

COMPARATIVE EVALUATION OF MICRO TENSILE BOND STRENGTH OF TWO TYPES OF ADHESIVE SYSTEMS AFTER DENTIN PRETREATMENT WITH CHLORHEXIDINE AND PROPOLIS UNDER IN-VIVO SIMULATING CONDITIONS

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ABSTRACT

This study aimed to evaluate the micro-tensile bond strength of the two-steps etch-and-rinse and one-step self-etch adhesive systems to dentin under *in-vivo* simulating circumstances including intra-pulpal pressure (IPP) and immersion in artificial saliva at 37 °C after treatment with chlorhexidine and propolis extract. 48 recently extracted sound human third molars were randomly divided into two main groups (n=24 teeth) according to the tested adhesive system: Group I: Adper™ Single Bond 2 adhesive system SB2 (Two-step etch-and-rinse adhesive system), Group II: OptiBond OB (one-step self-etch adhesive system) All-in-one adhesive system. Three subgroups (n=8 teeth) emerged from each main group based on the cavity disinfectant utilized: subgroup I: 2% CHX, subgroup II: 10% PRO, while subgroup III: no cavity disinfectant was applied (control group). Prior to receiving any treatments, all specimens were attached to the intra-pulpal pressure assembly and set for 24 hours at a pressure of 20 mmHg. Microtensile bond strength and mode of failure was tested. Results showed that SB2 adhesive treated with CHX showed higher statistically significant difference in all subgroups, while OB with no treatment showed lowest bond strength values. It was also concluded that bond durability of self-etch adhesive systems is worse than etch and rinse adhesives under simulated IPP regardless of the pre-treatment protocol pre-treatment of dentin with CHX or Propolis cavity disinfectants prior to the bonding have a positive impact on the micro-tensile of the resin-dentin bond that went through aging with the etch-and-rinse adhesive system.

KEYWORDS: Two-steps etch-and-rinse adhesive, one-step self-etch adhesive, chlorhexidine, propolis extract, micro-tensile bond strength.

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INTRODUCTION

Based on adhesion strategy, adhesives that have been used recently can be categorized into etch-and-rinse or self-etch adhesives. Etch-and-rinse adhesives requires complete phosphoric acid etching of the dental hard tissues (dentin and enamel). Monomers that comprise an acidic functional group can concurrently etch and prime dental tissue which eradicated the need for an acid etching step in the self-etching adhesives. Nowadays, clinicians have a choice between these two types of adhesives. Both categories of adhesives have performed effectively in both clinical and lab studies. ⁽¹⁾.

Despite successful immediate bonding, the durability of the adhesive interface may remain questionable due to the presence of a number of physical and chemical factors jeopardizing the interface of the adhesive. Matrix metalloproteinases (MMPs) and cathepsin cysteine proteases, which are found in dentin composition and are thought to be in charge of the hydrolysis of collagen fibers, the suppression of the hybrid layer's composite's attachment to mineralized dentin, the breakdown of the collagen matrix and the hydrolysis of polymerized hydrophilic resin to break down resin components, both affect how long an adhesive bonding to dentin lasts ⁽²⁾. Improvement of the bond durability by using MMPs inhibitors has been suggested by many authors. The most significant MMP inhibitor utilized in various investigations is chlorhexidine (CHX), which prevents critical ions (Ca and Zn) from binding to MMP enzymes. ⁽³⁾.

Secondary caries could result from complications including microleakage, which could allow oral cavity microorganisms to further harm the restorations' interfaces ⁽⁴⁾. A cationic-bisguanide, chlorhexidine (CHX) has bactericidal and bacteriostatic properties against both Gram+ and Gram-species. Long before the significance of CHX in restorative dentistry was understood, its antibacterial activity against *S. mutans* was established ⁽⁵⁾.

Honeybees process and gather propolis, a resinous material found in hives, from plant sources. More than 150 different kinds of compounds are present in it, including steroids, amino acids, phenolic aldehydes, polyphenols, flavonoids, and coumarins ⁽⁶⁾. Antioxidant, antibacterial, and antifungal properties against *Streptococcus mutans* are among the many pharmacologic activities of propolis. Propolis may be useful in preventing secondary caries and the enzymatic breakdown of dentin collagen because of its antimicrobial activity against cariogenic bacteria and antioxidant action that inhibits oxidative stress and lowers the expression and activity of MMPs by inhibiting free radicals ⁽⁷⁾.

