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EFFECT OF USING TWO DIFFERENT MOUTHWASHES ON MICROTENSILE BOND STRENGTH OF RESIN COMPOSITE BONDED TO DEEP DENTIN. AN IN-VITRO STUDY

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ABSTRACT

Aim: to evaluate the effect of using two different mouthwashes, either chlorohexidine or neem-based, on microtensile bond strength of resin composite bonded to deep dentin.

Materials and Methods: Twenty four intact freshly extracted human mandibular molars were selected for this study. All selected teeth were placed in acrylic blocks, then cross-sectioned horizontally leaving a flat deep dentin surface. Bonding protocol was applied to all teeth following the manufactures' instructions, followed by application of resin composite (Filtek Z250XT, 3M ESPE) to the exposed dentin. All teeth were randomly assigned into three equal groups (n=8) according to the mouthwash used for immersion. Group (1): teeth were immersed in distilled water for 12 hours (Control group), Group (2): immersion was in Chlorhexidine mouthwash for 12 hours and Group (3): immersion was in Neem (Azadirachata indica) mouthwash for 12 hours. Microtensile bond strength was evaluated after the immersion time using a universal testing machine.

Results: One-way ANOVA test revealed no statistically significant difference among the groups tested (p-value = 0.377).

Conclusions: various mouthwashes, used in this study, had no effect on microtensile bond strength of resin composite to deep dentin.

KEYWORDS: Chlorhexidine Mouthwash, Neem Mouthwash, Resin Composite, Microtensile bond strength.

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INTRODUCTION

Recently, people have started adopting a range of products to improve their oral hygiene as a result of growing awareness of oral health. It is well known that dental plaque is the direct cause of dental caries and periodontal problems, which are among the most common oral diseases. For this reason, many researchers and clinicians are now concentrating on finding efficient ways to reduce plaque and, in turn, preserve oral health[(1)].

Mouthwashes that can reduce microbial plaque are one of these methods. Chlorohexidine (CHX) is the most widely used mouthwash that has demonstrated an effective way to reduce dental plaque and pathogenic microorganisms, such as Streptococcus Mutans. In the majority of recent researches, CHX represents the gold standard and is considered as a positive control to investigate the performance of other products. However, staining of teeth and restorative materials, as well as unpleasant sensations like dryness and burning, are some of the drawbacks that cause patients to avoid using it [(2,3)].

The effectiveness of various plant extracts as antimicrobial agents was examined in multiple studies. The herb Azadirachta indica, also known as neem, is a member of the Melicea plant family and contains a component that has antibacterial properties against cariogenic bacteria. The growth of Streptococcus mutans was inhibited by neem extracts due to their antimicrobial, anti-inflammatory and antioxidant properties. Therefore, it's used today to treat periodontitis and to treat patients who are at high caries risk [(4)].

The necessity of understanding how various mouthwashing methods interact with restorative materials is increasing as their use becomes more common. The chemical composition of resin composites has changed multiple times since the advent of resin composite restorative materials and the advancement of etching techniques in order to enhance their mechanical qualities and, as a result, their clinical efficacy [(5)].

The inherent properties of the material and the environment to which it is exposed play a crucial role in influencing the longevity of resin composite restorative materials and their adhesion to tooth structure[(6)]. Chemical compounds included in saliva, food, drinks, and daily used mouthwashes can cause resin composite restorations to deteriorate even in the absence of mechanical stresses or abrasive forces [(7)].

The organic matrix and the bond of resin composite materials may deteriorate as a result of mouthwashes and beverages that contain alcohol and/or have a low pH [(8)]. Consequently, knowledge of these effects is crucial for the clinical longevity and indication of restorative materials [(9)].

Despite being essential for preserving a healthy oral environment, mouthwashes and other oral hygiene products may negatively impact the surface properties and bonding of restorative materials. The majority of mouthwashes include alcohol, and because their pH levels vary, using them excessively and frequently can cause negative effects on resin composite restorations [(10,11)].

The quality of the hybrid layer is correlated with the capacity of adhesive systems to adhere, which is impacted by the morphological and structural variations between superficial and deep dentin (DD). Because there are fewer collagen and intertubular fibrils, bonding to deep dentin is few and limited. Since there are more tubules close to the pulp chamber, the intrinsic wetness and moisture would increase, weakening the bond strength. Additionally, following age, bonding to deep dentin is more prone to deterioration than bonding to surface dentin [(12)].

