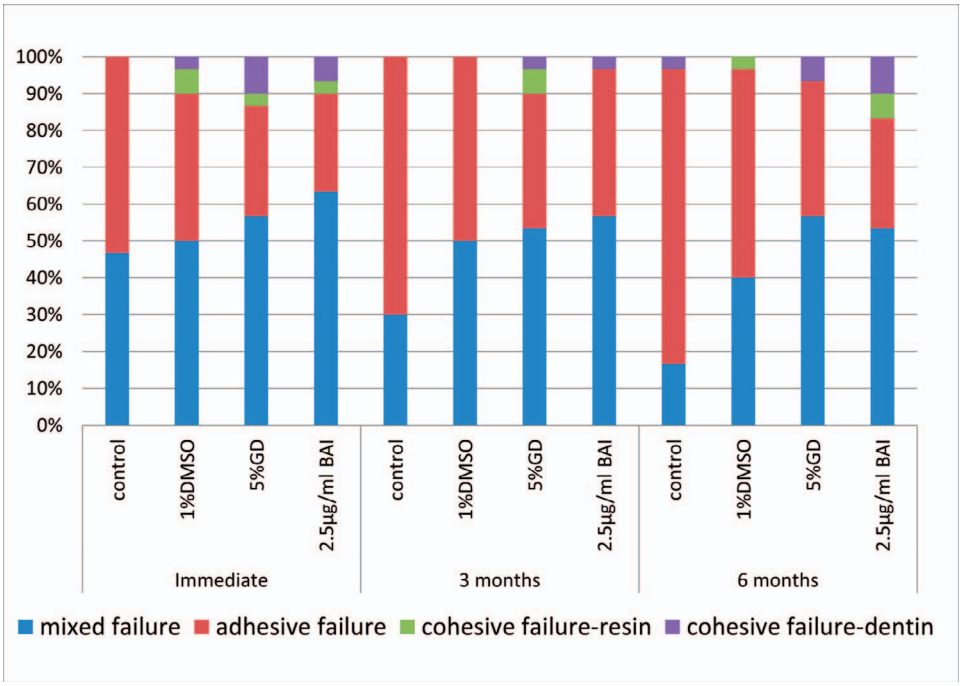

Figure 3. *Failure modes for each group immediately or after aging. Adhesive failures increased remarkably in the blank groups after aging, but mixed failure was still the predominant failure mode in the 2.5 μg/mL baicalein group after six months of aging.*

solutes increased the viscosity, which obstructed the infiltration of BAI into the dentin-collagen matrix and sequentially affected the combination with dentin-bond MMPs deep inside. In addition, the optimum concentration effects of BAI on gelatinase and collagenase were slightly different. This might be explained by the content or structure differences between these two types of proteases. However, the results of EnzChek gelatinase/collagenase assay kits only demonstrated that BAI could inhibit the total activities of dentin-bond gelatinase/collagenase; the inhibitory effect on definite MMP subtypes is still unclear; further research is, therefore, required.

Synthesizing the DC results and gelatinase/collagenase activities, we chose to apply 2.5 μg/mL BAI in an etch-and-rinse adhesive system to investigate its influence on bonding strength. According to the results of microtensile bond strength and nanoleakage testing, the 2.5 μg/mL BAI group had a significantly higher microtensile bond strength, less adhesive failure, and lower nanoleakage compared with the control and 1% DMSO groups, both immediately and after artificial saliva storage. This demonstrated the enhancement of resin-dentin bonding strength by BAI. Therefore, the null hypothesis of this study was rejected. We speculated that the BAI mechanism responsible for the enhancement of resin-dentin adhesive might be the coefficient of its MMP inhibitory effect and protein cross-linking property. Haslam[45] demonstrated that the amphiphilic structure of natural plants' polyphenol could help them cross-link with proteins. The principal driving forces toward complexation were "hydrophobic effects" from aromatic nuclei groups, and then enhancement by "hydrogen bonding" of phenolic hydroxyl groups. BAI has fewer phenolic hydroxyl groups and stronger hydrophobic performance compared with proanthocyanidins, implying that BAI might react with dentin collagen via hydrophobic bonding, mainly, similar to the bonding

