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The effect of transient proanthocyanidins preconditioning on the cross-linking and mechanical properties of demineralized dentin

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Abstract Proanthocyanidin-based preconditioners were prepared by adding powdered proanthocyanidins-rich grape seed extract to various solvents at different concentrations. Demineralized dentin specimens were preconditioned for 20, 30, 60 or 120 s, followed by the evaluation of their cross-linking degree, mechanical properties and micromorphology. The cross-linking degree of the demineralized dentin collagen exhibited concentration- and time- dependent increase after preconditioning treatment, irrespective of the preconditioner and the solvent. When treated for the same exposure time, specimens after 15% proanthocyanidins preconditioning resulted in the highest mean ultimate tensile strength compared with all the other groups tested. Five percent glutaraldehyde control group produced the highest cross-linking degree, but the ultimate tensile strength was lower than that of 15% proanthocyanidins group. The field emission scanning electron microscopy confirmed that the demineralized dentin collagen was in a homogeneous and regular arrangement after preconditioning and maintained expanding, regardless of the surface moisture conditions.

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1 Introduction

Dentin is a complex mineralized tissue arranged in an intricate three-dimensional frame, composed of 70% mineral (by weight), 20% organic component and 10% fluid. Type I collagen accounts for 90% of the dentin organic matrix and it is an essential molecule to provide tissues with tensile strength, form and cohesiveness. These physical characteristics are probably due to the presence of extensive, intermolecular, covalent cross-links in the fibrils [1, 2].

In restorative dentistry procedures, the formation and quality of a hybrid layer, composed of collagen fibrils from demineralized dentin matrix embedded with adhesive resin, play a pivotal role in creating good resin-dentin bonds. Despite of the significant progress in contemporary adhesives, incomplete resin infiltration in demineralized dentin cannot be always avoided, consequently resulting in a resin-deficient zone of unprotected collagen fibrils at the bottom of the hybrid layer. Meanwhile, resin degradation leads to greater collagen exposure. The denuded collagen fibrils within hybrid layers are susceptible to hydrolytic/enzymatic degradation, which is considered as one of the possible reasons responsible for the compromised durability of resin–dentin bonds [3]. To further stabilize and strengthen collagen fibrils in biological tissues, the induction of exogenous collagen cross-links has been proposed as a mechanism to improve the mechanical stability and reduce the biodegradation rates of collagen. Several synthetic chemicals [glutaraldehyde (GD), carbodiimide, etc.] and natural occurring agents [genipin, proanthocyanidins (PA), etc.] have been proved to be good crosslinkers for collagen [4–6].

PA is a group of polyphenolic natural products composed of flavan-3-ol subunits, widely available in fruits,

vegetables, nuts, seeds, flowers and barks. It has received an increasing attention due to its vast availability as dietary supplements and potential use in several medical fields, such as antioxidant, antimicrobial, anti-inflammatory agent, and inhibitor of some enzymes [7, 8]. Recently, PA from grape seed extract has been investigated in various studies as a potential collagen cross-linking agent to enhance the mechanical and chemical properties of demineralized dentin, and increase the resistance to enzymatic digestion [9–12]. As a type of natural cross-linkers, PA is superior to those synthetic ones owing to their moderate reaction rate, good biocompatibility and the variety of health-promoting actions [13, 14]. Consensus seems to exist about the use of PA on demineralized human dentin to improve the mechanical stability of the collagen matrix in hybrid layer. However, the mechanisms of interactions between PA and demineralized dentin matrix have not been fully determined yet. Moreover, the treatment duration of most previous studies (10 min or longer) is not clinically feasible, and the solvent used (for example, PBS solutions) for PA pretreatment differs from that of the present bonding systems [10, 11]. In other words, limited studies have been conducted about the effect of PA in different pure solvents within clinical feasible treatment duration on the properties of the demineralized dentin.

Therefore, in the present study, PA-based agents were prepared in different polar solvents, and preconditioning time was reduced to evaluate the effect of PA preconditioning on demineralized dentin matrix, using a more clinical relevant procedure. The null hypothesis tested was that the transient PA preconditioning would have no significant effect on the cross-linking degree and ultimate tensile strength (UTS), and make no notably morphological changes to demineralized dentin matrix.

2 Materials and methods

2.1 Tooth collection

Eighty extracted human molars were collected with the patients' informed consent, following a protocol approved by the Ethics Committee Board of the Fourth Military Medical University. The teeth were stored in physiological saline at 4°C and were used within 2 weeks after extraction.

