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Role of dentin cross-linking agents in optimizing dentin bond durability

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ABSTRACT

The present work compared the effects of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl) and ultraviolet (UV)- or blue light-activated riboflavin cross-linking agents on resin–dentin micro-tensile bond strength and nanoleakage in bonds produced using a two-step, etch-and-rinse adhesive after three storage intervals (24 h, 6 months, and 12 months). Forty eight extracted human third molars were used to investigate micro-tensile bond strength and interfacial nanoleakage in resin–dentin bonds created using Adper Single Bond 2, with or without pretreatment of acid-etched dentin surfaces. Either 0.5 M EDC-HCl or 1% riboflavin-5-phosphate activated by blue or UV light were used as cross-linking agents. Samples were evaluated after storage for 24 h, 6 months, or 12 months in distilled water at 37 °C. Statistical analyses revealed that 12 months of storage resulted in significant decreases in the bond strength of the controls ($p < 0.003$), with significant increases in their silver nanoleakage ($p < 0.05$), compared with the groups subjected to dentin pretreatment with either EDC-HCl or light-activated 1% riboflavin. Despite the significant drop in bond strength after 6 months' storage in all experimental groups compared with the 24-h bond strength ($p < 0.05$), there was a further non-significant drop in bond strength after 12 months in samples treated with EDC-HCl and UV-activated 1% riboflavin ($p > 0.05$). Dental collagen cross-linking induced by UV- or blue light-activated 1% riboflavin or EDC-HCl enhanced the durability and strength of the resin–dentin bond.

1. Introduction

In the current decade, adhesive dentistry and resin composite restorations have replaced amalgam as a preferred posterior restorative material. However, the authors of recent systematic reviews [1–3] have reported lower longevity and more replacements in resin composite restorations, compared with amalgam restorations. The main cause of resin composite replacement in these studies was secondary caries. Such caries are mostly localized gingivally in class II restorations, and are related to the restoration/tooth bond failure and the high level of cariogenic bacteria attached to the composite at these locations [3].

The challenges encountered during bonding to dentin are mainly due to its high residual water content [3] and the time-dependent hydrolysis of the hybrid layer and the collagen matrix caused by endogenous dentin collagenolytic enzymes, matrix metalloproteinases (MMPs), and cysteine cathepsins [4]. Studies have shown that the etching of dentin, followed by the application of self-etched or etch-and-rinse adhesives, exposes endogenous MMPs by removing the minerals, and activates protease enzymes owing to the acidity of the etchant and adhesive [5,6].

Considering the importance of dentinal MMPs in the long-term degradation of the hybrid layer [7–10], recent investigations have focused on the inhibition or inactivation of MMPs induced by various natural or chemical cross-linking agents to increase the integrity, durability, and strength of bonds [4,7,8,10–21].

Enzyme inhibitors and inactivators of endogenous dentin proteases have been discussed in recent publications [14,22–24]. Several investigations have confirmed that chlorhexidine digluconate and chlorhexidine methacrylate dramatically slow down collagen degradation by inhibiting collagenolytic enzymes [8,10,16,19,21,25,26].

The inactivation of proteolytic enzymes by cross-linking is non-specific. Such treatment cross-links all types of MMPs and cysteine

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cathepsins found in dentin [22,23] and collagen [14]. Most of the cross-links involve covalent bonds that are stable over time [14]. The covalent cross-links produced by various exogenous cross-linkers are very stable [21], and the results look promising in this regard [4,5,11–18,21,27,28]. Moreover, cross-linking improves the mechanical properties of dentin collagen and makes the collagen fibrils more resistant to degradation [11,20].

The available approaches to collagen cross-linking can be divided into two categories: chemical methods, such as the use of glutaraldehyde, genipin, proanthocyanidin (grape seed extract), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide [29–32]; and physical methods (photo-oxidative) that use light (blue or UV) to activate riboflavin (RF) [13,14,33,34]. Recently, fossilization of acid-etched MMPs via biomimetic remineralization has also been investigated [35,36].

Glutaraldehyde works well as a collagen cross-linker [20], but some regard it as toxic [29]. However, Scheffel et al. recently reported that a 0.4-mm thickness of dentin made even 10% glutaraldehyde nontoxic to MDPC-23 cells growing on the pulpal side of dentin disks [32]. Grape seed extract is also effective as a collagen cross-linker, but it stains dentin light brown [37].

