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The Effect of Zn-doped Adhesive alone or Combined with Calcium Phosphate Nanoparticles on the Integrity of the Bonded Resin-Dentin Interface

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ABSTRACT

Objectives: This study was designed to evaluate the ability of two experimental Zn-doped-adhesive (zinc chloride and zinc oxide nanoparticles) alone or with calcium phosphate nanoparticles to induce therapeutic effect on the bonded resin-dentin interface using two adhesive systems(total etch and self-etch). **Material and Method:** eighty human premolar dentin surfaces were assigned into four main groups (n=20) according to zinc doping (A1), the universal single bond doped with zinc chloride ($ZnCl_2$), (A2) the universal single bond doped with zinc oxide nanoparticles (NZnO), (A3) the universal single bond doped with zinc chloride +calcium phosphate nanoparticles (NACP) (A4) the universal single bond (as a control). Each group was further subdivided into tow subgroups according to the adhesive technique either etch and rinse (B1) or self-etch (B2). Prepared samples were stored in distilled water for 24h, and then submitted to microtensile bond strength testing. Two samples from each group were prepared for observation of nanoleakage by the scanning electron microscope (SEM). Data was collected and statistically analyzed. **Results:** In the total etch adhesive technique, the highest mean value was recorded in group(A4 B1), followed by (A2B1) and (A3B1) groups; with the least mean value for the (A1B1) group. Using the self-etch adhesive technique, the highest mean value was recorded in the (A2B2) and (A4B2) groups followed by (A3B2) group with the least mean value for (A1B2) group. Comparing the adhesive techniques, there was statistically significant difference with higher mean value for the etch and rinse adhesive technique except with NZnO group. **Conclusion:** Addition of zinc to the universal single bond significantly affected the microtensile bond strength except with ZnO nanoparticles group in the self-etch adhesive technique.

KEYWORDS

Zinc doping,
Adhesive technique,
Universal adhesives,
Nanoleakage.

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INTRODUCTION

Composite restorations are able to replace lost tooth tissue in an invisible way; however a good composite filling does not last long without good bonding to the remaining tooth structure [1]. The adhesion of resin-based restorative materials to dentin relies on the creation and stability of a microscopic interfacial structure composed of collagen fibrils reinforced by a resin matrix called the hybrid layer [2].

Two main strategies are employed in creating the hybrid layer. The first strategy involves the use of etch-and-rinse adhesive technique, which requires removal of the smear layer followed by the adhesive application [3]. The second strategy utilizes self-etch adhesive technique, which are based on polymerizable acidic monomers that simultaneously condition/prime and bond dentin [4]. Both bonding strategies may provide sites for collagen hydrolysis by endogenous enzymes such as matrix metalloproteinases (MMPs) which is evidenced by the loss of integrity of hybrid layers [5] and reduction of bond strength [6]. Thus, the activity of MMPs can importantly affect the long-term bonding durability of esthetic restorations.

Different approaches for inhibiting the enzymatic activity of MMPs have been studied in attempts to improve the stability of hybrid layers [7]. The incorporation of zinc into an etch-and- rinse adhesive not only exerted a protective effect on MMPs mediated collagen degradation, but also preserved bond strength, thus representing a promising and novel strategy for stabilizing resin-dentin bonds over time [8,9].

Approaches on the functionalization of adhesives have also been extensively studied to improve the long-term stability of hybrid layer. One approach is to incorporate calcium phosphate (Cap) particles into dental resins to promote remineralization and avoid demineralization. Recently, bonding agents containing nanoparticles of amorphous calcium phosphate (NACP) were developed. These bonding agents could release high levels of Ca and P ions to induce remineralization and combat caries [10].

Therefore, the aim of the present study was to evaluate the effect of Zn-doped Adhesive alone or combined with calcium phosphate nanoparticles on the Integrity of the bonded resin- dentin Interface by microtensile bond strength testing and observation of nanoleakage.

MATERIALS AND METHODS

Eighty sound human premolar teeth extracted for orthodontic reasons were used in this study. The teeth were cleaned and stored in 0.1% thymol solution at 4˚C and used within one month. Teeth were mounted in epoxy resin blocks using cylindrical Teflon mold (I5-mm diameter and 40-mm height), then stored in distilled water at 37˚C to prevent dehydration of the teeth till the next step. The occlusal surfaces of the teeth were cut just below the dentine-enamel junction by 1 mm to obtain flat occlusal dentin surfaces. Dentin surfaces were hand polished with 600 grit silicon carbide abrasive papers under running water to create a uniform surface.

