

Effect of Ozonated Olive Oil and Chitosan as Pretreatment Materials on the Bond Strength of Resin Composite to Dentin- In Vitro Study

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Abstract

BACKGROUND: Achieving strong bonding between resin and dentin is influenced by the condition and surface energy of the dentin. Chemical treatment can optimize the dentin's characteristics by increasing its surface area and energy, which enhances interaction with adhesive materials. This promotes improved micromechanical retention of resin. In adhesive dentistry, the main objective is to establish a reliable and long-lasting bond between the tooth and restorative materials.

AIM & OBJECTIVE: To compare and evaluate two different materials as cavity disinfectants on the shear bond strength of resin composite to dentin.

MATERIAL AND METHODOLOGY: Twenty-four sound human maxillary and mandibular premolars were used in this study. The crowns were separated from the roots 1 mm below the cemento-enamel junction, and the labial dentin was exposed with a fissure bur; all other surfaces were covered with nail varnish. The teeth were randomly divided into three groups of eight: Group 1 (Control, no pre-treatment), Group 2 (Chitosan nanoparticles), and Group 3 (Ozonated olive oil). After applying the respective pre-treatments, the exposed dentin surfaces were etched with 37% phosphoric acid, rinsed with water, and air-dried. Bonding agent was then applied, followed by placement of composite resin. All specimens were immersed in distilled water at 37°C for 24 hours to replicate oral conditions. Shear bond strength was assessed using a universal testing machine.

RESULTS: The control group showed the lowest mean shear bond strength (20.32 MPa), while Chitosan (42.40 MPa) and ozonated olive oil (39.60 MPa) groups recorded higher values. The Kruskal–Wallis test indicated no statistically significant difference among the groups ($p = 0.176$). Overall, both Chitosan and ozonated olive oil improved bond strength compared to control, but the difference was not significant.

CONCLUSION: Chitosan and ozonated olive oil enhanced shear bond strength compared to the control group, though the difference was not statistically significant. These findings suggest that both agents may have potential as dentin surface treatments without negatively affecting bond strength.

KEYWORDS: cavity disinfectant, chitosan, ozonated olive oil, bond strength, composite

INTRODUCTION:

The effectiveness of adhesive dentistry is primarily dependent on the ability to create a stable and durable bond between restorative materials and tooth structure. Despite substantial improvements in adhesive technologies, achieving predictable and long-lasting adhesion to dentin continues to be challenging

because of its complex structure, tubular architecture, and inherent moisture content¹. Moreover, the presence of residual microorganisms and organic remnants within prepared cavities may hinder proper infiltration of adhesive resins into dentin, thereby compromising the durability and clinical performance of restorations². As a result, considerable attention has been directed toward the use of cavity disinfectants and dentin pretreatment strategies aimed at improving both the biological condition of the cavity and the mechanical integrity of adhesive restorations.

Among the various approaches explored, ozone therapy has gained significant interest in dentistry because of its powerful antimicrobial activity and its ability to disrupt bacterial biofilms. Previous investigations have demonstrated that ozone is effective against cariogenic microorganisms such as *Streptococcus mutans*, leading to a reduction in the microbial load present in carious lesions³. In addition to its antibacterial action, researchers have evaluated the influence of ozone on adhesive procedures. Some studies have reported that ozone application does not adversely affect the mechanical characteristics of dental adhesive systems⁴. Furthermore, several investigations have indicated that the use of ozone as a cavity disinfectant produces little or no negative impact on the bond strength between resin materials and dentin, suggesting that it may serve as a useful adjunct during adhesive procedures^{5,6}. More recent studies continue to support the role of ozone in effective cavity disinfection while maintaining acceptable bonding performance^{8,9}.

Chitosan, a naturally occurring polysaccharide obtained from chitin, has also attracted attention as a potential biomaterial for dental applications. Due to its antimicrobial properties and excellent biocompatibility, chitosan has been investigated as a dentin pretreatment agent capable of enhancing the adhesive interface⁷. Evidence suggests that chitosan can interact with dentinal collagen fibrils, thereby contributing to stabilization of the hybrid layer formed during bonding procedures. In addition, chitosan has been reported to inhibit matrix metalloproteinases (MMPs), enzymes responsible for the degradation of collagen within the resin–dentin interface. By limiting enzymatic breakdown of collagen, chitosan may improve the long-term durability of adhesive bonds.

Among ozone-derived materials, ozonated olive oil has recently emerged as a novel delivery system that combines the antimicrobial benefits of ozone with the biological advantages of olive oil. In this formulation, olive oil acts as a stabilizing carrier that allows gradual and sustained release of ozone while also providing antioxidant and anti-inflammatory properties. Ozonated olive oil has been reported to reduce microbial contamination and promote dentin remineralization, indicating its potential application in conservative dental procedures.

Considering the growing interest in dentin pretreatment agents and the limited available literature comparing ozonated olive oil with chitosan, the present study was designed to evaluate and compare their effects on the bond strength of resin composite to dentin. This *in vitro* investigation aims to contribute to the development of strategies that enhance adhesion while maintaining a biologically favorable environment for restorative procedures.