Aim of the study

The aim of this study was to evaluate the micro-tensile bond strength of the two-steps etch-and-rinse and one-step self-etch adhesive systems to dentin under *in-vivo* simulating circumstances including intra-pulpal pressure (IPP) and immersion in artificial saliva at 37 °C after treatment with chlorhexidine and propolis extract. The current study's hypothesis was that the aged bond strength of the one-step self-etch adhesive and two-step etch-and-rinse adhesive systems to dentin would not be impacted by the application of propolis and chlorhexidine prior to hybridization.

MATERIALS AND METHODS

Materials tested in the current study

In the current study, we investigated two adhesive systems: the one-step self-etch adhesive system is OptiBond (OB) All-in-one and the two-step etch-and-rinse adhesive system is Adper TM Single Bond 2 (SB2). The Filtek TM Z350 (light-cured nano-hybrid resin composite) was applied using both adhesive techniques.

Preparation of the disinfectant solutions

- **Preparation of 2% chlorhexidine (CHX) solution**

2% CHX (Kahira Pharmaceutical and Chemical Industrial Company, Egypt) was used without dilution.

- **Preparation of 10% ethanolic extract Propolis (PRO) solution**

Propolis powder was obtained from a honey-bee Egyptian supplier (Emtenan, healthy shop, Egypt). Using a magnetic mixer, 25 g of powder was dissolved in 50 mL of 100% w/v dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, USA) for 24 hours at 37 °C. After that, sterile saline solution was used to generate working solutions at 10% concentrations ⁽⁸⁾.

Study design and selection of teeth

Forty-eight sound human third molars were extracted from patients aged 18 to 28 who were scheduled for tooth removal. Ethical approval for the study was obtained from the Nahda University Faculty of Oral and Dental Medicine Medical Research Ethics Committee, Egypt (No: 001-2-25-1), permitting the use of dentin tissue. In accordance with ISO 11405, teeth were kept in 0.5% chloramine-T for a week. After that, they were stored in distilled water at 4°C ⁽⁴⁾.

The teeth were randomly categorized into two primary groups (n=24) based on the adhesive system tested:

- Group I: SB2 {Adper TM Single Bond 2 (etch-and-rinse adhesive system)}.
- Group II: OB {OptiBond All-in-One (self-etch adhesive system)}.

Based on the cavity disinfectant used, groups were further split into three subgroups (n=8):

- Subgroup I: 2% chlorhexidine (CHX).

- Subgroup II: 10% propolis (PRO).

- Subgroup III: No disinfectant (control group).

Specimens preparation:

All teeth roots were severed 2 mm gingival to cemento-enamel junction. Using a #1 spoon excavator (Nordent Manufacturing Inc, Canada), the pulp tissue was carefully removed. The mid-coronal dentin was then revealed by grinding the occlusal enamel. A precision caliper was used to measure the thickness of the residual dentin that was available for bonding. In this investigation, only crown parts with 2 mm of dentin remaining were used. The dentin surfaces were then wet ground for one minute using 600-grit SiC paper to obtain a uniform smear layer ⁽⁹⁾.

To make sure all the debris was eliminated, the prepared dentin specimens were rinsed with an abundant amount of water coolant. A sterile cotton pellet was then used to plot dry them. The prepared crown segment was firmly fastened to a 150 mm diameter by 1 mm thick Teflon plate. In a central hole in the Teflon plate, a butterfly stainless steel needle (gauge 19) from Shanchuan Medical Instruments Co. Ltd., Zibo, China, was placed and securely fastened then both were embedded in chemically cured polyester resin until 1 mm below the cemento-enamel junction (CEJ). To replicate intraoral conditions and maintain tooth hydration before bonding, each specimen was attached to a 24-hour intra-pulpal pressure assembly that was set at 20 mmHg. In order to replicate the decrease in pressure that follows the administration of local anesthetic using a vasoconstrictor, the intra-pulpal pressure was decreased to 0–5 mmHg during the restorative treatments ⁽⁹⁾.

Application of cavity disinfectants:

Each dentin specimen received its assigned disinfectant and adhesive system according to the previously mentioned study design. For Group I,

etching of the dentin followed by thorough rinsing with copious amount of water, then blot drying of dentin was done before dentin treatment with CHX or PRO solutions. On the other hand, for Group II either CHX or PRO solution were applied to unetched dentin surface. Both disinfectant solutions were applied to dentin for 60s with a cotton pellet⁽¹⁰⁾.