2.2 Preparation of PA-based preconditioners

PA-based preconditioners were prepared by adding powdered grape seed extract (Acetar Bio-Tech Inc., Xi'an, China), rich in PA, to various solvents (aqueous ethanol $\geq$ 99.5%, aqueous acetone $\geq$ 99.9% or distilled water, Tian Yu Chemical Engineer Co. Ltd., Tianjin, China) at different concentrations, 5, 10 and 15%. The pH value of the slightly acidic solutions was adjusted to 7.2 using NaOH (HDB Chemical Engineer Co. Ltd., Beijing, China). Then the solutions were filtered before use.

2.3 Evaluation of cross-linking degree

Fifteen sound teeth were removed of enamel under running water and sectioned perpendicular to their long axis into slices with a thickness of 1.0 $\pm$ 0.1 mm, using a low-speed diamond saw (SYJ-160, Ke Jing Co., Shenyang, China). Then the slices were frozen in liquid nitrogen, pulverized and demineralized with 10% phosphoric acid for 5 h at 4°C. The insoluble residue was washed with distilled water by repeated centrifugation (4,000$\times g$ for 20 min) and lyophilized, composed mainly of dentin collagen (>90%) [15]. A 1-mg quantity of demineralized dentin powder was immersed in 5 ml of preconditioners (same preparation, solvents and concentrations described earlier) for different time (20, 30, 60, 120 s) at 37°C. 5% GD in distilled water was used as positive control.

After treatment, the specimens were extensively washed by repeated centrifugation (5 times), lyophilized, and subjected to ninhydrin assay [16]. The cross-linking degree was determined through the reaction of ninhydrin (2, 2-dihydroxy-1, 3-indanedione) with the primary amine groups of collagen according to the absorption spectroscopy method. Specimens were heated with ninhydrin solution (Ding Guo Biotechnology Ltd., Beijing, China) for 20 min, and then the optical absorbance was recorded with an enzyme-linked immuno-assay reader (Tecan Group Ltd, Männedorf, Switzerland) at 570 nm, using glycine at various known concentrations as the standard. Cross-linking degree was calculated as below:

$$\text{Cross - linking degree}(\%) = (M_0 - M_t)/M_0 \times 100\%.$$

M_0 is the amount of free amino groups in dentin matrix before cross-linking, and M_t is the amount of free amino groups in the matrix after cross-linking.

2.4 Measurement of ultimate tensile strength

Sixty teeth were sectioned parallel to their long axis into 0.5 $\pm$ 0.1 mm thick slabs (n = 6–8 beams per tooth) with a slow speed diamond under constant water irrigation. The sections were further trimmed to a rectangular dimension of 0.5 mm (thickness) $\times$ 1.7 mm (width) $\times$ 7.0 mm (length) in such a way that permitted the load applied in the testing in an orientation parallel to the dentin tubule long axis. An hourglass shaped neck area of 0.25 mm^2 was made at middle of the dentin specimens using a superfine cylindrical diamond bur. Specimens were fully

demineralized by 10% phosphoric acid solution (Tian Yu chemical engineer Co. Ltd., Tianjin, China) for 5 h, and complete demineralization was verified by X-ray [9, 11, 17]. Pure acetone, ethanol and distilled water were used as blank controls, and 5% GD was set as positive control. Specimens were immersed in the relative treatment solutions for 30, 60 or 120 s, respectively. Following thorough rinsing, the specimens were tested in a universal testing machine (EZ Test, Shimadzu, Japan) at a crosshead speed of 1 mm/min to measure the UTS (15 beams in each group). During the testing procedure, the specimens were maintained in a moist-to-wet state, with water used in a transfer pipette.

2.5 FESEM analysis

Dentin slices of about 1.0 mm thick were prepared from five teeth and were further cut into 4 equal blocks. The dentin surface were polished flat using 600, 800, 1,000 and 1,500 grit silicon carbide abrasive paper under running water. After etched by 37% Phosphoric Acid Etchant (Bisco Inc, Illinois, USA) for 15 s to superficially demineralize dentin, the specimens were randomly and equally assigned to one of the following treatments for different treatment duration (60 or 120 s): 15% PA in ethanol, 10% PA in ethanol, 15% PA in acetone, 10% PA in acetone, 5% GD and non-treated control. Then the specimens were rinsed with distilled water, and the excess water was blotted with the absorbent paper, leaving the surface visibly moist. For those treated for 120 s, specimens were divided into two subgroups in each preconditioning group, to observe the effects of different surface moisture conditions on the micromorphology of demineralized dentin with or without preconditioning treatment. For one subgroup, the dentin surface was commonly blot-dried as mentioned above. For the other, the surface was blot-dried followed by an additional air jet with an air-syringe for 5 s, 1 cm away from the dentin surface. The specimens were all fixed in 3% GD in 0.1 M PBS at pH 7.2 for 4 h at room temperature, and rinsed with 0.1 M PBS. After dehydration in an ascending ethanol series (25, 50, 75, 90, 95 and 100%), specimens were submitted to hexamethyldisilazane (Sigma Chemical Co., Louis, USA) drying. The processed specimens were sputter-coated with gold (E-1045, Hitachi, Tokyo, Japan), and the morphology of the demineralized collagen matrix was examined with a field emission scanning electron microscope (FESEM) (S-4800, Hitachi, Tokyo, Japan).

