AJDS ALAZHAR Al-Azhar Journal of Dental Science
Vol. 22- No. 1- 31:37- January 2019
Record 1110-2624 | the ISSN Portal
portal.issn.org

THE EFFECT OF DISINFECTANTS PRETREATMENT ON MICROTENSILE BOND STRENGTH OF NANOCOMPOSITE TO DENTIN (AN IN VITRO STUDY)

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ABSTRACT

This study evaluated the effect of three different anti-bacterial cleansing agents on tensile bond strength of composite to dentin. **Materials Methods***;* Flat dentin surface of 12 molars was prepared, the teeth were divided into four main equal groups according to surface pretreatment (three types of disinfectant pretreatment and one control); group chlorhexidine digluconate (CHX), group Cetyl pyridinium chloride (CPC), group Pomegranate peel extracts (PPE) and group Control (no pretreatment). Then, nanocomposite was built up bonded to the treated surfaces with an adhesive system. Then, the teeth were sectioned perpendicular to composite–teeth interface to obtain 120 rectangular sticks, and the tensile bond strength testing after storage in water for one day, one month, and three months. Finally, (random specimens) one specimen from each group of storage time (one day, and three months) were used for scanning electron microscope examination to evaluate the failure mode and the effect of various treatments on the dentin surface topography. **Result;** The highest tensile bond strength (27.139±2.72 MPa) was recorded for specimens treated with (CHX) after 1-day storage time while the lowest tensile bond strength (15.33±2.44 MPa) was recorded for specimens treated with (PPE) after 3 months storage time. **Conclusions**; CHX application before bonding with adhesive preserve bond strength over time. Using CHX, CPC and PPE have no adverse effect on the bond strength. Storage in water has a highly significant adverse effect on the bond strength to all groups especially with treated with PPE and with no surface treatment.

Keyword; disinfectant, microtensile, nanocomposite, dentin

INTRODUCTION

The resin composite was introduced commercially in 1960 by Bown as a direct esthetic restoration for the restoration of anterior teeth[1]. Since then, Resin composites are widely used in dentistry and have become one of the most commonly used esthetic restorative materials because of their adequate strength, excellent esthetics, ability to be bonded to tooth structure [2]. The increasing demand for esthetic dentistry has led to the development of resin composite materials for direct restorations with improved physical and mechanical properties, aesthetics and durability[3]. One of this development in the field has been the introduction of nanocomposite materials by combining nanometric particles and nanoclusters in a conventional resin matrix. Nanofilled materials are believed to offer excellent wear resistance, strength and ultimate esthetics [4,5]. It has been widely stated that resin-dentin bonds obtained with contemporary adhesive systems can deteriorate over time [6]. The loss of bond strength has been attributed mainly to the degradation of the hybrid layer at the dentin-adhesive interface. Clinicians have used disinfection materials to remove surface contaminants during cavity preparation[7]. It has been postulated that disinfection materials may negatively affect the shear bond strength of restorative materials. Therefore, large numbers of restorations may be predicted for early

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failure with the use of a disinfection protocol [8]. Matrix metalloproteinases (MMPs) are a family of host-derived proteolytic enzymes that are capable of degrading the organic matrix of demineralized dentin [9]. These MMPs can reach the exposed collagen fibrils at the base of the hybrid layer originated from the deficient resin infiltration within the demineralized dentin matrix, resulting in hydrolytic degradation and reduction of bond strengths [10, 11]. Dentin collagenolytic and gelatinolytic activities can be suppressed by protease inhibitors, indicating that MMP inhibition could be beneficial in the preservation of hybrid layers. This was demonstrated in a recent in vivo study, in which the application of chlorhexidine, known to have a broad-spectrum MMP-inhibitory effect, significantly improved the integrity of the hybrid layer in a six-month clinical trial [12, 13].

Chlorhexidine may also be a useful complementary method to other techniques of proven efficacy for rehydrating dried mineralized dentin and, therefore, preserving the humidity necessary for keeping the collagen network expanded [14]. The hypothesis of this study was directed to study the effect of different anti-bacterial cleansing agents on shear bond strength to dentin.

MATERIALS AND METHODS

All the materials compositions are listed according to the manufacturer's profile.