Carbodiimide is a stable isomer that works by activating the free carboxyl groups of glutamic and aspartic acids in proteins, which then react with the free amino groups present in adjacent protein molecules, forming covalent cross-links [20].

Carbodiimide has very low cytotoxicity and shows promising results in preventing dentin collagen degradation and preserving bond durability [12,38].

Few investigations have evaluated the influence of riboflavin (vitamin B_2) on dentin bond strength and durability [13–15,39]. Chiang et al. [13] and Fawzy et al. [15] showed that the pretreatment of acid-etched dentin with riboflavin improved bond strength and maintained bond durability by increasing biodegradation resistance after short-term water storage. Although ultraviolet (UV) light-photoactivated riboflavin had significantly more influence on bond strength and durability than blue light (BL)-photoactivated riboflavin, the results of BL activation were promising [15]. Blue lights are available in all dental offices, so more research on the activation of riboflavin using blue light should be considered.

Therefore, for better clinical application of dentin cross-linkers, research is needed to determine the most appropriate methodology for producing strong and durable bonds.

The aim of the present study was to investigate the effect of different cross-linking agent protocols (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl), UV- or BL-activated riboflavin-5-phosphate on the resin–dentin micro-tensile bond strength (μTBS) and nanoleakage exhibited by a two-step etch-and-rinse adhesive after three storage intervals (24 h, 6 months, and 12 months).

The null hypothesis was that the pretreatment of acid-etched dentin with EDC-HCl and UV- or BL-activated riboflavin-5-phosphate as cross-linking agents would not influence bond deterioration over time.

2. Material and methods

Detailed composition of the materials used in the study is presented in Table 1.

2.1. Specimen preparation for micro-tensile bond strength test

Extracted human third molars were collected from our institutional oral surgery clinic with the informed consent of the donors and approval from the King Abdulaziz University ethical committee (protocol # 004–15). A total of 48 sound molars were selected and stored in 0.5% chloramine T solution for less than one month. Occlusal enamel and superficial dentin were removed using a low-speed diamond saw under water irrigation (Micromet AG, Munich, Germany). The freshly cut middle dentin surfaces were finished with 360 grit silicon carbide papers to remove any enamel islands and to create a standardized smear layer-covered surface. Exposed coronal dentin surfaces were then etched with 32% phosphoric acid (etching gel, 3 M ESPE, St. Paul, MN, USA) for 15 s, rinsed with water for 10 s, and blot-dried using a mini-sponge. The etched teeth were divided randomly and equally into four groups according to the dentin cross-linking protocol to be used (N = 12 per group × 4 groups = 48 teeth).

The treatment groups were as follows: 1): Adper Single Bond 2: etched surfaces were bonded by Adper Single Bond 2 (3 M ESPE, St. Paul, MN, USA) according to the manufacturer instructions, without dentin surface pretreatment (control); 2): Single Bond 2 + EDC-HCl: acid-etched dentin surfaces were treated with aqueos 0.5 M EDC-HCl (ProteoChem, Loves Park, IL, USA) applied for 60 s, rinsed with water, then bonded with Adper Single Bond 2; 3):Single Bond 2 + BL RF: acid-etched dentin surfaces were pretreated with 1 wt % riboflavin-5-monophosphate sodium salt hydrate (R-5-PO_4) (Sigma, St. Louis, USA) for 60 s, and activated by BL (light-emitting diode curing unit; 3 M ESPE, Elipar, Seefeld, Germany) delivering 1200 mW/cm^2, at 430–480 nm for 20 s, then bonded with Adper Single Bond 2; and 4): Single Bond + UV RF: acid-etched dentin surfaces were pretreated with 1 wt % (R-5-PO_4) activated by UV (Camag UV-Cabinet II, Switzerland) delivering ~10 mW/cm^2, at 366 nm for 20 s, then bonded with Adper Single Bond 2. All lights were held 3 mm above the specimen.

