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Original Article

Evaluating mesoporous zinc oxide nanoparticles for dentin pretreatment: Synthesis, characterization, and bond strength performance with a universal adhesive

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ABSTRACT

Background: This study aimed to synthesize mesoporous zinc oxide nanoparticles (ZnO NPs) and evaluate their effect as dentin pretreatments on the microshear bond strength (μSBS) of a universal adhesive.

Materials and Methods: This *in vitro* experimental study used 100 extracted human molars sectioned to expose mid-coronal dentin. Samples were divided into five groups (n = 20) based on pretreatment: no treatment, chlorhexidine (CHX), calcined mesoporous ZnO NPs, noncalcined mesoporous ZnO NPs, and ZnO NPs. Each group was subdivided into two subgroups (n = 10) based on the universal adhesive application mode: etch-and-rinse (E and R) or self-etch (SE). Pretreatments were applied for 1 min. Composite resin was bonded using a universal adhesive. After 24 h of storage in distilled water at 37°C, μSBS testing was performed. Statistical analysis included the Shapiro–Wilk test for normality, two-way analysis of variance with Tukey's *post hoc* test, and *t*-tests, with significance set at $P < 0.05$.

Results: The noncalcined mesoporous ZnO NP group showed the highest μSBS, followed by the ZnO NP group, with significant differences compared to other groups ($P < 0.05$). Lower μSBS values were observed in the calcined mesoporous ZnO NP, CHX, and untreated groups. The adhesive application mode had a significant effect only in the untreated and calcined mesoporous ZnO NP groups ($P < 0.05$), with SE yielding higher μSBS than E and R.

Conclusion: Noncalcined mesoporous ZnO NPs enhanced dentin bond strength more effectively than other pretreatments, including CHX, indicating their potential as a promising alternative in adhesive dentistry.

Key Words: Dentin-bonding agents, nanoparticles, shear strength, zinc oxide

INTRODUCTION

In recent years, the use of nanoparticles (NPs) in dentistry has gained attention due to their notable antibacterial properties and enhanced ability to penetrate deeply into dentinal tubules, surpassing conventional antibacterial irrigants.[1] Previous research has shown that zinc oxide NPs (ZnO NPs) are effective in combating

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various dental plaque bacteria, including *Streptococcus mutans*.[2] Recently, mesoporous materials with pore sizes ranging from 2 to 50 nm have attracted attention and found applications in various dental fields.[3-5] The compositions, pore sizes, and structures of mesoporous materials can be adjusted during the synthesis process.[6] These materials possess distinct properties, including favorable biocompatibility, well-defined ordered mesoporous pores, and exceptionally high specific surface areas.[5,6] Furthermore, the surface or internal structure of mesoporous materials can be modified or functionalized to enhance their properties.[6] Among the mesoporous materials investigated in numerous therapeutic areas, mesoporous ZnO NPs have garnered significant interest.[7] Studies have reported the exceptional properties of mesoporous ZnO NPs, including high surface area, crystallinity, substantial porosity volume, and strong potential antibacterial effects attributed to their large specific surface areas.[7]

The latest generation of adhesive systems, known as universal adhesive bonding systems, can be applied in either the etch-and-rinse (E and R) or self-etch (SE) modes, hence earning them the names "multi-mode" or "multi-purpose" adhesive bonding systems.[8] Given the even higher specific surface areas of mesoporous ZnO NPs in comparison to regular ZnO NPs, there is potential for mesoporous ZnO NPs to be used as effective antibacterial irrigants in dentistry.[9]

To the authors' knowledge, no previous studies have examined the impact of dentin pretreatment using mesoporous ZnO NPs as an antibacterial irrigant on the bond strength of a universal adhesive bonding system to dentin. Thus, the objective of this study was to synthesize and characterize mesoporous ZnO NPs and assess the influence of dentin pretreatment with these NPs on the microshear bond strength (μSBS) of a universal adhesive bonding system to dentin.

MATERIALS AND METHODS

This study was designed as an *in vitro* experimental investigation. The research protocol was approved by the Research and Ethics Committee of Shiraz University of Medical Sciences (Protocol # IR.SUMS.DENTAL.REC.1401.097). Mesoporous ZnO NPs were synthesized and characterized specifically for this study. ZnO NP solutions with a particle size of 50 nm were obtained from ASEPE Company, located in Tabriz, Iran.