TABLE (1) Dentin disinfectant materials:

Cavity disinfectants	Composition & action	Manufacturer
Chlorhexidine digluconate (CHX)	Chlorhexidine gluconate (2%), (1,1-bis hexamethylene 5-chlorphenyl biguanide di-D-gluconate), which is a commercial product as a mouthwash, CHX has a broad spectrum of action against both Gram-positive and Gram-negative microbes.	Pure Chlorhexidine digluconate, Mora-fresh, Pharma Mix, Egypt.
Cetyl pyridinium chloride (CPC)	Cetylpyridinium chloride is a quaternary ammonium compound and is the active chemical in some human mouthwashes in the market. CPC has bacteriostatic and bactericidal effects against Streptococcus mutans.	Pure Cetyl pyridinium chloride, B Fresh, Pharo Pharma, Egypt.
Pomegranate peel extracts (PPE)	PPE (hydroalcoholic extract (HAE)) from Punica granatum (pomegranate fruits) activity on dental plaque microorganisms rich in polyphenols have been demonstrated to exhibit a strong radical scavenging effect, and also antibacterial activity against gram-positive and gram-negative bacteria.	Pure Pomegranate Peel Extract, Aquamint, Roxeen, Egypt

Preparation of the specimens:

A-Fabrications of the molds:

Specially fabricated cylindrical Teflon molds were fabricated to create standardized acrylic blocks in which the root of teeth was embedded. Each mold has 23mm height, 19mm in external diameter and 14mm in internal diameter, then the tooth was individually mounted in polymethyl-methacrylate (PMMA) resin blocks, vertically along their long axis to the level of cementoenamel junction.

B- Preparation of the dentin surface and application of the restorative material:

The flat dentin surface perpendicular to the long axis of the tooth was produced using a special abrasive stone under coolant, just passing the dentin-enamel junction. A graduated periodontal probe was used to confirm the depth of preparation on all occlusal surface of the crown. The 12 molars were randomly divided into four equal main groups according to the three tested disinfectant agents (the first group dentin surface was subjected to Chlorhexidine (CHX) pretreatment, the second group were pretreated with Cetyl pyridinium chloride (CPC) to dentin surface, the third group received Pomegranate peel extracts (PPE) pretreatment) and the fourth group did not receive disinfectant pretreatment (control group). In the three groups that subjected to disinfections pretreatment, the dentine surfaces were disinfected by the application of disinfection with a plastic syringe and left it in contact with the dentine surface for 60 seconds then it was rinsed off. All the prepared teeth were pre-treated by 37% phosphoric acid gel for 15 seconds. Rinsed for five seconds and gently air-dried with a cotton pellet to remove excess water and keep the tooth surface moist. Two consecutive coats of Adper Single Bond 2 were then applied to the etched surfaces, left for 10 seconds and gently thinned with air and light-cured for 20 seconds. Finally, the nanocomposite was built up to approximately 4 mm in height in two increments (each increment about 2mm thickness) and using a Teflon coated instruments. Each increment was light-cured for 40 seconds and an addition cure for 40 seconds was done to the lateral side of specimens. The final specimens of approximately 4mm height were polished with 300-grit SiC paper to achieve uniform thickness throughout the specimens and then stored in distilled water at 37˚C. The teeth with nanocomposite were sectioned perpendicular to composite–teeth interface under copious water using an Isomet diamond saw (Isomet 1000, Buehler Ltd, Lake Bluff, IL, USA) in two planes, 90˚ to each other, to obtain 120 rectangular sticks (1mm wide; 8 mm long). Out of each tooth, ten beams with a cross-sectional area 1mm for microtensile bond strength testing.

Testing procedure:

Tensile bond strength testing was performed on the specimen using a computerized testing machine (Lloyed testing England) at a speed of 0.5mm/min. This was achieved by placing the samples in a specially designed and precisely constructed test jig which consists of two metallic plates. Each plate with dimensions of 10x 10x 2cm, they are screwed together with a screw to permit hinge movement of the two parts and ensure application of a pure tensile force to microbars. Both plates are grooved where the samples are glued to it by cyanoacrylate adhesive. The upper plate has a hole with 1 cm in diameter at which the machine descends its load through it to the lower plate. This will allow the microbar components to be retrieved from each other and in this way, the tensile load applied to the specimen. The test jig was used to secure the cemented samples in place to the universal testing machine during tensile stress application and the force separate the samples were recorded in Newton and then transferred into MPa according to the following equation:

$$\text{Bond} = \frac{\text{Force till debonding in Newtons}}{\text{Surface area of microbar}}$$

Then the data were presented as mean and standard deviation (SD) values.

Scanning electron microscope examination:

Finally, (random specimens) one tooth from each group that used for disinfectant treatment at the end of storage time (one day, and three months) were used for scanning electron microscope examination to evaluate failure mode distribution to study the effect of various treatments on the dentin surface topography.