2.6 Statistical analysis

Means and standard deviations of cross-linking degree and UTS values were calculated. As values were normally distributed (confirmed by Kolmogorov–Smirnof test), data were statistically analyzed using a two-way ANOVA and Tukey's post hoc test with 95% confidence level.

3 Results

3.1 Evaluation of cross-linking degree

Cross-linking degrees of demineralized dentin after preconditioning are presented in Table 1. The results showed that cross-linking degrees exhibited concentration- and time-dependent increase, irrespective of the preconditioner and the solvent. The 5% GD group showed the highest cross-linking degree, especially for the specimens treated for 120 s (62.27 ± 0.64%), followed by specimens treated by 15% PA in ethanol for 120 s. Those treated by 5% PA in distilled water for 20 s presented the lowest cross-linking degree (5.63 ± 2.03%). Among the PA preconditioning groups, those with ethanol or acetone as the solvent showed significantly higher cross-linking degree than those of distilled water group when treated at the same concentration and for the same treatment duration ($P < 0.05$). There were no significant differences in the cross-linking degree between specimens treated for 20 and 30 s, when using the same preconditioner at the same concentration ($P > 0.05$).

3.2 Measurement of ultimate tensile strength

The mean UTS values of demineralized dentin after cross-linking treatment were summarized in Table 2. No statistically significant interaction was found between the factors studied (treatment vs. time). Mean baseline UTS in distilled water group was 12.70 MPa, which had no significant difference with those in ethanol and acetone groups. UTS values rose along with time extension in treatment groups. Specimens treated by 5% GD, 10% PA or 15% PA for 120 or 60 s presented significantly higher UTS than those treated for 30 s and blank controls ($P < 0.05$). The 15% PA-based preconditioners resulted in the highest mean UTS values compared with all the other groups when exposed for the same treatment duration. There were no significant differences in UTS for dentin specimens preconditioned for 30 s, regardless of the preconditioner type ($P > 0.05$).

3.3 FESEM analysis

Representative FESEM micrographs of the preconditioned demineralized dentin surface in the secondary electron mode were shown in Figs. 1, 2, 3, 4. The demineralized dentin matrix of non-treated specimens showed slender collagen fibrils in a randomly loose arrangement (Fig. 1a).

Table 1 Cross-linking degree of demineralized dentin matrix treated by PA-based preconditioners at different concentrations overtime

Cross-linking degree (%, mean ± SD)					
Treatment (solvent)	Concentration of PA (wt%)	Treatment duration			
		120 s	60 s	30 s	20 s
5%GD(DW)	–	$62.27 \pm 0.64^{A,a}$	$45.60 \pm 5.78^{B,a}$	$37.95 \pm 5.84^{C,a}$	$28.07 \pm 1.04^{D,a}$
PA(DW)	5	$31.53 \pm 2.84^{A,b}$	$17.39 \pm 1.43^{B,b}$	$6.85 \pm 2.15^{C,b}$	$5.63 \pm 2.03^{C,b}$
	10	$42.28 \pm 0.89^{A,c}$	$23.74 \pm 2.46^{B,c}$	$15.15 \pm 3.96^{C,c}$	$11.26 \pm 1.67^{D,c}$
	15	$44.16 \pm 1.86^{A,c}$	$25.40 \pm 1.24^{B,c}$	$21.00 \pm 2.12^{C,d}$	$15.58 \pm 1.45^{D,d}$
PA(ET)	5	$42.50 \pm 1.14^{A,c}$	$25.11 \pm 1.64^{B,c}$	$23.88 \pm 1.63^{B,d}$	$14.72 \pm 1.45^{C,d}$
	10	$44.52 \pm 6.17^{A,c}$	$25.11 \pm 1.52^{B,c}$	$22.44 \pm 2.25^{B,d}$	$19.84 \pm 1.27^{C,e}$
	15	$55.92 \pm 3.62^{A,d}$	$32.18 \pm 1.75^{B,d}$	$30.45 \pm 1.36^{B,e}$	$27.92 \pm 2.93^{C,a}$
PA(AC)	5	$34.63 \pm 5.91^{A,b}$	$26.84 \pm 1.22^{B,c}$	$23.59 \pm 4.08^{B,d}$	$15.58 \pm 2.01^{C,d}$
	10	$43.29 \pm 4.56^{A,c}$	$32.32 \pm 2.75^{B,d}$	$27.27 \pm 1.06^{C,e}$	$22.29 \pm 0.85^{D,e}$
	15	$48.99 \pm 1.67^{A,e}$	$32.97 \pm 2.73^{B,d}$	$26.84 \pm 3.00^{C,e}$	$25.69 \pm 2.80^{C,a}$