Preparation of mesoporous zinc oxide nanoparticles

Mesoporous ZnO NPs were synthesized using zinc acetate (Zn (Ac) 2·2H2O) and cetyltrimethylammonium bromide (CTMAB) as the precursor and template, respectively. Zinc acetate and CTMAB were obtained from Merck Company (Germany). CTMAB (1.0 g) was mixed with 480 mL of deionized water and stirred at 80°C, followed by the addition of zinc acetate (4.92 g). The pH was adjusted with NaOH maintained at 80°C for 2 h. The resulting product was filtered, washed, and dried to obtain noncalcined mesoporous ZnO NPs. Calcined mesoporous ZnO NPs were produced by heating the powder sample to 500°C for 4 h.

Characterization of mesoporous zinc oxide nanoparticles

Mesoporous ZnO NPs were characterized using multiple techniques to assess their morphology, composition, and structure. Surface morphology and particle size were evaluated by Transmission Electron Microscopy (TEM) using a Philips CM120 microscope at 80 kV and Field Emission Scanning Electron Microscopy (FESEM) with a TESCAN Mira II instrument, after dispersing the powder in water, drying, and coating it with a thin layer of gold. Crystal structure and phase identification were performed by X-Ray Diffraction (XRD) using a Philips PW1730 diffractometer, with nickel-filtered Cu Kα radiation. Surface area and porosity were measured using nitrogen adsorption/desorption isotherms at 77.3 K using a BELSORP-mini II adsorption porosimeter. Specific surface area and pore size distribution were calculated using the Brunauer–Emmett–Teller (BET) and Barrett–Joyner–Halenda (BJH) methods, respectively, after degassing samples at 200°C under vacuum for 1 h.[10] Fourier Transform Infrared Spectroscopy (FTIR) was performed by compressing a blend of 2 mg mesoporous ZnO NPs with 200 mg potassium bromide into a pellet and analyzed using a Bruker Tensor 27 spectrometer at 4 cm^{-1} resolution.[11] Elemental composition and distribution of zinc and oxygen were confirmed by energy-dispersive X-ray mapping. Samples were stored in a dark plastic bag in a cool, dry place before to analysis.

Preparation of the dentin specimens

In this study, one hundred healthy human third molars were collected from patients undergoing orthodontic or periodontal treatment and stored in a 0.5% chloramine T solution at 4°C for up to 1 month

after obtaining informed consent. A trained, blinded, and calibrated operator performed all procedures. The teeth were cleaned with a periodontal curette, and the roots were separated from the crowns at the cementoenamel junction using a low-speed cutting machine with water cooling. The occlusal thirds of the crowns were removed to expose smooth dentin surfaces at a right angle to the long axis of the teeth. The teeth were then embedded in acrylic resin blocks, with dentin surfaces perpendicular to the base, and ground gently with 320-grit silicon carbide papers for 1 min to produce a consistent smear layer. Finally, the dentin surfaces were washed and dried using an air-water syringe.

Experimental groups

The specimens were divided into 10 experimental groups (n = 10) according to dentin pretreatment type and adhesive bonding mode (E and R or SE). All-Bond Universal adhesive (ABU, Bisco Inc., Schaumburg, IL, USA) was used for bonding in all groups. The specific dentin pretreatment assigned to each group is detailed in Table 1.

Groups 1 and 2 served as controls, receiving universal adhesive bonding without any dentin pretreatment. In Group 1, the E and R mode was applied, while in Group 2, the SE mode was used.

For groups 3–10, dentin surfaces were pretreated by applying the respective agents using a foam pellet for 1 min and gently removing any excess material. The pretreatment agents used were as follows:

- Groups 3 and 4 received 2% chlorhexidine (CHX) solution (Consepsis, Ultradent Inc., South Jordan, UT, USA)
- Groups 5 and 6 were treated with the calcined mesoporous ZnO NP solution
- Groups 7 and 8 were treated with the noncalcined mesoporous ZnO NP solution
- Groups 9 and 10 were treated with ZnO NP solution.

