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Photochemical crosslinking of caries-affected dentin combined with total- or self-etch systems

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Abstract: Purpose: To evaluate the effects of collagen crosslinking with riboflavin 0.1% and ultraviolet-A (UVA) 5.4 J on bond strength of total-etch or self-etch adhesives on caries-affected dentin. Methods: Sixty human caries-affected molars were randomly divided into three groups: control (C), riboflavin (R), and riboflavin + 3 minutes of UVA (R+UVA). After each treatment, either total-etch or self-etch adhesives were applied following the manufacturer's instructions, and composite stubs were built up on the treated surfaces. They were de-bonded in tension to measure bond strength. Twelve extra molars were used for scanning electron microscope (SEM) analysis. Results: We observed that R+UVA-treated group yielded significantly higher bond strengths for carious dentin when the total-etch adhesive was applied. For the self-etch adhesive, no statistical differences were observed between the three pretreated-groups. Conclusion: Our results, for the first time, are suggesting that etching with phosphoric acid potentialized the benefits of R+UVA crosslinking on carious dentin. R+UVA dentinal collagen crosslinking improves bond strength for caries-affected dentin when using a total-etch adhesive, but did not affect it when using a self-etch adhesive.

Keywords: Bond strength, crosslinking, riboflavin, UVA, collagen, dentin

Introduction

Millions of dental restorations are placed every year in the United States and more than 50% of those are replacements due to failed restorations [1]. The factors and mechanisms involved in the premature failure of the adhesive bond to dentin are not fully understood but it has been suggested that this may be due to the weakness of the collagen in caries affected dentin matrix [2]. From a clinical perspective, it is very important to be able to prevent the degradation of collagen by collagenolytic enzymes and to increase bond strength. Due to its heterogeneity, dentin can be described as a dynamic substrate for bonding [3-5]. Several approaches have been used to improve bond durability, including modification of dentin substrate with different crosslinking agents to strengthen the dentin-restoration interfaces [6-8].

The main type of collagen present in the teeth is the collagen type I, similarly to the eye's cornea. In ophthalmology, a photochemical meth-

od using riboflavin (R) and ultraviolet-A (UVA) radiation is clinically used to improve corneal collagen crosslinking and increase corneal stiffness [9, 10]. Although the mechanisms of action are still not fully understood, it is suggested that when riboflavin is exposed to UVA radiation, it interacts directly with the collagen molecules and indirectly through generation of singlet oxygen creating new bonds that result in the increase of corneal stiffness [11]. Because of riboflavin's efficacy and lack of toxicity, collagen crosslinking induced by R+UVA has been widely used as a successful treatment for human ophthalmic diseases such as keratoconus [10, 12, 13], wherein the recovery of collagen stiffness is necessary.

The use of R+UVA to successfully improve the mechanical properties of sound dentin has been reported [14]. However, to be clinically relevant, the improvement in bond strength needs to be investigated in carious dentin. The structures of collagen and minerals are altered by the caries process [15] and bond-strength of

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cariou affected dentin is usually lower than sound dentin [16]. The smear layer and the reduced permeability observed in carious dentin [17, 18] may affect the efficacy of the crosslinking method. The purpose of this study is to evaluate the ability of R+UVA to crosslink dentinal collagen on caries-affected dentin and improve bond strength of self-etch (SE) and total-etch (TE) adhesives.

Materials and methods

Experimental design

Sixty human caries-affected molars were randomly selected for this study. The teeth were ground parallel to the occlusal surface with 60-grit SiC paper (Carbimet Paper Disc, Buehler, Lake Forest, IL, USA) immediately before bonding on a polishing machine (Ecomet 6, Buehler, Lake Forest, IL, USA) to expose enamel and then finished with 600-grit SiC paper (Carbimet Paper Disc, Buehler, Lake Forest, IL, USA). Molars were randomly distributed into three groups. In Group C (control group), riboflavin or UVA was not applied and molars were treated with the either total- or self-etch bonding agents according to the manufacturer's instructions. Group R (riboflavin group) was treated with riboflavin 0.1% for 4 minutes prior to the application of the different bonding agents. Group R+UVA (UVA activated Riboflavin group) was treated with R 0.1% for 4 minutes + 3 minutes of UVA (30 mW/cm²) prior to the application of the different bonding agents.

