

Fig 1 Illustration of dentin specimen preparation for testing the modulus of elasticity, swelling ratio, and mass change.

in vitro studies have shown proven efficacy of UVA riboflavin in collagen cross linking,^{12,16} the harmful effects of UV light may be a limiting factor which might not allow this method to gain popularity in routine clinical dentistry.

Synthetic agents involve the use of glutaraldehyde (GA) or 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide (EDC). Glutaraldehyde is a potent synthetic collagen cross linker.¹¹ However, its harmful effect on cells has made it unsuitable for routine use in clinical dentistry. Conversely, EDC is a unique collagen cross linker that has gained popularity in dentistry in recent years.^{6,42} Carbodiimide, commonly known as a zero-length cross linker,²⁸ does not introduce additional linkage groups for collagen cross linking. It forms covalent peptide bonds between proteins by activating the free carboxyl groups of glutamic and aspartic acids present in protein molecules.^{43,48} These covalent peptide bonds result in the formation of an O-acylisourea intermediate, which reacts with the epsilon amino group of lysine or hydroxylysine in a neighboring polypeptide chain to form a stable, covalent amide bond. This chemical reaction produces urea as a byproduct²⁸ that could easily be eliminated from dentin by rinsing with water.

Unlike glutaraldehyde, EDC is not reported to be cytotoxic. Recently, EDC has been shown to possess some inhibitory activities against matrix-bound proteases.^{37,42} This capacity of EDC is due to its cross-linking effect on MMPs, which occurs even more rapidly than its cross linking in dentin collagen.⁴² These properties make EDC a suitable alternative for cross linking of dentin collagen.

Previous studies have mainly used EDC with water as a solvent. Recently, Bou-Akl et al⁸ have shown that acetone could be a better solvent for EDC, as the collagen cross-linking potential of EDC is greater in acetone than in water. This was demonstrated by a significant improvement in the elastic modulus and ultimate tensile strength of collagen fibers cross linked with EDC in acetone.⁸ So far, no studies have examined the role of solvent on the dentin collagen cross-linking effect of EDC. Therefore, the aim of this study was to compare the dentin collagen cross-linking potential of EDC prepared in the three most commonly used solvents in dental adhesive systems, ie, water, ethanol, and acetone. The cross-linking potential of EDC in water, ethanol, and acetone was compared by examining the mechanical properties of demineralized dentin after treatment with the respective EDC solutions.

The null hypotheses tested were that there is no difference in the solvents used in the preparation of EDC solutions on (i) the modulus of elasticity, (ii) the resistance of dentin matrix against enzymatic degradation, (iii) the swelling ratio and (iv) the mass change of demineralized dentin matrix.

MATERIALS AND METHODS

Thirty-eight freshly extracted caries-free human molars were collected after the patients' informed consent was obtained under a protocol reviewed and approved by the local Institutional Review Board (No: UW 14-177). The teeth were first kept in 1% chloramine-T solution at 4°C and utilized within 3 months from the time of extraction.

Modulus of Elasticity Measurement

Twenty-four dentin beams (0.5 mm × 1.7 mm × 6.5 mm) were prepared from coronal dentin of 8 sound extracted human molars (Fig 1) under water cooling (Isomet, Buehler; Lake Bluff, IL, USA). The beams were fully demineralized using 10% phosphoric acid for 5 h following the protocol by Bedran-Russo et al.³ The beams were thoroughly washed with deionized water and randomly divided into 3 test groups (n = 8) for treatment with their respective cross-linking solutions: group 1, 0.3 M EDC in water; group 2, 0.3 M EDC in 90% acetone; group 3, 0.3 M EDC in 90% ethanol.

The EDC salt used in this research project was manufactured by International Laboratory (South San Francisco, CA, USA). The specimens were treated with their respective cross-linking solutions following the protocol by Bou-Akl et al.⁸ Accordingly, the demineralized dentin specimens were treated with the cross-linking solutions for 24 h. In group 1, 0.12 M N-hydroxysuccinimide (NHS) (Sigma-Aldrich; St Louis, MO, USA) was added to the 0.3 M EDC solution, because NHS has been shown to prevent hydrolysis of the carboxyl groups of dentin collagen that were activated by EDC.²² However, NHS was not used in groups 2 and 3, as only 10% water was used in the preparation of EDC solutions in these groups; thus, the risk of such hydrolysis is negligible. Furthermore, with the use of acetone or ethanol as co-solvents, water can be easily removed from demineralized dentin because of their high vapor pressures.