The bonding mode was then applied according to the designated protocol for each group:

- In the E and R mode (Groups 1, 3, 5, 7, and 9), dentin was first etched using a 35% phosphoric acid gel (Bisco Inc., Schaumburg, IL, USA) for 15 s. The surface was thoroughly rinsed, and excess water was removed by blotting with an absorbent pellet or using high-volume evacuation for 1–2 s, to ensure the dentin remained visibly moist. The adhesive was then applied following the SE technique described below
- In the SE mode (Groups 2, 4, 6, 8, and 10), the adhesive was applied directly to unetched dentin. The application procedure involved dispensing 1–2 drops of adhesive into a clean well, followed by scrubbing the preparation with a microbrush for 10–15 s per coat, with two separate coats applied. The solvent was then evaporated thoroughly by air-drying for at least 10 s to ensure no visible movement of the adhesive. A uniform glossy surface appearance was confirmed before light curing for 10 s.

The experimental procedures strictly followed the manufacturer's instructions for All-Bond Universal to ensure consistency in adhesive application across all groups.

Micro shear bond strength, failure mode analysis, and statistical analysis

Adhesive tape with a punched hole was used to determine the bonding area. A translucent PVC microtube (0.5 mm height, 0.7 mm internal diameter) was placed on the hole. Adhesive bonding was cured using a VIP Junior light-curing unit (Bisco, Schaumburg, IL, USA) at 600 mW/cm² intensity. Microtubes were filled with Z250 composite resin (3M ESPE, St. Paul, MN, USA) and cured with the

Table 1: Experimental groups and corresponding pretreatment methods and bonding modes

Group	Dentin pretreatment	Pretreatment time	Bonding mode	Acid etching (35% phosphoric acid)
1	No pretreatment	NA	Etch and rinse	Yes
2	No pretreatment	NA	SE	No
3	CHX	1 min	Etch and rinse	Yes
4	CHX	1 min	SE	No
5	Calcined mesoporous ZnO NPs	1 min	Etch and rinse	Yes
6	Calcined mesoporous ZnO NPs	1 min	SE	No
7	Noncalcined mesoporous ZnO NPs	1 min	Etch and rinse	Yes
8	Noncalcined mesoporous ZnO NPs	1 min	SE	No
9	ZnO NPs	1 min	Etch and rinse	Yes
10	ZnO NPs	1 min	SE	No

NA: Not available; ZnO NPs: Zinc oxide nanoparticles; CHX: Chlorhexidine; SE: Self-etch

same light-curing unit. Specimens were soaked in distilled water at 37°C for 24 h. μSBS tests were performed using an Instron universal testing machine (Z020, Zwick Roell, Germany) at 1 mm/min crosshead speed (MPa). The experimental setup is depicted in Figure 1. Failure mode analysis used a Carl Zeiss Inc. stereomicroscope (Oberkochen, Germany) at ×40 magnification. Failure modes are classified as: (A) adhesive failure within the interface, (B) cohesive failure within resin or dentin, and (C) mixed failure involving both adhesive and cohesive failures.

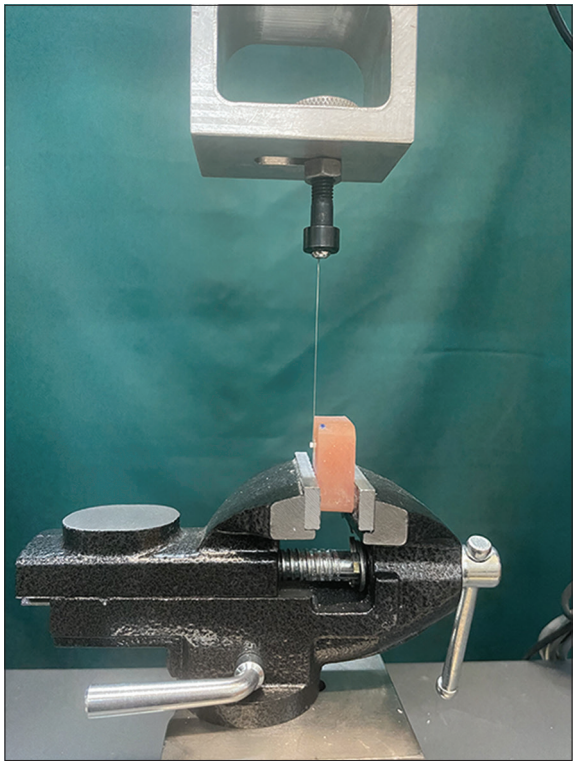

Figure 1: A composite resin micro-cylinder secured at its base with a stainless-steel ligature wire on a sample being tested using a universal testing machine.