Different upper and lower case letters indicate statistically significant ($P < 0.05$) differences for rows and columns, respectively

DW distilled water, *ET* ethanol, *AC* acetone

Table 2 The UTS of demineralized dentin treated by PA-based agents

UTS (MPa, mean ± SD)				
Treatment (solvent)	Concentration of PA (wt%)	Treatment duration		
		120 s	60 s	30 s
DW	–	$12.70 \pm 3.67^{A,a}$	–	–
ET	–	$13.15 \pm 3.28^{A,a}$	$12.71 \pm 4.18^{A,a}$	$12.65 \pm 4.04^{A,a}$
AC	–	$14.93 \pm 3.96^{A,a}$	$14.93 \pm 3.96^{A,a}$	$13.60 \pm 4.34^{A,a}$
5% GD(DW)	–	$18.92 \pm 5.16^{A,b}$	$16.96 \pm 5.88^{A,b}$	$13.59 \pm 4.08^{B,a}$
PA(DW)	5	$13.80 \pm 5.40^{A,a}$	$12.93 \pm 4.70^{A,a}$	$12.69 \pm 3.50^{A,a}$
	10	$20.16 \pm 6.28^{A,b}$	$16.37 \pm 6.42^{B,b}$	$12.40 \pm 3.47^{C,a}$
	15	$24.42 \pm 7.02^{A,c}$	$20.51 \pm 5.98^{B,c}$	$15.88 \pm 5.73^{C,a}$
PA(ET)	5	$15.61 \pm 6.41^{A,a}$	$13.28 \pm 4.30^{B,a}$	$12.80 \pm 4.26^{B,a}$
	10	$20.85 \pm 7.75^{A,b}$	$16.98 \pm 7.57^{B,b}$	$13.01 \pm 3.57^{C,a}$
	15	$24.13 \pm 5.82^{A,c}$	$21.06 \pm 3.44^{B,c}$	$15.99 \pm 5.90^{C,a}$
PA(AC)	5	$15.17 \pm 5.88^{A,a}$	$13.44 \pm 3.51^{B,a}$	$12.95 \pm 3.82^{B,a}$
	10	$21.22 \pm 6.87^{A,b}$	$17.33 \pm 6.80^{B,b}$	$13.95 \pm 3.70^{C,a}$
	15	$24.78 \pm 8.64^{A,c}$	$21.34 \pm 5.96^{B,c}$	$16.19 \pm 6.32^{C,a}$

Different upper and lower case letters indicate statistically significant ($P < 0.05$) differences for rows and columns, respectively

DW distilled water, *ET* ethanol, *AC* acetone

When the surface was intentionally dried, the denuded collagen matrix appeared to collapse (Fig. 1b). In contrast, the collagen matrix with homogeneous dimension arranged regularly after PA preconditioning, exhibiting interlaced and porous structure. There were no notably alterations in specimens no matter whether the dentin surface was kept moist or intentionally dried (Figs. 2, 3). Although the dentin surface pretreated by 5% GD displayed highly cross-linked, the matrix appeared rumpled and shrunk, especially the specimens treated for 120 s. Moreover, intense spherical particles or clusters were detected on the surface of collagen fibrils network biomodified by GD (Fig. 4).

4 Discussion

Although the reaction mechanisms of PA with biological tissue have not been well understood, the induction of exogenous collagen cross-links has been proposed to help maintain, restore and improve tissue function, and also produce a mechanically and enzymatically resistant collagen scaffold [18–20]. The current study quantitatively evaluated the cross-linking degree of PA preconditioned demineralized dentin matrix, and investigated its relationship with UTS. As reported in previous studies [10, 11] that PA treatment could increase the mechanical properties of demineralized dentin in a concentration- and

