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THE EFFECT OF DENTIN PRE-TREATMENT WITH PROANTHOCYANIDIN ON SHEAR BOND STRENGTH OF DIFFERENT RESTORATIVE MATERIALS IN PRIMARY TEETH (IN VITRO STUDY)

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ABSTRACT

BACKGROUND: Bond stability relies on the mechanical characteristics of dentin collagen fibers. Grape seed extract (GSE), which is rich in proanthocyanidin (PA), has the ability to enhance dentin stiffness by stimulating collagen crosslinking.
OBJECTIVES: The study was conducted to measure the effect of 6.5% PA on the dentin shear bond strength of resin-modified glass ionomer cement (RMGIC) and composite resin and to evaluate its effect on the dentin bonding interface by scanning electron microscopy (SEM).
MATERIALS AND METHODS: Twenty-four primary teeth were collected, randomly and equally allocated into 2 groups according to the pre-treatment of dentin: Group I (study): Dentin was pretreated with 6.5% PA before applying the restorative materials, and group II (control): Dentin was not pretreated with 6.5% PA. The teeth were divided equally and restored with either RMGIC or composite resin. Each sample was subjected to universal testing machine to measure shear bond strength. Then the debonded area was then examined under a stereomicroscope to assess the mode of failure. Additionally, sixteen teeth were collected for assessment of the bonding interface by scanning electron microscopy.
RESULTS: Regarding the shear bond strength, there was no statistically significant difference detected in the application of PA compared to the control group using both restorative materials, while there was a statistically significant difference found between the different tested restorative materials. Regarding the mode of failure assessed in the fractured specimens, mixed and adhesive failure patterns were predominantly observed in all groups, with no significant difference observed between them (P_{MC}=0.72).
CONCLUSION: PA did not compromise the dentin shear bond strength of composite resin and RMGIC.
KEYWORDS: Proanthocyanidin, collagen, dentin, cross-linking, primary teeth.

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INTRODUCTION

One of the most frequent chronic conditions in children is dental caries. Caries affects nearly 50% of preschool children in various countries (1). It is a disease induced by an ecological alteration in the structure and behaviour of bacterial biofilms when exposed to fermentable carbohydrates over time (2). One of the main treatment goals for children is to restore their carious deciduous teeth (3).

Glass ionomer cement systems (GICs) have emerged as essential restorative materials in pediatric dentistry (4). They were first presented at the beginning of the 1970s and have been applicable for almost 5 decades now (5). It has been recommended to be used as a convenient restorative material in primary teeth (6). The material showed many advantageous features, such as the cariostatic effect of fluoride discharge, bonding to the tooth structure, which allows preservation of the sound tooth structure, and biocompatibility (7). The drawbacks are moisture susceptibility or lack of hydration during the initial setting, retarded rate of hardening, poor fracture toughness and low wear resistance (8).

Resin-modified glass ionomer cements (RMGICs) were generated for mechanical characteristic enhancement and contain 4.5-6% resin. These materials have more working time, a superior resistance to wear, and improved fracture toughness. Glass ionomer setting occurs by acid-base reaction. By contrast, when RMGICs are exposed to visible light, the resin polymerizes first, followed by further setting through an acid-base reaction (7).

Adhesive resin composite systems proved their importance in aesthetic dentistry, and most of the missing dental tissues in the aesthetic region could be restored. Composite restorations depend on adhesive systems to restore lost structures of teeth. However, clinicians have found it difficult to accomplish durable bonds to the underlying tooth structure, as the average survival time of composites was assessed from 5 to 7 years (9).

Adhesive systems have been markedly enhanced. However, the weakest area of adhesive restorations is the bonding interface, which includes the combination of organic dentin matrix and residual hydroxyapatite crystals in addition to bacterial products, internal proteolytic enzymes and oral fluids. Moreover, a layer of denuded collagen fibers forms at the bonding interface after acid etching of dentin and is at high risk of degradation by matrix-bound proteases, such as matrix metalloproteinases (MMPs) (10,11).