Data normality was verified using the Shapiro–Wilk test. A two-way analysis of variance (ANOVA) assessed the impact of universal bonding application mode and dentin pretreatment type, with Tukey's tests for inter-group μSBS value comparisons. *t*-tests were employed to compare adhesive application modes within each dentin pretreatment group. Statistical analyses were conducted using SPSS software version 17, with significance established at $P < 0.05$.

RESULTS

The mesoporous ZnO NPs, observed via FESEM [Figure 2a], exhibited spherical shapes with distinct grain boundaries and particle sizes of 70–100 nm. These NPs were densely packed, forming a compact configuration with uniformly dispersed smaller particles. EDX-map analysis [Figure 2b] confirmed the even distribution of oxygen (O) and zinc (Zn) elements within the NPs. TEM analysis [Figure 2c] of the synthesized ZnO NPs revealed an average size of 10–15 nm, with quasi-spherical shapes, surface porosity, uniform and aggregated distributions, and inter-agglomeration forming larger pores and voids, as confirmed by BJH analysis. The XRD patterns [Figure 3a] revealed well-defined diffraction peaks at 31.772°, 34.642°, 36.442°, 47.792°, 56.842°, 63.092°, 66.592°, 68.242°, 69.342°, 72.792°, and 77.292°. These peaks correspond to the crystal planes [100], [002], [101], [102], [110], [103], [200], [112], [201], [004], and [202], respectively. The presence of these sharp peaks indicates a high crystallinity in the mesoporous ZnO NPs, aligning with standard values reported in JCPDS Card no. 036-451. Low-angle XRD patterns in the 2-theta range of 0.85°–10° [Figure 3b] showed no distinct peaks.

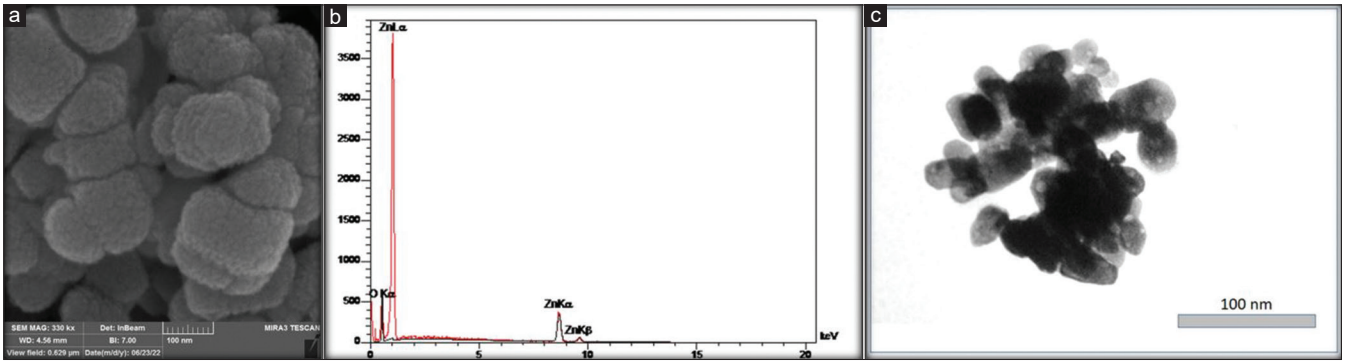

Figure 2: (a) Field emission scanning electron microscopy image of mesoporous zinc oxide nanoparticles (ZnO NPs); (b) EDX spectrum confirming the elemental composition of the nanoparticles, with characteristic peaks for Zn and O; (c) Transmission electron microscopy image of the prepared mesoporous ZnO NPs.

