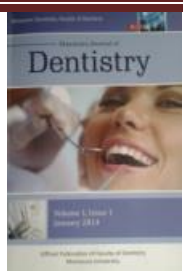

Impact of Two Different Application Times of A Natural Collagen Crosslinker on The Bonding Quality to Caries Affected Dentin

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Abstract:

Objectives: The current study was conducted to evaluate a natural collagen crosslinker as a dentin pretreatment step for improving the bond strength to caries affected dentin (CAD) at two different application timing.

Materials and Methods: Fifteen molars had their coronal dentin exposed followed by artificial caries induction. Following, dentin was etched for 10 seconds and treated with either 6.5% grape seed extract (GSE) or phosphate buffered solution (PBS) for either 10 minutes or 1 hour. Samples were bonded and restored with Single Bond Universal Adhesive (SB) and Z250 XT nanohybrid composite resin. The samples were sectioned into resin dentin sticks and tested for microtensile bond strength immediately and after thermocycling. Data were statistically analysed using three way-ANOVA followed by t-test ($\alpha = 0.05$).

Results: Higher immediate μTBS were observed for 1 hour GSE when compared to 10 minutes GSE and control groups, but non-significant ($P > 0.05$). After thermocycling, results showed non-significant reduction in μTBS for either times of GSE treatment ($P > 0.05$). In contrast, significant reduction in μTBS for control group was observed ($P \leq 0.05$).

Significance: GSE may be effective in enhancing the bond strength to CAD with increasing the concentration and application time. Both treatment times were effective in maintaining the bond strength after thermocycling.

Introduction

The concept of caries removal and cavity preparation has impressively changed in the last decade. With the advent of effective adhesive systems and the subsequent developments in minimal cavity design, changes in the concepts of surgical removal of diseased tooth now aim at conserving as much of the tooth as possible.1 Partial caries removal procedures are used clinically in an attempt to conserve tooth structure and prevent pulp damage.2 Carious dentin entails two distinctive layers: an outer layer of bacterially infected dentin and an inner layer of affected dentin. The outer layer [caries infected dentin (CID)] was characterized as being highly demineralized, physiologically unremineralizable and showing irreversible denatured collagen fibrils with a virtual disappearance of cross-linkages. The inner layer [caries affected dentin (CAD)] is uninfected, partially demineralized and physiologically remineralizable, hence should be preserved during clinical treatment.3

Within this approach of partial caries removal, the CID is removed, and the partially demineralized CAD is preserved.2 Consequently, in cavity preparation for an adhesive restoration after removal of caries-infected dentin, large areas of the cavity floor are composed of CAD. Therefore, it forms the main bonding substrate to the bonded restorative materials.3 Attaining stable and reliable bonding of composite resin to CAD is a big challenge in restorative dentistry and becomes more problematic when the bonding substrate is not normal dentin.4

CAD produces lower bond strengths than normal dentin does, regardless of the type of adhesive system used (etch-and-rinse system or self-etch system) or its number of clinical application steps.5-7 The reduction in the cohesive strength of CAD and the change in its chemical and morphological characteristics would be main reasons for lowering the bond strength values.8-11 The mineral phase of CAD is less crystalline and has lower mineral content than normal dentin.12 Mineral crystals in CAD are scattered and randomly distributed, with larger apatite crystallites and wider intercrystalline spaces compared with intact dentin.13 There is also a reduction in collagen cross-linking, but this change is reversible.14

For further stabilization and strengthening of dentin collagen fibrils, which play a crucial role in the formation and quality of the hybrid layer, composed of collagen fibrils from demineralized dentin matrix embedded with adhesive resin, the induction of exogenous collagen crosslinks has been suggested as a mechanism to enhance the mechanical stability and diminish the biodegradation rates of collagen. Numerous synthetic chemicals [glutaraldehyde (GD), carbodiimide, etc.] and natural agents [genipin, proanthocyanidins (PA), etc.] have been demonstrated to be good crosslinkers for collagen. The low cytotoxicity of natural crosslinking agents compared with synthetic ones has made them more feasible for clinical use.15

So, this study was conducted to evaluate a natural collagen crosslinker in an attempt to improve the quality of CAD and subsequently increase its bond strength to adhesive resin materials.

MATERIALS AND METHODS

The materials used in this study are presented in Table 1.