Bond stability relies on the mechanical characteristics of dentin collagen fibers. So, any attempts to preserve collagen constituents and improve collagen features can have a direct impact on the longevity of bonds (12). The organic dentin matrix contains approximately 90% collagen fibril type I and approximately 10% noncollagenous proteins including proteoglycans as well as phosphoproteins (13). It is expected that mechanical stability will be increased, and the rate of collagen biodegradation will decrease by the stimulation of cross-linking of exogenous collagen (14).

It has been documented that artificial agents like glutaraldehyde and natural agents, for instance genipin as well as proanthocyanidin stimulate cross-linking of exogenous collagen (15). Glutaraldehyde can potentially stabilize collagen, but its disadvantages, such as toxicity, restrict its use (16). Proanthocyanidins (PAs) have been shown to be beneficial in a variety of clinical applications and are also used as nutritional supplements (17). Owing to hydrogen and covalent bonds, proanthocyanidin, a natural cross-linker of collagen, has the ability to stimulate proline-rich protein precipitation, such as collagen (15). The main sources of proanthocyanidin are grape seeds, vegetables, cocoa, various fruits, flowers, and nuts. The mechanical characteristics of demineralized dentin have been shown to be improved by proanthocyanidins (18). Moreover, PA was revealed to enhance collagen production, stimulate collagen conversion from the soluble to insoluble form, significantly increase dentin resistance to collagenase digestion, and act as a MMP inhibitor which improves the dentin collagen stability and increases the bond durability (10,19,20).

In a research conducted by Han B et al., the use of proanthocyanidin derived from grape seed extract to repair biological tissues revealed that a 6.5% PA solution increased the strength of demineralized dentin and its modulus of elasticity owing to the increased collagen cross-links (21). Therefore, this present study was designed to evaluate the effect of PA derived from GSE at a concentration of 6.5% on the shear bond strength of RMGICs and composite resin restorations to dentin of primary teeth. The null hypothesis of this present study is that PA will have no effect on the shear bond strength to the dentin of primary teeth of the different tested restorative materials.

MATERIALS AND METHODS

The current study was an in vitro experimental study designed to investigate the effect of PA derived from GSE at a concentration of 6.5% on the shear bond strength of RMGICs and composite resin to dentin of primary teeth. The study was approved by the Scientific Research Ethical Committee at the Faculty of Dentistry (IRB 00010556-IORG 0008839), Alexandria University, Egypt. Informed consent was obtained from the parent/legal guardian of the participant prior to the collection of the teeth. The study was performed in the Department of Pediatric Dentistry and Dental Public Health, Department of Oral Biology in Faculty of Dentistry, Department of Dental Materials in Faculty of Dentistry and Faculty of Science, Alexandria University. Egypt.

Sample size was determined using the assumptions that alpha error= 5% and study power= 80%. Atabek and Özden (22) reported the medians and ranges of shear bond strength, which were used to calculate the mean and standard deviation (SD) for RMGIC with and without 6.5% proanthocyanidin (PA) (23). According to Srinivasulu et al, (24) the mean ± SD shear bond strength of the composite when 6.5% PA derived from GSE was applied and when it was not applied= 27.57 ± 0.92 and 17.84 ± 0.56. Based on the comparison of pairs of means using the largest SD to ensure statistical power, the sample size was determined to be 8 for each group, and this was raised to 12 to compensate for laboratory processing errors. The total sample size required to compare the effect of 6.5% PA on RMGIC and composite resin shear bond strength equals the of groups multiplied by the number per group= 2 X 12= 24 (25,26). After applying load from universal testing machine the mode of failure was qualitatively evaluated using stereomicroscope. Eight teeth were added to each group to qualitatively evaluate the bonding interface using scanning electron microscope.

The total teeth involved in the study were forty anterior primary teeth (24 teeth assigned to shear bond strength test and 16 teeth assigned to SEM assessment) that had the following inclusion criteria (12)

Teeth with intact dentin

Teeth devoid of cracks
Teeth without caries and/or restorations
Teeth devoid of developmental defects.
Teeth were randomly assigned to one of the two groups using a computer-generated random number (Sealed Envelope Ltd.2021.Simple randomization service). Teeth were cleaned with fluoride-free prophylaxis paste using brushes at low speed and preserved in distilled water until utilized.