Considering the growing interest in dentin pretreatment strategies and the limited literature comparing ozonated olive oil with other biomodifiers, the present study aims to evaluate and compare the effect of ozonated olive oil and chitosan on the bond strength of resin composite to dentin. By investigating these materials, this *in vitro* study seeks to identify potential approaches for enhancing adhesion while creating a biologically favorable environment for restorative procedures.

The increasing interest in ozone-based cavity disinfectants, previous investigations have evaluated the effect of ozone application on dentin bonding. Ali et al. reported that pretreatment of dentin with ozone

gas did not adversely affect the shear bond strength of composite resin and produced a continuous hybrid layer, suggesting its safe use as a cavity disinfectant prior to adhesive procedures¹⁰. In addition, clinical studies have demonstrated that ozonated olive oil exhibits strong antimicrobial properties and promotes dentin remineralization, indicating its potential usefulness in conservative and restorative dentistry¹¹. Considering the limited literature on ozonated olive oil and the growing evidence supporting chitosan, this study aims to evaluate and compare their effects as pretreatment agents on the bond strength of resin composite to dentin. By investigating these novel materials, this in vitro study seeks to contribute to the development of strategies that optimize adhesion while promoting a biologically favorable restorative environment.

METHODOLOGY:

The study was conducted in the Department of Conservative Dentistry and Endodontics at KVG Dental College and Hospital, Kurunjibagh, Sullia, India, during July 2025.

Inclusion Criteria

The research included freshly extracted human maxillary and mandibular premolars that were non-carious.

Exclusion criteria

The study excluded human extracted teeth that had carious lesions, developmental abnormalities, irregular root formation, attrition, or cervical abrasion.

Specimen preparation:

A total of 24 intact human maxillary and mandibular premolars were included. The crowns of the samples were sectioned from the roots 1 mm apical to the cemento-enamel junction and prepared by exposing the dentin on the labial surface using a fissure bur, while all other surfaces were coated with nail varnish.

Sample grouping:

The specimens will be randomly assigned into 3 groups, each comprising 8 teeth: Group 1: No pre-treatment (Control), Group 2: Chitosan nanoparticles and Group 3: Ozonated olive oil.

Group 1 (Control): No pretreatment was carried out. 37% phosphoric acid was used to etch the exposed dentinal surfaces, followed by rinsing with water. The dentinal surfaces was air-dried, bonding agent was applied and following which composite resin was placed.

Group 2 (Chitosan): Chitosan was applied as pretreatment on the dentin surface using an locator tip for 1 minute followed by which surface was rinsed with water and air-dried. 37% phosphoric acid was used to etch the exposed dentinal surfaces, followed by rinsing with water. The dentinal surfaces was air-dried, bonding agent was applied and following which composite resin was placed.

Group 3 (Ozonoid): Ozonoid was applied as pretreatment on the dentin surface using an applicator tip for 1 minute followed by which surface was rinsed with water and air-dried. 37% phosphoric acid was used to etch the exposed dentinal surfaces, followed by rinsing with water. The dentinal surfaces was air-dried, bonding agent was applied and following which composite resin was placed.

Assessment of Shear Bond Strength:

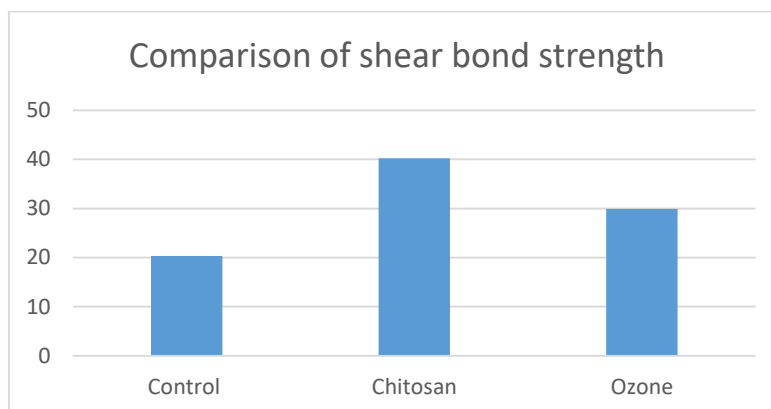
For 24 hours, all samples were stored in distilled water at 37°C, to simulate oral environment. A universal testing equipment was utilized to test the samples for shear bond strength.

RESULTS:

Table 1 and Graph 1 present the mean shear bond strength values of the three groups. The control group demonstrated the lowest mean value (20.32 ± 16.10 MPa), whereas both experimental groups—Chitosan

(40.2 ± 38.5 MPa) and ozonated olive oil (29.8 ± 30.5 MPa)—recorded comparatively higher values. Among the groups, the highest mean bond strength was observed with Chitosan pretreatment, followed closely by ozonated olive oil.

Table 2 summarizes the intergroup comparison using the Kruskal–Wallis test. Although higher mean values were recorded in the experimental groups, statistical analysis revealed no significant difference in bond strength between control, Chitosan, and ozonated olive oil groups ($p = 0.176$).



