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THE EFFECT OF TWO NANO PROANTHOCYANIDIN-BASED DENTIN BIOMODIFIERS ON MICRO TENSILE BOND STRENGTH OF DENTIN TREATED WITH TWO ADHESIVE MODES

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ABSTRACT

Aim: The current study was performed to investigate the effect of using two nano PA-based cross-linkers on microtensile bond strength of superficial dentin to total-etch and self-etch adhesive modes using two nano proanthocyanidins cross-linkers in an attempt to improve the bond strength.

Materials and Methods: Sixty sound human molars were used in this study. Teeth were divided into3 main groups (n= 20) according to the cross-linker used. Group 1: Teeth treated with 1%nano pine bark extract gel. Group 2: Teeth treated with 1% nano cocoa seed extract gel. Group 3: Teeth had no treatment (control group). Each group was subdivided into two subgroups according to the adhesive system applied total-etch and self-etch. All specimens were subjected to a micro tensile bond strength test.

Results: The highest micro tensile bond strength values were recorded for group 1 either with total-etch mode (42.3±0.99) or with self-etch mode (34.49±1.05) with a significant difference between groups ($p \leq 0.05$).

Conclusion: Natural dentin bio modifiers enhanced the micro tensile bond strength while the 1% nano pine bark uniquely affected the micro tensile bond strength.

KEYWORDS: Natural dentin bio modifiers, proanthocyanidin, pine bark, cocoa seed

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INTRODUCTION

Adhesion to dentin is a thought-provoking process that may be affected by many factors. These factors include hydrolytic degradation through water sorption, inadequate infiltration of resin monomers, and collagen lysis by endogenous matrix metalloprotease enzymes (MMPs) [1]. The MMPs are enzymes in the organic part of dentin that cleave the unprotected collagen fibrils by hydroxyapatite or resin. MMPs are formed by odontoblasts and kept trapped inside the calcified matrix as an inactive form until caries, erosion, and acid etching for adhesive restoration cause release and activate them [2]. The apatite crystals on collagen fibrils and the non-collagenous proteins could block the access of the catalytic site located on the collagen molecule and keep the MMPs inactive [3].

Many strategies are introduced to inactivate this enzymatic degradation. Applying natural exogenous cross-linkers can strengthen the matrices against the MMPs attack. Proanthocyanidin (PA) is a natural dentin cross-linker offered by fruits, vegetables, nuts, seeds, flowers, leaves, and bark. PAs' sourcing, extraction, and preparation are fundamental for their potentiality [4]. PAs can improve the viscoelasticity of the collagen due to the reformation of protein-solvent complex and by formatting hydrogen bonds, which was the primary interaction between the phenolic hydroxyl group (OH group) of PAs and the amide carbonyl groups of collagens. PA also can enhance collagen synthesis and increase the alteration of soluble collagen to insoluble collagen throughout the development [5].

The adhesive used could highly affect the longevity of the dentine bond, the acid-demineralized collagen fibers' structural integrity, the mechanical properties, and the collagen stability [1]. The mechanical properties of collagen can be improved by increasing intramolecular, intermolecular, and inter-microfibrillar cross-links [7].

Consequently, the study aimed to investigate the effect of using two nano PA-based cross-linkers on the micro tensile bond strength (mTBS) of superficial dentin treated with total-etch and self-etch adhesive modes to improve the bond strength. The null hypothesis was no difference in mTBS between groups treated with nano PA pine bark or coca seed with total-etch or self-etch adhesive modes.

MATERIALS AND METHODS

Materials

a. 1%nano pine bark gel and 1%nano coca seed gel (table 1)

b. Filtek™z250XT composite resin (3M ESPE, USA)

c. Adper Single Bond (3M ESPE, USA)

d. Clearfil ™ SE protect (Kuraray, Japan) mild acidic of pH 2.

e. A 37% phosphoric acid (Denfil, Vericom, Korea)

TABLE (1) Describes the nano PA-based cross-linkers applied in this research.

Materials	Manufacture	Composition	Lot number
1% nano pine bark gel	Hamburg, Germany	Nanoparticles of 95% procyanidin in1%w/v distilled water with 4%w/v poly carboxymethyl cellulose	J403509611
1%nano coca seed gel	Nur. fit, Germany	Nanoparticles of 95% procyanidin in1%w/v distilled water with 4%w/v poly carboxymethyl cellulose	X00146TXN7

Methods:

Study design:

This experimental study collected sixty anonymous molars teeth following extraction for periodontal reasons. Teeth were checked for defects and thoroughly cleaned from calculus and tissue deposits using an ultrasonic scaler (Cavitron, Dentsply, USA) [8, 9]**.** Teeth were randomly divided into three groups Group 1: teeth treated with nano pine bark, Group 2: teeth treated with nano cocoa seed, and Group 3: teeth had no treatment (control group) (n = 20). Each group was subdivided into two subgroups according to the adhesive system applied total-etch and self-etch. All specimens were subjected to a micro tensile bond strength test. This in vitro study was approved by the Research Ethics Committee (REC) of the Faculty of Dentistry, Sinai University, Egypt, on 18-12-2022 with code (REC-OP-22-02).

