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Effect of zinc oxide nanoparticles with matrix metalloproteinase inhibitors on the shear bond strength of a universal dentin bonding agent to caries affected dentin

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Abstract

Objective: This study evaluated the effect of zinc oxide (ZnO) nanoparticles combined with matrix metalloproteinase (MMP) inhibitors on the shear bond strength (SBS) of a universal bonding agent to caries-affected dentin (CAD).

Methods: Forty extracted molar teeth were ground to expose dentin. CAD was induced by immersing specimens in 10% citric acid for 4 hours. Samples were divided into five groups (n=8) based on the surface treatment following acid etching: Group 1: CAD treated with hesperidin, Group 2: CAD treated with ZnO nanoparticles and hesperidin, Group 3: CAD treated with grape seed extract, Group 4: CAD treated with ZnO nanoparticles and grape seed extract, and Group V: Control (no surface treatment). G-Premio Bond was applied and then composite resin cylinders were bonded to the dentin surface. After thermocycling, the bond strength of the specimens was measured using a universal testing machine. The data were analyzed by ANOVA and Tukey's post hoc test, at a significance level of $P < 0.05$.

Results: A significant difference in SBS was observed between the study groups ($P < 0.001$). Group 2 demonstrated the highest SBS (9.73 ± 0.37 MPa), followed by group 4 (8.76 ± 0.11 MPa), group 1 (7.68 ± 0.27 MPa), and group 3 (6.47 ± 0.34 MPa). The lowest bond strength was observed in the control group (4.32 ± 0.46 MPa). All pairwise intergroup comparisons were statistically significant ($P < 0.05$).

Conclusions: Surface treatment with ZnO nanoparticles and MMP inhibitors enhances the bond strength of universal bonding agents to caries-affected dentin.

Keywords: Dental caries, Hesperidin, Grape seed extract, Matrix metalloproteinase inhibitors, Zinc oxide, Nanoparticles

Introduction

Dentin is a mineralized, cross-linked collagen matrix, containing approximately 20% organic compounds (mainly type I collagen) and 10% water. Nakajima et al. described caries-affected dentin (CAD) as an uninfected and partially demineralized layer, which retains the ability to undergo physiological remineralization. As such, it should be preserved during clinical treatment (1). CAD's ability to withstand acid etching while preserving the integrity of collagen fibrils makes it a suitable substrate for restorative interventions (2).

The total-etch concept, introduced by Fusayama, demonstrated that applying acid to dentin followed by a resin adhesive forms a resin-infiltrated collagen network known as the hybrid layer (3). Bond strength reflects the quality of hybridization and plays a crucial role in the durability of restorations. Because of its remineralization ability, CAD is often included in restorative preparations after the removal of infected dentin (4). Nano-leakage within the hybrid layer, caused by water-rich spaces and un-infiltrated collagen fibrils, presents a significant challenge to the durability of restorations. These exposed collagen fibrils are particularly vulnerable to enzymatic degradation by matrix metalloproteinases (MMPs). MMPs are calcium-dependent zinc-containing proteases, which are capable of degrading all kinds of extracellular matrix proteins.

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This activity can undermine the integrity and durability of the hybrid layer over time (3,5).

To address the challenges associated with collagen degradation, the use of MMP inhibitors has been suggested. MMP inhibitors enhance the longevity of restorations by reducing the enzymatic degradation of collagen fibrils (6). By preventing the breakdown of intra- and inter-molecular collagen cross-links, MMP inhibitors help maintain the integrity of the collagen network.

Both synthetic and natural MMP inhibitors can be applied to dentin before bonding to counteract the adverse effects of MMP activity (7). Commonly used MMP inhibitors include chlorhexidine, hesperidin (HPN), and grape seed extract (GSE).

Natural MMP inhibitors derived from fruits, flowers, and nuts, enhance the mechanical and structural properties of dentin. Hesperidin (HPN), a citrus-derived flavonoid, strengthens the collagen network by inhibiting the breakdown of cross-links (3). Similarly, grape seed extract (GSE), composed of up to 97% proanthocyanidins, effectively inhibits MMP enzymatic activity, thereby preserving the collagen network and enhancing adhesive bonding. (7).