The pretreated dentin surfaces were bonded using Adper Single Bond 2 (3 M ESPE) according to the manufacturer instructions, and Universal Restorative nano-hybrid composite (Filtek Z350 XT, 3 M ESPE) was used to incrementally build up a 6-mm core. Each 2-mm increment was cured with BL for 40 s. The restored teeth in each group (n = 4) were stored in distilled water at 37 °C for 24 h, 6 months, or 12 months.

2.2. Microtensile bond strength evaluation

After each storage interval (24 h, 6 months, or 12 months), the samples were sectioned through the resin composite buildup and dentin into 1 × 1 mm ± 0.1-mm thick beams (n = 16) using an IsoMet saw (Allied High Tech Products, Inc., Canada) following the technique described by Shono et al. [40]. Sixteen beams from each tooth were stored at 37 °C in separate containers containing distilled water.

Fifteen beams from each of the 4 teeth in each group (n = 4 × 4 treatment groups = 16 teeth) were randomly selected for each storage period; one beam from each tooth in each group was used for nanoleakage evaluation.

Before micro-tensile testing, the dimensions of beams were recorded using a digital caliper (accurate to 0.01 mm) to calculate the bond strength. Beams were bonded to a jig using cyanoacrylate adhesive (Zapit, Dental Ventures of America Inc., Corona, CA, USA). The jig was mounted on a micro-tensile tester machine (Bisco Inc.) and subjected to tensile failure at a crosshead speed of 1 mm/min. All debonded surfaces were examined using a stereomicroscope (Meiji Techno Co. Ltd., Tokyo, Japan) at 50X after micro-tensile bond strength testing, to classify the modes of failure, into adhesive, cohesive, or mixed failures. Debonded samples before testing was also recorded, but was not included in the statistical analysis because all premature failures occurred during beam preparation.

2.3. Nanoleakage evaluation

One beam from every tooth in every group was utilized for nanoleakage assesment (N = 4 per group, i.e., four beams from each bonding group at every time point). Specimens were prepared for nanoleakage evaluation, following the procedures described by Tay et al. [41]. In brief, coating of selected beams with two layers of nail varnish was applied, leaving only 1 mm free at the interface. Beams were soaked in a 50 wt% ammoniacal silver nitrate ($AgNO_3$) solution (pH

Table 1
Composition of the materials used in the study.

Materials	Manufacturer	Composition
Etching gel	3M ESPE, St. Paul, MN, USA	32 wt % *phosphoric acid*
Adper Single Bond 2	3M ESPE, St. Paul, MN, USA	bisGMA, HEMA, dimethacrylates, ethanol, water, polyacrylic and polyitaconic acids 10 wt % 5 nm silica particles.
Carbodiimide (EDC-HCl)	ProteoChem, Loves Park, IL, USA	0.5 M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
Riboflavin (RF)	Sigma, St. Louis, USA	1 wt % riboflavin-5-monophosphate sodium salt hydrate
Filtek Z350 XT	3M ESPE, St. Paul, MN, USA	bis-GMA, UDMA, TEGDMA, and bis-EMA(6), PEGDMA. Agglomerated/non-aggregated 20 nm silica filler, non-agglomerated/non-aggregated 4 −11 nm zirconia filler, and aggregated zirconia/silica cluster filler 20 nm silica and 4 −11 nm zirconia particles

9.5) for 24 h, then removed and rinsed with water for 5 min and placed in a photo-developing solution (Eastman Kodak Co., Rochester, NY, USA) for 8 h under fluorescent light to reduce the diamine silver ions ($[Ag(NH_3)_2]+$) to metallic silver grains. The prepared beams were polished with SiC paper of increasing fineness (600–1200 grit), polished with a soft cloth, with a 0.05 mm alumina particle suspension (Buehler, Lake Bluff, IL, USA), and ultrasonically cleaned in distilled water for 30 min (Ultrasonic Cleaning System 2014, L & R Manufacturing, Kearny, NJ, USA). Analysis of the resin–dentin interfaces was carried out in an environmental scanning electron microscope (Quanta 200 ESEM, FEI France, Mérignac, France) operated in the backscattered electron mode at 1000X magnification. Quantitative analysis of the amount of silver nitrate that penetrated the hybrid layer was performed using image analysis software (NIH Image, Scion Corp. Fredrick MD, USA) [41].