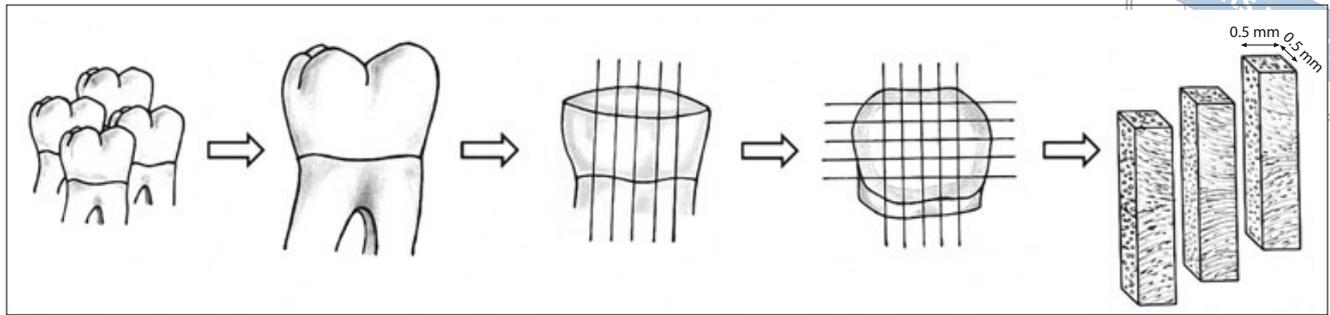


Fig 2 Illustration of dentin specimen preparation for testing the ultimate tensile strength (UTS) and the collagen degradation resistance.

After cross linking, the cross-linked dentin specimens in group 1 were washed with 0.1 M sodium phosphate-dibasic (Na_2HPO_4) (International Laboratory) for 2 h. Subsequently, the specimens were washed for four times with deionized water and dried overnight at room temperature. For groups 2 and 3, after treatment of the demineralized dentin beams with their respective cross-linking solutions for 24 h and before washing them with 0.1 M sodium phosphate-dibasic (Na_2HPO_4) for 2 h, the beams were treated with 90% acetone or 90% ethanol for 24 h, followed by 50% acetone or 50% ethanol for 4 h. The modulus of elasticity (MOE) was measured using the three-point bending test. The pre-treatment and post-treatment measurements were made on the same specimens, as they were not allowed to exceed their elastic limit. The span distance was fixed at 4 mm and the specimens were tested while they were hydrated at a crosshead speed of 0.5 mm/min. The load/displacement curves were converted to stress-strain curves and the apparent MOE were calculated at 3% strain using the following formula:²⁶

$$D = \epsilon L^2 / 6T$$

where ϵ is strain, L is support span length, and T is thickness of the specimen. The apparent elastic modulus (E) of the specimens was expressed in MPa and calculated using the following formula:²⁶

$$E = PL^3 / 4DbT$$

where P is the maximum load, L is the support span length, D is the displacement, b is the width of the specimen, and T is the thickness of the specimen. As the normality and homoscedasticity assumptions of the data appeared to be valid, the modulus of elasticity was analyzed using two-way ANOVA (time vs solvents used in the preparation of EDC solutions). Multiple comparisons were performed using the Student-Newman-Keuls (SNK) test. The statistical significance was set at 5%.