Nanoparticles, defined as particles smaller than 100 nm, provide advantages due to their size, surface area, and energy (8). Zinc oxide (ZnO) nanoparticles, widely utilized in dental adhesives, can penetrate the collagen fibrillar network and inhibit MMP activity (9,10). The zinc ion has been shown to have an inhibitory effect on MMP activation. By increasing the amount of zinc ions, binding sites on MMPs are occupied, resulting in spatial deformation and ultimately inhibiting MMP activity (10). In this way, ZnO nanoparticles can significantly improve the durability and strength of the resin-dentin adhesion (6).

Previous studies have consistently shown that bonding to CAD results in lower bond strengths than bonding to sound dentin, underscoring the need for improved bonding protocols in such clinical scenarios (1,11,12). Although several studies (7-10) have evaluated the individual and combined effects of ZnO and natural MMP inhibitors on sound dentin, there is limited research on their impact on shear bond strength to caries-affected dentin. Therefore, this study aimed to assess the effect of ZnO nanoparticles in combination with HPN and GSE on the shear bond strength of a universal denting bonding agent to CAD.

Materials and methods

This in vitro study utilized extracted human teeth following approval from the Institutional Review Board (DPU/71028/2022). Inclusion criteria included permanent maxillary or mandibular molars extracted for periodontal reasons, with no prior restorations, caries, or cracks affecting the experimental area. Teeth with visible caries or structural damage and those kept in inappropriate storage conditions were excluded.

The sample size was calculated using G*Power Software (version 3.1.9.4; Dusseldorf, Germany) with an effect size of 5.15, an alpha level of 5%, and a power of 80%. A minimum of 40 specimens were calculated as the required sample size, with 8 specimens allocated to each group.

Soft tissue and calculus were mechanically removed from the root surfaces, followed by disinfection with 3% sodium hypochlorite solution (Prime Dental Products, Mumbai, India). The samples were stored in saline until further preparation. The buccal and lingual surfaces of each tooth were ground using a carborundum disc to expose dentin over an area of 4 × 6 mm, indicated by a yellowish color. Specimens were then immersed in a 10% citric acid solution (Pioneer, Tokyo, Japan) for 4 hours to simulate caries-affected dentin (CAD) by reducing inorganic content (13).

The 40 specimens were then divided into five groups as follows: Group 1 consisted of CAD treated with hesperidin; Group 2 included CAD treated with a combination of ZnO and hesperidin; Group 3 comprised CAD treated with GSE; Group 4 involved CAD treated with ZnO and GSE; and Group 5 served as the control group, consisting of untreated CAD.

The exposed dentin surfaces were etched using 37% orthophosphoric acid (Prime Dental Products) for 20 seconds, followed by thorough rinsing and gentle air drying to prevent dehydration.

Two surface treatment solutions, hesperidin (Yucca Enterprises, Mumbai, India) and grape seed extract (INLIFE Pharma Pvt. Ltd, Hyderabad, India), were prepared by dissolving 6.5 g of powdered extract in 100 mL of distilled water to achieve 6.5% hesperidin (HPN) and 6.5% grape seed extract (GSE) solutions (14). For zinc oxide (ZnO) nanoparticle surface treatment material, 1 gram of ZnO nanoparticle powder (Nano Research Lab, Jamshedpur, India) was mixed with 100 mL of distilled water under continuous stirring to achieve a homogeneous solution with a uniform color and consistency.

Surface treatment solutions were applied using a micro-brush in two layers. Each layer was left on the dentinal surface for 30 seconds and was gently air-dried

Figure 1. Dentin surface treatment in the study groups: (A) Acid etching of the exposed dentin surface; (B) Different surface treatment solutions; (C) Treatment of the exposed dentin with HPN, (D) Treatment of the exposed dentin with GSE, and (E) Treatment of the exposed dentin with ZnO.

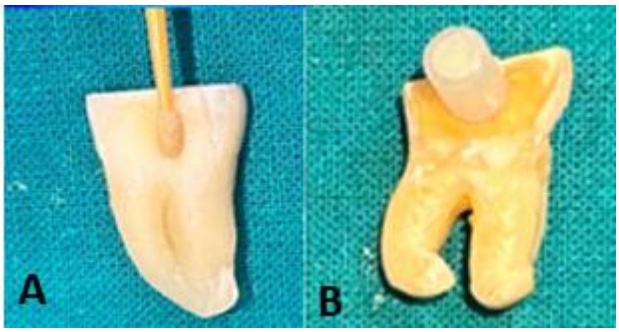

Figure 2. (A) Application of G-Premio Bond, followed by drying for 10 seconds and curing for 20 seconds; (B) Restoration of the exposed dentin surface with composite cylinders.