Samples were divided into four main groups (20 each) according to zinc doping (A), the universal single bond doped with zinc chloride ($ZnCl_2$) (Sigma-Aldrich, Germany) (A1), with zinc oxide nanoparticles (NZnO) (Nanotech Egypt for Photo Electronics) (A2), with zinc chloride +calcium phosphate nanoparticles (NACP) (Nanotech Egypt for Photo Electronics) (A3) and the universal single bond (as a control) (A4). Each group was further subdivided into tow subgroups according to the adhesive technique (B) either etch and rinse (B1) or self-etch (B2).

1. Preparation of adhesive solutions:

For mixing the single bond universal adhesive with zinc compounds, the universal single bond was put into tubes wrapped with dark adhesive tape to protect it from exposure to the light. For the control group, the adhesive was used as supplied

by the manufacturer. The amount of each material to be mixed with the bond was calculated according to the following equation: [11]

$$\frac{\text{weight}}{\text{volume}} \% = \frac{\text{weight of solute}}{\text{volume of solution}} \times 100$$

The weight of $ZnCl_2$ (2 wt. %) was 0.05gm.and the weight of NZnO and NACP (10 wt. %) was also calculated and the result was 0.25gm for each. An electronic balance (A&D Company Limited, Japan) was used to measure the amount of each material. Each material was transferred to its specific tube and covered securely to be ready for mixing. This process was done in the developing dark room. Each tube was given a number corresponding to each group.

To achieve complete dissolution of $ZnCl_2$ and dispersion of the nanoparticles, the adhesive mixtures were vigorously shaken for 60 seconds by Vortex Wizard (RX3 Velp Scientifica, Milan, Italy) [8] .

2. Preparation of the teeth for the microtensile bond strength test:

2.1. Application of the adhesive system and composite build up:

For the etch and rinse technique**,** Scotch bond etchant gel was applied to the dentin surface for 15 second then, rinsed with water using a three way syringe for 10 seconds, and excess water was blotted using a piece of gauze until the surface appeared glistening without pooling of water. The previously prepared adhesive solutions were applied to the etched dentin and rubbed using a disposable applicator, gently air thinned for 5 seconds until it was no longer moved, and light cured for 10 seconds using LED light curing unit (Woodpecker light cure unit Led-F (LK-G38), China). Another layer was applied by the same manner and cured.

For the Self-etch technique, the adhesive was applied to the dentin surface and was rubbed for 20 seconds using a disposable applicator, gently air thinned with for 5 seconds until it no longer moved. Then, it was light cured for 10 seconds as mentioned previously. Another layer was applied by the same manner and cured.

A cylindrical composite build-up (4 mm high) was incrementally performed o with a resin composite (Filtek Z350XT) by using specially constructed Teflon mold with internal dimensions of 4 mm height and 4 mm diameter. Each increment of composite (2 mm) was light-activated for 40 s using LED light curing unit. Resin-bonded specimens were then stored in distilled water at 37˚C for 24 h.

2.2. Preparation of the beams for microtensile bond strength testing:

Specially designed L-shaped gripping attachment was used to hold the acrylic blocks with mounted teeth firm in place, parallel to the sectioning direction. Then mounted into the diamond saw machine (Isomet 4000, Buehler Ltd., Lake Bluff, IL, USA). The restored teeth were serially sectioned, using a 0.3-mm thick diamond coated disc under copious water coolant. Serial sectioning was done in bucco-lingual direction then rotated 90° clockwise and sectioned in mesio-distal direction. The blocks with the attached beams were then removed from the attachment. A final horizontal cut at level of cemento-enamel junction was done to obtain beams of (1 mm x 1 mm) thickness. The central beams from each specimen were selected and their thickness was checked using a digital caliper. Beams with different thickness were excluded.

2.3. Microtensile bond strength measurement:

Each beam was stressed by tensile forces to the composite dentin attachment line at a cross head speed of 1mm/minute until failure occur. Data were recorded using computer software (Bluehill Lite software, Instron instruments). Specimens which showed premature debonding during testing were excluded. The load required for debonding of each stick was recorded in MPa. Microtensile bond strength (MPa) values were determined by

computing the ratio of maximum load (MPa) by the adhesion area in mm^2.

3. Ultramorphological analysis and nanoleakage evaluation:

Two sticks from each resin-tooth block were set aside for evaluation of nanoleakage at the tooth/ restoration interface with a scanning electronic microscope (SEM). Two nail varnish layers were used to coat the beams leaving only 1mm from the adhesive interface. After rehydration in distilled water for 10 minutes, the beams were immersed in the tracer solution 50% (wt.%) ammoniacal silver nitrates for 24 hours [12]. The silver-impregnated beams were rinsed with running water for 5 minutes and placed in photo developing solution for 8 hours under a fluorescent light. The beams were rinsed thoroughly for 5 minutes under running water in order to guarantee the removal of any traces of the photo developing solution.