N2 adsorption/desorption analysis [Figure 4a] confirmed the mesoporous properties of the ZnO NPs, showing a Type IV isotherm with an H3 hysteresis loop. The specific surface area was calculated as 5 m^2/g using the BET method. BJH analysis [Figure 4b] identified two distinct pore sizes – around 8.5 nm and 27 nm – in the sample. FTIR analysis [Figure 4c] of the calcined sample confirmed ZnO formation, with characteristic Zn-O stretching vibrations showing peaks at 508 cm^{-1} and 433 cm^{-1}, supporting ZnO presence in the mesoporous NPs.

Mean μSBS values (MPa) and their standard deviations for all 10 experimental groups are detailed in Table 2. Two-way ANOVA results [Table 3] revealed significant variations in mean μSBS values across all groups

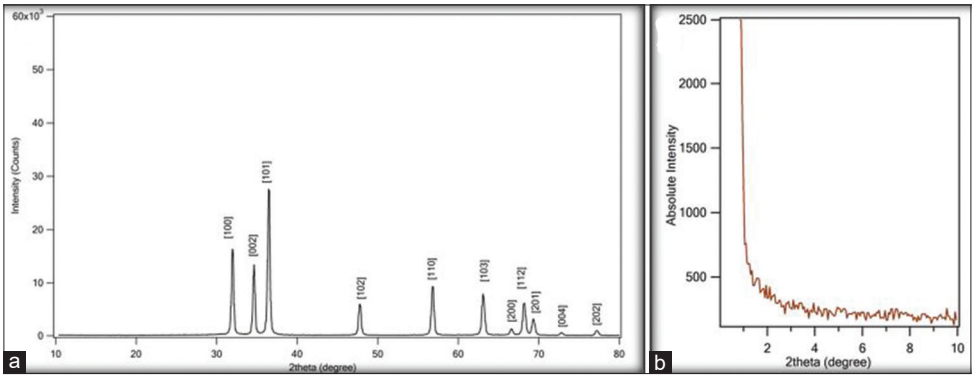

Figure 3: (a) Wide-angle X-ray diffraction (XRD) pattern of the prepared mesoporous zinc oxide nanoparticles (ZnO NPs); (b) low-angle XRD pattern of the prepared mesoporous ZnO NPs.

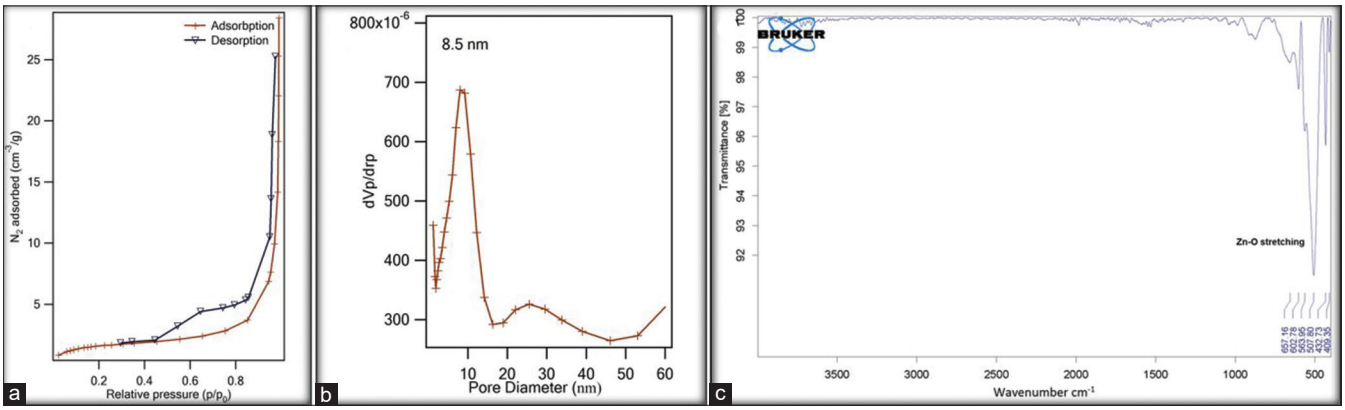

Figure 4: (a) Nitrogen adsorption-desorption isotherm; (b) pore size distribution plots obtained from the Barrett–Joyner–Halenda model for the adsorption/desorption branch isotherm; (c) Fourier transform infrared spectroscopy spectra of mesoporous zinc oxide nanoparticles.