Sample size calculation:

According to Vase et al. (2021), the Mean ± SD of SBS values (MPa) varied between (14.60± 1.09), in comparison to 16.78 ± 1.34 and 15.76 ± 1.10. It was based on Vase et al. (2021) and Used the G power statistical power Analysis program (version 3.1.9.4) for sample size determination. A total sample size of 60; (subdivided into 20 in each group) n=20 would be sufficient to detect a large effect size (f) =0.42, with an actual power (1-β error) of 0.8 (80%) and a significance level (α error) 0.05 (5%) for the two-sided hypothesis test (10).

Nanoparticles preparation, characterization, and nano-gel formation:

Nanoparticles of pine bark extract and nano cocoa seed extract were obtained using the ball milling technique (planetary-ball-mill-pm-400, JAPAN) [1]. The particle morphology and size of both extracts were examined by Transmission Electron Microscope (TEM) on JEOL JEM-2100 high resolution at an accelerating voltage of 200 kV (model JEM-1230, Jeol, Tokyo, Japan) [8].

Then the nanoparticles of both extracts were dispersed in distilled water at 1%w/v with stirring (hot plate and magnetic stirrer, thermo-scientific, UK) to get 1% Nano agents [9] and then gradually adding poly carboxymethyl cellulose (Loba-Chimie, India) 4%w/v to the solution to get 1% nano gel form of the two agents were prepared by (Nano Gate pharmaceutical laboratory, Cairo, Egypt).

Sample preparation:

Each tooth was vertically implanted into a specially fabricated self-curing acrylic resin block (Acrostone Dental Factor, England) up to the level of the cervical line to hold the tooth in place in a centralized position. They were then using (Isomet Linear Precision Saw, Buehler, USA) to remove the occlusal surfaces of the selected teeth under continuous water coolant at 2.5 mm deep from the cuspal tip and perpendicular to their long axis to expose flat dentin substrate. An indentation of 2.5mm in depth was made in the tooth. The indentation depth was guided using a rubber stopper glued to the shaft after grinding dentin to check that only 2.5mm was removed from the total height of the tooth [11].

Pretreatment of dentin surfaces:

Nano pine bark group:

Both subgroups' specimens were treated with nano pine bark 1%pine bark gel. The 1% pine bark gel was coated with a micro brush onto the dentin surface with rubbing motion for one minute [3], then gently rinsed for 10 seconds with water and dried for 5 seconds.

Nano coca seed group:

Both subgroups' specimens were treated with nano pine bark 1%pine bark gel. The 1% coca seed gel was coated with a micro brush onto the dentin

surface with rubbing motion for one minute (3), then gently rinsed for 10 seconds with water and dried for 5 seconds.

Adhesive system procedures:

Both adhesive system modes were applied according to the manufacturer's instructions.

Total-etch mode:

Ten specimens from each group were conditioned with 37% phosphoric acid (Denfil, Vericom, Korea) for 15 seconds and rinsed for another 15 seconds. Then the following treatments were done on acid-etched dentin surface with corresponding pretreatment gels (groups 1 and 2) except for the control group. Then, two coats of (Adper Single bond) were implemented in two coats for 15 seconds, using a micro brush, and the solvent was vaporized via a 5-second gentle airflow. The specimens were then light-cured for 10 seconds.

Self-etch mode:

Ten specimens from each group were coated with the corresponding pretreatment gels (groups 1 and 2) except for the control group, followed by a two-step self-etch adhesive system (ClearfilTM SE protect) application. Primer was applied with a disposable micro brush attached to the holder and left in place for 30 seconds. Excess solvent was removed by air drying for 5 seconds. Then the bond was applied using a disposable brush, followed by gentle air drying for 5 seconds and light cured for 20 seconds.

Restorative procedure:

Cubical composite blocks (6x6 mm in diameter and 4 mm high) were prepared in the space occupying the center of a specially constructed Teflon mold using a visible light-activated nanohybrid restorative resin composite Filtek™ Z250 XT Universal restorative (3MESPE, ST. Paul, MN, USA). After removing the split Teflon mold, the resin composite blocks were further light cured for 20 seconds on each side. The bonded specimens were left at room temperature for 30 minutes to secure the final polymerization [8].