(Figure 1). For the combination material groups, two separate layers of each material were applied sequentially following the same protocol.

Following surface treatments, G-Premio Bond adhesive (GC Corp., Tokyo, Japan) was applied according to the manufacturer's instructions. A single coat was employed, agitated, and left for 20 seconds to penetrate the dentin. It was then gently air-dried for 10 seconds and light-cured for 20 seconds. Cylindrical composite resin (Filtek Z250, 3M ESPE, St. Paul, MN, USA) restorations were fabricated on the exposed dentin using a plastic mold, measuring 4 mm in diameter and a height of 6 mm (Figure 2). The composite was light-cured for 40 seconds to ensure proper polymerization.

Specimens were subjected to thermocycling at 5°C and 55°C for 500 cycles with a dwell time of 1 minute at each temperature. Samples were then mounted on acrylic blocks, with the roots embedded in acrylic and the crowns exposed. Shear bond strength (SBS) was measured using a universal testing machine (Unitest-10; ACME Engineers, Maharashtra, India) at a crosshead speed of 1 mm/min. The SBS values were measured on the buccal or lingual crown surfaces of each specimen. The mean and standard deviation for each group were calculated, with the results reported in megapascals (MPa).

Statistical analysis

Group comparisons were performed using one-way ANOVA, while pairwise comparisons were conducted by Tukey's post hoc test. Statistical analyses were performed using SPSS software (version 23; IBM Corp., Armonk, NY, USA), with statistical significance set at $P < 0.05$.

Results

Table 1 presents the distribution of shear bond strength (SBS) across the study groups. Group 2 (ZnO and HPN) exhibited the highest mean SBS (9.73 ± 0.37 MPa), followed by group 4 (ZnO and GSE) with a mean SBS of 8.76 ± 0.11 MPa. The lowest SBS was observed in group 5 (control), with a mean SBS of 4.32 ± 0.46 MPa.

One-way ANOVA revealed a statistically significant difference in SBS between the study groups ($P < 0.001$). Pairwise comparisons using Tukey's post hoc test showed statistically significant differences between all study groups ($P < 0.05$) in the following SBS order:

Table 1. The mean and standard deviation (SD) values of shear bond strength (MPa) in the study groups

Group	Surface treatment	Mean ± SD
1	Hesperidin	7.68 ± 0.27[a]
2	ZnO nanoparticles + hesperidin	9.73 ± 0.37[b]
3	Grape seed extract	6.47 ± 0.34[c]
4	ZnO nanoparticles + grape seed extract	8.76 ± 0.11[d]
5	Control (no surface treatment)	4.32 ± 0.46[e]
P-value	<0.001	

ZnO: Zinc oxide
The groups that have been defined by different lowercase letters indicate statistically significant differences at P<0.05.

ZnO + HPN > ZnO + GSE > HPN > GSE > control

Discussion

The present study evaluated the effects of natural matrix metalloproteinase (MMP) inhibitors, including hesperidin (HPN) and grape seed extract (GSE), either alone or in combination with zinc oxide (ZnO) nanoparticles, on the shear bond strength (SBS) of caries-affected dentin (CAD). All experimental groups in this study demonstrated significant improvements in SBS compared to the control group. This improvement is primarily due to the chemical modifications induced by MMP inhibitors, which enhanced bond strength at the resin-dentin interface.

In this study, the highest bond strength was observed in the group treated with HPN (a natural MMP inhibitor) in addition to ZnO nanoparticles. The group treated with a combination of GSE and ZnO nanoparticles showed slightly lower bond strength compared to HPN and ZnO, followed by groups treated with only HPN, and only GSE. All groups showed significant differences in pairwise comparisons. The findings of this study highlight the potential of combining ZnO nanoparticles with MMP inhibitors to enhance bond strength to CAD.

Caries-affected dentin is distinct from caries-infected dentin, as it retains the ability for remineralization. Therefore CAD layer is suggested to be preserved during clinical treatments (1). The leathery texture of CAD distinguishes it from infected dentin, which is soft and less resistant to pressure (15). On the other hand, CAD's higher degree of demineralization makes it more susceptible to acid etching and prone to the collapse of collagen fibrils. Achieving reliable bonding to CAD remains challenging due to its structural and biochemical properties.