Resistance of Dentin Matrix to Enzymatic Degradation

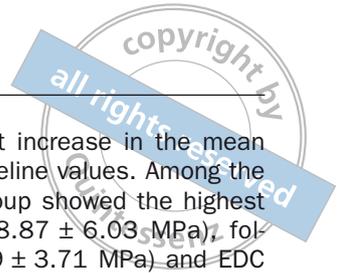
Ten extracted caries-free human molars were used for this part of the study. The cusps of the teeth were ground flat using 600-grit SiC paper under running water. The teeth were then sectioned 1 mm below

the cemento-enamel junction and the roots were removed. The crown was further sectioned under water cooling (Isomet, Buehler) to obtain 0.5 ± 0.1 mm by 0.5 ± 0.1 mm thick dentin beams ($n = 6$) (Fig 2).⁵ The beams were completely demineralized with 10% phosphoric acid for 5 h and were washed thoroughly with the deionized water. The demineralized dentin specimens were randomly allocated to the three test groups ($n = 20$) for cross linking of dentin collagen as in the previous section.

To test the collagen degradation resistance of the cross-linked dentin specimens, 50% of the cross-linked specimens in each group were subjected to treatment with bacterial collagenase, while the remaining 50% of the specimens were kept as controls and were not subjected to such a challenge. This was achieved by immersion of 10 cross-linked dentin specimens per group in phosphate buffer solution (PBS) with bacterial collagenase (*Clostridium histolyticum*, Sigma-Aldrich) (100 $\mu\text{g}/\text{ml}$) and the remaining 10 specimens in that group in PBS without bacterial collagenase for 24 h. The specimens were then thoroughly rinsed with deionized water and tested for their ultimate tensile strengths (UTS) using a Bencor Multi-T device (Danville Engineering; San Ramon, CA, USA) attached to a universal testing machine (Model 4440, Instron; Canton, MA, USA) operated at a constant crosshead speed of 1 mm/min. The bacterial collagenase treatment of the dentin collagen is comparable to the proteolytic effect of the matrix-bound enzymes. As the normality and homoscedasticity assumptions of the data appeared to be valid, the ultimate tensile strength was analyzed using two-way ANOVA (solvents used in the preparation of EDC solutions vs collagenase challenge). Multiple comparisons were performed using the SNK test. The statistical significance was set at 5%.

Swelling Ratio

Thirty dentin beams ($0.5 \text{ mm} \times 1.7 \text{ mm} \times 6.5 \text{ mm}$) were prepared from coronal dentin of 10 sound extracted human molar (Fig 1). The beams were fully demineralized using 10% phosphoric acid for 5 h and were thoroughly washed with deionized water. The specimens were randomly divided into 3 test groups ($n = 10$) for treatment with their respective cross-linking solutions for 24 h. After treatment, the specimens were allowed



to swell in water and were treated overnight in PBS (at pH 7.4) at room temperature. The specimens were then blotted with lint-free tissues (Kimwipes, Kimberly-Clark; Irving, TX, USA) to remove the excess water from their surfaces and were weighed immediately with an analytical balance with an accuracy of 0.1 mg. Dentin samples were then placed in an excess of deionized water to remove the buffer salts and were dried in the air until they reached a constant weight. The swelling ratio (Q) was calculated as the ratio of the weight of swollen sample to that of dry sample.¹⁰ One-way ANOVA was used to test the effect of “solvents used in the preparation of EDC solutions” as the independent factor on the swelling ratio as the dependent factor. Multiple comparisons were performed with the SNK test. The statistical significance was set at 5%.

Mass Change

Thirty dentin beams (0.5 mm × 1.7 mm × 6.5 mm) were prepared from coronal dentin of 10 sound extracted human molars (Fig 1). The beams were fully demineralized using 10% phosphoric acid for 5 h and were thoroughly washed with deionized water. The demineralized dentin specimens were dried in a vacuum desiccator containing anhydrous calcium sulfate for 24 h at room temperature and weighed (M_1) with an analytical balance with an accuracy of 0.1 mg. The specimens were randomly divided into 3 test groups ($n = 10$) for treatment with their respective cross-linking solutions for 24 h. The demineralized cross-linked dentin specimens were thoroughly washed with deionized water, dried in the vacuum desiccator as mentioned before, and reweighed (M_2) with the same balance. The mass change ($W_{mc}\%$) for each specimen (which could be a gain or loss) was calculated based on the following formula:¹

$$W_{mc}\% = [(M_2 \times 100)/M_1] - 100$$

where M_1 is the demineralized dentin matrix mass before dentin biomodification and M_2 is the biomodified dentin matrix mass. One-way ANOVA was used to test the effect of “solvents used in the preparation of EDC solutions” as the independent factor on “mass change” as the dependent factor. Multiple comparisons were performed with the SNK test. The statistical significance was set at 5%.