Bonding agents

Groups C, R, and R+UVA were treated with either a total-etch adhesive (All-Bond 3 TE adhesive, Bisco, Inc., Schaumburg, IL, USA) or a self-etch adhesive (All-Bond SE, Bisco, Inc., Schaumburg, IL, USA). For each carious-affected molar treated with total-etch adhesives, 35% phosphoric acid was applied before the application of Riboflavin or R+UVA. For group R, the dentin surfaces were treated with 1 drop of 0.1% riboflavin-5-phosphate (Sigma-Aldrich, Saint Louis, MO) every minute, for 4 minutes, and then bonded according to the manufacturer's instructions. Group R+UVA was treated with riboflavin 0.1% for 1 minute and exposed to UVA for 3 minutes while applying a new drop of riboflavin each minute, and then bonded accordingly to the manufacturer's instructions.

Room light was kept reduced during the procedure to avoid external activation of the riboflavin.

A specialized jig was placed on top of each bonded molar teeth and composite (Aelite All-Purpose Body, Bisco, Inc., Schaumburg, IL, USA) stubs (5 mm height/19.6 mm² area) were built up on the treated surfaces. Bonded specimens were stored in water at 37°C for 24 hours before debonding in tension. They were then de-bonded under shear load with a universal testing machine (Instron Model 4465, Instron Corp., Canton, MA, USA) at a crosshead speed of 0.5 mm/min. Bond strengths were reported in MPa. Failure modes were determined based on the standard criteria [19].

Scanning electron microscopy

Twelve additional sound and carious extracted human molars were ground with 600-grit SiC paper (Buehler) on the occlusal surface to expose dentin. The specimens were sectioned perpendicular to the adhesive interface with a slow-speed diamond saw (Isomet, Buehler) to produce slices, each 1-2 mm thick. One slice was acid-etched with 5 N HCl for 30 s, followed by 5% NaOCl for 30 min, and then rinsed thoroughly with distilled water. The other slice was fractured perpendicularly to the interface. Each slice was then dehydrated with 33% ethanol for 30 min, 67% ethanol for 30 min, 85% ethanol for 30 min, and 100% ethanol for 1 h. The specimens were left overnight to dry and then mounted on 12-mm aluminum stubs and sputter-coated with gold-palladium alloy. The specimens were viewed from 1000 to 2500× magnification in a NeoScope JCM-5000 SEM at a high vacuum. Analyses of the dentin-adhesive interface characteristics were based on at least 20 images taken along the length of the interface.

Statistical analysis

Results are presented as mean ± SEM of *n* independent observations. Statistical comparisons were made using GraphPad Prism 7.04 statistical software. A *p*-value < 0.05 was considered statistically significant. The dependent variable was the mean bond strength, expressed in MPa. As specified in the figure legends, one-way ANOVA followed by Tukey's *post hoc* test, was performed to compare the mean bond strength, between different 3 pretreated-

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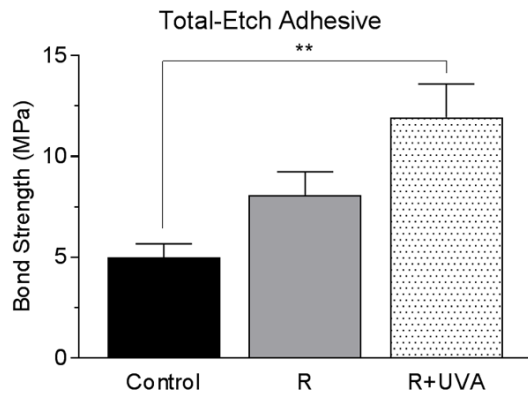