Table 2: Means and standard deviations of microshear bond strength to dentin (MPa) in the experimental groups

Group number	Experimental condition	μSBS to dentin (MPa), mean±SD
1	Etch and rinse (without dentin pretreatment)	13.70±0.98
2	SE (without dentin pretreatment)	15.20±1.35
3	CHX + etch and rinse	12.02±0.86
4	CHX + SE	12.55±0.83
5	Calcined mesoporous ZnO NP + etch and rinse	10.38±0.80
6	Calcined mesoporous ZnO NP + SE	11.69±0.60
7	Noncalcined mesoporous ZnO NP + etch and R	18.11±0.79
8	Noncalcined mesoporous ZnO NP + SE	18.58±0.69
9	ZnO NP + etch and rinse	16.05±0.98
10	ZnO NP + SE	16.49±0.72

CHX: Chlorhexidine; SE: Self-etch; ZnO NP: Zinc oxide nanoparticle; SD: Standard deviation; μSBS: Microshear bond strength

(P = 0.001), with a statistically significant interaction effect between dentin pretreatment type and mode of universal adhesive application (P = 0.001). The influence of dentin pretreatment type is significant (P = 0.001), while the mode of universal adhesive application does not notably affect μSBS values (P > 0.05). Tukey's test compared group results, with noncalcined mesoporous ZnO NPs resulting in the highest μSBS values, followed by ZnO NPs, both showing significant differences (P < 0.05) from other groups. Conversely, calcined mesoporous ZnO NPs with CHX treatment yielded the lowest μSBS values, differing significantly (P < 0.05) from other groups. Comparing adhesive application modes for each pretreatment, statistical significance was evident only in untreated dentin and calcined mesoporous ZnO NPs groups (P < 0.05), where the SE mode exhibited a significant μSBS increase over the E and R mode. Figure 5 illustrates failure modes, with mixed failure (adhesive and cohesive) predominating across all groups.

DISCUSSION

This study investigated the impact of mesoporous ZnO NPs on dentin pretreatment within a universal adhesive bonding system. Noncalcined mesoporous ZnO NPs significantly enhanced μSBS relative to other groups, independent of the adhesive application method. In groups without dentin pretreatment or those pretreated with calcined mesoporous ZnO NPs, the adhesive application mode significantly influenced outcomes, with the SE mode exhibiting a notable μSBS increase over the E and R mode.

The μSBS test is a robust and efficient method to assess bond strength, improving experimental throughput by enabling multiple specimens to be tested from a single tooth. Unlike macroshear tests, μSBS testing mitigates issues such as non-uniform stress distribution and mixed loading modes, thereby reducing the likelihood of cohesive failure within the dentin substrate instead of at the restorative material–dentin interface.[12] In this study, the μSBS test was used to assess the impact of

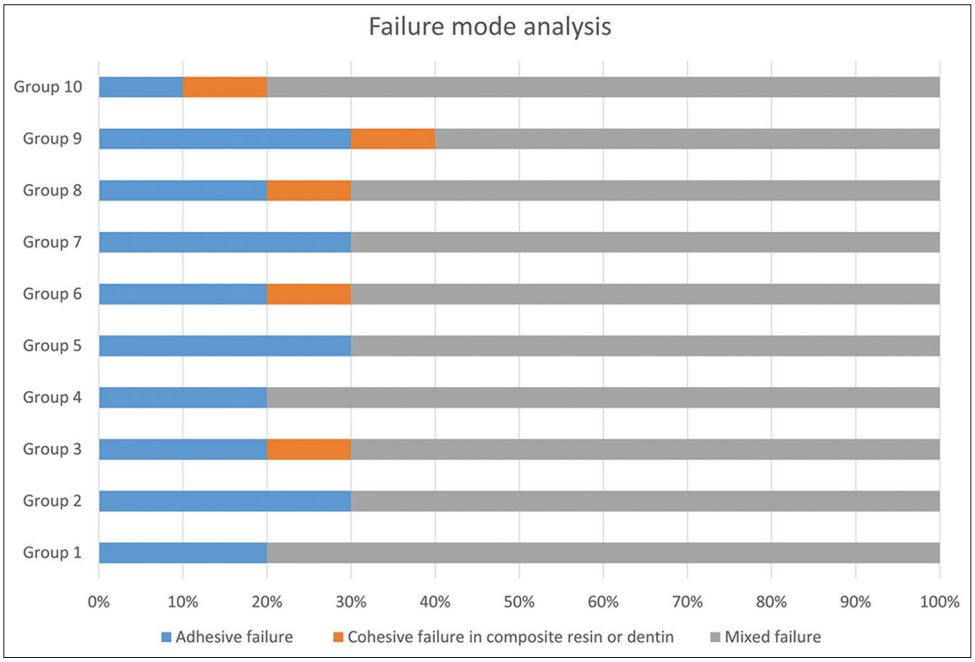

Figure 5: Frequency distribution of the different failure modes.