The beams were then demineralized for 30 seconds with 6N HCl, rinsed again, deproteinized with 2.5% NaOCl for 10 minutes, and then serially dehydrated with 25, 50, 75, 95 and 100% ethanol (Radovic et al., 2006). Next, the specimens were mounted on aluminum stubs, sputter- coated with gold and examined under SEM Model Quanta 250 FEG (Field Emission Gun) attached with EDX Unit (FEI Company, Netherlands). Observations were carefully performed on the entire adhesive interface of the stick, at X 1000. Images of the most representative area of each specimen were then taken. Differences in silver deposition and penetration were observed.

4. Statistical analysis

Statistical analysis was performed using SPSS software program (SPSS 19; SPSS, Chicago, IL, USA). The differences between groups were evaluated by one way analysis of variance (ANOVA) test. Tukey's post hoc test was used when ANOVA yielded positive results. Significance of the difference between both adhesives was compared using unpaired t test. The level of significance was set at $P < 0.05$.

RESULTS

Micro-tensile bond strength results:

The zinc addition variable caused a significant difference between groups ($p \leq 0.05$). Moreover, the adhesive technique variable caused a significant difference between subgroups ($p<0.0001$). Furthermore, the interaction of variables resulted in a significant difference in mean micro- tensile bond strength (MPa) (Table 1 and Figure1).

Table (1) *Effect of adhesive technique on mea n micro-tensile bond strength*

Zinc addition	Adhesive technique	Mean	Std. Dev	Std. Error	Min	Max	t value	P value
No zinc (control)	Etch and rinse	34.54	9.04	2.61	24.50	50.70	2.883	0.00389*
	Self- etch	25.12	8.19	2.05	11.8	43.6		
Zinc Chloride	Etch and rinse	17.18	10.12	2.26	2.30	39.20	2.299	0.0143*
	Self- etch	9.39	3.17	2.13	2.3	25.1		
Zinc oxide Nano particles	Etch and rinse	24.46	8.4	1.88	10.50	40.10	0.66	0.25596^{ns}
	Self-etch	26.83	13.63	3.04	6.5	55		
Zinc chloride +calcium phosphate nanoparticles	Etch and rinse	22.91	11.4	2.55	7.00	46.50	4.22	0.000073*
	Self-etch	10.86	3.36	1.28	1.4	27.9		

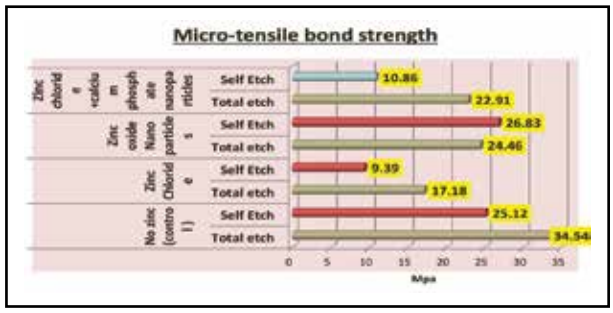

Fig. (1) A bar chart illustrating mean micro-tensile bond strength (MPa) using both adhesive techniques in different zinc groups

II. Results of SEM:

The observation of the photomicrographs resulting from backscattered electron mode at magnification of 1000X revealed that in all the specimens nanoleakage was manifested by silver penetration that had different patterns and different degrees. { Figures (2-9)}

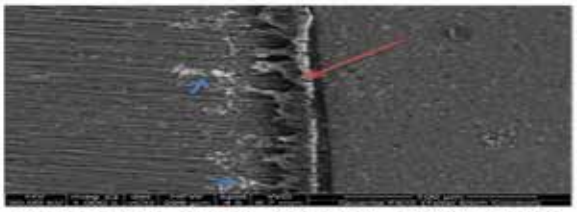

Figure (2) group (A4B1): SEM photo micrograph of nanoleakage at adhesive interface of universal single bond using etch and rinse adhesive technique.

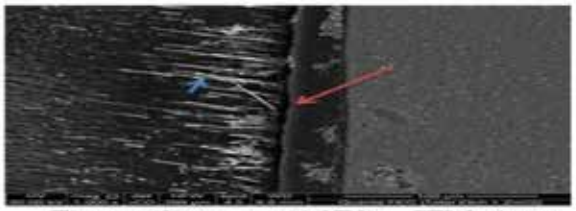

Figure (3) group (A1B1): SEM photo micrographs of nanoleakage at adhesive interface of universal single bond doped with zinc chloride using etch and rinse adhesive technique.