As controversial findings are included in the literature, there are no clear data on the effect of different mouthwashes on bond strength of resin composites to deep dentin. Thus, this study was held to evaluate the effect of using two different mouthwashes, either chlorohexidine or neem-based, on microtensile bond strength of resin composite bonded to deep dentin

The study's null hypothesis was that CHX-based and neem-based mouthwashes would not differ in their impact on microtensile bond strength of resin composite bonded to deep dentin.

MATERIALS AND METHODS

Sample size calculation

The sample size was determined through power analysis using microtensile bond strength (MPa) as the primary outcome. The effect size f = (1.179174) was based on the findings of Jang et al. 2010 [13]. It was assuming that the standard deviation within each group = 3.85, using alpha level of 5% and beta level of 95% i.e. power = 95%. The minimum required sample size was calculated to be 24 samples in total (eight samples per group). Sample size calculation was done using G*Power version 3.1.9.2.

Sample Selection and Preparation

Twenty four human mandibular molars extracted due to periodontal reasons were selected for this study. The selected teeth fulfilled the inclusion criteria which were; permanent mandibular sound molars and intact enamel surface. While the exclusion criteria included; primary mandibular molars, presence of cracks, developmental defects, carious teeth and presence of restoration [14]. All teeth were radiographically examined to assure matching the inclusion criteria.

The selected teeth were washed under running water and cleaned from any debris and attached soft tissue using a tweezer and a scalpel. Scaling with ultrasonic scaler device was also performed to remove any calculus deposits. Teeth were polished with a rubber cup (Kenda, Liechtenstein) and polishing pumice (grain size 50 μm, Imicryl, Turkey) with a low-speed hand piece then stored in saline that was changed daily to prevent dehydration until the study beginning.

The teeth were allocated into three groups (n=8) based on the immersion media; **Group I:** distilled water **(DW)**; **Group II:** chlorohexidine-based mouthwash **(CM)** (Orovex, Macro Group pharmaceuticals, Egypt); **Group III:** Neem (Azadirachata indica) -based mouthwash **(NM)** (Neem Mouthwash, Theraneem Naturals, USA). The composition and manufacturer of the materials used in this study are illustrated in **Table (1)**.

TABLE (1) The composition and manufacturer of the materials used in this study

Product	Composition	Manufacturer
Adper, single bond 2 Adhesive Agent	Bis-GMA, HEMA, dimethacrylates, polyalkenoic acid copolymer, 10 vol% of 5 nm silica nanofiller, initiators, water, ethanol.	**3M ESPE Dental Product St. Paul, MN,USA**
Filtek Z250 XT Nanohybrid Resin Composite	**Resin monomers:** Bis - GMA, UDMA, Bis-EMA, PEGDMA and TEGDMA. **Fillers:** (82% by weight) Zirconia/silica, non-agglomerated/ non-aggregated 20 nanometer surface-modified silica particles.	**3M ESPE Dental Product St. Paul, MN,USA**
Orovex Mouthwash	Thymol, Menthol, Glycerine, Sodium Saccharine, Sodium Fluoride, Chlorhexidine	**MacroGroup Pharmaceutical, Egypt**
Neem Mouthwash	Deionized Water, Aloe Barbadensis gel, Sorbitol, Glycerine Vegetarian, Poloxamer 407, peppermint leaf, Spearmint leaf, Clove, Illicium verum oil, Azadirachta Indica (Neem) Bark, Thymus Serphyllum leaf oil, Ascorbic acid, Xylitol, Potassium Sorbate	**Theraneem Naturals, USA**

Teeth Embedding in Acrylic Resin Molds

The occlusal surfaces of the selected samples were positioned upwards in self-cured acrylic resin (Acrostone Dental & Medical Supplies, Cairo, Egypt), with 2 mm of the cemento-enamel junction (CEJ) was positioned above the surface of acrylic resin.

Teeth Flattening to Expose Deep Dentin

To standardize deep dentin level, the occlusal surface of each tooth was removed, by using automated diamond saw machine (Isomet 4000, Buehler Ltd., Germany), until removal of enamel and exposure of a flat layer of dentin surface (superficial dentin). A 2 mm from the flat occlusal surface was measured by a graduated periodontal probe and marked all over the circumference of the tooth. Teeth were remounted to the automated diamond saw machine to cut off this 2 mm in order to expose deep dentin [15]. The dentin surfaces were smoothed using a 600 grit silicon carbide paper under wet condition for 10 seconds to standardize the smear layer, then all teeth were stored in normal saline solution at room temperature until the time of use [16].