RESULTS

Data were presented as mean and standard deviation (SD) values. One-way ANOVA was used to compare between the four groups as well as to compare between different storage periods. Least Tukey's post-hoc test was used to determine significant differences between the means when ANOVA test is significant. The significance level was set at $P \leq 0.05$. Statistical analysis was per-

® SPSS, Inc., Chicago, IL, USA.

formed with SPSS 16.0® (Statistical Package for Scientific Studies) for Windows.

Comparison between the four groups: Figure(1) Mean shear, standard deviation (SD) values, results of ANOVA and Tukey's tests for comparison between shear bond strength of the four groups; chlorhexidine digluconate (CHX), cetyl pyridinium chloride (CPC), pomegranate peel extracts (PPE) and control(no pretreatment) revealed that;

After 1 day, (CHX) group showed the statistically significantly highest mean tensile bond strength. There was no statistically significant difference between (CPC), (PPE) and the control group which showed the statistically significantly lowest means tensile bond strength.

After 1 month, (CHX) group showed the statistically significantly highest mean tensile bond strength. There was no statistically significant difference between (CPC), (PPE) and control groups which showed the statistically significantly lowest means tensile bond strength.

After 3 months, (CHX) group showed statistically significantly highest mean tensile bond strength. There was no statistically significant difference between (CPC), (PPE) and control groups which showed the statistically significantly lowest means tensile bond strength.

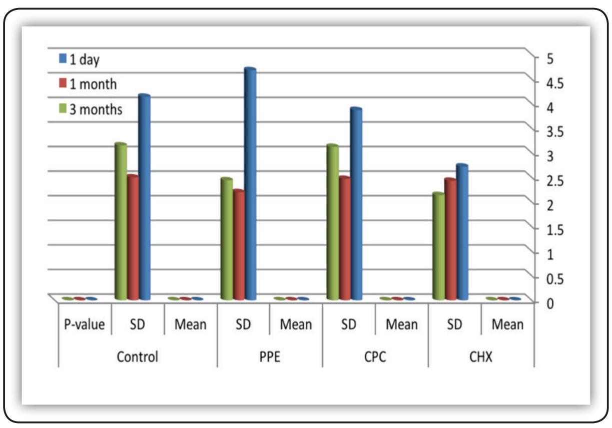

FIG (1) Mean tensile, standard deviation (SD) values: *: Significant at P ≤ 0.05.

Scanning Electron Microscopy SEM observations of failure mode:

The samples that subjected to scanning electron microscopic examination at various magnifications ranging from 500X to 2000X. SEM showed the dentin surface topography produced by different treatment procedures: disinfection with (CHX), (CPC), (PPE), and control (no treatment).

Cohesive failure = cohesive failure of bonding resin or restorative material. Mixed failure = failure in which part of resin was seen remained on dentin surface (failure in composite). Adhesive failure = failure in which no resin was seen on the dentin surface. Scanning electron microscopic observations of the resin-dentin interface revealed that, the mode of failure of CHX treated specimens show mixed failure, the mode of failure of CPC treated specimens show mixed and adhesive failure, the mode of failure of PPE treated specimens show mixed, cohesive and adhesive failure and the mode of failure of control specimens show cohesive and adhesive failure.

DISCUSSION

The durability of bonds between resins and tooth substrates is significant importance for the clinical longevity of adhesive restorations. However, the long-term stability of the resin bonded dentin is still questionable [15, 16]. Chlorhexidine appears to be an interesting substance, since it has been used for disinfecting cavity preparations, acting as an efficient method for inhibiting bacterial growth, while simultaneously preserving the integrity of the resin-dentin interface when used before application of the adhesive system [17]. Analysis of the present data apparently indicates that the use of other antibacterial as CPC and PPE is as effective as CHX due to its beneficial effects on the preservation of dentin bond strength as an MMP inhibitor when applied prior to bonding with no further rinsing [18].

1- Effect of disinfection on tensile bond strength to dentin:

The results of this study revealed that the tensile bond strength of samples treated with chlorhexidine were the highest values. These results may be related to the fact that chlorhexidine partially removed the smear layer and even slightly exposed the underlying dentinal tubules. Chlorhexidine has cationic properties, thereby enabling it to binds to phosphorate groups in apatite, and produces a strong affinity for tooth surfaces[19]. Chlorhexidine (CHX) has been shown to inhibit MMP-2, -8, and -9 activities directly. These are the same MMPs that have been shown to be present in human dentin[20]. They speculated that the CHX-saturated matrix becomes sequestered from interstitial fluids by resin tags that occlude dentinal tubules, by adhesive resin coating collagen fibrils, and by an overlying adhesive layer, which may produce prolonged retention of CHX and inhibition of MMPs [21].