Table 3: Results of the two-way analysis of variance test

Source	SS	df	Mean square	*F*	*P*
Type of dentin pretreatment	696.288	4	174.072	221.725	<0.001*
Mode of application of adhesive bonding (SE or etch and rinse)	2.716	1	2.716	3.459	0.066
Type of dentin pretreatment × mode of application of adhesive bonding	20.651	4	5.163	6.576	<0.001*
Error	70.657	90	0.785	-	-
Total	21,758.51	100	-	-	-

*Significant at P<0.05. SS: The sum of squares; SE: Self-etch; df: Degrees of freedom; *F: F* statistic

adhesive application modes and dentin pretreatments on dentin bond strength.

ZnO NPs have garnered considerable attention in dentistry owing to their potent antibacterial properties.[13-15] Additionally, they possess antioxidant properties and confer several benefits to dentin, including prevention of demineralization, mitigation of decalcification during orthodontic treatment, and promotion of remineralization.[16] ZnO provides sustained inhibition of collagen degradation in demineralized dentin and reduces biofilm formation on tooth surfaces by up to 85%.[17,18] Recently, mesoporous materials have attracted significant interest across multiple medical fields due to their high surface area and drug delivery capabilities. Mesoporous calcium-silicate nanoparticles (MS NPs) loaded with CHX exhibit low cytotoxicity, *in vitro* remineralization potential, and anti-*Enterococcus faecalis* activity, rendering them promising candidates for treating bone defects and root canal applications.[19] Incorporation of CHX-loaded MS NPs into composite resins effectively inhibits *Lactobacillus casei* and *S. mutans*.[3] Mesoporous ZnO NPs have attracted interest for their potential antibacterial effects, high surface area, and crystallinity, positioning them as promising agents for antibacterial irrigation in dentistry and for ureteral stent fabrication.[20] This study aimed to synthesize and characterize mesoporous ZnO NPs and assess their impact on μSBS when applied as a dentin pretreatment.

Continuous research efforts aim to optimize adhesive bonding and enhance the integrity of restorations across different dental substrates, highlighting ongoing advancements toward improving the durability and reliability of bonded and adhesive restorations.[21,22] Universal adhesive bonding systems represent the latest advancement in adhesive technology.[8] This study investigated the effect of adhesive application mode (E and R versus SE) on bond strength to dentin surfaces pretreated with various agents. The study demonstrated that, except for the group treated with calcined mesoporous ZnO NPs and the untreated group, bond strength values did not significantly differ between E and R and SE modes for dentin pretreated with noncalcined mesoporous ZnO NPs or ZnO NPs. However, in these exceptions, the SE mode yielded significantly higher bond strength to dentin. The superior bond strength observed with the SE mode in the absence of dentin pretreatment may be attributed to risks inherent to dentin etching.[18] Insufficient infiltration of resin monomers into exposed collagen fibrils during E and R mode can lead to gap formation, rendering the dentin bond susceptible to degradation.[23] Pretreatment with noncalcined mesoporous NPs or ZnO NPs resulted in comparable bond strength between E and R and SE application modes. The nanoscale dimensions of these particles likely enabled them to occupy gaps within collagen matrices, potentially inhibiting matrix metalloproteinase activity and enhancing bond durability.

Acid etching alters dentin surface energy, exposing collagen fibrils and promoting close nanoparticle adhesion, which facilitates enhanced diffusion of small molecules within partially demineralized dentin – thereby compensating for discrepancies in demineralization depth and adhesive penetration.[24] Consequently, both E and R and SE modes of the universal adhesive demonstrated equivalent efficacy on dentin pretreated with mesoporous ZnO NPs or ZnO NPs.