Table 4: *Percentage of Nanoleakage (%, Means and Standard Deviations) of Different Treatment Groups at Different Aging Time Points*[a]

	Control	1% DMSO	5% GD	2.5 μg/mL BAI
Immediate	10.69 ± 3.12 Aa	7.26 ± 2.48 Ba	8.16 ± 3.11 ABa	3.38 ± 1.26 Ca
3 mo	19.72 ± 3.59 Ab	11.49 ± 3.52 Bb	8.10 ± 2.9 7BCa	7.33 ± 3.32 Cb
6 mo	34.96 ± 6.87 Ac	19.41 ± 6.10 Bb	9.62 ± 3.59 Ca	10.72 ± 4.71 Cb

Abbreviations: BAI, baicalein; DMSO, dimethyl sulfoxide; GD, glutaraldehyde.
[a] *Different uppercase letters indicate significant differences between different treatment groups. Different lowercase letters indicate statistically significant differences between different aging time points ($p<0.05$). Control = distilled water.*

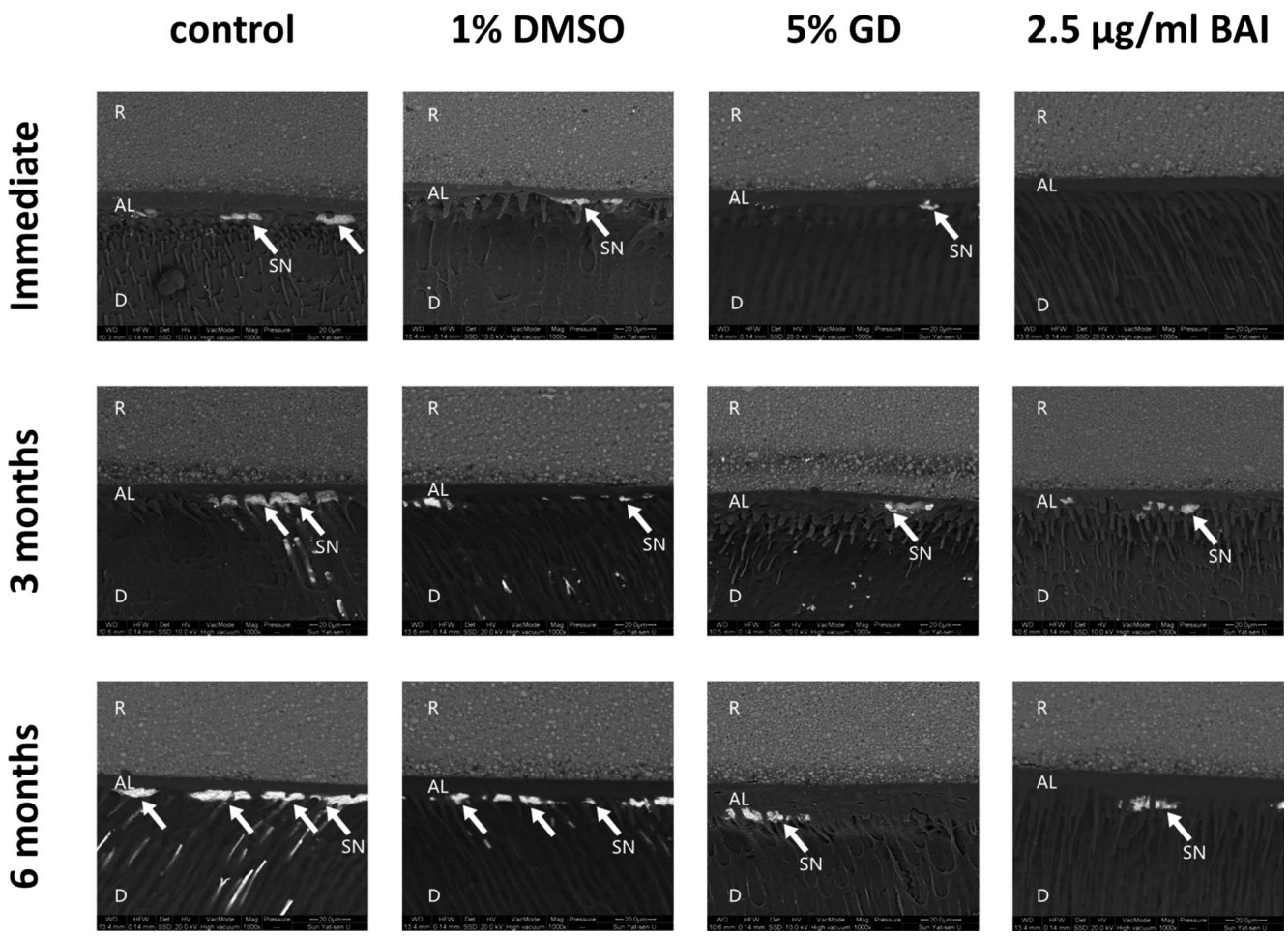

Figure 4. *Nanoleakage images of different groups immediately or after aging under SEM (1000×) showed that the baicalein group had a significantly lower nanoleakage percentage than the control group at each time point. (p<0.05). R, resin; AL, adhesive layer; D, dentin; SN, silver nitrate.*

mode of BAI and human serum albumin.[42] Consequently, BAI might improve the mechanical properties of dentin collagen by forming hydrogen bonds with the latter to increase the cross-linking and prevent the collagen fibrils network from extending and slipping. Furthermore, according to the cross-linking mechanism of proanthocyanidins,[46,47] the stronger hydrophobic performance of BAI might decrease water absorption and the swelling of collagen, thereby minimizing sensitivity to hydrolysis and protecting collagen from degradation.

In addition, DMSO had a certain effect on bond strength improvement in the present study. The findings of other studies[48-50] also reached an agreement about the enhancement of DMSO on dentin adhesives. It is worth mentioning that the μTBS and nanoleakage of the 1% DMSO group at all time points were significantly superior to those of the control group, but they were inferior to those of the 2.5 μg/mL BAI group, indicating that 1% DMSO has a