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EFFECT OF COLLAGEN CROSS-LINKERS ON MICRO TENSILE BOND STRENGTH OF TOTAL-ETCH ADHESIVE TO DENTIN

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ABSTRACT

Purpose: To evaluate the efficiency of collagen cross-linkers on micro-tensile bond strength of total-etch adhesive with and without thermal cycling.

Materials and methods: The occlusal surfaces of twenty-four caries-free permanent human molars were ground flat to obtain a uniform surface free of enamel exposing the mid thickness of dentine. The prepared speciemens were randomly divided into four equal test groups according to the type of dentin pretreatment (n=6). Group I; control group: the exposed dentin did not receive additional treatment after acid etching. Group II; the etched dentin was treated with Gluma Desensitizer(GA). Group III; the specimens surface was painted with 5% glutaraldhyde solution (GD). Group IV; the specimen surface was painted with 15% proanthocyanidin (PA). Single Bond Universal adhesive was applied to the dentin surface of all the test groups and the dentin specimens were restored with resin composite. The specimens were sectioned to obtain beams of 0.9±0.1 mm in thickness and 5.5±1 mm in length and half of the resultant beams of each group were subjected to thermo cycling. Tensile load was applied until bonding failure of the specimen occurred and micro tensile bond strength was calculated in Mega Pascal. Comparison between all test groups was done using One Way ANOVA followed with Tukey's post hoc test when the ANOVA test was significant. p value was ≤0.5. Comparison between the thermocycled and none thermocycled groups was done using unpaired student t test.

Results: The highest statistically significant microtensile bond strength values were found in the Gluma and GD surface pretreated groups, with no statistical significant difference between them. The lower micro tensile bond strength values were found in the control and PA groups with no significant difference between them, while thermocycling did not decrease the microtensile strength values of the PA group.

Conclusions: 1-Gluma and glutaraldehyde used as cross-linkers increased the microtensile bond strength of dental adhesive to dentin and maintain collagen stability with thermocycling. 2-The PA cross-linkers did not improve microtensile bond strength but maintain bond stability with thermocycling increasing the longevity of the adhesive bond.

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INTRODUCTION

The achievement of a successful and stable bond between the resin composite restorations and dentin structure is and will remain a challenge for every operator in the restorative dentistry [1,2]. Dentin structure is the most abundant mineralized tissue in the human tooth. It is a complex mineralized tissue arranged in an intricate three-dimensional frame, composed of 70% mineral (by weight), 20% organic component and 10% fluid. Type I collagen contribute with 90% of the dentin organic matrix and it is a main molecule to provide tissues with tensile strength, form and cohesiveness. These physical characteristics are probably due to the presence of extensive, intermolecular, covalent cross-links in between the fibrils [3, 4].

The current adhesive systems available in the market produce a bond between the resin composites and the dentin structure through a micro- mechanical mechanism based on the formation of a hybrid layer [5,6]. Stability of this hybrid layer, a collagen-resin interface, represents the most vulnerable portion of the bonded interfaces where stress tends to concentrate and most failures happens [7,8].

Usually, failure of this bond is due to degradation that happens at the dentin-adhesive interface over time. The continuous research is on going to improve the mechanical properties and stability of this interface. [9]The tensile properties of the collagen are due to the cross-links between the collagen molecules. Using extrinsic cross-linking agents might induce additional formation of inter and intra-molecular cross-links enhancing the ultimate tensile strength and stiffness [10,11]Glutaraldehyde (GD), is a synthetic cross-linking agent, is commonly used as a fixative agent. It could potentially increase stability and biodegradation resistance. However, there have been few studies to actually evaluate the effect of cross linked collagen on its biodegradation resistance. [12]Proanthocyanidine (PA) is a natural product extracted from the grape seeds has been shown to safely and effectively cross-link collagen in both in vitro and in vivo models [13] It has also been shown to promote bone formation in the mandibular condyles of rats, [14]increase the stiffness of demineralized dentin by improving its mechanical properties, [15] and inhibit the progression of artificial root caries.[16,17]Desensitizing agents incorporated in the bonding systems has been an interesting topic in research as it reduce the postoperative sensitivity and also enhance the bond strength associated with light activated resin composite restorations.The application of Gluma desensitizer after etching of dentin has been shown to improve the efficacy of dentin bonding system and have been reported in a few other studies[18,19,20].