2.4. Statistical analysis

The experimental design is presented in Table 2, which shows the sample distribution of the subgroups and the different storage periods. The statistical unit was the tooth, not the beam. A beam from the same tooth cannot be considered the experimental unit because it does not fulfill the analysis of variance (ANOVA) requirements.

The μTBS and nanoleakage data were collected and analyzed using a two-way ANOVA design, with the dentin treatment and storage time as the two-factors. Statistical significance was set at 0.05.

The data were normally distributed and had equal variances. The response variables were μTBS and nanoleakage.

The fracture modes were analyzed using the chi-square test to delineate the differences among the groups. The interaction between the variables was tested.

3. Results

3.1. Microtensile bond strength test (μTBS)

The interaction between factors was significant ($p = 0.002$, $F = 4.443$). Therefore, the two-way ANOVA for bond strength was re-run using pairwise comparison to compare the means of the different dentin treatment groups at the same storage time, and to compare means after different storage times for each dentin surface treatment.

Table 3 shows the mean values of μTBS for Single Bond 2 in the control group after 24 h, 6 months, and 12 months of storage at 37 °C. There was no significant difference in μTBS between the control group and the experimental groups treated with either EDC-HCl or riboflavin (BL- or UV-activated) after 24-h storage ($p > 0.05$) (Table 3).

However, after 6 months of storage in water at 37 °C, the control group (Single Bond 2) showed a significant decrease ($p < 0.001$) in bond strength (approximately 10 MPa) compared with the 24-h bond strength. In contrast, EDC-HCl and riboflavin-treated dentin surfaces

Table 2
Flow chart of experimental design showing number of teeth vs beams for microtensile bond strengths and nanoleakage.

Adper Single Bond 2	Tooth number (beams)	Bond strength		Nanoleakage
		μTBS	Mean μTBS	
Time 24 h	#1 (16)	15	Tooth #1 mean μTBS	1 beam
	#2 (16)	15	Tooth #2 mean μTBS	1 beam
	#3 (16)	15	Tooth #3 mean μTBS	1 beam
	#4 (16)	15	Tooth #4 mean μTBS	1 beam
	4 teeth × 16 beams/tooth = 64 beams	60 beams used for μTBS	24 h: Grand mean ± SD	4 beams used for 24 h nanoleakage*
Time 6 months	#5 (16)	15	Tooth #5 mean μTBS	1 beam
	#6 (16))	15	Tooth #6 mean μTBS	1 beam
	#7 (16)	15	Tooth #7 mean μTBS	1 beam
	#8 (16)	15	Tooth #8 mean μTBS	1 beam
	4 teeth × 16 beams/tooth = 64 beams	60 beams used for μTBS	6 months: Grand mean ± SD	4 beams used for 6 m nanoleakage
Time 12 months	#9 (16)	15	Tooth #9 mean μTBS	1 beam
	#10 (16)	15	Tooth #10 mean μTBS	1 beam
	#11 (16)	15	Tooth #11 mean μTBS	1 beam
	#12 (16)	15	Tooth #12 mean μTBS	1 beam
	4 teeth × 16 beams/tooth = 64 beams	60 beams used for μTBS	12 months: Grand mean ± SD	4 beams used for 12 m nanoleakage

Table 2 can be extended to include the 3 other experimental groups: Single Bond 2 + EDC-HCl, Single Bond 2 + BL RF, and Single Bond 2 + UV RF. These were not included to save space.

Each subgroup contained 4 teeth or 60 beams for bond strength and 4 beams for nanoleakage. There were 3 subgroups (24 h, 6 months, 12 months), and 4 experimental groups received different dentin surface pretreatments (12 teeth in each group × 4 surface treatment groups = total of 48 teeth).

The mean value for μTBS for 24 h = the grand mean of 15 beams per tooth. All 4 teeth in each subgroup were averaged to obtain a grand mean ± SD, the statistical unit was tooth and not beam.*The nanoleakage values of the 4 beams per subgroup were averaged to give a mean nanoleakage score for each time period in each material subgroup for each substrate.

Abbreviations: BL = blue light (430–380 nm); UV = ultraviolet light (366 nm); RF = 1% riboflavin-5-monophosphate; EDC-HCl = 0.5 M 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; μTBS = micro-tensile bond strength.