RESULTS

Modulus of Elasticity

Results of two-way ANOVA showed that both factors, “time” ($p < 0.001$) and “solvents used in the preparation of EDC solutions” ($p < 0.01$), had a significant effect on MOE. The interaction between the two factors was also significant ($p < 0.01$). The results of the multiple comparisons are shown in Table 1. The mean baseline MOE did not differ significantly among the tested groups. However, after 24 h of cross linking, all

the groups showed a significant increase in the mean MOE when compared to the baseline values. Among the test groups, EDC in acetone group showed the highest increase in the mean MOE (18.87 ± 6.03 MPa), followed by EDC in ethanol (10.19 ± 3.71 MPa) and EDC in water (10.04 ± 3.59 MPa) groups.

Resistance of Dentin Matrix to Enzymatic Degradation

Results of two-way ANOVA showed that both factors “solvents used in the preparation of EDC solutions” ($p < 0.001$) and “collagenase treatment” ($p < 0.001$) had a significant effect on UTS. However, interaction between the two factors was not significant ($p > 0.05$). The results of the multiple comparisons are shown in Table 2. The UTS of demineralized dentin was significantly higher following treatment with EDC in acetone (8.08 ± 1.32 MPa) and EDC in ethanol (6.88 ± 1.24 MPa) than after treatment with EDC in water (5.02 ± 1.07 MPa). However, the mean UTS of the groups treated with EDC in ethanol (5.41 ± 1.35 MPa) and EDC in water (3.81 ± 0.51 MPa) dropped significantly after the collagenase treatment. In contrast, the UTS of the EDC in acetone group (6.88 ± 1.17 MPa) was maintained, even after the treatment with collagenase, showing the resistance of dentin matrix to enzymatic degradation.

Swelling Ratio

Results of one-way ANOVA showed that the factor “solvents used in the preparation of EDC solutions” had a significant effect ($p < 0.001$) on the swelling ratio. The results of the multiple comparisons tests (Table 3) show that the swelling ratio of the specimens cross linked by EDC in acetone (2.15 ± 0.19) and EDC in ethanol (2.27 ± 0.17) was significantly lower than those specimens cross linked by EDC (2.52 ± 0.15) in water ($p < 0.05$).

Mass Change

Results of one-way ANOVA showed that the factor “solvents used in the preparation of EDC solutions” had a significant effect ($p < 0.001$) on the mass change. The results of the multiple comparisons tests (Table 4) show that the mass change (%) of the EDC in acetone group ($10.28 \pm 4.12\%$) was the highest and that of the EDC in water group the lowest ($0.25 \pm 4.58\%$) ($p < 0.05$).

DISCUSSION

Recently, Scheffel et al³⁵ showed that treatment of acid-etched dentin with EDC did not cause transdentinal cytotoxic effects in odontoblast-like cells, providing evidence that the use of EDC to enhance the quality of resin-dentin bonding is safe. The results of this study show that a higher modulus of elasticity of demineralized dentin was observed after cross linking with EDC in acetone than EDC in ethanol and EDC in water; hence, the first null hypothesis that there is no difference in

Table 1 Modulus of elasticity of demineralized dentin specimens treated with EDC prepared with different solvents

Dentin matrix treatment	Modulus of elasticity	
	Baseline	After 24 h of cross linking
EDC in water	3.99 (2.10) ^a	10.04 (3.59) ^b
EDC in acetone	4.18 (2.56) ^a	18.87 (6.03) ^c
EDC in ethanol	4.48 (2.55) ^a	10.19 (3.71) ^b

Values are mean ± standard deviation in MPa. Values with the same superscript letter are not significantly different.