Research Aims

1- Investigate the ability of dentin treatment using gluma, glutaraldehyde and proanthocyanidin to cross link dentin collagen and improve microtensile bond strength of total-etch adhesive.

2- Evaluate the stability of adhesive interface following treatment of etched dentin with collagen cross-linkers through the effect of thermal cycling on dentin bond strength.

Research hypothesis

Collagen cross-linkers may enhance microtensile bond strength and promote durability of total-etch adhesive to dentin.

MATERIALS AND METHODS

This study investigated the effect of Gluma® Desensitizer, 5% w/v Glutaraldhyde (GD) and 15% w/v proanthocyanidin (PA) on the micro-tensile bond strength to dentine.

A solution of 5% glutaraldehyde (GD) was prepared with a 25% aqueous solution of GD, while proanthocyanidin (PA) was prepared from the dietary supplement Proanthocyanidin grape seed

extract by Preventive Nutrition®. The grape seed extract powder was dissolved in aqueous ethanol 99.5% to obtain an extract of concentration 15%. Finally, the solution was filtered through paper filter n°6 and ready to be used. The pH of the prepared solutions was tested and adjusted 7.2 using NaOH.

Specimen selection and preparation

Twenty-four caries-free permanent human molars were collected and used in the study. The teeth were examined with magnifying lens and any debris was cleaned off using a periodontal scalar. The teeth were stored in distilled water after extraction. The occlusal surface was ground flat using a low speed diamond saw with water coolant to obtain a uniform surface free of enamel exposing the mid thickness of dentine. All the exposed dentin surfaces were polished for one minute using silicon carbide paper (600 grit Si C paper). The prepared teeth were randomly divided into four equal test groups according to the type of dentin pretreatment (n=6).

The exposed dentin of all the specimens was acid etched with phosphoric acid 35% (Scothchbond Universal Etchant), rinsed with water, air dried with oil-free three-way syringe. Group I; control group: the etched dentin did not receive additional treatment. Group II; Gluma group: the etched dentin was treated with minimal amount of Gluma Desensitizer according to manufacturer's instructions for 60 secs and then gently air dried till the dentin surface no longer appears shiny. Group III; the specimens surface was painted with 5% GD solution for 60 secs, then rinsed off for three minutes with distilled water and air dried. Finally, group IV; the specimen surface was painted with 15% PA for 60 secs, and then rinsed off with distilled water and air dried. The adhesive (Single Bond Universal) was applied to the dentin surface of all the test groups according to manufacturer instructions and light cured for 20 secs. All the treated dentin specimens were restored with resin composite Z-250 (3M-ESPE Dental Products) as a 4mm resin composite blocks built up incrementally (2mm) where each increment was light cured for 20 secs.

Materials used in the study are presented in table 1

Each bonded specimen was serially sectioned (using a 0.3-mm thick diamond coated disc (Buehler, IL, USA), at 2050 rpm; 8.8 mm/min feeding rate under copious coolant. Serial sectioning was done in bucco-lingual direction then rotated 90°clockwise and sectioned in mesio-distal. A final horizontal cut at level of cemento-enamel junction was done to obtain beams perpendicular to the bonded interface.Resultant beams were 0.9±0.1 mm in thickness and 5.5±1 mm in length. A digital caliper (Mitutoyo, Tokyo, Japan) was used to check the thickness and length of all beams. Each beam was stored in distilled water at room temperature in a tight-seal plastic cone labeled according to subgroup and tooth of origin. Half of the produced beams from each group were thermocycled in water baths held at 5°c and 55°c with a dwell time of one minute each for 1000 cycles prior to MT testing. Thermocycling was performed using THE-1100 SD Mechatroniksthermocycler (Germany) cold cycle 20sec and hot 20 secs with a resting time of 5 secs in between.

The produced beams from each group were mapped and visually examined then fixed using a cyanoacrylate adhesive to a jig onto the universal testing machine (*Instron,* MA, USA). Tensile load was applied with a load cell of 500 N, at a cross-head speed of 0.5 mm/min, until bonding failure of the specimen occurred. Bond strength was calculated in Mega Pascal (Bluehill Lite software, Instron, MA, USA). Specimen fragments were carefully stored in their corresponding labeled plastic container until examination of failure mode.