Bonding Resin Composite to Deep Dentin

For all groups, deep dentin was etched by the application of 35% phosphoric acid (FineEtch 37, Spident, Korea) for 15 seconds according to the manufacture instructions. Deep dentin surface was rinsed with distilled water for 15 seconds. The surfaces were dried with a mini sponge gently keeping the surface moist. The adhesive agent (Adper, single bond 2, 3M ESPE Dental Product St. Paul, MN, USA) was applied. Gentle air blast for 5 seconds was done to evaporate the adhesive solvent followed by 20 seconds of light curing by a LED curing unit (Blue phase N, Ivoclar Vivadent, Schaan, Liechtenstein, Germany). Flattened teeth were matriced with a circumferential metal matrix (Ivory matrix no. 8, AR instrumed, Pakistan) holded by a tofflemier matrix holder (AR instrumed, Pakistan) to standardize a 4 mm thickness of resin composite. This thickness was selected as a prerequisite for microtensile bond strength test [14]. Before applying resin composite, a graduated periodontal probe was used to check the 4 mm height of the matrix above deep dentin surface. Nanohybrid resin composite (Filtek Z250XT, 3M ESPE Dental Product St. Paul, MN, USA) was applied in increments (increment was nearly 2 mm thickness) using a gold platted instrument to fill the 4 mm resin composite thickness, then resin composite was light cured for 20 seconds. Resin composite thickness was confirmed using a graduated periodontal probe. Finally, for all tested teeth, margins of the 4 mm resin composite were finished and polished using Optidiscs (Kerr Corporation, California, USA) using a low-speed hand piece following manufacturers' instructions.

Teeth Immersion

According to their corresponding group, teeth were immersed in their immersion media, either distilled water, chlorohexidine-based mouthwash (CM) (Orovex, Macro Group pharmaceuticals, Egypt) or Neem (Azadirachata indica)-based mouthwash (NM) (Neem Mouthwash, Theraneem Naturals, USA) for 12-hour immersion period corresponding to 1 minute of mouthwash use twice a day for 1 year [17]. For each immersion, teeth were individually inserted in a container containing 15 mL of the respective immersion medium.

After 12-hour immersion period, teeth were stored in DW at 37°C till time of evaluation microtensile bond strength.

Microtensile Bond Strength (μTBS) Testing

Longitudinal sectioning of the teeth was carried out to obtain resin composite-deep dentin beams with a surface area of 0.9 mm x 0.9 mm (±0.1 mm for both dimensions) and a height of 5.5±1 mm. To ensure that the sectioning was perpendicular to the flat occlusal surface of the teeth, a specially designed gripping attachment was used to secure the acrylic blocks with mounted teeth in alignment with the sectioning direction, maintaining a perpendicular angle between the cutting disc and the occlusal surface **[Figure (1)]**.

To standardize the location of beam extraction from all teeth, the central 12 beams (3 buccolingually and 4 mesiodistally) were marked with permanent ink, ensuring that the central beams were distinguishable from the peripheral ones (14) **[Figure (2)]**. A final horizontal sectioning was performed at the level of the cementoenamel junction to separate the beams.

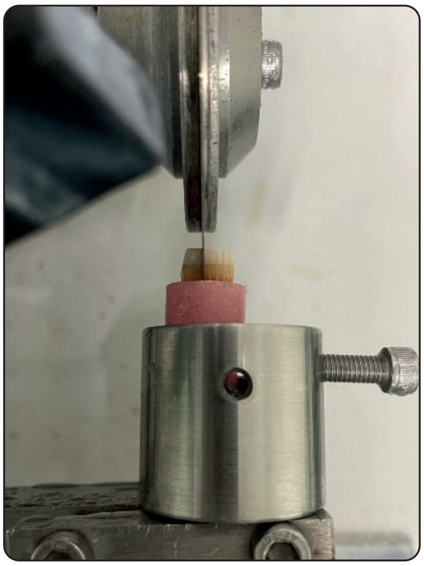

Fig. (1) Sectioning of tooth using automated diamond saw machine (Isomet 4000, Buehler Ltd, Germany).

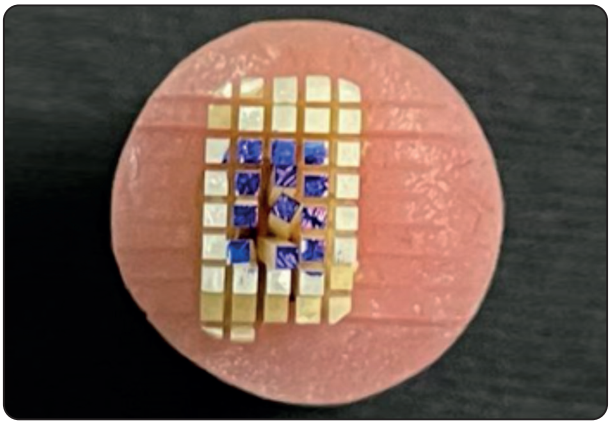

Fig. (2) The central 12 beams marked with a permanent ink.