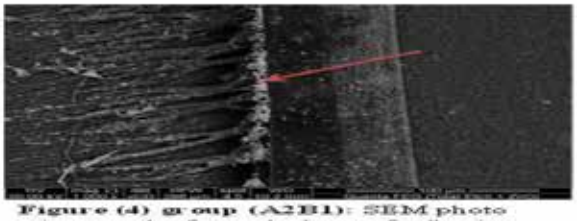

Figure (4) group (A2B1): SEM photo micrograph of nanoleakage of adhesive interface of universal single bond doped with zinc oxide nanoparticles using etch and rinse technique

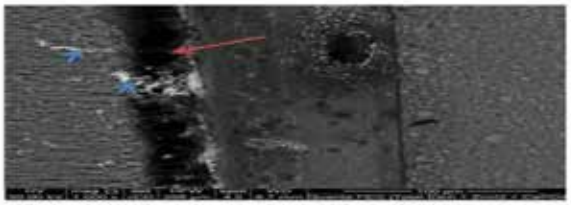

Figure (5) group (A3B1): SEM photo micrograph of nanoleakage at adhesive interface of universal single bond doped with zinc chloride plus calcium phosphate nanoparticles using etch and rinse technique.

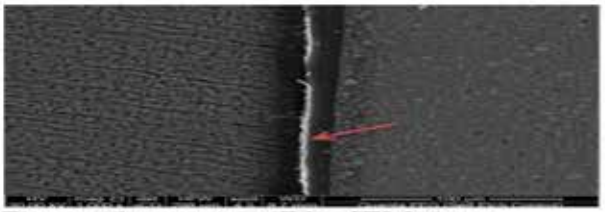

Figure (6) group (A4B2): SEM photo micrographs of nanoleakage at adhesive interface of universal single bond using self-etch technique.

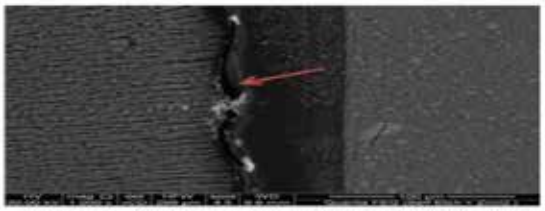

Figure (7) group (A1B2): SEM phot micrographs of nanoleakage at adhesiv interface of universal single bond dope with zinc chloride using self-etc technique.

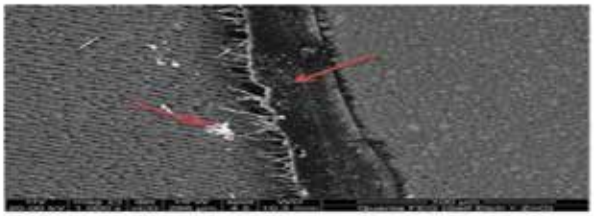

Figure (8) group (A2B2): SEM photo micrograph of nanoleakage of adhesive interface of universal single bond doped with zinc oxide nanoparticles using self-etch technique.

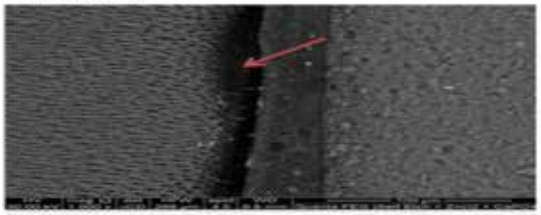

Figure (9) group (A3B2): SEM photo micrograph of nanoleakage at adhesive interface of universal single bond doped with zinc chloride plus calcium phosphate nanoparticles using self-etch technique.

DISCUSSION

Adhesion to dentin takes place mainly through hybrid layer formation between the adhesive resin and exposed dentin matrix. Thus, resin-dentin interface can be referred to as a biological composite which involves the interaction between resin monomer, dentin organic matrix and residual hydroxyapatite crystals. Optimal integrity of the interface necessitates complete infiltration of the resin monomer into the collagen-rich demineralized dentin surface to preserve the collagen fibers and inhibit their enzymatic degradation [6].

This study focused on the adhesive dentin interface as it is the main affected substrate by enzymatic degradation by host derived [13]. Several matrix metalloproteinases (MMPs) and cysteine cathepsins have been identified in dentin, and suggested to be responsible for the digestion of collagen fibrils exposed at the adhesive interface. This has led to several tentative approaches to prevent such enzymatic activity [14]. These approaches aim to modify dentin chemically by biomodification strategies, such as increasing collagen crosslinking, biomimetic remineralization and inhibition of MMPs to improve the tissue properties and stability[15].