This study demonstrated that pretreatment with noncalcined mesoporous ZnO NPs or ZnO NPs significantly improved universal adhesive bond strength to dentin compared to untreated groups or those pretreated with CHX. The NPs likely enhanced surface free energy and wettability by infiltrating spaces between partially demineralized collagen fibers.[24] Furthermore, the hydrophilic properties of the NPs used may have modulated the dentin surface tension, thereby enhancing receptivity to the universal adhesive's dual bonding mechanisms – both micromechanical and chemical. However, this study did not directly evaluate the nanoparticles' effects on dentin surface free energy and wettability; therefore, further investigations are warranted.

Clinically, the choice of universal adhesive application mode is influenced by cavity characteristics, with enamel pre-etching shown to improve long-term restoration durability.[25] For predominantly dentin surfaces, selective-etch or SE techniques are recommended to minimize sensitivity and preserve the resin–dentin interface.[23] Previous studies suggest phosphoric acid etching of enamel prior to adhesive application to achieve stronger bonding, particularly when using the E and R mode.[23,26,27] However, achieving selective enamel etching without compromising dentin remains challenging.[23] This study found that dentin pretreatment with mesoporous

ZnO NPs or ZnO NPs produces comparable bond strengths across both adhesive application modes. Applying these NPs prior to selective enamel etching may mitigate adverse effects on dentin bond strength while providing additional antimicrobial benefits. Furthermore, in situations where selective phosphoric acid application is hindered by cavity size, employing the E and R technique with universal adhesives in conjunction with mesoporous ZnO NPs or ZnO NPs pretreatment may be beneficial. These NPs likely enhance dentin wettability and surface energy following acid etching, potentially facilitating improved resin monomer infiltration.

Pretreatment with mesoporous ZnO NPs and ZnO NPs improves dentin wetting properties, thereby influencing the efficacy of the universal adhesive system evaluated in this study. ABU contains 10-methacryloxydecyl dihydrogen phosphate (10-MDP) as its functional monomer. Upon application to dentin, 10-MDP partially demineralizes the surface at the submicron scale and forms nanoscale 10-MDP-calcium salt layers within the hybrid layer through interaction with released calcium ions.[28] SE adhesives depend on water to generate hydrogen ions facilitating dentin demineralization and monomer infiltration, with water content being critical for optimal bonding and varying according to adhesive formulation.[29] Adhesive water content influences pH, which is essential for monomer ionization and self-etching efficacy; SE adhesives with lower water content generally contain less aggressive resin monomers.[30] In this study, the universal adhesive possessed <3% water content and a pH of 3.2.[29] Following acid etching in the E and R mode, adhesive water content becomes critical for ionization and dentin wetting; insufficient moisture may hinder collagen network re-expansion and compromise bond strength.[29] Mesoporous ZnO NPs and ZnO NPs could aid in enhancing dentin wettability, potentially assisting in re-expanding the collagen network following ABU application.

The study examined two forms of ZnO – ZnO NPs at 10 mg/mL and mesoporous ZnO NPs at the same concentration – in both calcined and noncalcined variations. Calcined mesoporous ZnO NPs, obtained by heating the noncalcined NPs to remove volatiles, exhibited lower bond strength, potentially due to the effects of heating on the NPs. This study suggested that mesoporous ZnO NPs enhanced bonding compared to universal adhesives alone, offering potential applications as antimicrobial agents in dentistry. Study limitations should be acknowledged. As an *in vitro* experiment, these findings may not directly translate to *in vivo* settings. The study focused solely on the effects of mesoporous ZnO NPs on dentin bond strength using a universal adhesive within a 24-h timeframe, indicating the need for investigations over longer periods. Future research should explore the wettability characteristics of mesoporous ZnO NPs on dental structures, their sustained antibacterial efficacy, and their compatibility with diverse universal adhesive systems.

CONCLUSION

Pretreating dentin with noncalcined mesoporous ZnO NPs or ZnO NPs improved bond strength compared to no pretreatment. The SE technique showed higher bond strength without pretreatment; however, with ZnO NPs pretreatment, the adhesive performed well regardless of the etching mode. Noncalcined mesoporous ZnO NPs could serve as an alternative to CHX in dental applications.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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