Table 3
Mean values and standard deviations (SD) of micro-tensile bond strength (μTBS) in MPa before and after dentin pretreatment for different storage intervals.

Dentin surface treatment	Storage time at 37 °C		
	24 h	6 months	12 months
Single Bond (SB)	$44.7^{A,a}$ (2.1)	$34.5^{B,b}$ (1.4)	$29.8^{C,c}$ (2.0)
Single Bond + EDC (SB + EDC)	$43.9^{A,a}$ (2.0)	$41.8^{A,a,b}$ (2.1)	$39.5^{A,b}$ (2.5)
Single Bond + BL / riboflavin (SB + BL-RF)	$43.2^{A,a}$ (1.1)	$40.9^{A,a}$ (1.7)	$35.4^{B,b}$ (2.1)
Single Bond + UV / riboflavin (SB + UV-RF)	$45.7^{A,a}$ (2.0)	$43.2^{A,a,b}$ (1.6)	$40.2^{A,b}$ (1.6)

The upper case letters indicate a statistically significant difference ($p = 0.05$) in the same vertical column; the lower case letters indicate a statistically significant difference in the same horizontal row.

Table 4
Means and standard deviations (SD) of nanoleakage (%) before and after dentin pretreatment for different storage intervals.

Dentin surface treatment	Storage time at 37 °C		
	24 h	6 months	12 months
Single Bond (SB)	$51^{A,a}$ (3.0)	$62.7^{A,a}$ (4.2)	$84^{B,b}$ (2.8)
Single Bond + EDC (SB + EDC)	$52.2^{A,a}$ (6.9)	$54^{A,a}$ (6.4)	$56.5^{A,a}$ (4.6)
Single Bond + BL Riboflavin (SB + BL-RF)	$53.3^{A,a}$ (4.5)	$60.2^{A,a,b}$ (4.8)	$65^{A,b}$ (7.2)
Single Bond + UV Riboflavin (SB + UV-RF)	$48.7^{A,a}$ (5.3)	$55^{A,a,b}$ (8.4)	$62^{A,b}$ (6.2)

The upper case letters indicate a statistically significant difference ($p = 0.05$) in the same vertical column; the lower case letters indicate a statistically significant difference in the same horizontal row.

showed no significant change in bond strength compared with the 24-h mean values ($p > 0.05$) (Table 3).

After 12 months of storage, there was a further significant decrease in bond strength in the control Single Bond 2 group, compared with both the 24-h and 6-month storage intervals ($p < 0.001$, $p = 0.01$, respectively). The bond strength values of samples treated with EDC-HCl decreased significantly after 12 months of storage compared with 24-h storage ($p = 0.015$), whereas the mean bond strength values were not significantly different ($p > 0.05$) from the 6-month strength values. Similarly, dentin treatment with UV-activated riboflavin-5-phosphate resulted in a small significant decrease in bond strength after 12 months, compared with the 24-h value ($p = 0.003$), with a non-significant difference compared with the 6-month bond strength ($p > 0.05$). However, dentin treatment with BL-activated riboflavin-5-phosphate resulted in a large significant decrease in bond strength after 12 months compared with both the 24-h and 6-month bond strength values ($p < 0.05$) (Table 3).

After 12 months of storage, the bond strength in the Single Bond 2 group (the control group) was significantly lower than that in the experimental groups treated with the tested protocols ($p < 0.003$). The bond strength of the dentin treated with BL-activated riboflavin-5-phosphate after 12 months of storage was significantly higher than that of the control group ($p = 0.003$), but was significantly lower than that of the EDC-HCl- and UV-activated riboflavin treatment groups ($p = 0.023$ and $p = 0.009$, respectively). Therefore, surface treatment of acid-etched dentin with EDC-HCL, or with either UV- or BL-activated riboflavin, increased the durability of the bond, compared with the untreated controls.

The power of the test for μTBS at alpha = 0.05 was 1.00 for storage time, 1.00 for dentin treatment, and 0.96 for storage time X dentin treatment.

3.2. Nanoleakage evaluation

The interaction between factors was significant ($p = 0.006$, $F = 3.674$). Therefore, the two-way ANOVA for nanoleakage was re-run using pairwise comparisons to test the nanoleakage means of the different dentin treatment groups at the same storage time, and to compare means for different storage times for each dentin surface treatment.