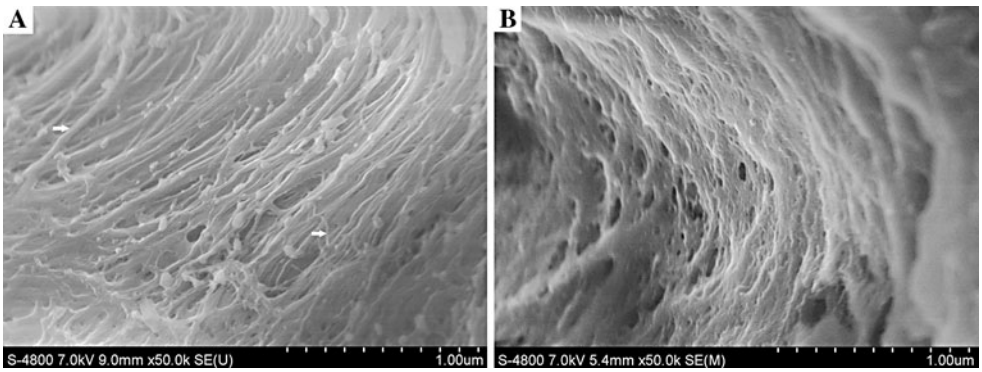

Fig. 1 Representative FESEM images of non-treated demineralized dental matrix (control group). **a** In moist condition: the demineralized dentin collagen fibrils of different dimension (arrowheads) were in a randomly loose arrangement. **b** In air-dry condition: the denuded collagen matrix appeared to collapse

time- dependent manner, higher concentration of PA-based agents and shorter treatment duration were used in this study to make it more clinically relevant as well as effective.

Ninhydrin assay is a rapid and precise microtiter plate method to quantify total proteins based on the amount of alpha-amino acid of protein hydrolysates. It is several times more sensitive than the Coomassie reaction and linear over a greater range of protein concentration [21, 22]. The current study used the assay to detect and quantify the amino acids in demineralized dentin and further calculate the cross-linking degree. According to the results, the application of PA-based preconditioners at a higher concentration (10 or 15%) for 30 s or longer highly increased the cross-linking degree compared to the blank control group, and the UTS of the dentin rose correspondingly. While the UTS of dentin pretreated by 5% PA-based agents were not significantly increased as that of the previous studies [9, 11]. It may be due to the limited treatment duration.

As a type of nature derived agents, PA forms a complex subgroup of the flavonoid compounds. Factors such as solvents, the extracted process of the products, pH and temperature may also influence the structure of PA and its overall cross-linking potential [7, 8]. Therefore, it is important to analyze the effect of the PA-based preconditioners dissolved in different polar solvents on the properties of dentin matrix, and further optimize the preconditioning conditions for potential clinical applications. The solvents used in this study was based on that (water, water/ethanol or water/acetone) of the contemporary adhesive systems. In addition, previous studies [23, 24] had proved that the mechanical properties of demineralized dentin matrix could be increased because of its dehydration by the solvent. So the specimens treated by neat solvents were tested as controls. It was found that there were no significant differences between different neat solvent groups. But PA preconditioning in ethanol and acetone solvents made more notable improvement to the cross-linking degree and UTS of the dentin matrix, especially when exposure time was longer than 60 s. This may be related to the hydrogen bonding ability of these solvents, as measured by the Hansen solubility parameter for hydrogen bonding, δ_H [25]. The δ_H values of ethanol and acetone, were lower than that of distilled water, so less hydrogen bonding sites would be occupied by the weaker bond forming solvent. These additional, available hydrogen bonding sites, coupled with the collagen structure, may then permit more new hydrogen bonding between PA-collagen or collagen–collagen molecules, which induced collagen cross-linking and resulted in stronger mechanical properties [26]. In addition, Hagerman et al. [27] reported that ethanol rather than acetone could decrease the dielectric constant of the media and stimulate PA and collagen interactions. So ethanol may be a preferable solvent for PA-based preconditioners. This result was not in accordance with the previous study [28], reporting that PA dissolved in water showed stronger effects and acetone–water mixture was the best extraction solvent for monomeric, oligomeric or polymeric PA from grape seeds. It is possibly due to the different solvent used in the extraction process of PA.

GD had been well known as a potential collagen cross-linking agent, but its toxicity was the main reason for its limit in clinical application [29, 30]. In the present study, dentin specimens pretreated by 5% GD were used as positive controls. The results indicated that the cross-linking degree of dentin matrix with GD treatment longer than 20 s was significantly higher than those of the other groups. However, the UTS values of demineralized dentin matrix pretreated with PA at higher concentration (10 or 15%) were superior to that treated with 5% GD for the same time. It appears that a proper degree of cross-linking is helpful for the maintenance of dentin collagen matrix,