Another explanation, the mechanical stability of resin-dentin bonds may be controlled by the durability of resin-infiltrated collagen fibrils in the matrix. They speculated that chlorhexidine remains bound on collagen, beneath resin that infiltrated interfibrillar spaces. In the CHX-treated cavities, the MMPs would remain inhibited for as long as CHX remained bound to the matrix [22].

Recent studies revealed the contributions of host-derived MMPs to the breakdown of collagen matrices and have suggested that incomplete infiltrated zones within hybridized dentin may be degraded by MMPs in the dentin matrix, in the absence of bacterial enzymes. MMPs are a family of zinc-dependent proteolytic enzymes that are capable of degrading the dentin organic matrix after demineralization. Enzymes with gelatinolytic (MMP-2 and MMP-20) activities are present within the intact dentinal matrix and in carious dentin [23]. Since release and activation of host-derived MMPs may be associated with the degradation of acid-demineralized denuded collagen fibrils, these enzymatic activities may also occur within incomplete resin-infiltrated subsurface regions of hybrid layers created by contemporary adhesives, despite the better surface seal achieved with these adhesives [24]. This is in agreement with Türkün et al, [25] they concluded that a chlorhexidine gluconate-based disinfectant did not adversely affect the tensile bond strengths of dentin after using self-etching dentin bonding systems. Furthermore, Hebling et al, [26] have carried out an in vivo study and observed by means of Transmission Electron Microscopy that the teeth treated with CHX 2% showed hybrid layer with normal structural integrity of the collagen network, while the control group showed progressive disintegration of the fibrillar network.

These results were in disagreement with the results obtained by Soares CJ, [27] they evaluated the influence of different disinfectants applied prior to the adhesive systems and showed that a CHX-based disinfectant determined higher levels of microleakage, suggesting that the use of cavity disinfectants with composite resin restorations appears to be material specific regarding their interactions with various dentin bonding systems. This opinion also supported by Tulunoglu et al,[28] who observed lower bond strength values and higher microleakage associated with CHX. This may be due to self-etch adhesives are applied on a less permeable surface when compared with etch-and-rinse adhesives due to the presence of smear layer, the adhesion can have been disturbed by the flowing of serum through the dentin. It is known that the adhesive layer acts like a micro permeable layer, [29, 30] so the outward movement of dentinal fluid under positive pulpal pressure can permeate the adhesive layer and reach the adhesive surface [31].

2- The effect of storage time on tensile bond strength:

The result of this study showed that, highest the tensile bond strength after 1 day and the lowest after

3 months. This may be related to, the CHX applied on exposed collagen fibrils, then sealed in place with adhesive resin/resin composites, may protect the collagen against a dentinal collagenolytic attack, thereby delaying the typical degradation seen when using contemporary dental adhesives [32]. So significant reductions in the tensile bond strength were seen within the adhesive and hybrid layers after 3 months of water storage in all groups [33].

This is in agreement with the result by Carrilho and others, [34] which revealed a significantly lower percentage of failure mode in the hybrid layer, especially in the bottom section after six months of chlorhexidine treatment, indicating that the higher bond strengths observed in this group reflected the preservation of a hybrid layer collagenous matrix, especially in the bottom zone, where partially exposed collagen fibrils are most prone to initial enzymatic degradation.

Another explanation for drop the tensile bond strength values*,* after 3 months as result from hydrolytic degradation of the resin and collagen fibers in the submicron spaces of the hybrid layer increase with increasing exposure to water[35,36]. In fact, during long-term water storage, the resin absorbs a significant amount of water and consequently swelling of the resin may result in the closure of any space between the bonding resin and dentin surface. Conversely, stresses my simultaneously be induced at the bonding resin-dentin interface, which may pull the collagen fibers into the hybrid layer and resin, leading to tearing along the bonded interface [37].

CONCLUSIONS

According to the circumstances of this investigation concluded that:

1- Chlorhexidine application before bonding with self-etch adhesive preserve bond strength over time.

2- Using antibacterial as Cetyl pyridinium chloride and Pomegranate peel extracts has no effect on the bond strength.

3- Storage in water for long periods has a highly significant adverse effect on the bond strength to all groups especially with surface treated with pomegranate peel extract and with no surface treatment.

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