Teeth were randomly assigned into two groups. Group I: (study group) included (20 teeth) where dentin was pretreated with 6.5% PA. Group II: (control group) included (20 teeth) where dentin was not pretreated with 6.5% PA. Both groups were subdivided into 2 subgroups according to the restorative materials used; subgroup A RMGIC and subgroup B Composite resin.

Preparation of 6.5% proanthocyanidin

Grape seed extract (Indena S.p.A., Milan, Italy) at an amount of 1.3 grams was weighed using an electronic balance (AS 220-R2, RADWAG Wagi Electroniczne. Poland 2015) and then mixed with 20 ml distilled water using a magnetic stirrer (F91T, Falc. Italy 2018). A pH meter (HQ411D, Hach, USA) was used to record the pH 4.3 of the 6.5% PA solution (Department of Dental Materials, Faculty of Dentistry).(22)

Shear bond strength test (SBS)

Each tooth was mounted individually on acrylic blocks with the buccal surface facing upward perpendicular to the long axis of the block. The buccal surfaces were grounded flat to reveal the underlying dentin (22).

For subgroup IA (RMGIC + PA): Dentin surfaces were conditioned with dentin conditioner (Cavity Conditioner, GC, Tokyo, Japan) for 20 seconds, rinsed and dried. Then, 6.5% PA was applied for 5 minutes, rinsed and dried. RMGIC (GC Fuji II LC Capsule, GC, Tokyo, Japan) was adapted to the dentin surfaces and light cured for 20 seconds (Cromalux-E, halogen light, Mega-Physik, Germany) (24,27).

For subgroup IB (composite resin + PA): Etching of the flat surfaces was performed using 37% phosphoric acid (Eco-Etch gel Ivoclar Vivadent, Schaan) for 15 seconds, rinsed with water, and blot dried. The etched dentin surfaces were treated with 6.5% PA for 5 minutes, rinsed with water and blot dried. Then, adhesive bonding (G-premio Bond, GC, Tokyo, Japan) was applied and light cured for 20 seconds followed by composite buildup (G-aenial, GC, Tokyo, Japan) and light curing for 40 seconds (24).

For the control group (subgroup IIA and subgroup IIB), dentin was not pretreated with PA, and the restorative materials were applied equally as mentioned before in accordance with the manufacturer's instructions.

The restorative materials were placed to the flattened buccal surface by using a ready-made cylindrical plastic mold (3 mm in height and 3 mm in diameter) after etching and conditioning of the dentin. The plastic mold was placed perpendicular and centralized over the dentin surface and stabilized by sticky wax. After hardening of the materials, the plastic molds were removed carefully. All specimens were kept in distilled water at 37°C for 1 day in an incubator (BST 50 20, VEB MLW Dental Fabrik. Leipzig, Germany) (28).

Shear bond strength was determined using a Universal Testing Machine (5ST, Tinius Oslen England 2018). Each tooth was placed in a particular attachment. A shear strength was applied at a crosshead speed of 0.5 mm/minute in a parallel direction to the bonded interface. By dividing the load at failure by the adhesive surface area, the bond strength was estimated in Mega Pascals (MPa) (mm2). Failure modes of the debonded surfaces were assessed by a single operator using a stereomicroscope at x18 magnification (OLYMPUS SZ II. Olympus Optical Co. Tokyo, Japan) to determine the location and type of failure and categorized as follows: cohesive (failure occurred within the substrate of the restorative material), adhesive (failure occurred between restorative material and dentin), mixed (adhesive and cohesive failures occurred simultaneously) (22).

Scanning Electron Microscopy (SEM)

An additional sixteen teeth (8 teeth for each group) were selected according to the same criteria previously mentioned and examined for qualitative assessment by scanning electron microscopy (JSM-IT200, Joel Japan) at x1000 magnification (Electron microscope unit at Faculty of Science, Alexandria University). The specimens were randomly and equally assigned to groups and restored as previously done in the shear bond strength test.

The specimens were sectioned buccolingually perpendicular to the bonding dentin interface and preserved in distilled water for 1 day in an incubator. Then, they were etched with 35% phosphoric acid gel for 15 seconds, washed for 15 seconds and dried. The specimens were soaked in 5.25% sodium hypochlorite solution for 20 minutes and rinsed under running water for 5 minutes (29). Then, the specimens were vacuumed and gold sputter coated with a gold-palladium layer before examination under SEM to study the bonding interface (30).