Table 1. Materials used in the study

Materials	Chemical form or composition	Batch number	Manufacturer
Grape Seed Extract	95% Proanthocyanidins (PAs) content	W0000264	Mega Natural, Madera, USA
3M™ Scotchbond™ Universal Etchant Gel	37% Phosphoric Acid	41263	3M ESPE, Seefeld, Germany
3M™ Single Bond Universal Adhesive	MDP Phosphate Monomer, Dimethacrylate resins, HEMA, Vitrebond Copolymer, Filler, Ethanol, Water, Initiators, Silane	41266	
Z250 XT, Nanohybrid Composite Resin	BIS-GMA, UDMA, BIS-EMA, PEGDMA, TEGDMA, Zirconia, and Silica Fillers	1470A3	

Methods

1. Specimen preparation

Fifteen intact recently extracted human lower first molars were collected, cleaned from debris and stored in 0.1% thymol for 1 month. After that, they were stored in distilled water at 4 oC until use. The occlusal enamel was sectioned perpendicular to the tooth long axis in a low-speed diamond saw machine (Isomet 4000; Buehler Ltd., Lake Bluff, IL, USA) (Figure 10) to expose the subjacent dentin. Dentin was wet ground flat with 340- and 600-grit silicon carbide paper until a uniform enamel-free dentin surface was obtained. The root of each tooth was removed about 2 mm above the cementum-enamel junction using the same saw machine cut parallel to the occlusal surface. Following, the teeth were randomly divided into three groups (n=5).16

2. Artificial caries induction by pH cycling

Artificial caries was conducted following the procedure described by Marquezan et al.17

3. Grouping of specimens

Three groups were obtained according to dentin pretreatment protocol prior to the bonding procedure. Group I is a control group. Groups II & III are GSE treated groups.

4. Pretreatment procedure

Prior to surface treatment of CAD, acid etching using phosphoric acid (37%) was conducted for 10 seconds within all groups. Dentin pretreatment was performed using GSE 6.5% (w/v) for 1hour and 10 minutes. In control group, dentin pretreatment was performed using phosphate buffered solution (PBS) for the used two time intervals (10 minutes and 1 hour).

5. Restorative procedure

After dentin pretreatment, composite restoration was performed for each specimen as follows; the adhesive system (3M™ Single Bond Universal Adhesive, 3M ESPE) primarily, was used following manufacturer's instructions. Later, a nanohybrid resin composite restorative material (Z250 XT, 3M ESPE) was placed over the bonded surfaces incrementally till reaching 5-6 mm resin composite full thickness to allow for gripping during the tensile testing. Increment thickness was limited to 1.5-2 mm, and curing was accomplished for 20 seconds per increment (Elipar S10; 3M ESPE Co., Seefeld, Germany).18

6. Microtensile bond strength (μTBS) test

After the restoration procedures, all specimens were stored in distilled water at 37 °C for 24 h. Each specimen was sectioned perpendicular to the bonding interface area to obtain beams with a cross section area of approximately 1.0 mm2 using a water-cooled diamond blade in a low-speed saw machine (Isomet 4000; Buehler Ltd., Lake Bluff, IL, USA). The cross-sectional area of the bond interface of each beam was measured using a digital caliper (Mitutoyo Corporation, Tokyo, Japan). Beams at specimen peripheries were discarded. The beams of each group were further divided into 2 groups. Half of the beams were measured immediately and the other Half were thermal cycled in distilled water for 10,000 cycles at 5 and 55ºC in a water bath (Thermocycling, Robota-eg) (Figure 11), with a dwell-time of 15 seconds and a transfer time of 5 seconds before microtensile measurements. The beams were submitted to μTBS test in a universal testing machine (Instron Co., Canton, MA, USA) (Figure 12) with a load cell of 50 kgf at a crosshead speed of 0.5 mm/min until failure. The μTBS was expressed in MPa.16, 19

7. Statistical analysis

The data obtained were tabulated for statistical analysis, which was conducted using statistical, package SAS 9.1.3. Means and standard deviations were calculated and expressed in MPa. Data were statistically analysed using three way-ANOVA followed by t-test ($\alpha = 0.05$).

RESULTS

The results of three-way ANOVA are represented in Table 2. Means and standard deviations of μTBS (immediate and after thermocycling) and the results of t-test are shown in Tables 3-5. A significant effect of dentin pretreatment materials as well as thermocycling was detected ($P \leq 0.05$). However, no significant effect of treatment application time was observed ($P > 0.05$). Three-way ANOVA revealed no interaction between factors ($P > 0.05$). Data analysis for immediate μTBS revealed that GSE 1 hour pretreatment showed the highest μTBS values, which did not differ statistically from both GSE 10 minutes or control groups ($P > 0.05$). All groups showed a decrease in μTBS results after thermocycling when compared to immediate evaluation. The decrease in μTBS after

thermocycling was significant for control group ($P \leq 0.05$), but non-significant for GSE groups ($P > 0.05$).

Table 2. Three-way ANOVA showing the effect of time, dentin pretreatment material, thermocycling and their interactions on microtensile bond strength.

Source of variance	DF	Sum of Squares	Mean Squares	F-value	P val
Treatment	1	411.81	411.81	12.56	0.0(
Time	1	18.01	18.01	0.55	0.4(
Thermocycling	1	892.71	892.71	27.23	0.0(
Treatment*Time	1	9.91	9.91	0.30	0.5(
Treatment*Thermocycling	1	64.97	64.97	1.98	0.1(
Time*Thermocycling	1	3.04	3.04	0.09	0.7(
Treatment *Time* Thermocycling	1	3.16	3.16	0.10	0.7
Error	112	3671.38	32.78		
Total	119	5075.02			

Table 3. Means and standard deviations of immediate microtensile bond strength (MPa) and results of t-test.