Figure 1. Effect of pretreatment with riboflavin or the combination of riboflavin and UVA on mean bond strengths of carious dentin treated with Total-Etch Adhesive. All Bond 3 TE (Total-Etch Adhesive) was applied subsequently in a carious molar control group (black bar), riboflavin-(R, 0.1%; gray bar) or riboflavin 0.1% combined with 5.4 J UVA (R+UVA; dotted bar)-treated groups. Twenty-four hours after the application of Total-Etch Adhesive, comparison between the mean bond strength was made in all 3 groups, by Instron testing machine, and expressed in MPa. We found that the Total-Etch Adhesive markedly increased the mean bond strength in the R+UVA-treated group ($F_{(2,27)} = 7.965$, $**P = 0.0019$, when the control-, the R- and the R+UVA-treated groups are compared 24 hours after Total-Etch Adhesive application; one-way ANOVA followed by Tukey's *post hoc* test. $N = 10$ carious molars per treated-group). These findings indicate that the pretreatment with riboflavin combined with UVA has potential to translate into a clinically relevant step in the treatment of carious tooth, increasing bond strength and prolonging the durability of the restorations.

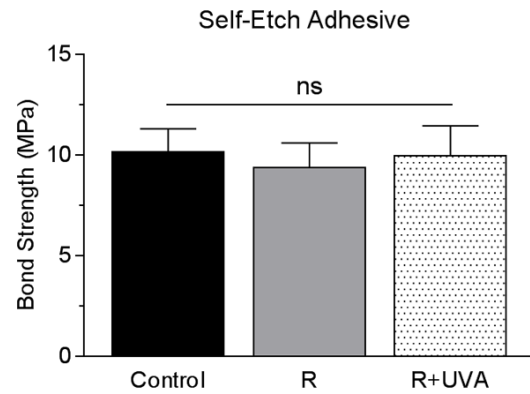


Figure 2. Pretreatment with riboflavin or riboflavin + UVA did not change the mean bond strength of carious dentin treated with Self-Etch Adhesive. Carious molar groups pretreated with control (Control; black bar), riboflavin (R; gray bar) or the combination of riboflavin and UVA (R+UVA; dotted bar) followed by the application of All Bond SE (Self-Etch Adhesive). Bonded specimens were stored in water at 37 °C for 24 hours before debonding in tension. They were de-bonded under shear load with a universal testing machine (Instron Model 4465, Instron Corp., Canton, MA, USA) and the mean bond strength was compared between groups. In all 3-pretreated groups, Self-Etch Adhesive did not affect, significantly, the mean bond strength (MPa) ($F_{(2,27)} = 0.1028$, $P = 0.9027$; one-way ANOVA followed by Tukey's *post hoc* test. $N = 10$ carious molars per treated-group).

groups that received Total- or Self-Etch Adhesives.

Results

Mean bond strengths for groups treated with All-Bond 3 TE are shown in **Figure 1**. Among the caries-affected molars bonded with total-etch adhesive, the highest mean bond strength was observed in Group R+UVA, suggesting that R+UVA dentinal collagen crosslinking is effective in increasing bond strength for molars treated with the total-etch adhesive system. The caries-affected molars treated with All Bond SE (**Figure 2**) resulted in similar mean bond strengths across the treatments groups, suggesting that they were not affected by either treatment of Group R or Group R+UVA.

The SEM micrographs were analyzed for specimens that were bonded with All-Bond 3 TE

(**Figure 3**). Group R+UVA with carious dentin, which had the highest bond strength out of all the conditions, showed numerous resin tags on the dentin/adhesive interface with a thick hybrid layer of approximately 6.5 μm (**Figure 3B**). On the other hand, SEM micrograph of Group C with carious dentin showed numerous long dentin resin tags with a thinner hybrid layer of approximately 2 μm (**Figure 3C**) compared to Group R+UVA with carious dentin.