Table (1): Materials used in the study

Product name	Specification	Composition	Manufacturer
Scotchbond ™ Universal Etchant	Enamel and dentine etching gel	32% wt phosphoric acid	3M Deutschland GmbH Dental Products Carl-Schurz-Str. 1 41453 Neuss - Germany
Gluma® Desensitizer		2-hydroxyethyl methacryalte, gluteraldhyde, purified water	HeraeusKulzer GmbH GrunerWeg 11 63450 Hanau Germany
5% Glutaraldhyde	Synthetic cross linking agent	An aqueous solution of 25% GD	Analytical laboratory faculty of Pharmacy (Misr university for Science and Technology)
Proanthocyanidin Grape seed Extract	Dietary supplement	Grape seed extract, microcrystalline cellulose, vegetable cellulose	Preventive Nutrition, Nutra manufacturing inc. 1050 Woodruff Rd. Greenville, SC29607. USA
Single Bond Universal	Adhesive resin	MDP phosphate monomer, Dimethacrylate resins, HEMA, Vitrebond™ copolymer, filler, ethanol, water, initiators, silane.	3M Deutschland GmbH Dental Products Carl-Schurz-Str. 1 41453 Neuss - Germany

RESULTS

Mean±SD of microtensile bond strength values in MPa for all tested groups:

Surface pretreatments	Control	Gluma	GD	PA	F value	Pvalue
Without thermocycling	**16.32±4.932 B**	26.03±7.488 A	24.43±7.403 A	17.24± 5.694 B	8.721	P<0.0001
With thermocycling	**12.15±4.718 C**	24.86±9.590 A	22.25±5.557 AB	17.36± 7.096BC	9.672	P<0.0001
P value	0.0248	0.7138(ns)	0.3692 (ns)	0.9588 (ns)		

Different letters indicate statisticallysignificant within the same row, comparison within the same column was done using student t test.

Data was analyzed using Graph Pad Prism 5, comparison between the surface pretreated groups (Eitherthermocycled or not) was done using One Way ANOVA followed with Tukey's post hoc test when the ANOVA test was significant. p value was ≤0.5. Comparison between the thermocycled and non thermocycled groups was done using unpaired student t test

The results showed thatthere was no statistical significant difference between the tested groups in both the non-thermocycled and thermocycled tested groups at F values 8.721, 9.672 respectively and P Value P<0.0001. In the non-thermocycled group the highest statistically significant microtensile bond strength values were found in the Gluma (GA) and GD surface pretreated groups, with no statistical significant difference between them. The lower micro tensile bond strength values were found in the control and PA groups with no significant difference between them.

In all tested groups, thermocycling did not statistically affect the recorded bond strength values, as there was no statically significant difference between the thermocycled and non-thermoclyed Gluma (GA), GD and PA treated groups. On the other hand, the control thermocycled group showed a statically significant lower microtensile bond strength values compared to non-thermocycled group.

DISCUSSION

In our current study we have evaluated the efficiency of different collagen cross-linkers agent as gluma, glutaraldehyde and proanthocyanidins, on microtensile bond strength of total-etch adhesive with and without thermal cycling. The results were in agreement with the research hypothesis. These agents were applied onetched dentin for 60seconds which is considered a clinically relevant time period before applying the bonding agent. The treated dentin specimens with the Gluma and the glutaraldehyde have shown the highest micro tensile bond strength values with no statistically significant difference between the two groups (26.03±7.488 and 24.43±7.403) followed by the PA (17.24± 5.694) then the control group (16.32±4.932) with no statistically significant difference between these two groups. This could be attributed to the fact that glutaraldehyde increases type I collagen covalent bonding by bridging the amino groups of lysine and hydroxylysineremnants of different collagen polypeptide chains by monomeric or oligomeric cross-links, affecting the demineralized dentine properties leading to improved resin bonding to substrates. The aldehyde group of glutaraldehyde cross-links primarily with the e-amino groups of lysine and hydroxylysine residues in dentin collagen resulting in protein fixation which demonstrates that glutaraldehyde may bond to dentin collagen fibrils.This process could possibly stabilize the collagen layer and thus contribute to improved bond strengths, as explained by *Reiter etal in 2000*[19].The microtensilebond strength values of GA group was slightly higher than the GD group although not statistically significant which could be attributed to the presence of HEMA that being hydrophilic is an excellent adhesion-promoting monomer. By enhancing wetting of dentin, it significantly improves bond strengths [20]. On the other hand, PA-based agents have been shown to interact with proline-rich proteins by covalent interaction, ionic interaction, and hydrogen and hydro- phobic bonding interactions as explained by previous studies [10,22]. It was estimated that PA has stronger interaction ability with collagen than GD and improves the mechanical properties of dentin more greatly [19,21].