Table 2 Resistance of demineralized dentin matrix to enzymatic degradation: ultimate tensile strength

Dentin matrix treatment	Ultimate Tensile Strength		
	Dentin matrix treatment		
	EDC in water	EDC in acetone	EDC in ethanol
Buffer	5.02 (1.07) ^b	8.08 (1.32) ^c	6.88 (1.24) ^c
Collagenase/ buffer	3.81 (0.51) ^a	6.88 (1.17) ^c	5.41 (1.35) ^b

Values are mean ± standard deviation in MPa. Values with the same superscript letter are not significantly different.

Table 3 Swelling ratio of demineralized dentin treated with EDC prepared with different solvents

Dentin matrix treatment	Swelling ratio
EDC in water	2.52 (0.15) ^a
EDC in acetone	2.15 (0.19) ^b
EDC in ethanol	2.27 (0.17) ^b

Values are mean ± standard deviation in %. Values with the same superscript letter are not significantly different.

Table 4 Mass change of demineralized dentin treated with EDC prepared with different solvents

Dentin matrix treatment	Mass change (%)
EDC in water	0.25 (4.58) ^a
EDC in acetone	10.28 (4.12) ^c
EDC in ethanol	4.46 (3.90) ^b

Values are mean ± standard deviation. Values with the same superscript letter are not significantly different.

the solvents used in the preparation of EDC solutions on the modulus of elasticity of demineralized dentin matrix had to be rejected. The in vitro enzymatic degradation assay revealed that the UTS values of the EDC in acetone group were not significantly affected by collagenase exposure, while a significant drop in UTS was found in the groups EDC in ethanol and EDC in water; thus, the second hypothesis that there is no difference in the solvents used in the preparation of EDC solutions on the resistance of dentin matrix to enzymatic degradation was also rejected.

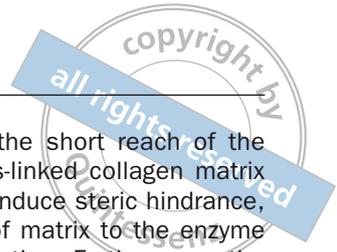
Treatment of demineralized dentin with EDC in ethanol or acetone significantly reduced the swelling ratio when compared to EDC in water; hence, the third null hypothesis had to be rejected. The fourth null hypothesis also had to be rejected, because the percentage of mass change of demineralized dentin after treatment with EDC in acetone was significantly higher than EDC in ethanol or water.

In this study, we chose not to have a negative control group, as EDC is a proven dentin collagen cross linker.^{2,6} Also, it has been shown that the stiffness of decalcified dentin matrix could be significantly lowered when it is wet with water, compared to its stiffness after dehydration with solvents.²³ However, the literature does not answer the question of whether any potential role of solvents exists in the enhancement of dentin collagen cross-linking potential of EDC. Therefore, in this study – aside from the

test groups EDC in 90% acetone and EDC in 90% ethanol – only a positive control group (EDC in water) was included. The concentration of EDC was chosen to be 0.3 M in this study, which has been shown in previous studies^{6,24} to have a significant dentin collagen cross-linking potential. All the mechanical tests used in this study, ie, change in MOE, UTS, resistance of dentin matrix to enzymatic degradation, swelling ratio, and mass change, are standard methods used to assess the dentin collagen cross-linking potential of a natural or synthetic collagen cross linker.^{1,5,6,23} Recently, Scheffel et al³⁶ showed that EDC treatment of demineralized dentin, along with a significant increase in collagen stiffness and decreased MMP activity, could also increase collagen's thermal denaturation temperature and the degradation resistance of collagen.

Cross linking by EDC occurs in dentin collagen as well as in dentin matrix-bound MMPs. Tezvergil-Mutluay et al⁴² and Scheffel et al³⁷ reported that when demineralized dentin was treated with collagen cross linkers, cross linking occurred more rapidly in MMPs than in collagen. This could be explained by better accessibility of carboxyl and amino groups in MMPs than in collagen.^{29,33} This shows that EDC is a potent MMP inhibitor and that its MMP inhibitory effect is much faster than its collagen cross-linking effect.