Table 4 shows the mean values of nanoleakage for the control group bonded with Single Bond 2 after 24 h, 6 months, and 12 months of storage at 37 °C. After 24 h and 6 months of storage, there was no significant difference between the Single Bond 2 (control) group and the 6 months groups subjected to the various dentin treatments ($p > 0.05$). However, after 12 months of storage, nanoleakage in the control group increased significantly ($p < 0.05$) compared with the 24-h or 6-month controls.

Twelve months of storage time resulted in a significant increase in nanoleakage in the control group compared with the other groups treated with EDC-HCL or BL/UV-activated riboflavin ($p < 0.05$).

Moreover, nanoleakage in the samples treated with riboflavin-5-phosphate (BL/UV- activated) after 12 months of storage was significantly higher than after 24 h ($p < 0.05$), but did not differ significantly compared with after 6 months ($p > 0.05$).

No significant differences were detected in nanoleakage between the different storage times (24 h, 6 months, 12 months) when the dentin surface was treated with EDC-HCL ($p > 0.05$) (Table 4). The power of the test for the nanoleakage results at alpha = 0.05 was 1.00 for storage time, 0.981 for dentin treatment, and 0.920 for storage time X dentin treatment.

3.3. Fracture modes

The fracture modes for all the groups are shown in Table 5. The chi-square test was used to compare the various failure modes over storage time and dentin treatment, and it revealed no significance differences among the test groups ($p = 0.089$).

4. Discussion

The results of the current work demonstrate that treatment of acid-etched dentin with 0.5 M EDC-HCL for 60 s or 1% riboflavin-5-phosphate photoactivated for 20 s, prior to the application of Adper Single Bond 2, significantly reduced deterioration in bond strength over long-term storage periods (6 and 12 months), and significantly reduced nanoleakage in the hybrid layers over time. Therefore, the results of this study require the rejection of the tested null hypothesis.

The use of EDC-HCl or UV-activated 1% riboflavin-5-phosphate resulted in greater preservation of bond integrity over time compared with BL-activated 1% riboflavin-5-phosphate.

Our findings that pretreatment of acid-etched dentin with EDC-HCl improves bond stability over time supports the results from other studies [12,17,18], in which the use of EDC-HCL did not affect immediate bond strength to dentin but demonstrated preservation of initial bond strength after 1 year.

The ability of EDC-HCL to preserve bond integrity has been implied in previous reports on its ability to cross-link collagen, and its ability to inactivate MMPs and reduce their collagenolytic activity in the hybrid layer [12,17,18,38], preventing degradation of bond strength over time.

Carbodiimide cross-links proteins non-specifically by activating the free carboxylic acid groups of glutamic and aspartic acids, forming an O-acyl isourea intermediate, which reacts with the Ɛ-amino groups of lysine or hydroxylysine to form amide cross-links [38]. Tezvergil-Mutluay et al. [38] speculated that EDC-HCL also has the ability to cross-

Table 5
Failure modes in the three groups over time.

Surface treatment	Single bond			Single bond + EDC			Single bond + BL-activated riboflavin			Single bond + UV-activated riboflavin		
Fracture mode	24 h	6 m	12 m	24 h	6 m	12 m	24 h	6 m	12 m	24 h	6 m	12 m
Adhesive Failure	20	24	20	12	20	24	16	20	28	8	16	24
Cohesive in composite	4	0	4	0	4	4	4	8	4	4	8	4
Cohesive in dentin	0	0	4	4	0	0	0	0	0	8	0	0
Mixed	36	36	32	44	36	32	40	32	28	40	36	32
Total	60	60	60	60	60	60	60	60	60	60	60	60

link peptide chains in MMPs and cysteine cathepsins bound to dentin matrices. This would decrease the molecular mobility of the catalytic sites in these enzymes, suppressing enzyme function [7].

The present study confirmed that the use of 0.5 M carbodiimide-HCl for 60 s as a pretreatment of acid-etched dentin is a practical clinical procedure for the preservation of bond integrity over time.