Even though the three conditions analyzed were bonded with TE adhesives, only Group R+UVA with carious dentin showed a distinct hybrid layer of 6.5 μm . Also, while SEM micrographs of each condition showed resin tags, Group C with carious dentin showed the most resin tags (**Figure 3B**). The hybrid layer is an important factor of the bonding mechanism in total-etch adhesives, but the presence and thickness of the hybrid layer did not correlate with the bond strengths. In addition, the length of resin tags did not correlate with the bond strengths because the carious dentin control group showed the most numerous and long resin tags (**Figure 3B**).

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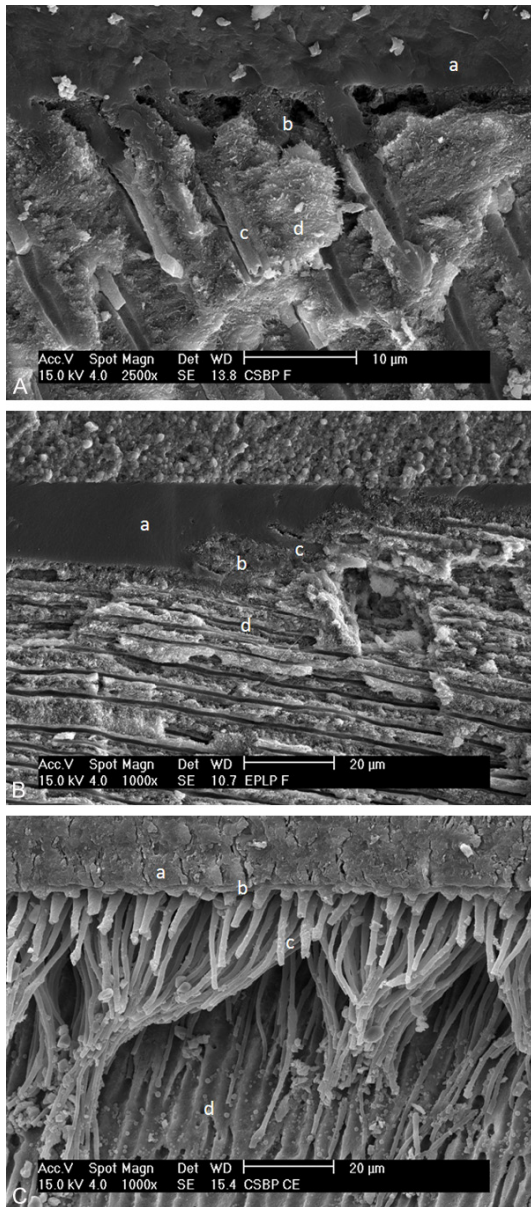


Figure 3. Scanning Electron Microscopy of molar specimens bonded with Total-Etch Adhesive. A. Sound molar specimen pretreated with riboflavin 0.1% and UVA 5.4 J; B. Carious molar specimen pretreated with riboflavin 0.1% and UVA 5.4 J; C. Carious molar specimen with no pretreatment (control). a = adhesive, b = hybrid layer, c = resin tags, d = dentin.

Discussion

In this study we investigate, for the first time, the biomechanical effects of pretreating caries-affected molars with riboflavin 0.1% combined with 5.4 J UVA followed by restoration with total- or self-etch adhesives. When comparing the groups treated with All Bond 3 TE,

the R+UVA-treated group yielded highly statistically significant ($P < 0.0019$) increase in bond strengths on carious-affected molars. There was no difference between the caries affected groups bonded with All-Bond SE. Our findings are indicative that the pretreatment with riboflavin combined with UVA (R+UVA) crosslinks dentinal collagen on caries-affected molars, increasing bond strength when using a total-etch adhesive, but it was not effective on carious dentin when using a self-etch adhesive.