Statistical Analysis

All quantitative variables were verified for normality using descriptive statistics, plots (histograms and boxplots), and normality tests. All variables showed a normal distribution, so means and standard deviations (SD) were estimated, and parametric tests were used. Frequencies and percentages were calculated for all qualitative variables.

One-way ANOVA was used to compare shear bond strength between the research groups, followed by multiple pairwise comparisons with Bonferroni adjusted significance levels. Comparison of failure modes between the study groups was performed using the chi-square test with the Monte Carlo corrected significance level. Significance was inferred at p value < 0.05. Data were analyzed using IBM SPSS for Windows (Version 23.0).

RESULTS

1- Shear bond strength

Shear bond strength (SBS) in the study group represented a mean of 1.02 MPa and 4.06 MPa for RMGIC + PA (study) and composite resin + PA (study), respectively, while SBS in the control group represented a mean of 1.12 MPa, and 3.38 MPa for RMGIC (control) and composite resin (control), respectively. Using one-way ANOVA, there was a statistically significant difference between the different tested restorative materials (P<0.001), as shown in Table 1 and Figure 1.

Post hoc multiple pairwise comparisons are showed in Table 2. There was a statistically significant difference between the composite and RMGIC + PA (P<0.001) and also there was a statistically significant difference between the composite and RMGIC (p<0.001), while no statistically significant difference was detected between the composite and composite + PA (p=0.66).

There was a statistically significant difference between RMGIC and composite + PA (p<0.001), while there was no statistically significant difference between RMGIC and RMGIC + PA (p =1.00).

Comparing composite + PA and RMGIC + PA, there was a statistically significant difference between them (p<0.001)

Regarding the mode of failure using stereomicroscope, the adhesive mode of failure was found in 4 specimens (66.7%) of RMGIC + PA, 4 specimens (66.7%) of composite + PA, 2 specimens (33.3%) of RMGIC, and 4 specimens (66.7%) of composite. A cohesive mode of failure was not detected in the specimens of either group. A mixed mode of failure was found in 2 specimens (33.3%) of RMGIC + PA, 2 specimens (33.3%) of composite + PA, 4 specimens (66.7%) of RMGIC, and 2 specimens (33.3%) of composite. Comparison of failure modes among the groups was performed using the chi-square test using the Monte Carlo corrected significance level, which revealed that no statistically significant difference was found between the study groups. (P_{MC}= 0.72) as shown in table 1, Figure 2.

2- Scanning Electron Microscope

Examination of the dentin bonding interface using SEM at magnification of X1000 revealed the following:

For subgroup IA (RMGIC + PA) (study)

Examination of the specimens by scanning electron microscopy showed funnel-shaped tags of cement extending for a small distance along the exposed dentinal tubules. However, the deeper dentin preconditioned with polyacrylic acid showed obvious dissolution of its tubules. An ill-defined hybrid layer with resin tags was observed between RMGIC and dentin, as shown in Figure 3a.

For subgroup IIA RMGIC (control)

Examination of the specimens by scanning electron microscopy showed that a clear gap was detected at the dentin-RMGIC interface with no resin tags seen within the dentinal tubules. However, a distinct hybrid layer was identified compared to RMGIC + PA, as shown in Figure 3b.

For subgroup IB composite + PA (study)

Examination of the specimens by scanning electron microscopy showed numerous, well-formed uniform condensed resin tags penetrating deep into the dentin with no gaps found between dentin and composite resin. A well-formed distinct hybrid layer was clearly identified, as shown in Figure 4a.

For subgroup IIB composite (control)

Examination of the specimens by scanning electron microscopy showed that the resin tags were fewer in number and shorter than composite + PA with a less defined hybrid layer. Gaps of variable widths were seen along the interface between the dentin and composite resin, as shown in Figure 4b.

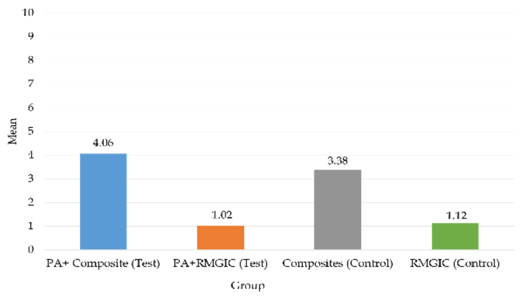

Figure 1: Shear bond strength in the study groups.