Previous studies indicated that properly etched dentin allows for effective resin infiltration into the demineralized collagen network, creating a stable and durable bonding framework (16). However, in the case of CAD, incomplete resin infiltration into the demineralized collagen network usually occurs due to collagen collapse, which weakens the bond strength. Furthermore, the presence of MMPs in dentinal tubes can lead to collagen degradation, compromising the resin-dentin bond over time (17).

MMP inhibitors enhance the mechanical properties of collagen fibers by strengthening the collagen network. They prevent the breakdown of intra- and inter-molecular bonds by MMPs (18-20). Commonly used natural MMP inhibitors include HPN, and GSE, which are derived from fruits (20, 21). In the present study, the application of HPN or GSE, either alone or in combination with ZnO nanoparticles, enhanced bond strength to CAD. The best outcomes, however, were observed in the combined ZnO nanoparticles and MMP inhibitor groups. This improvement may result from a synergistic effect, where the properties of each component complement the others and lead to a stronger bonding.

Hesperidin, a natural flavonoid MMP inhibitor derived from citrus fruits, has shown effectiveness in enhancing bond strength in the resin-dentin interface (21). Similarly, GSE improves the mechanical and physical properties of demineralized dentin by preventing the degradation of collagen cross-links (21, 22). However, in this study, HPN was more effective than GSE in strengthening the bond at the resin-dentin interface. This difference is likely due to the larger molecular weight of GSE, which limits the diffusion of its molecules within the dentin matrix.

This study found that surface treatment of CAD with a combination of ZnO nanoparticles and HPN resulted in the highest shear bond strength, followed by the combination of ZnO and GSE. This improvement can be attributed to the nanoparticles' ability to penetrate the collagen matrix, inhibit MMP activity, prevent the degradation of cross-links, and stabilize the hybrid layer. These findings are consistent with previous studies that showed ZnO nanoparticles can enhance dentin adhesion and hybrid layer stability by inhibiting MMP-mediated collagen degradation (23–27). Their antimicrobial properties offer an additional advantage, making them a promising component in dental adhesives without compromising bond strength.

In the present study, HPN was found to be more effective than GSE in enhancing the bond strength at the resin-dentin interface. This result is consistent with the findings of Islam et al. (28), who reported that HPN significantly improved bond strength, whereas GSE did not cause a similar enhancement in the mechanical properties of bonded surfaces. The reduced efficacy of GSE may be due to the complexity of its molecular composition, which includes monomers, oligomers, and polymers. These components, with their larger molecular weight, hinder material diffusion through the dentinal matrix, limiting their ability to interact with collagen fibrils.

In this study, G-Premio Bond was selected for its well-established efficacy in promoting bond strength, owing to its formulation containing 10-MDP (10-methacryloyloxydecyl dihydrogen phosphate), acetone, silica fillers, and 4-MET (4-methacryloxyethyl

trimellitate). As a universal adhesive, it is widely recognized for its high bond strength and compatibility with both self-etch and dual-cure systems, making it suitable for a variety of restorative procedures (29). Thermocycling was employed to replicate the thermal cycling conditions that adhesive restorations undergo in the oral environment. This procedure provides an accurate assessment of the adhesive's durability and long-term performance under simulated clinical conditions.

This study has several limitations, including its in vitro design, which cannot fully replicate the complexity of the oral environment. Another limitation is that the effect of ZnO nanoparticles on SBS was not assessed as a separate group. Clinical trials are warranted to establish standardized protocols for managing CAD and optimize bonding techniques in real-world settings.

Conclusions

Within the limitations of this study, surface treatment with the HPN (a natural MMP inhibitor) combined with ZnO nanoparticles achieved the highest shear bond strength on caries-affected dentin, followed by the combination of ZnO and GSE. The use of natural MMPs alone also produced significant improvement in bond strength compared to the control group. These findings suggest that the incorporation of natural MMP inhibitors and ZnO nanoparticles may help clinicians improve the longevity and durability of restorations on caries-affected dentin.

Acknowledgments

None to report.

Conflict of interest

There is nothing to declare.

Authors' contributions

D.A., S.S.R., S.A., N.L., and P.I. contributed equally to the conception, design, data acquisition, analysis, and interpretation of the study. All authors were involved in drafting the manuscript, revising it critically for important intellectual content, and approving the final version for publication.

Ethical approval

This study was conducted as an in vitro investigation, and therefore, ethical approval was not required. The ethics committee waived the need for ethical clearance due to the non-human subject nature of the research.

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