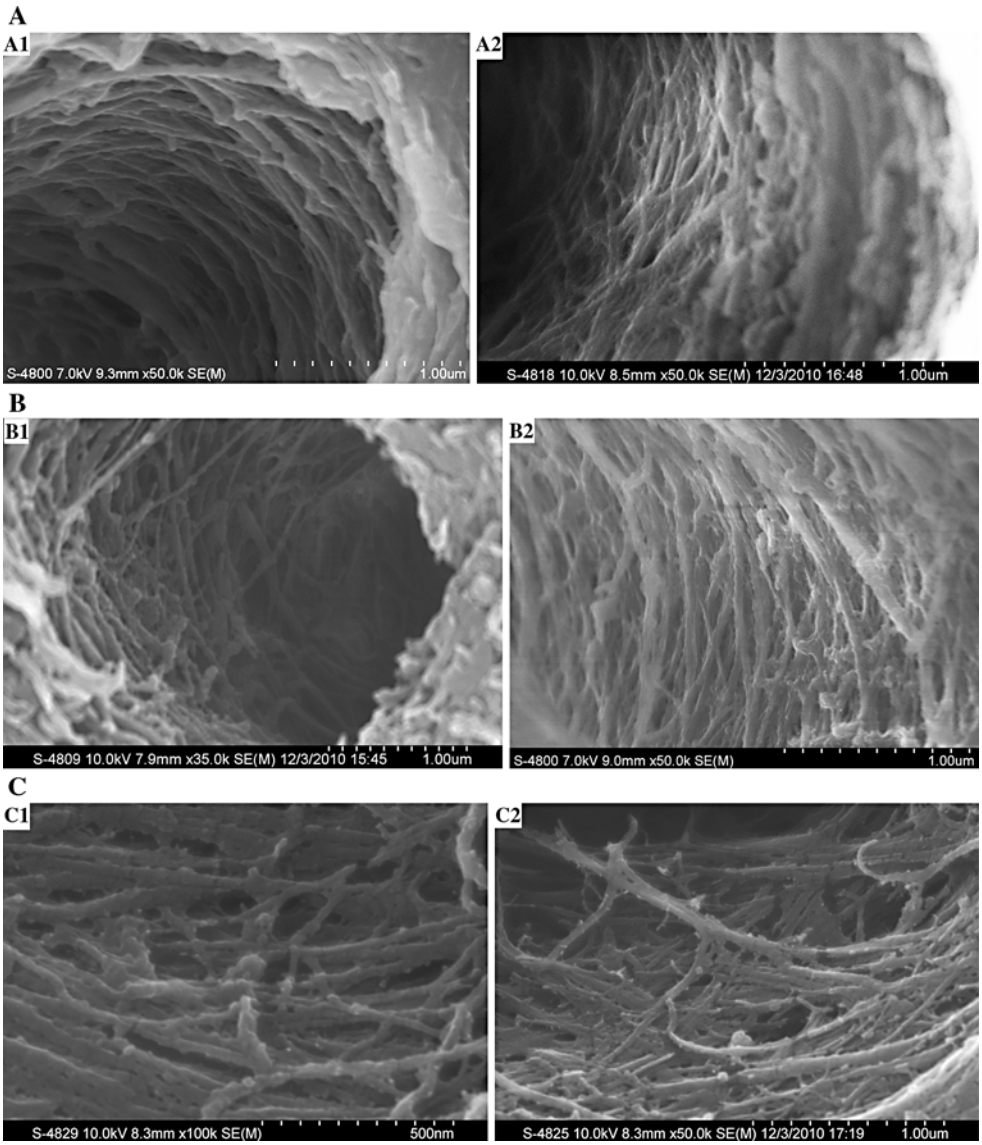

Fig. 2 Demineralized dentin matrix pretreated by PA in ethanol: **a** for 120 s in moist condition, **b** for 120 s in air-dry condition, **c** for 60 s in moist condition. 1–15% PA, 2–10% PA. The dentin collagen fibrils with homogeneous dimension were in an orderly arrangement, especially for the 15% PA group. There were no notably alterations in the same concentration treatment group whether the dentin surface was kept moist or intentionally dried

while excessive cross-linking may compromise the integrity of dentin matrix and negatively affect the UTS of the dentin. In which degree or at which range of cross-linking is optimal remains still uncertain and needs further studies. Besides, other possible reason is that PA plays a role in stabilizing collagen in a distinct way from GD. GD increases type I collagen covalent bonding by bridging the amino groups of lysine and hydroxylysine residues of different collagen polypeptide chains by monomeric or oligomeric cross-links, while PA-based agents have been shown to interact with proline-rich proteins by covalent interaction, ionic interaction, and hydrogen and hydrophobic bonding interactions [7, 17]. It is speculated that PA has stronger interaction ability with collagen than GD and improves the mechanical properties of dentin more greatly.