Zinc was used as it was recently demonstrated that the quality and the longevity of the resin–dentin interface may be increased by using innovative dental adhesives containing zinc within their composition [7]. Several studies incorporated calcium phosphate (Cap) nanoparticles into dental resins to promote remineralization and avoid demineralization. These bonding agents could release high levels of Ca and P ions to induce remineralization and combat caries, and hence are promising to improve the longevity of the restorations [10]. For this reason Cap nanoparticles was chosen.

The adhesive system utilized in the study was Single Bond Universal Adhesive. It was claimed by the manufacturer that this adhesive system could be used in either etch and rinse or a self-etch approach. This is due to the change in the phosphorylated monomers used. The Universal adhesive uses methacryloxydecyl phosphate (MDP) instead of methacryloxyhexyl phosphate (MHP) [16]. So it was important to compare the adhesive techniques (etch and rinse and self-etch techniques) as they interact differently with dentin.

On comparing the mean microtensile bond strength (MPA) between different groups of zinc regardless of the adhesive technique; it was found that zinc-doping of the adhesive reduced the bond strength in comparison to the control groups. This is in agreement with a study which assessed the Chemical interaction of 10-MDP in zinc-doped self-etch adhesives. They found that presence of zinc (Zn^{2+}), a positively double-charged ion similar to calcium (Ca^{2+}), may negatively influence the performance of MDP in forming MDP–Ca salts. The chemical interaction of MDP proved the formation of MDP–zinc and Ca–MDP–Zn salts rather than MDP– Ca. MDP–zinc salts also interfered with the optimal chemical interaction of MDP-containing self-etch adhesives to dentine [8]. As the single bond universal adhesive has MDP in its composition, this might explain the results.

When ZnO nanoparticles were added to the universal single bond there was an increase in the bond strength mean values than with $ZnCl_2$. Also when calcium phosphate nanoparticles were added with $ZnCl_2$, the bond strength values increased compared with $ZnCl_2$ alone. It can be speculated that ZnO and ACP nanoparticles were not completely solubilized into the adhesive blends, thereby performing as fillers [17].

When comparing the adhesive techniques, the results showed that the adhesive technique significantly affected the bond strength. The microtensile bond strength values of etch and rinse adhesive

technique recorded a higher mean value than self-etch technique in all groups. It is known that the smear layer constitutes a true physical barrier and makes it extremely difficult for the bonding and hybrid layer formation to be fully integrated with the dentine. After preliminary etching with phosphoric acid, the smear layer is removed and superficial dentine is demineralized. This increases impregnation by the adhesive, allowing the creation of a well impregnated hybrid layer [18].

On comparing SEM images of etch and rinse and self-etch control groups, nanoleakage pattern showed more dense silver deposition along the adhesive-dentin interface in self-etch control group. This may be due to the fact that the self-etch adhesive technique does not totally remove the smear layer thus the presence of water in the smear layer may lead to incomplete polymerization of the adhesive and further water hydrolysis leading to nanoleakage[19]. In the zinc chloride and zinc chloride + calcium phosphate groups there were a gap at the adhesive dentin interface in both total and self-etch adhesive techniques, indicating no infiltration of adhesive resin into dentin. As a result, silver deposited in the acid etched dentin along dentinal tubules in etch and rinse group while there was no silver observed in the self-etch group. It seems that zinc chloride interfered with action of the adhesive leading to failure to infiltrate dentin due to the problems mentioned before with zinc chloride.

In the zinc oxide group with etch and rinse adhesive technique, silver deposition was observed along the base of the hybrid layer with no obvious difference from etch and rinse control group. In the self-etch adhesive technique a thin layer of silver was observed which was very thin than that of self-etch control group indicating better hybridization. This may be attributed to being nanosized so induced better infiltration and hybridization.

CONCLUSIONS

Under the conditions of the present study, the following conclusions can be delivered:

1. Addition of zinc to the universal single bond significantly affected the microtensile bond strength except with ZnO nanoparticles group in the self-etch adhesive technique.
2. Total etch adhesive technique has higher microtensile bond strength than self-etch adhesive technique except with ZnO nanoparticles group in the self-etch adhesive technique.

RECOMMENDATION

1. Further in vivo studies should be done to evaluate the effect of zinc addition to universal single bond by different methods of incorporation and tests.
2. The insertion of Zn in adhesive although having promising results, requires further reviews, due to the possibility of changing adhesive systems' biological, physico- chemical and mechanical properties, and, consequently, their clinical performance.

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