Application of the adhesive systems:

• Application of SB2 adhesive system

Using a fully saturated applicator, SB2 adhesive system was applied in two to three successive layers as directed by the manufacturer. The application was then softly air-thinned for five seconds using oil-free air to promote solvent evaporation. Finally, a light curing device (RTA MINI S, Woodpecker, China) running at an intensity of 600 W/cm² was used to light-cure for 10 seconds.

• Application of OB adhesive

OB adhesive was applied following the manufacturer's instructions in two successive layers, with each layer scrubbed for 20 seconds using a brush was initially dried with gentle air, followed by medium air for 5 seconds using oil-free air to aid solvent evaporation. Finally, light-cured for 10 seconds.

Application of resin composite:

Two increments of Filtek Z350 composite were layered onto the bonded specimens into Filtek Z350 blocks subsequent to the application of the adhesive systems. The was applied in, with each layer being light cured for 20 seconds as per the manufacturer's guidelines.

The restored teeth underwent an aging process for six months under simulated intra-pulpal pressure, which was maintained at 20 mmHg. All specimens were stored in artificial saliva at 37°C. The artificial saliva formulation (mmol/L) included KCl (30), NaN₃ (0.3), CaCl₂ (0.7), MgCl₂ (0.6), H₂O (0.2), KH₂PO₄ (4.0), and HEPES buffer (20). Sodium

azide was added to stop bacteria from growing, as indicated by the artificial saliva's ability to stay clear throughout the trial time. ⁽¹¹⁾.

- Microtensile bond strength (μTBS) testing

A 0.5 mm thick low-speed diamond disc (FLEXIFLEX®, Germany) was used for the sectioning procedure. The first step involved vertical slicing of resin specimen blocks into slabs, each of which had a thickness of roughly 1±0.05 mm to create multiple rods from the restored teeth with an approximate cross-sectional surface area of about 1±0.05 mm². The specimens were then rotated 90 degrees, and they were sectioned longitudinally to create many bar-shaped rods, each with a surface area of around (1±0.05) mm². The cross-sectional area was confirmed using a digital precision caliper (Mitutoyo, Japan). Only rods with the same cross-sectional area were used for standardization, and the study only included rods with the same dentin thickness. In total one hundred and twenty rods from six subgroups were opted, 20 from each class. Prior to testing, all specimens were kept in distilled water for a full day.

A cyanoacrylate adhesive (Rocket Heavy, Dental Ventures of America, CA, USA) was used for microtensile bond strength attachment of each rod. A universal Lloyd testing machine (Lloyd Instruments Ltd, an Ametek firm, UK) was then used to apply tensile stress to the specimens until they failed, using a crosshead speed of 0.5 mm/min. was achieved using. A universal Lloyd testing machine (Lloyd Instruments Ltd, an Ametek firm, UK) was then used to apply tensile stress to the specimens until they failed, using a crosshead speed of 0.5 mm/min. The maximum load (N) divided by the bonded surface area (mm²) yielded the microtensile bond strength (MPa).

- Failure mode analysis:

Using a 50x stereomicroscope (Nikon, SMZ-2 Japan), the fragmented portions of each rod (both sides, tooth and composite) were examined in order to identify the mode of failure (adhesive, cohesive,

or mixed). For every subgroup, each mode of failure's frequency was expressed as a percentage. Gold-sputter-coated aluminum stubs containing representative specimens of each failure type were examined using an environmental scanning electron microscope (ESEM) (FEI Quanta 200 ESEM, France) and captured on camera at 100× magnification.

Failure mode was assigned into the following types ⁽¹²⁾:

Type 1: Dentin adhesive failure (AD)

Type 2: The adhesive layer's cohesive failure (CA)

Type 3: Mixed failure, which includes both cohesive failure in the adhesive layer (CA) and adhesive failure at the dentin (AD).

Type 4: Mixed failure, which includes cohesive failure in the resin composite (CC) and cohesive failure in the adhesive layer (CA).

Type 5: Mixed failure, which includes cohesive failure in the resin composite (CC), adhesive failure at the dentin (AD), and cohesive failure in the adhesive layer (CA).