The FESEM images presented that there were dimensional changes in the matrix of demineralized dentin treated with 5% GD. White spot substances in high density were observed on the surface of dentin specimen modified by GD. It may be the non-collagenous proteins, such as proteoglycans, which were also detected by Bedran-Russo et al. [17] under transmission electron microscopy. They proposed that the cross-linking agent may also interact with the non-collagenous proteins, such as proteoglycans. PA-rich grape seed extract exhibited glycosaminoglycan removal function, which may play a role in the superior

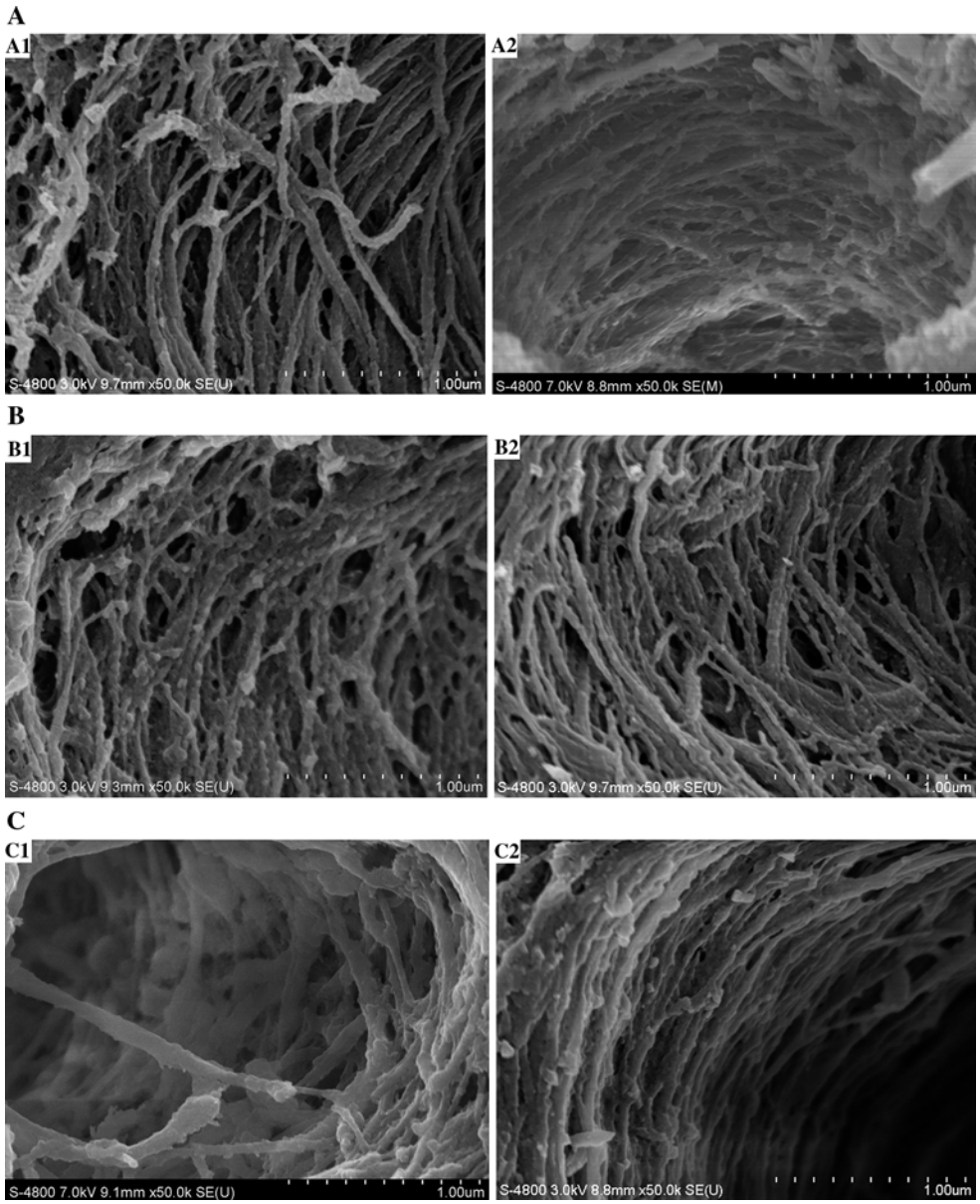

Fig. 3 Demineralized dentin matrix pretreated by PA in acetone: **a** for 120 s in moist condition, **b** for 120 s in air-dry condition, **c** for 60 s in moist condition. 1–15% PA, 2–10% PA. The morphology of collagen showed homogeneous, some degree of shrinkage and in an orderly arrangement, despite of hydration or dehydration