synergistic effect on bonding improvement with BAI. The effect of DMSO might be explained as follows. First, the amphiphilic character of DMSO could help dissolve hydrophobic monomers,[51] including BAI, for improved diffusion and collagen encapsulation. Secondly, DMSO has the ability to break down water's self-associative tendency,[52] establishing a stable hydrophobic microenvironment to enhance cross-linkage between BAI and collagen. In addition to its other abilities, such as collagen moisturization and hydrogen-bond modification,[48] and its influence on the surface wetting behavior of resins,[53] DMSO was capable of synergistically improving bonding quality with BAI in the resin-dentin adhesive procedure.

A limitation of this study is that it only investigated the effect of BAI on the Adper™ Single Bond 2 etch-and-rinse adhesive system with a certain preconditioning time (120 seconds). The potential difference in findings regarding other adhesive

systems and treatment periods is still unclear. Further experiments should focus on the influence and mechanism of BAI on definite subtypes of MMPs while using BAI in other adhesive systems for clinical exploration.

CONCLUSIONS

BAI used as a preconditioner in an etch-and-rinse adhesive system could effectively improve resin-dentin bonding durability and decrease interface nanoleakage under the premise that it has no significant influence on the curing behavior of the adhesive agent. The effect of BAI in dentin adhesion might be related to its anti-MMP function and cross-linking property, which enhance the collagen matrix and stabilize the hybrid layer synergistically with DMSO during the bonding process.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Ethics Review Committee, Guanghua School of Stomatology, Hospital of Stomatology, Institute of Stomatological Research, Sun Yat-sen University. The approval code for this study is ERC-[2014]-23.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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