Statistical analysis

For every test, the mean and standard deviation values were determined for every group. Using the Shapiro-Wilk and Kolmogorov-Smirnov tests, the data were examined for normality and revealed a parametric (normal) distribution. In unrelated samples, comparisons between more than two groups were made using a one-way ANOVA and the Tukey post hoc test. Two groups in unrelated samples were compared using the independent sample t-test. To examine the impact of interactions between various variables, a two-way ANOVA was employed. A significant threshold of $P < 0.05$ was established. IBM® SPSS® Statistics Version 20 for Windows was used to conduct the statistical analysis.

RESULTS

- Microtensile bond strength results

A two-way ANOVA was conducted to assess the impact of various factors on micro-tensile bond strength. The type of adhesive system had a statistically significant influence ($p < 0.001$), according to the data. Furthermore, there was a substantial impact from the dentin preparation technique ($p = 0.014$). Nevertheless, there was no statistically significant influence from the interaction between these two variables ($p = 0.975$). When comparing the tested adhesive systems, as presented in Table 1, Group I SB2 adhesive exhibited a significantly higher mean bond strength compared to Group II OB adhesive system across all subgroups (No treatment, CHX, and PRO), with ($p < 0.001$).

While comparing each dentin treatment protocol within each adhesive material, the results showed that with the SB2 adhesive, a statistically significant difference was revealed between (No treatment), (CHX) and (PRO) groups where ($p = 0.012$). There was a statistically significant difference between the groups who received no therapy and those that received CHX and PRO, with p values of 0.010 and 0.020, respectively. At $p = 0.573$, there was no discernible statistical difference between the (PRO) and (CHX) groups. On the other hand, with OB adhesive, no statistically significant difference was shown between (No treatment), (CHX) and (PRO) groups where ($p = 0.224$).

- Mode of failure analysis results

Figure 1. displayed the mode of failure percentage for each group. Failure mode analysis revealed that while Type 1 (adhesive failures at dentin) was the most common failure mode for the control group, SB2 adhesive groups had a higher percentage of mixed failure for groups that had received CHX and PRO pretreatment. However, with OB adhesive systems, the most common failure mode across all groups was type 2 (cohesive failure at the adhesive layer), which was followed by mixed type failures, as seen in Figure 2.

TABLE (1) Micro-tensile bond strength mean, standard deviation (SD) values of different groups

Variables	Microtensile bond strength				p-value
	SB2 Adhesive		OB Adhesive		
	Mean	SD	Mean	SD	
No treatment (control)	19.22 ^{aA}	2.58	9.47 ^{bA}	1.13	<0.001*
Chlorhexidine (CHX)	24.43 ^{aB}	3.53	11.50 ^{bA}	4.20	<0.001*
Propolis (PRO)	23.56 ^{aB}	4.19	10.20 ^{bA}	2.86	<0.001*
p-value	0.012*		0.224ns		

A statistically significant difference is indicated by different capital letters in the same column and distinct lower-case letters in the same row: *, significant ($p < 0.05$); ns; non-significant ($p > 0.05$).