Dentin pretreatment	Timing of application				t-value	P-value
	10 minutes		1 hour			
	Mean	SD	Mean	SD		
Control	18.71	6.14	18.90	6.24	0.09	0.9325
GSE (6.5%)	20.04	5.27	22.04	6.57	0.92	0.3674
t-value	0.64		1.34			
P-value	0.5288		0.1916			

Table 4. Means and standard deviations of microtensile bond strength (MPa) after thermocycling and results of t-test.

Dentin pretreatment	Timing of application				t-value	P-value
	10 minutes		1 hour			
	Mean	SD	Mean	SD		
Control	11.78	3.67	11.98	3.87	0.15	0.882(
GSE (6.5%)	16.70	6.24	17.41	6.84	0.30	0.770(
t-value	2.63		2.67			
P-value	0.0137		0.0124			

Table 5. Means, standard deviations and results of t-test of immediate and after thermocycling microtensile bond strength (MPa).

Dentin Pretreatment	Immediate		After Thermocycling		t-value	P-value
	Mean	SD	Mean	SD		
Control (10 min)	18.71	6.14	11.78	3.67	3.75	0.0008
Control (1 h)	18.90	6.24	11.98	3.87	3.64	0.0011
GSE (6.5%) (10 min)	20.04	5.27	16.70	6.24	1.58	0.1249
GSE (6.5%) (1 h)	22.04	6.57	17.41	6.84	1.86	0.0694

DISCUSSION

Type-I collagen is existing in tissues as fibrils that are stabilized by lysyl-oxidase-mediated covalent intermolecular cross-linking.4 Intrinsic collagen cross-links are responsible for the tensile properties of collagen molecules. It has been reported that extrinsic chemical cross-linkers could further stabilize collagen fibrils in several connective tissues by the way of induction of additional formation of inter and intramolecular cross-links of dentin collagen. Selective cross-linking agents have been proven to increase the elastic modulus and the ultimate tensile strength of demineralized dentin, which in turn could improve the stability of collagen organic matrix thus, diminishing the susceptibility to enzymatic degradation.20

Glutaraldehyde (GA) had been well known as a potential collagen crosslinking agent, but its toxicity was the main reason for its limit in clinical application.15 Proanthocyanidins (PAs), broadly exist in fruits, vegetables, seeds, flowers, and widely used as food supplements, are potent antioxidant crosslinking agents with free radical scavenging capacity and high affinity to protein. In recent times, the use of a Grape seed extract (GSE), mainly composed of PA, has been revealed to induce exogenous crosslinks within dentin collagen matrix with subsequent enhancement of the mechanical properties of dentin.18, 21 It is speculated that PA has stronger interaction ability with collagen than GA and improves the mechanical properties of dentin more greatly.15 Besides, Grape seed extract has been reported to be a low-toxicity compound.4

The present study demonstrated that CAD biomodification with GSE did not result in a significant increase in μTBS with both application times. However, it has a significant effect on CAD bonding stability. Results of this study disagreed with a previous study that stated that the bond strength to CAD could be enhanced by the use of GSE crosslinker.4 This difference may be due to a number of factors that include; the difference in the procedure of obtaining CAD, sample size and application procedure.

Grape seed extract (GSE) is composed principally of proanthocyanidin, a naturally existing crosslinking agent, which have the potential to give rise to stable hydrogen bonded structures and create non-biodegradable collagen matrices with subsequent enhancement in the mechanical and

physical properties of dentin. GSE interacts with proteins to induce crosslinks through four different mechanisms: covalent interaction, ionic interaction, hydrogen bonding interaction or hydrophobic interactions. Proline-rich proteins resembling collagen have a tremendously high affinity to proanthocyanidin, yielding strong bonds. This interaction predominantly occurs through hydrogen bonding between the phenolic hydroxyl group of the crosslinking agent and the protein amide carbonyl group of the collagen.4, 18, 21

These interactions between dentin collagen and PA can explain the stabilization of the dentin bonding after thermocycling.22 PA has also been stated to be an effective agent in reducing the matrix metalloproteinases (MMPs) activity.21, 23 The biodegradation of the resin dentin interface is quite multipart and encompasses a cascade of events, beginning with the withdrawal of resins that have infiltrated the dentin matrix, followed by an enzymatic attack on exposed collagen fibrils. Proteases such as MMPs and cysteine cathepsins are supposed to be responsible for enzymatic degradation of the collagen fibrils via hydrolysis. In addition, the exogenous crosslinks of the dentin matrix by PA leads to dehydration of the collagen fibrils. This hydrophobic effect result in less water and fluid sorption, better adhesive infiltration and subsequently less denuded fibrils within the hybrid layer. Overall, the outcome of these factors is a hybrid layer that is less prone to the MMPs' activity with subsequent enhanced bond stability.21

CONCLUSIONS

GSE may be effective in enhancing the bond strength to CAD with increasing the concentration and application time. Both treatment times were effective in maintaining the bond strength after thermocycling.

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