Collagen cross linkers have the potential to render the cross-linked collagen resistant to enzymatic degrada-



tion.^{18,39} Scheffel et al³⁷ reported that the collagen cross linkers increased the stiffness of the collagen, which made them more difficult to unwind for degradation by proteases. The degradation resistance of cross-linked dentin collagen to matrix-bound endopeptidases is important for the durability of resin-dentin bonded interfaces and consequently the success of a bonded restoration. In vitro studies have adopted the method of testing degradation resistance of cross-linked dentin collagen using bacterial collagenases.^{3,10} The bacterial collagenase extracted from *Clostridium histolyticum* was used in our study, as this enzyme has been shown to hydrolyse the triple helical sites of collagen under normal conditions.²¹ This method closely resembles the enzymatic challenge to the denuded collagen fibrils at the bottom of the hybrid layer by matrix-bound proteases. It was used in the present study so that degradation resistance of the cross-linked dentin collagen could be assessed within a short period of time.

The changes in MOE of demineralized dentin collagen specimens is a very good method to measure and compare the stiffness of dentin collagen before and after cross linking with the tested collagen cross linker in different solvents. Because this is a non-destructive method, the same dentin specimens could be used for pre- and post-treatment comparisons when the strain is 3%.²³ Demineralized dentin cross linked by EDC in acetone showed a 4.5-fold increase in MOE compared to specimens cross linked with EDC in ethanol and EDC in water, which only showed a 2.5-fold increase in their MOE. An improved cross linking of dentin collagen with less water in the dentin matrix would have made the specimens stiffer in the acetone group, compared to specimens cross linked by EDC in ethanol or water.

The mean UTS of the group cross linked by EDC in ethanol did not differ significantly from that cross linked by EDC in acetone. However, after treatment with the collagenase enzyme, there was a significant reduction in the mean UTS of the group cross linked by EDC in ethanol. This shows that dentin matrix cross linked by EDC in ethanol did not exhibit resistance to enzymatic degradation, and therefore the mechanical stability of these cross-linked collagen fibrils is questionable.

The degree of collagen cross linking by EDC depends on the stability of the activated carboxyl groups, which are prone to hydrolysis from the aqueous solution as well as from water present in the demineralized dentin. There are several advantages associated with the use of acetone as a solvent for EDC. Firstly, acetone has a much higher vapor pressure (184 mmHg) at room temperature than do ethanol (40 mmHg) and water (17 mmHg). Acetone and water are miscible, and acetone effectively facilitates the removal of water from demineralized dentin; acetone has thus been termed a water chaser.^{27,44} Due to its dehydrating potential, acetone helps to prevent hydrolysis of the activated carboxyl group of dentin collagen. Secondly, following removal of water from demineralized dentin by acetone, the amino acids of the collagen come into close proximity, improving cross linking.^{7,9,17} This is particularly important for a zero-length

cross linker like EDC, due to the short reach of the cross links.⁸ The densely cross-linked collagen matrix formed by EDC in acetone may induce steric hindrance, which blocks the accessibility of matrix to the enzyme and reduces collagenase adsorption. Furthermore, the dense matrix network may also mask the enzyme cleavage sites on the collagen and improve matrix resistance to enzymatic degradation.¹⁰

To prevent hydrolysis of the carboxyl groups of dentin collagen activated by EDC, Olde Damink et al²⁸ suggested the addition of N-hydroxysuccinimide (NHS) to EDC when using water as a solvent. N-hydroxysuccinimide stabilizes the activated carboxyl groups and improves the cross-linking potential of EDC. Therefore, the third advantage of using acetone as a solvent in the preparation of EDC solution for dentin collagen cross linking is that the need for NHS is eliminated, as there would be very little/no water in demineralized dentin collagen. Therefore, the chance for the carboxyl groups activated by EDC, which are required for collagen cross linking, to be hydrolyzed would be minimized. This would not only eliminate an extra step in the preparation of the cross-linking solution, but also reduce the cost of cross-linking treatment.