Descriptive Statistics

	N	Mean	Std. Deviation
Control	8	20.3263	16.10023
Chitosan	8	40.2325	38.58631
Ozone	8	29.8900	30.59002
Valid N (listwise)	8		

Kruskal-Wallis Test

Test Statistics^{a,b}

	Chitosan	Ozone
Chi-Square	6.250	6.917
df	6	6
Asymp. Sig.	.396	.329

These findings indicate that both Chitosan and ozonated olive oil pretreatments enhanced the shear bond strength of resin composite to dentin compared to the control, but the differences were not statistically significant.

DISCUSSION:

The present in vitro study evaluated the effect of two pretreatment agents, Chitosan and ozonated olive oil on the shear bond strength of resin composite to dentin. The findings demonstrated that both experimental groups produced higher mean bond strength values compared to the control group. Specifically, Chitosan recorded the highest mean value (40.20 MPa), followed closely by ozonated olive oil (29.8 MPa), whereas the control group exhibited the lowest (20.32 MPa). However, statistical analysis using the Kruskal–Wallis

test revealed no significant difference among the groups ($p = 0.176$). These outcomes indicate that both agents can enhance the bonding process without negatively affecting adhesion, though their superiority over conventional bonding could not be confirmed statistically.

Bond strength testing is a critical parameter in adhesive dentistry, as it helps determine the durability and reliability of resin–dentin interfaces. In the present study, a shear bond strength test was employed due to its ability to provide clinically relevant information regarding interfacial behaviour.

The application of cavity disinfectants prior to bonding has gained increasing attention, as residual microorganisms can compromise restoration longevity. Ozone, in particular, has shown strong antimicrobial efficacy against cariogenic species such as *Streptococcus mutans*². Johansson et al.³ highlighted ozone's bactericidal properties, while Magni et al.⁴ reported that ozone gas did not significantly impair the mechanical performance of adhesive systems. Similarly, Cangul et al. found that the use of ozone as a cavity disinfectant did not significantly affect bond strength when applied in conjunction with antibacterial bonding agents.⁵ These findings align with the present results, where ozonated olive oil, a stabilized form of ozone delivery, enhanced bond strength compared to control without detrimental effects.

Ozonated derivatives have also been investigated in caries management and restorative procedures. Nisar et al.⁶ reported that ozonated water application improved bond strength in deciduous teeth compared to untreated dentin, further confirming ozone's role in promoting a favorable bonding environment. More recently, Ali et al.⁹ demonstrated that ozone gas alone and in combination with natural agents (such as mushroom extracts) enhanced bond strength of resin composites to dentin. These findings suggest that ozone-based pretreatment agents, including ozonated olive oil used in the present study, may represent a promising adjunct in adhesive dentistry.

Chitosan, on the other hand, has gained recognition for its multifunctional role as a biocompatible, antibacterial, and bioactive material. Its ability to interact with dentin collagen fibrils enhances the stability of the hybrid layer, thereby improving the integrity of resin–dentin bonds⁷. Moreover, chitosan has been shown to inhibit matrix metalloproteinases (MMPs), enzymes responsible for collagen degradation at the adhesive interface, thereby prolonging bond durability. Sharafeddin and Aghaei demonstrated that chitosan pretreatment significantly improved shear bond strength compared to control, consistent with the present findings.⁷

The comparative outcomes of the present study suggest that while Chitosan yielded the highest mean bond strength, ozonated olive oil performed similarly, indicating both agents can serve as effective dentin pretreatments. In a comprehensive review of novel cavity disinfectants, emphasized that natural bioactive agents not only enhance antimicrobial action but also improve adhesive performance without compromising mechanical properties. This perspective supports the inclusion of agents like Chitosan and ozonated olive oil in restorative protocols.

LIMITATIONS AND FUTURE RESEARCH:

This was an in vitro study, and results may not fully replicate the complex intraoral conditions such as pulpal pressure, saliva, and masticatory stresses. The study evaluated bond strength only after 24 hours of water storage, without long-term aging procedures like thermocycling or mechanical loading. The study assessed only immediate bond strength and did not evaluate the durability of the resin–dentin interface over time.

Future research should therefore focus on studies with larger sample sizes and incorporate long-term aging

protocols, including thermocycling and mechanical loading, to better replicate clinical conditions. Comparative analyses using different adhesive systems, such as self-etch and total-etch approaches, would also provide valuable insights into the interaction of pretreatment agents with bonding strategies. Advanced analytical techniques, including scanning electron microscopy, should be employed to evaluate the ultrastructural integrity of the resin–dentin interface. Ultimately, well-designed clinical trials are necessary to confirm the biological safety, antimicrobial efficacy, and long-term effectiveness of Chitosan and ozonated olive oil as dentin pretreatment agents in restorative dentistry.

CONCLUSION:

Within the limitations of this in vitro study, both Chitosan and ozonated olive oil pretreatments enhanced the shear bond strength of resin composite to dentin compared to the control group. Although the differences were not statistically significant, the results indicate that these agents can be safely used as dentin surface pretreatments without negatively compromising adhesion. Their antimicrobial and bioactive properties further suggest potential clinical benefits, warranting future long-term in vivo and clinical studies to confirm their effectiveness and durability in restorative dentistry.

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