Storage of samples

The specimens were then stored in distilled water at 37°C for 24 hours for completion of polymerization before immediate testing.

Micro tensile bond strength assessment:

All teeth were sectioned with the IsoMet saw (low-speed Isomet 1000, Buehler, Lake Bluff, IL, USA) to obtain resin-dentin beams of (1mm x 1 mm) in the area; four central beams were selected from each tooth [6]. To measure the dimensions of each stick, a digital caliper was used. Each resin-dentin beam was attached to the testing apparatus with a cyanoacrylate adhesive and loaded until failure under tension using a universal testing machine (DL 200MF, Emic Instron, Brazil) at a cross-head speed of 0.5 mm/min. The micro tensile bond strength mTBS was calculated as the maximum load at failure divided by the cross-sectional area and was expressed in MPa [5].

RESULTS

Nanoparticles characterization result:

The morphology of the nanoparticles was observed using TEM. The micrographs for nano pine bark particles and nano coca seed particles showed that spherical-shaped unagglomerated particles were produced and characterized by smooth surfaces with narrow size distributions having average diameters of 0.12 nm and 90.25 nm, respectively (fig.1).

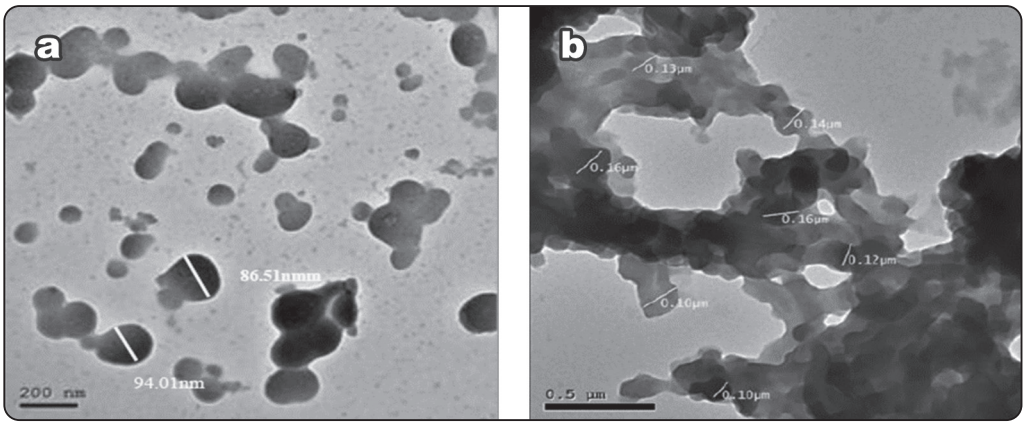

Fig. (1): a; TEM of pine bark, b; TEM of coca seed.

Micro tensile bond strength results:

A-Comparison of all subgroups (table 2)

The highest mean value was recorded in nano pine bark (total-etch) (42.3±0.99), followed by nano pine bark (self-etch) (34.49±1.05), then nano coca seed (total-etch) (22.51±6.77), then nano coca seed (self-etch) (18.14±5.5), then the control group (total-etch) (14.43±1.28), followed by the control group (self-etch) (13.73±1.07). The difference between all groups was statistically significant (p=0.000). Post hoc test revealed no significant difference between both control subgroups (total and self-etch).

B-Comparison between groups using the same adhesive system mode (table 3)

Using the total-etch mode: The highest mean value was recorded in nano pine bark (total-etch) (42.3±0.99), followed by nano coca seed (total-etch) (22.51±6.77), then the control group (total-etch) (14.43±1.28), with a statistically significant difference between each of the groups (p=0.000).

Using the self-etch mode: The highest mean value was recorded in nano pine bark (self-etch) (34.49±1.05), followed by nano coca seed (self-etch) (18.14±5.5), then the control group (self-etch) (13.73±1.07), with a statistically significant difference between each of the groups (p=0.000).

TABLE (2) Comparison of micro tensile bonds in all subgroups (ANOVA test)

Groups			95% Confidence Interval		P value
	Mean	Std. Dev.	Lower Bound	Upper Bound	
Nano Pine Bark Group	38.397	5.52	36.576	40.217	0.00*
Nano Coca seed Group	20.322	3.09	18.675	21.969	
Control Group	14.082	.49	12.354	15.809	

Significance level p≤0.05, *significant

TABLE (3) Comparison of micro tensile bonds in the same etching modes (independent t-test):

Etching			95% Confidence Interval		P value
	Mean	Std. Dev.	Lower Bound	Upper Bound	
Total-etch	25.75	12.24	21.18	30.32	0.00*
Self-etch	21.57	9.41	18.06	25.09	

Significance level p≤0.05, *significant

c-Comparison between etching mode within the same group (table 4)

Nano pine bark group: A significantly higher mean value was recorded in (total-etch) (42.3±0.99), in comparison to self-etch (34.49±1.05), with a mean difference (of 7.81±0.48). This difference was statistically significant (p=0.00).