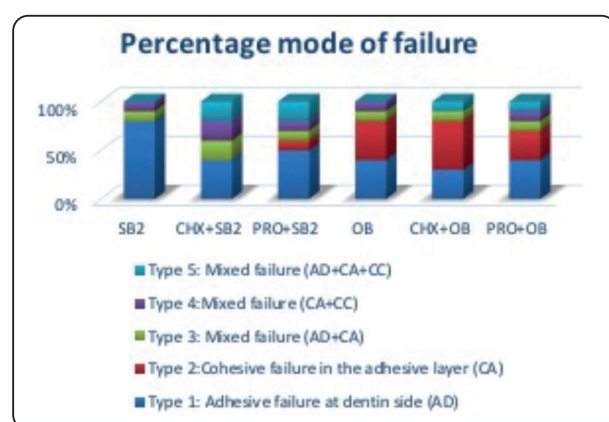


Fig. (1) Percentage mode of failure of different groups

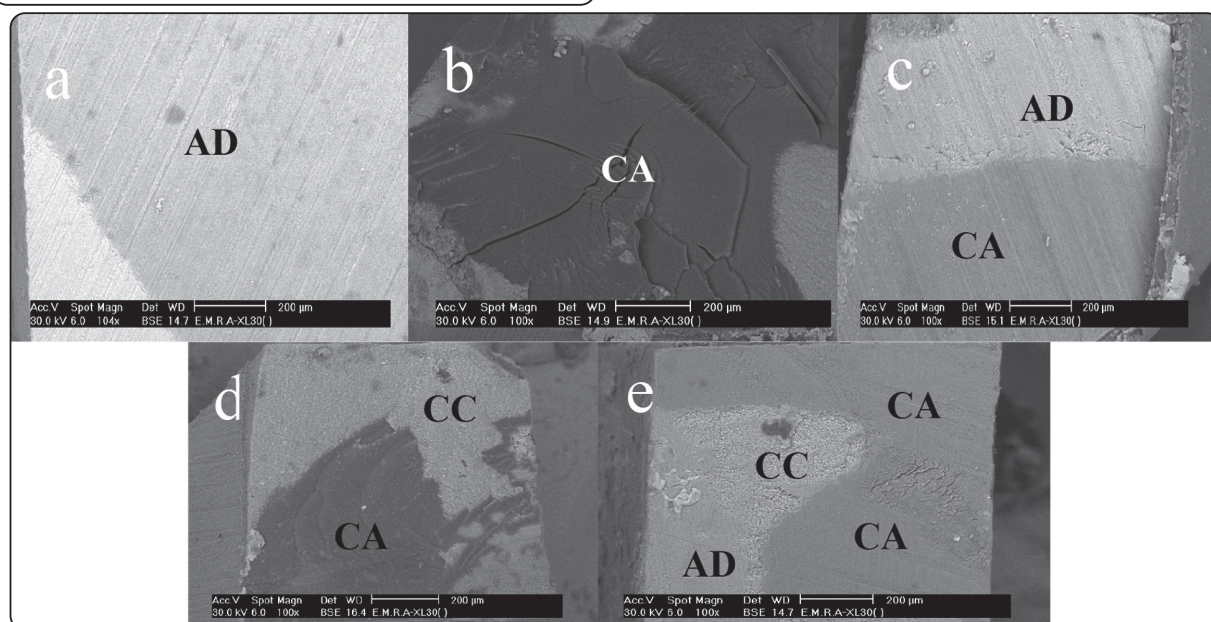


Fig. (2). SEM photomicrograph showing a sample of fractured specimens. Type 1 failures include adhesive failure at the dentin (adhesive failure at dentin side (AD)); Type 2 failures include cohesive failure in the adhesive layer (CA); Type 3 failures are mixed failures (cohesive failure in the adhesive layer (CA) and adhesive failure at the dentin side (AD)); Type 4 failures are mixed failures (cohesive failure in the adhesive layer (CA) and cohesive failure in resin composite (CC)); and Type 5 failures are mixed failures.

DISCUSSION

In this study, oral environmental conditions were simulated to assess bond durability under simulated intra-pulpal pressure (IPP). To minimize discrepancies between *in vivo* and *in vitro* results, the teeth were stored in artificial saliva. Both dentin moisture content and pulpal pressure play a crucial role in dentin bonding procedures. Positive intra-pulpal pressure causes an increase in water outflow to the dentin surface, which results in lower bond strengths. *In vitro* and *in vivo* studies have shown that fluid droplets transude across polymerized adhesives attached to dentin. Dentinal fluid appears to infiltrate polymerized hydrophilic dentin bonding agents when there is a modest positive pulpal pressure ⁽¹¹⁾.

In certain bond strength experiments, palpal pressure is employed to replicate *in vivo* circumstances. Pulpal pressure has been shown in numerous investigations to reduce μ TBS in a variety of bonding systems. Additionally, it was shown that simulated pulpal pressure had varying effects on the resin cements' long-term adhesive ability, with the effects often varying depending on the kind of tested adhesive ⁽¹³⁾.

According to the current study's findings, the two-step etch-and-rinse adhesive system (SB2) had the highest mean micro-tensile bond strength when compared to the OB (one-step self-etch) adhesive system, regardless of the dentin pretreatment.

Bond strength tests are widely used for initial screening and comparison of adhesives. The underlying principle is that a stronger adhesive bond between the restorative material and tooth structure translates to better resistance against stresses arising from resin polymerization and routine oral function ⁽¹⁴⁾. One of the most common ways to measure and test bond strength between dental materials and substrates is through microtensile tests (μ TBS), this manner of testing enables a larger number of sample measurements from one tooth, including a regional study of adhesion ⁽¹⁵⁾.