In the current study, swelling ratio was measured to assess the extent of water sorption of demineralized dentin. A reduction in the swelling ratio following exogenous cross linking of dentin matrix has been reported previously,¹⁰ as the dense cross-linked collagen matrix impaired water sorption. The decreased in the swelling ratio of demineralized dentin cross linked by EDC in acetone could be explained by the greater ability of the collagen fibrils to form interpeptide bonds when saturated with acetone. When water is removed from demineralized collagen matrix by acetone, its plasticizing effect is lost and would therefore lead to stiffening of the collagen matrix. This stiffening together with the interpeptide bond formation between collagen fibrils could have led to the decrease in swelling ratio.²³ Wang et al^{45,46} stated that the cross-linked collagen matrix was dense and therefore less prone to creep rupture⁴⁵ or cyclic fatigue rupture,⁴⁶ even after extended periods of intraoral function.

The mass change reflects the collagen cross-linking potential of EDC in different solvents. The results of this study showed that demineralized dentin cross linked by EDC in acetone had significantly higher mass change than the other two groups. The stiffening of the collagen fibrils from cross linking could have increased the mass of the specimens. As the specimens cross linked by EDC in acetone were stiffer than the specimens cross linked by EDC in water or ethanol, a greater change in the mass % was observed.

The dentin matrix-bound collagenolytic enzymes MMPs and cysteine cathepsins are hydrolases and require water for their collagenolytic action on demineralized dentin collagen. In this respect, it would be logical to use water-chasing solvents, such as acetone and ethanol, which have high vapour pressures. Such an approach would avoid or at least reduce the collagenolytic actions of these endogenous enzymes, thus promoting durable resin/dentin interfaces and successful bonded restorations.

In general, acetone in dental adhesives offers several clinical advantages. With a high vapor pressure (184.48 mmHg at 20°C), it can remove water from demineralized dentin during bonding procedures with resin adhesives. It is less viscous than ethanol (0.316 cP vs 1.10 cP at 25°C), making it better able to penetrate into the demineralized dentin.¹⁵ It has been shown that acetone-based adhesives could significantly reduce cervical dentin hypersensitivity.⁴⁰

The use of acetone also presents a few drawbacks. The hydrogen bonding [H-bonding] capacity of acetone [δ h: 7 (J/cm³)^{1/2}] is much lower than that of dried demineralized collagen [δ h: 42.3(J/cm³)^{1/2}], and as a result, acetone-based dental adhesives cannot re-expand demineralized collagen that collapsed due to drying. This in turn leads to poor infiltration of resin monomers into the demineralized dentin.³⁰ Hence, it is strictly recommended to apply water-free acetone-containing dental adhesives only to moist demineralized dentin surfaces.⁴¹

Acetone evaporates easily from the dental adhesive container during frequent use and even storage, affecting its shelf life. The high vapor pressure of this solvent is the reason for such loss.³² The risk of solvent loss from containers due to evaporation demands multiple coats of acetone-based adhesive application.¹³ Some manufacturers have added an extra fraction of acetone in order to compensate for its potential loss from evaporation. When products containing an extra fraction of acetone are used, it is still necessary to apply multiple coats, this time for better adhesive infiltration into the demineralized dentin substrate.

There are potential clinical implications of this study. First, the superior cross linking of demineralized dentin collagen by EDC in acetone would be able to produce improved collagen fibers with enhanced physical properties that can not only help in formation of a quality resin-dentin interdiffusion or hybrid layer, but can also act as a strong anchor that connects the hybrid layer and restoration lying above it to underlying mineralized dentin. Second, the increased resistance to enzymatic degradation could also protect the anchor from proteolytic degradation of dentin matrix-bound proteases. Clinically, this means that the demineralized dentin collagen that is cross linked by EDC in acetone can produce stronger and more durable bonded restorations.

Further research should be performed with more clinically applicable protocols to study the effect of solvents in enhancing the dentin collagen cross-linking potential of EDC. Studies should also be performed to find the effect of EDC in the tested solvents on soluble and dentin matrix-bound proteases.

CONCLUSIONS

Within the limitations of this study, it may be concluded that the dentin collagen cross-linking potential of EDC is enhanced by using acetone as a solvent, and dentin collagen cross linked by EDC in acetone instead of water or ethanol is more resistant to enzymatic degradation.

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Clinical relevance: The use of acetone as solvent could enhance the cross-linking potential of the demineralized dentin collagen by EDC. This could lead to more stable resin/dentin bonded interfaces and therefore improve the durability of the bonded restorations.