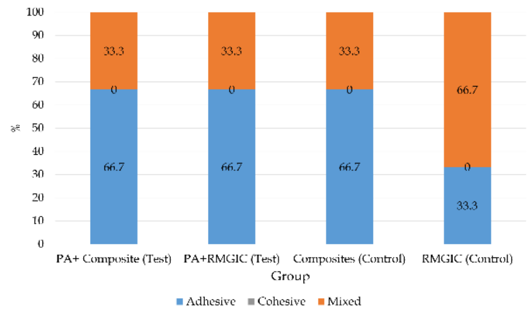

Figure 2: Failure mode in the study groups.

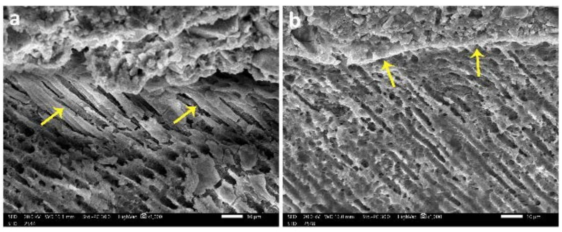

Figure 3: a. Representative SEM images of the dentin bonding interface of RMGIC + PA showing funnel-shaped tags of RMGIC with an ill-defined hybrid layer (x1000). **b.** Representative SEM images of the dentin bonding interface of RMGIC showing a thin definite hybrid layer (x1000).

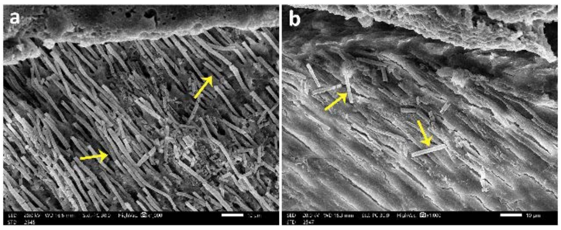

Figure 4: a. Representative SEM images of the dentin bonding interface of composite + PA showing long resin tags penetrating deep into the dentin (x1000). **b.** Representative SEM images showing the dentin bonding interface of the composite showing short and few resin tags seen within the dentinal tubules (x1000).

Table 1: Shear bond strength and mode of failure in the two study groups.

	Group I (study)		Group II (control)		F of ANOVA P value
	RM GIC + PA	Composite + PA	RM GIC	Composite	
	Mean ± SD				
Shear bond strength (MPa)	1.02 ± 0.68[b]	4.06 ± 0.80[a]	1.12 ± 0.64[b]	3.38 ± 0.69[a]	F= 29.36 P <0.001*
Mode of failure					X^2 P value
Adhesive	4 (66.7%)	4 (66.7%)	2 (33.3%)	4 (66.7%)	X^2= 2.06 P_{MC} = 0.72
Cohesive	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Mixed	2 (33.3%)	2 (33.3%)	4 (66.7%)	2 (33.3%)	

*Statistically significant at p value <0.05.
a, b: Different letters denote statistically significant differences between groups using Bonferroni adjusted significance levels, X^2: Chi-square test, P_{MC}**:** Monte Carlo corrected p value.

Table 2: Post hoc multiple pairwise comparisons using Bonferroni adjusted significance levels.

Group	Compared to	P value
Composite (Control)	RMGIC (Control)	<0.001*
	Composite + PA	0.66
	RMGIC + PA	<0.001*
RMGIC (Control)	Composite + PA	<0.001*
	RMGIC + PA	1.00
Composite + PA	RMGIC + PA	<0.001*

*statistically significant using Bonferroni adjusted significance levels

DISCUSSION

Bonding to dentin is considered to be a complex process owing to the partially exposed collagen in the hybrid layer that is prone to hydrolytic and enzymatic damage, which results in collagen degradation at the bonding interface. The dentin bond durability is challenging due to the breakdown of collagen by matrix-bound proteases, such as cysteine cathepsins and matrix metalloproteinases (MMPs), which are exposed in the dentin during acid etching. Bond durability may be enhanced by pretreatment of dentin with MMP inhibitors and collagen crosslinkers (31).