In caries-affected dentin, the smear layer consists of large and irregularly shaped debris while the smear layer of sound dentin is homogeneous [17]. When bonding with total-etch adhesives, phosphoric acid is needed to interact with the dentin substrate and to remove the smear layer, which possibly allowed riboflavin to better penetrate and interact with dentinal collagen during the UVA crosslinking treatment. However, in self-etch adhesives, the smear layer is not removed, and it possibly impairs the penetration of riboflavin and the crosslinking to occur. Thus, the irregularity of the smear layer in caries-affected dentin may have hindered the penetration of riboflavin in the dentinal tubules. A reduction in the local availability of oxygen or in the local delivery of UVA caused by the smear layer are less probable but could also contribute to a reduced crosslinking effect.

Modifications to facilitate the usage of this crosslinking method should be taken into consideration. Besides its peak of absorption at 366 nm, riboflavin has other peaks of absorption in the UVB and blue spectrum [20]. UVB rays are not suitable for clinical use because of deleterious and carcinogenic effects [21]. On the other hand, riboflavin's absorption peak at 436 nm (blue spectrum) makes it possible to activate riboflavin by halogen, white and blue LED light sources, which are commonly used in dental clinic [20].

Fawzy and colleagues [22] studied the effects of R+UVA on sound dentin after acid etching and reported a significant enhancement on tensile bond strength at 24 h in the group treated with riboflavin + UVA (10 mW/cm² for 20 s) but not in the group treated for 20 seconds with a blue-light (BL) tungsten/halogen-lamp. Their relatively short light exposure time may have influenced their results. In our study, we used a custom 365 nm UVA LED light source with an

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irradiance of 30 mW/cm² for 3 minutes, corresponding to a 5.4 J total dose, similar to the total dose used in corneal collagen crosslinking. The potential of oxygen enrichment to improve dentinal collagen crosslinking should also be considered, however the results of oxygen enrichment for corneal collagen crosslinking are controversial [23, 24]. There is also a potential to combine this method with the application of mineralizing agents. Bortolotto and colleagues reported a significant increase in the elastic modulus in dentin specimens treated with riboflavin plus a calcium phosphate-based product (Teethmate®) treated with blue light for 60 seconds [25].

Since our study has only tested the bond strength after a 24 hours storage period, future studies should examine various storage times, testing the longevity of the treated carious-affected molars and the durability of the bond in long-term water storage.

The SEM analysis suggested that the length of resin tags do not correlate with the bond strengths in molars treated with the total-etch adhesive. This finding correlates with previously results reported by Pinzon and colleagues, who showed that improved bond strengths were not correlated with the formation of the hybrid layer and long, numerous resin tags [26]. Other authors have reached similar conclusions studying the correlation between the hybrid layer thickness, resin tag length and the bond strength of conventional adhesive system [27].

It is interesting to note that the idea of photochemical corneal collagen crosslinking emerged two decades ago in a dental office, when an ophthalmologist (Dr. Theo Seiler) observed his dentist using a curing light over a restoration material [28]. Seiler decided to investigate the possibility of using photosensitizers on corneal collagen and successfully described a photochemical crosslinking technique using a non-toxic vitamin (riboflavin). Now may be the time for dentists to observe what was learned over two decades of corneal crosslinking treatment and bring the idea back to the dental office, optimizing the R+UVA method to crosslink dentinal collagen.

In conclusion, dentinal collagen crosslinking using a combination of riboflavin 0.1% and UVA

(30 mW/cm² for 3 minutes) improves bond strength on caries-affected dentin when using a total-etch adhesive system. Removal of the smear layer using phosphoric acid is important and caries-affected dentin may not benefit from the R+UVA method if followed by a self-etching adhesive. This method has potential to translate into a clinically relevant step in the treatment of carious tooth, increasing bond strength and prolonging the durability of the restorations. Future studies should optimize the parameters and confirm the safety and efficacy of R+UVA dentinal collagen cross-linking method in clinical trials.

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Disclosure of conflict of interest

None.

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