Nano coca seed group: A significantly higher mean value was recorded in (total-etch) (22.51±6.77), in comparison to (self-etch) (18.14±5.5), with a mean difference (4.37±2.63). This difference was not statistically significant (p=0.11).

Control group: A significantly higher mean value was recorded in (total-etch) (14.43±1.28), in comparison to (self-etch) (13.73±1.07), with a mean difference (of 0.7±0.53). This difference was not statistically significant (p=0.20).

TABLE (4) Comparison between etching modes within the same group (independent t-test):

Groups	Adhesive system mode	Mean	Std. Dev	Difference				t	p
				Mean	Std. Dev	C.I. lower	CI. upper		
Nano Pine Bark Group	Total-etch	42.30	.99	7.81	.48	6.79	8.84	16.18	.000*
	Self-etch	34.49	1.05						
Nano Coca seed group	Total-etch	22.51	6.77	4.37	2.63	-1.11	9.86	1.66	0.11 ns
	Self-etch	18.14	5.50						
Control group	Total-etch	14.43	1.28	.70	.53	-.41	1.80	1.32	0.20 ns
	Self-etch	13.73	1.07						

Significance level p≤0.05, *significant

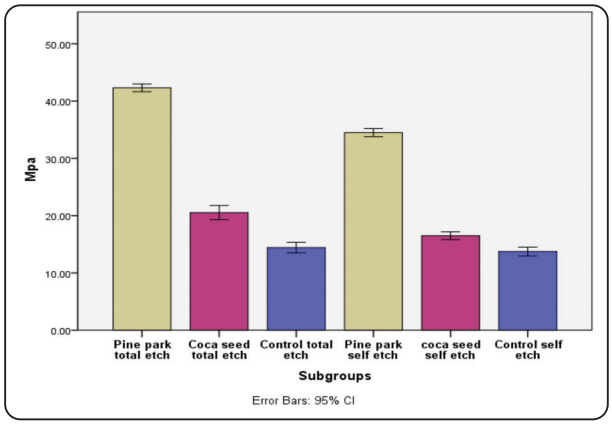

Fig. (2): Bar chart illustrating mean micro tensile bond strength in all subgroups

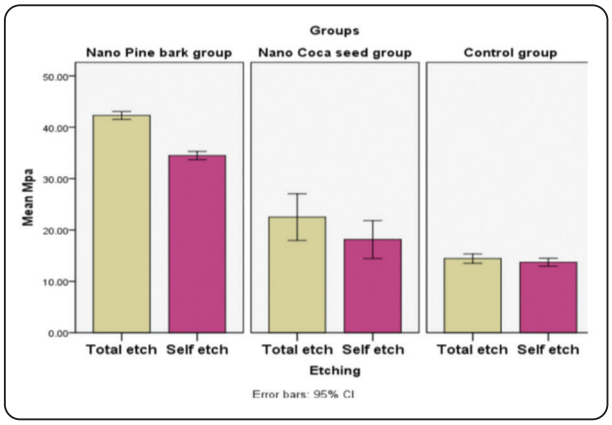

Fig. (3): Bar chart illustrating mean micro tensile bond strength using total-etch and self-etch modes in each group

DISCUSSION

Several strategies are considered to decrease dentine-adhesive interface degradation during the bonding process, aiming to avoid the loss of collagen either by collagenolytic enzyme inhibition or by enhancing the collagen resistance towards degradation through the cross-linking mechanism[2]. Cross-linking of dentin matrix collagen is a natural mechanism occurred in dentin. Hence, researchers aimed to improve this mechanism using chemical substances with cross-linking properties, making the collagen scaffold less susceptible to proteolytic attack [4].

Naturally occurring compounds have attracted interest in their characteristics like low toxicity and sustainable availability [8]. The Proanthocyanidins (PACs) are condensed tannins, highly hydroxylated structures able to form an insoluble complex with carbohydrates and proteins [9]. These interactions between PACs and the dentin matrix explain their clinical usage and make them an up-and-coming class of bioactive agents [5]. The sourcing of PAs is fundamental for their dentin bioactivity [12]; the extract level in the material used is considered the key to the action [3]. The present study used pine bark and coca seed extracts as examples of PA sources. The null hypothesis of this study was rejected because of the statistically significant differences in micro tensile bond strength values between groups.