Each marked beam for each tested tooth was placed on Geraldeli's jig, positioned in the jig's centre groove, and secured at both ends with cyanoacrylate-based glue (Zapit, DVA Inc, USA). Zapit accelerator was used to accelerate the glue's hardening process. After that, the jig was put into a universal testing device with a 500 N load cell (Instron, MA, USA). Until bonding failure, a tensile load was applied at a cross-head speed of 0.5 mm/min. The Bluehill Lite program (Instron, MA, USA) was used to record each beam's microtensile bond strength in MegaPascals (MPa).

Statistical Analysis

All data were collected, tabulated and statistically analyzed. Normality was assessed using the Shapiro-Wilk test to evaluate the data distribution. Since the data followed a normal distribution, they were presented as means, standard deviations (SD), and 95% confidence intervals for the mean values.

A one-way ANOVA was conducted to compare the groups. If statistical significance was found, Tukey's post-hoc test was applied for pairwise comparisons.

A significance level of $P \leq 0.05$ was considered. Statistical analysis was carried out using IBM® SPSS® Statistics Version 20.

RESULTS

Statistical analysis of the assessment between microtensile bond strength among the groups are provided in Table (1) and Figure 1. The one-way ANOVA test revealed no statistically significant difference between the groups (p-value = 0.377). Group 1 (Control group) exhibited the highest mean microtensile bond strength (34.86 MPa), followed by Group 3 (Neem Mouthwash) with a mean value of 33.62 MPa. Group 2 (Chlorhexidine Mouthwash) had the lowest mean value at 31.26 MPa, with no significant differences observed between the groups.

TABLE (2) One-way ANOVA test comparing microtensile bond strength of all tested groups.

	Sum of Squares	df	Mean Square	F-Value	P-Value
Between Groups	40.077	2	20.039	1.211	0.325
Within Groups	248.224	15	16.548		
Total	288.301	17			

Table (3): Means and standard deviations of microtensile bond strength (MPa) of all tested groups.

Immersion Media	Mean	SD	95% CI		P-value
			Lower bound	Upper bound	
DW	34.86	2.25	32.50	37.21	
CHX	31.26	5.49	25.49	37.02	0.377
NM	33.62	4.75	28.63	38.61	

Significant at p-value ≤ 0.05.

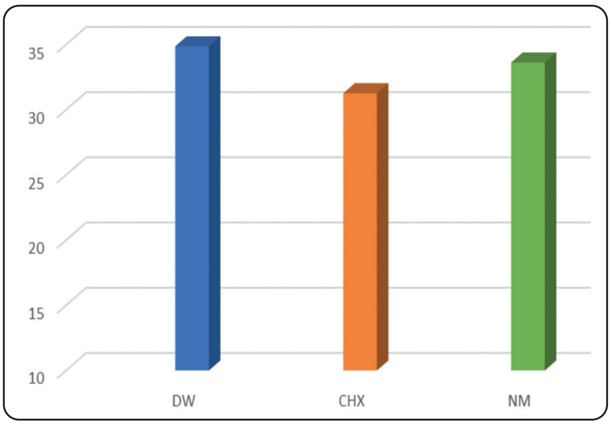

Fig. (3) Bar-chart showing mean microtensile bond strength values for all groups.

DISCUSSION

Establishing a strong, long-lasting bond between the restorative material and the tooth structure is the main objective of adhesive dentistry. The resin-to-dentin adhesion occurs through the formation of a hybrid layer which is the result of the infiltration and polymerization of hydrophilic resins inside the collagen matrix that is exposed by the acid decalcification of dentin [(18)].

Apatite crystals rich in carbonate are scattered throughout collagen fibrils to form the biological structure known as dentin. Dentin depth affects the density of dentinal tubules. Superficial dentin comprises fewer tubules and more intertubular dentin, while deep dentin is formed of massive funnel-shaped dentinal tubules with significantly less intertubular dentin. It has been established that the intertubular dentin has a distinct impact on the creation of hybrid layers. Additionally, the water concentration varies with dentin levels, with superficial dentin having a lower water content than deep dentin [(12)]. According to reports, bonding to deep dentin is challenging because of its high water content, poor intertubular dentin content, and low collagen fibril content [(19)]. Therefore, bonding to deep dentin represents a challenge, thus deep dentin was selected as a substrate to be tested in this study.