Increasing concentrations of PA have been shown to decrease the degradation rate of the dentin matrix after bacterial collagenase treatment, indicating an inverse relationship between concentration of PA and collagen stabilization. As PA induced collagen inter-microfibrilar cross-links at high concentration, modifying the tensile strength properties of the dentin matrix. Along this line, dentin treatment with PA has been reported to limit the matrix degradation by specific bacterial proteases through induction of collagen inter-microfibrilar cross-links by removing and replacing GAG (glucose amino glycans) by PA[10,24,25].Although PA used in our study did not increase microtensile dentin bond strength as compared to the control group which may be attributed to its lower concentration as compared to previous studies that used higher concentration (90%PA mixed with 5.0 wt%)[34]it remarkably prevented bond strength from being decreased by the effect of thermocycling increasing the longevity of the adhesive bond and confirming its ability to stabilize bond strength by increasing collagen resistance to biodegradation.

The use of these natural derivatives are affected by many factors such as solventsused for preparation, the source of these natural products, the extracted process of the products, pH, time of application and

temperature may also affect the structure of PA and its overall cross-linking potential [13,18]. Therefore, it is important to analyze the effect of the PA-based pre-conditioners dissolved in different polar solvents on the properties of dentin matrix, and further optimize the conditions for potential clinical applications, having a direct effect on the tensile bond strength values.

The results of our study revealed that the PA group prepared with ethanol solvents did not decrease microtensile bond strength values compared to the control group moreoverthe tensile strength values were maintained after thermocycling which confirm the fact that PA prepared in ethanol solvents cross-linked dentin collagen, a proper degree of cross-linking is helpful for the maintenance of dentin collagen matrix, preventing bond degradation therefore increasing dentin bond durability. This was in disagreement with *Ruirui et al*[24] who found that the PA-based pre-conditioners prepared in ethanol solvents as prepared by previous studies using the PA in the adhesive systems [34] showed lower values of bond strength inducing less cross-linking effect in the dentin collagen matrix.

Testing the dentin conditioned surfaces durability by thermal cycling by simulating the introduction of hot and cold extremes in the oral cavity, shows the relationship of the linear coefficient of thermal expansion between dentin and the resin composites. Thermal cycling stresses the bond between resin and the tooth and depends on the adhesive system which may affect the bond strength values, this is in accordance to previous studies [23,24,26]. The tensile bond strength values were decreased after thermocycling with significant values in the control group. This could be explained by water sorption of the resin composite after thermocycling. This procedure causes hygroscopic expansion as well as chemical degradation of the materials. The tested specimens of resin composites that were thermocycled absorb more water than those that were not thermocycled. [27,28]. The water might also infiltrate and negatively affect the mechanical properties of the polymer matrix, by swelling and reducing the frictional forces between the polymer chains, a process known as 'plasticization'. Hydrolysis might result from thedegradation of resin or tooth structure collagen. It has been reported that the hydrolytic degradation of the bond can influence the bonding efficacy (bond strength and marginal seal) of the bond on the long run [29,30]

Which is not the case for the dentine specimens treated with the cross-linkers used in our study (GA), (GD) and (PA) and this might be explained by the more stabilization of the adhesive bond to the collagen, minimizing the degradation without jeopardizing the adhesive polymerization in accordance with the previous studies [31,32,33]

CONCLUSIONS

Within the limitations of this in vitro study that simulated clinical situations, it was concluded that:

1. Gluma and glutaraldehyde used as collagen cross-linkersincreased the microtensile bond strength of dental adhesive to dentin and maintain collagen stability with thermocycling.
2. The PA collagen cross-linkersdid not improve microtensilebond strength but maintain bond stability with thermocycling increasing the longevity of the adhesive bond.

RECOMMENDATIONS

1. PA collagen cross-linkers being a safe compound can be used on etched dentin prior to bonding procedure as a potential approach to increase the longevity of the adhesive bond.
2. Further studies should be performed using different concentrations of grape seed extract to reveal their impact on the physical properties of the adhesive/dentin interface on the long term.

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