SB2 is a two-step adhesive technique, which may account for its better binding strength. The smear layer is entirely demineralized and rinsed away using the etch-and-rinse technique, which necessitates using phosphoric acid in a separate stage to demineralize the tooth structure and smear layer. Comprised of loose, porous collagen and organic waste that binds mineral particles, the dentin smear layer has a complicated structure. According to reports, the properties of the smear layer can significantly affect how well self-etch adhesives adhere to dentin ^(16,17).

Moreover, SB 2 contains hydroxyethyl-methacrylate (HEMA), which is the principal hydrophilic monomer in most adhesives, this enhances the adhesive wettability by favoring diffusion through the collagen fibers of the dentin. Additionally, HEMA prevents phase separation occurrences by guaranteeing that hydrophilic and hydrophobic monomers coexist in the same formulation. But it can also cause the rody layer to hydrolyze and sorb water ⁽¹⁸⁾. Because it contains ethanol, it is assumed that the adhesive's low viscosity solution facilitates improved substrate penetration and wetting. Water's role in the infiltration of hydrophobic monomer into wet dentin is also anticipated to be diminished since ethanol has the effect of dislodging water ⁽¹⁹⁾.

Additionally, SB 2 incorporates Vitrebond, a poly alkenoic acid copolymer. This functional methacrylate copolymer, which was initially utilized in the formulation of VitrebondTM Glass Ionomer (3M ESPE), is a blend of poly-acrylic and polyitaconic acids. It has been suggested that Vitrebond copolymer is responsible for a chemical adhesion with hydroxyapatite ⁽²⁰⁾.

On the other hand, the One-step self-etch OB adhesive showed a statistically significantly lower bond strength than SB 2 adhesive system. This might be due to the low acidity of OB adhesive system (pH > 2.5), which is an ultra-mild self-etch adhesive. It might not have been able to etch dentine

that was only superficial. Its hydrophilic qualities might be another factor. One-step self-etching adhesives are known to draw more water and to be more hydrophilic than two-step self-etching adhesives. Due to the difficulty of evaporating from these adhesives, water will rapidly diffuse back into the adhesive resin from the bonded dentine, resulting in a reduced mechanical strength⁽²¹⁾. The presence of the restored specimens under IPP may have increased the amount of water sorption and solubility in the current study, which impacted the endurance of the dentin bond of this one-step self-etch adhesive. The current study's findings indicate that using CHX and PRO before applying adhesive systems had no appreciable negative effects on the in-vitro aged μ TBS, and yet they improved it with the etch-and rinse adhesive system. On the other hand, no positive or negative effect was recorded on the OB (one-step self-etch) adhesive.

The most significant MMP inhibitor utilized in various investigations is chlorhexidine (CHX), which prevents critical ions (Ca and Zn) from binding to MMP enzymes⁽³⁾. It is an aqueous solution that significantly reduces oral bacteria, including streptococcus mutants in oral cavities, by inhibiting MMPs (particularly MP 2, 8, and 9) and cysteine cathepsins⁽²²⁾. In the present study, CHX increased the μ TBS of Single bond 2 adhesive group. By raising the free energy of the enamel and dentin surfaces, CHX's two potent, positive ionic charges improve their binding to the negative charges of the carboxylate groups in the collagenous matrix or the phosphate groups in the mineralized dentin crystallites. CHX enhances the hybrid layer's binding strength and long-term durability in mineralized and demineralized dentin substrates by harnessing the inhibitory actions of MMPs⁽²²⁾.

MMPs are released by all self-etching dental adhesives because they are acidic (pH 1.5–2.7). According to a report, MMPs are activated by adhesives with low pH, which results in adhesive bond breakdown. These MMPs that get activated can slowly hydrolyze unshielded collagen fibrils

of hybrid layers which is thought to be responsible for the evidence of thinning and Collagen fibrils in aged, bonded dentin vanished from partially penetrated hybrid layers, while CHX usage showed suppression of dentin proteolytic activity⁽²³⁾.

Regarding the results of the failure mode, all single bond 2 groups revealed a number of dentin adhesive failures, but this was the most frequent mode of failure for the control group. The rate of mixed failure rose for groups that had previously received CHX and PRO. The bonding system's failure mechanism made it clear that the bond strength had increased, which may have contributed to the rise in the proportion of mixed failure. On the other hand, propolis pretreatment of dentin revealed non-statistically significant different bond strength mean values compared to CHX group. In contrast to the control group, it displayed a more statistically significant difference in bond strength mean values.