The influence of UV-activated riboflavin in preserving bond integrity and reducing nanoleakage over time has been reported previously [13,14].

The UV/VIS spectrum of riboflavin shows four absorbance: two in the UV (230, 260 nm), one at 370 nm and one at 450 nm [42,44]. Thus, UV light at 366 nm would match the riboflavin 370 nm peak, while the blue light emission maximum at 470 nm would match the 450 nm absorbance peak of riboflavin.

The use of 20 sec of blue light to activate riboflavin in the current study was identical to that used by Fawzy et al. [15]. Those investigators also used a 20 sec UVA exposure to activate riboflavin. Thus, we duplicated their exposures to allow comparison of their results with ours, which were very similar.

The use of UVA to activate riboflavin was first used in ophthalmology [42,43] to crosslink corneal collagen fibrils. The presence of excess riboflavin in the corneas absorbs about 95% of the UV light, resulting in a nontoxic dose of UVA to the corneal endothelium [45–47].

The U.S. Food and Drug Administration approved the use of riboflavin and UVA irradiation to crosslink corneal collagen in vivo in April 18, 2016. Hopefully similar safety on the use of riboflavin and UVA-irradiation to cross-link hybrid layers in teeth, in vivo will be done so that it can be used in clinical dentistry.

The use of 1% riboflavin in the Fawzy et al. study [15], activated with either BL or UV for 20 seconds, showed significant improvement in biodegradation resistance to collagenolytic enzymes; UV-activated riboflavin showed more significant improvement compared with BL-activated riboflavin. These differences between UV and BL were confirmed in the present study in which UV-activated riboflavin showed significantly higher preservation of bond integrity than the BL-activated material. However, BL-activated riboflavin resulted in significant preservation of bond integrity compared with the control group, which did not receive any dentin pretreatment. Moreover, both riboflavin activation techniques resulted in a significant reduction in nanoleakage compared with the control group.

In previous studies [42,44], it has been confirmed that the riboflavin absorbs light over a wide spectral range from UV to visible light, with four maximum absorption peaks at 230, 260, 370, 366, and 450 nm. In the current study, we used a BL-emitting diode as a visible light source. The maximum wavelength peak output of blue light source was 455 nm ± 10 nm, which may not have matched the maximum absorption peak of riboflavin in the visible spectrum. However, the UV light source used in the present study had a maximum emission wavelength peak at 366 nm, which perfectly matched the 370 nm absorption peak of riboflavin. This could explain the slight difference observed between the results for UV- and BL-activated riboflavin.

Nanoleakage at the resin–dentin interface results from either hydrolytic degradation of the adhesive resin induced by water sorption, or enzymatic degradation of the collagen fibrils present in the hybrid layer. These processes can be observed by the uptake of ammoniacal silver nitrate, which traces the distribution of water-filled channels within dentin matrices [41].

In the present work, the nanoleakage results were similar to those of previous investigations [13,14,17], in which carbodiimide or photo-activated riboflavin significantly reduced nanoleakage over time, compared with the untreated controls. In the current work, the carbodiimide pretreatment was more effective at reducing nanoleakage than the riboflavin pretreatments.

These results imply that the cross-linkers preserve the integrity of collagen fibers by cross-linking and inhibiting collagenolytic enzyme activity over time. Alternatively, cross-linkers may increase the stiffness of collagen fibrils, affecting the ability of collagenases to unwind the collagen peptides [48]. Therefore, the authors of this work encourage clinicians to consider the routine pretreatment of acid-etched dentin using either BL-activated riboflavin or the less demanding cross linker, EDC-HCL, for preserving bond integrity over time. However, further investigations are required, especially with regard to challenging boding locations, such as cervical margins of class V restorations, in which bonding is mainly to dentin, or in minimally invasive caries removal aimed at preserving the natural tooth structure for extended periods.

5. Conclusions

The results of the present study demonstrate that the application of carbodiimide or photoactivated riboflavin as a cross-linking pretreatment for acid-etched dentin is a clinically practical technique for the long-term preservation and optimization of adhesive bond integrity. Further research is required to optimize the blue light activation of riboflavin for stronger inhibition of adhesive bond degradation.

Conflict of interest

The authors have no financial conflicts of interest to declare regarding the materials discussed in this manuscript.

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