Primary total-etch bonding systems and self-etch adhesives activate the MMPs via dentin bonding, reducing the bond longevity [11]. So, the surface treatment with a nano PA-based cross-linking agent can hinder the collagenolytic enzymes and could improve the bond strength [13]. In this study, we investigated the micro tensile bond strength of superficial dentin to total-etch and self-etch adhesive modes using two nano PAs extracts to increase the bond strength [10].

The study was conducted on sound dentin substrates as caries-affected dentin is disorganized, wet, and porous with different collagen alteration levels and demineralization, which may affect the tested material's result [13]. Nanoparticles could quickly and smoothly go toward the dentinal tubules. The current study utilized nano-formulated agents to bio-modify the dentin matrix to enhance the material's effect [14].

The clinically rapid biomodification time is an essential part of the possibility of using cross-linker agents. Several studies used considerably long biomodification protocols or added it as an extra step for resin composite restorations [15]. Researchers record the application time ranged from 10 minutes to 1 hour, which is impractical in the restorative procedure [11]. It was found that rinsing the bio modifier after one-minute application could clear the surface from the smear particles and allow diffusion of the monomer into the dentin [4]. Concentrations up to 1 wt% of PA could be added before the dental adhesive resin without interfering with the resins' mechanical properties or solubility [16].

Regarding the results, the nano pine bark-treated group recorded the highest micro tensile bond strength values: dentin treated with total-etch (42.30) or self-etch (34.49). While the nano coca seed group had lower values than the nano pine bark group, either dentin treated with total-etch (22.51) or self-etch (18.14). This result could be attributed to the higher content of PA in the nano pine bark extract. This result agreed with a previous study that compared two sources of PA extract, one from Vitis vinifera and the other from Pinus masssoniana [17]. It was concluded that the higher the PA content recorded, the higher the bond strength could be. This low level of PA could slow down or even decrease their ability to interact with collagen [18] [19]. Another explanation could be related to the structural composition of coca seed extract with High phase liquid chromatography (HPLC). Studies showed that cocoa seed has more monomers and oligomers up to pentamers with short-length molecules and poor infiltration of resin monomers [18]. This may explain the stabilized bond strength despite their exposure to different adhesive modes in the nano pine bark samples [15].

Regarding the results, the control group in both the self-etch and the total-etch adhesive systems showed the lowest micro tensile bond strength values could be due to the enzymatic activity that destroys the exposed collagen fibrils at the base of the hybrid layer. The absence of enzyme inhibitors in the control group could have resulted in low bond strength.

In the total-etch approach, all the smear layers are removed after etching to dentin, and the adhesive monomers are responsible for infiltration in the

porous etched dentine [19], resulting in strong micro tensile bond strength. This could explain the highest values recorded in groups using the total-etch mode, disregarding the dentine pre-treatment agents used. On the other hand, stable bonds can only be achieved if the adhesive infiltrates uniformly the etched dentine in order to prevent the different degrees of incomplete infiltration [20] that, if happens, a trapped demineralized zone at the bottom of the hybrid layer (HL) will occur [21].

In contrast, in the self-etch mode, adhesive stability depends on the effective coupling of the co-monomers with the infiltrated dentine as acidic co-monomers uniformly demineralize and infiltrate dentin [22]. The high percentages of hydrophilic monomers were used for the mild self-etch adhesives to increase the infiltration and the adhesive's permeability. Unfortunately, this could be resulted in Nanoleakage and decrease the quality and stability of the hybrid layer (HL) [23] [24], which explains the lower micro tensile bond strength values with the self-etch mode treated groups.

Although the resin monomers uniformly infiltrate the demineralized dentin and do not create a region of unprotected fibrils [2]; but, small areas of incomplete monomer infiltration could be seen with the use of self-etching adhesives. Also, degradation of the hydrophilic resin monomers could be observed, leading to more collagen liable to hydrolysis [27] [28].

CONCLUSIONS

Regardless the limitation of this study, it was concluded that; natural dentin biomodifiers enhanced the micro tensile bond strength, while 1% nano Pine Bark was the most effective.

List of Abbreviations

PA: proanthocyanidin**; mTBS:** micro tensile bond strength; **TEM:** Transmission electron microscope; **REC:** The Research Ethics Committees; **TEGDMA:** triethylene glycol dimethacrylate; **UDMA:** urethane dimethacrylate **HL**: hybrid layer.

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