Dentin morphological changes during the various bonding processes and the adhesive chemistry are both necessary for a successful bond. For adhesion, etch and rinse bonding techniques have been regarded as the gold standard [(20)]. The permeability of this kind of adhesives, even with careful water removal, may results into decrease of bond strength. This relates to "over-wetting phenomena," wherein an inability to completely remove water from deeply etched dentin may dilute the resin monomer and prevent it from properly infiltrating [21]. Therefore, a two-step etch & rinse bonding agent was used in this study due to their technique sensitivity during application which is considered a real challenge to most of dental clinicians.

Chemical stimulants might have the potential to degrade the adhesive substance and weaken the strength of bonding. Consequently, clinicians can choose materials more effectively if they are aware of how mouthwashes affect the clinical performance of adhesive restorations [10]. Therefore, to expand on existing literature, assessing the impact of current mouthwashes on the bonding efficacy of resin composite to dentin might provide great value.

Because it is the most widely used antibacterial mouthwash in preventive dentistry, chlorohexidine mouthwash (Orovex) was also chosen for this study. Even though chlorohexidine has become more popular as reported in the literature, resin-based restorations may deteriorate if it is used for an extended period of time [22,23].

While neem mouthwash, on the other hand, was chosen to represent a newly introduced natural extract mouthwash, which has been claimed for its high antimicrobial effect with minimal shortcomings that were encountered with other artificially prepared chemical mouthwashes [24]. Thus, this study was performed to evaluate the impact of using Chlorhexidine and Neem Mouthwashes on the microtensile bond strength of resin composite to deep dentin.

The immersion time of our samples in this study was twelve hours. This time of immersion was calculated to simulate the manufacturer instructions of mouthwashes usage - 1 minute of mouthwash use twice a day for 1 year [18].

The results of this study demonstrated no significant difference in the mean microtensile bond strength values across the three immersion media. However, Group 1 (DW) showed the highest mean bond strength (34.86 MPa), followed by Group 3 (NM) at 33.62 MPa, and Group 2 (CHX) with the least mean bond strength (31.26 MPa). Although these results were statistically insignificant but still considered comparable results.

Regarding group (CHX), the effect of CHX on deep dentin bond strength was comparable to that of DW, as it is well known that CHX is a potent, non-specific matrix-metalloproteinase (MMP) inhibitor and has a great effect on the prevention of degradation of resin dentin hybrid layer increasing the longevity and durability of the bonded interface [25]. Such effect was evident in low concentrations.

Such MMP inhibitory effect of CHX may have been augmented by the presence of sodium fluoride (which is present in Orovex mouthwash). This is in agreement with Brackett et al. who found that fluoride diffusion into the underlying hybrid layer may have a MMPs inhibitory effect in deep dentin, therefore preventing the proteolytic activity of such enzymes on the exposed collagenous fibers at the base of the resin-dentin hybrid layer [26].

Such MMPs inhibitory effect is evident in small concentration of CHX, yet the prolonged use of CHX contained materials may participate in increasing its concentration at the adhesion junction [27].

Other studies found that increasing the concentration of CHX in the hybrid layer may lead to deterioration in the mechanical properties of methacrylate-based filled polymers, as a result of hydrolysis and degradation of polymeric chains and influencing the sorption level of the polymer increasing penetration into the matrix leading to its solubility and hydrolysis and attacking the resin-filler interface [28-30]

For Group 3 (Neem Mouthwash), the results showed higher mean value (33.62 MPa). Its antioxidant and antibacterial properties may be attributed be due to the presence of bioactive components such as nimbidin and nimbolide [31,32]. Also it has been reported that in addition to its ability to inhibit Streptococcus mutans, MMPs 2 and 9 were also found to be inhibited by neem, which improves the bond strength and adds to the durability and longevity of the bond [33-35]. However, there is limited data in literature addressing its long term effect on the different available resin-based materials.

Finally, the null hypothesis of this study was accepted as there was no difference between CHX-based and Neem-based mouthwashes regarding to their effect on microtensile bond strength of resin composite bonded to deep dentin.

CONCLUSION

It could be claimed that various mouthwashes used in this study, had no impact on the bonding of resin composite to deep dentin.

Recommendations

More researches are needed to discuss

1. The effect of neem-based mouthwashes on bonded resin-based restorations.
2. The effect of various mouthwashes on different dentin bonding agents.

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