Collagen type-1 integral stiffness is obtained from extracellular collagen crosslinking, which improves the dentin bond strength. Proanthocyanidins interact with proteins through different mechanisms, such as ionic interactions, hydrophobic interactions, hydrogen bonding and covalent interactions, to form collagen cross-links. Cross-links are thus considered to form when a collagen-rich dentin matrix is pretreated with PA derived from GSE, leading to demineralized dentin collagen stabilization (32).

The results determined in the current study revealed that there was no statistically significant difference found between the test group and the corresponding control with regard to the shear bond strength of different tested restorative materials.

The possible explanation of this finding is that there are many challenges that could influence these natural derivatives affecting GSE structure and its cross-linking tendency. Many factors influence these natural products, including the solvents involved in preparing the extract, natural component origin, method of extraction, time of dentin pretreatment, concentration, pH, temperature and various storage times. Furthermore, differences in the selected teeth (in this study, anterior primary teeth were selected) and varied adhesive and restorative materials might have affected the study results (33).

The findings of the present study were in agreement with Balakrishnan et al. in 2021 (34), who demonstrated that 5% PA had no impact on the composite resin shear bond strength. Furthermore, Shafiei et al. in 2019 (27) claimed that applying 6.5% PA for one minute did not raise the shear bond strength of RMGIC to dentin. Moreover, AbuElmagd et al. in 2018 (33) observed that 15% PA did not increase the microtensile dentin bond strength of composite resin in comparison to the control group. This was also in line with the findings of the research done by Paulose et al. in 2017 (29), who found no significant improvement in bond strength with 6.5% PA pretreatment. Feiz et al. (2017) (32) additionally revealed that the prepared 20% and 100% PA solutions did not increase the bond strength between adhesive and dentin.

In contrast, Srinivasulu et al. in 2012 (24) observed that dentin pretreatment with PA for 10 minutes greatly enhanced the composite resin bond strength to deep dentin. Moreover, the results of the present study are in disagreement with the findings found by Macedo et al. in 2009 (35), who revealed that applying PA to etched dentin considerably enhanced the immediate dentin bond strength and stability of dentin collagen of sound dentin and caries-affected dentin. However, in their studies, dentin pretreatment with 6.5% PA was performed for 1 hour. However, such application techniques take prolonged time and have been considered clinically impractical.

Regarding the mode of failure observed in the fractured specimens, mixed and adhesive failure patterns were predominantly observed in the specimens with no significant difference between them (P_{MC}=0.72), while no cohesive mode of failure was detected. These results revealed that PA pretreatment did not disturb the retention of the composite resin RMGIC to dentin.

Shear bond strength represented a mean of 1.02 MPa in RMGIC + PA, 4.06 MPa in composite + PA, 1.12 MPa in RMGIC, and 3.38 MPa in the composite, which coincided with the results of the SEM examination, where composite + PA showed the absence of a gap between composite resin and dentin and a well-formed distinct hybrid layer with the densest, most numerous and uniform deeply penetrating resin tags into dentin, while RMGIC (control) showed no resin tags and a thin hybrid layer with a gap between RMGIC and dentin.

These findings are explained by the fact that the amount of resin constituent in the RMGIC formulation is lower than that in the composite resin, and the polyacrylic acid conditioner is a weak acid that exposes less collagen than phosphoric acid. Moreover, RMGIC has a high molecular weight that makes resin tag penetration into dentin difficult (36).

The limitation of this study is that it was performed in vitro using sound-extracted primary teeth, and it was difficult to simulate all environmental oral factors. Therefore, the effect of PA under the influence of various clinical situations was not investigated. In vivo studies are recommended to evaluate the impact of PA on the bond strength and durability of different restorative materials.

CONCLUSION

In accordance with these study results, it was concluded that 6.5% proanthocyanidin dentin pretreatment did not compromise the dentin shear bond strength of resin-modified glass ionomer cement and composite resin, accepting the null hypothesis.

Further clinical study should be performed for evaluation of PA effect on durability of restoration.

Conflict of Interest

The authors declare that they have no conflict of interest.

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