Propolis was selected for this investigation because it is a non-toxic substance with antibacterial and antioxidant properties. It is a naturally occurring biocompatible material that is safe to use in oral goods. The phytogeographic circumstances surrounding the hive mostly determine the composition of propolis, although it also fluctuates periodically within the same location. Both flavonoids and iso-flavonoids were present in the propolis ethanol extract. Polyphenols (flavonoids and phenolic acid) can slow down the breakdown of collagen by fortifying the collagen chain and increasing the quantity of crosslinks in collagen fibrils⁽⁷⁾.

Propolis's flavonoids and phenolic acid have been demonstrated to possess antioxidant properties in addition to antibacterial, antiviral, and antifungal properties. One of the main components of propolis' biological action is flavonoids. Four categories can be used to categorize flavonoids' biological effects: their capacity to scavenge free radicals, bind to biological polymers, bind heavy metal ions, and act as a catalyst for electron transport. This may also be

connected to the way that propolis inhibits MMP9 since it contains phenyl ester and caffeic acid ⁽⁶⁾. Propolis may also be able to counteract the effects of metalloproteinases due to its high polyphenol content. ⁽⁷⁾

In the current study propolis solution was prepared using dimethyl sulfoxide (DMSO). DMSO is utilized as a solvent in the dental adhesives due to its ability to infiltrate the biological substrates without producing extracellular matrix collagen dissociation. This infiltration ability is either due to the DMSO capability of transformation of the collagen interfibrillar spacing on a submicron scale or due to competition of DMSO with H₂O molecules at the interpeptide hydrogen bonds within the dentin collagen matrix ⁽²⁴⁾.

Regarding the results of the OB adhesive system, CHX or propolis pretreatment to dentin didn't influence the in-vitro aged resin-dentin microtensile bond strength. The CHX pretreatment has no influence on the bond strength of dental adhesives, several researchers have examined the impact of CHX on the bond strength of dental adhesive solutions on dentin. However, additional research has shown that CHX slows down the pace of resin-dentin bond dissolution by acting as an inhibitor of MMPs. Additionally, it was said that applying 2% CHX before self-etch adhesive solutions was detrimental to bond strength ⁽²⁵⁾.

A prior study assessed the effects of 2% CHX on the μ TBS of an etch-and-rinse and a self-etch adhesive following nine months of aging. The authors found that CHX stopped the etch-and-rinse's μ TBS from decreasing with age, but not the self-etch adhesive's, which may activate MMPs and increase their negative activity, eventually leading to the dentin bond's degradation ⁽²⁶⁾.

It seemed that in self-etch adhesive systems with mild acidity, the discharge of the endogenous collagenolytic MMPs does not happen as with the etch-and-rinse adhesive systems where the discharge of endogenous enzymes is major and

considerable. Thus, factors as water sorption and hydrolytic degradation of the adhesive layer might be the major causative factor for the worsening of the hybrid layer ⁽²⁵⁾. These findings were supported by the mode of failure analysis of OB adhesive groups (Figure 2), which showed increased type 2 mode of failure (cohesive at adhesive layer).

SUMMARY AND CONCLUSION

This study aimed to evaluate the micro-tensile bond strength of the two-steps etch-and-rinse and one-step self-etch adhesive systems to dentin under *in-vivo* simulating circumstances including intra-pulpal pressure (IPP) and immersion in artificial saliva at 37 °C after treatment with chlorohexidine and propolis extract. All specimens were connected to the intra-pulpal pressure assembly adjusted at 20 mmHg pressure for 24h before receiving any treatments. Microtensile bond strength and mode of failure was tested. Results showed that AB 2 adhesive treated with CHX showed a higher statistically significant difference in all subgroups, while OB adhesive with no treatment showed lowest bond strength values. In conclusion, pre-treatment of dentin with CHX or Propolis cavity disinfectants prior to the bonding procedures with the etch-and-rinse adhesive system positively influences the resin-dentin micro-tensile bond that went through aging.

According to the results of this current study, it was also concluded that bond durability of self-etch adhesive systems is worse than etch and rinse adhesives under simulated IPP regardless of the pre-treatment protocol and pre-treatment of dentin with CHX or Propolis cavity disinfectants prior to the bonding procedures positively influences micro-tensile bond strength of the resin-dentin bond that went through aging with SB2 etch-and-rinse adhesive system but both had no effect on the aged self-etch OB adhesive system's bond strength.

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