mechanical properties and perhaps biodegradation rates of biomodified dentin matrix tissues by changing the hydration rates and molecule diffusivity through the tissue [31]. Further studies are essential to elucidate the interaction of non-collagenous proteins with PA and their roles in dentin matrix stabilization and degradation. Moreover, the collagen matrix pretreated with 15% PA or 5% GD did not collapsed after the dentin surface was desiccated. This may because PA induced exogenous cross-link in dentin matrix, which leaded to a decrease in the swelling ratio of matrix. Low swelling ratio may indicate that the network would be dense, and prevent water/collagenase absorption and maintain the structure of dehydration condition [32, 33]. It suggests that applying exogenous cross-linking agents on etched dentin surface can minimize the risk of collagen network collapse, resulted from intentionally air drying. Therefore, this preconditioning step may minimize the technique sensitivity of wet bonding when using total-etch bonding systems [34]. Whether it will be a potential protective mechanism over a long period of time needs to be further tested.

The present study demonstrated that transient PA preconditioning increased the collagen cross-linking degree and UTS of demineralized dentin. Recently, some studies

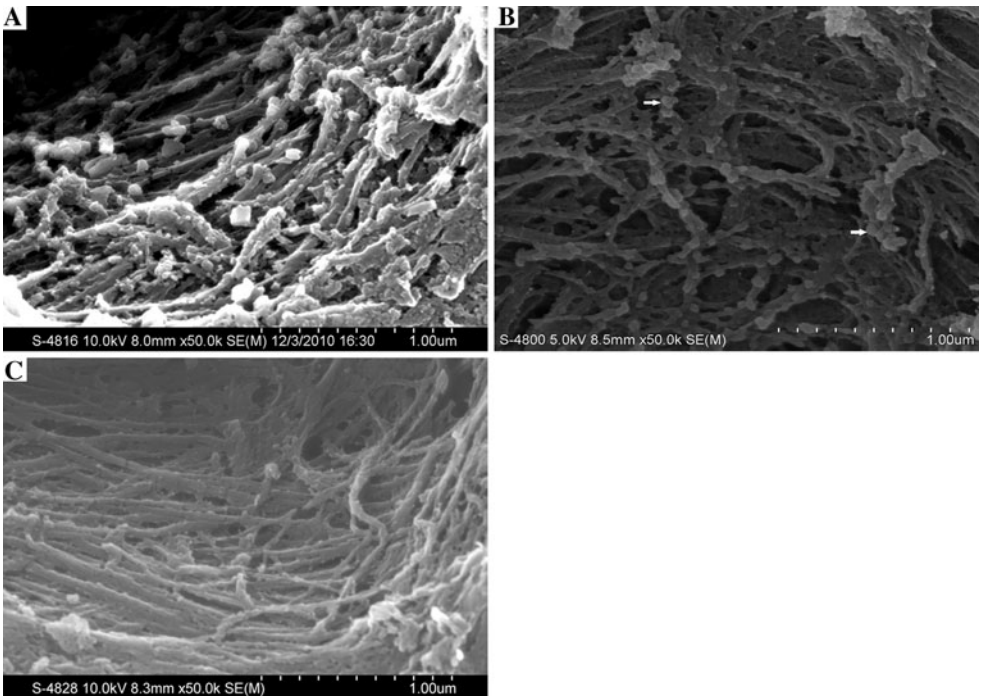

Fig. 4 Demineralized dentin matrix pretreated by 5% GD: **a** for 120 s in moist condition: the matrix appeared rumpled and shrunk due to the fixation of GD. **b** for 120 s in air-dry condition: many spherical particles or clusters were clearly observed and may be the non-collagenous proteins (*arrow heads*). **c** for 60 s in moist condition: the collagen fibrils with homogeneous dimension were in sandwich arrangement

reported that the demineralized dentin treated with 6.5% PA-based agents for 10 min significantly decreased collagenase degradation and reduced water absorption. Collagen crosslinks induced by PA may be resistant to endogenous collagenases and provide more durable collagen layer than untreated controls [35]. Therefore, the goal of ongoing efforts is to characterize the effect of PA on the resin-dentin bond strength and the stability of the adhesive restorative interface using clinically appropriate techniques with appropriate concentration, solvent and treatment duration.

5 Conclusion

Transient collagen cross-linking treatment by PA-based preconditioners increased the cross-linking degree and UTS of demineralized dentin. The changes to the dentin matrix after cross-linking treatment were both concentration- and time- dependent. Clinical application of collagen cross-linkers during adhesive restorative procedures may be a potential approach to improve dentin bond strength.

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