ORIGINAL RESEARCH

Effect of Mushroom, Ozone Gas, and Their Combination as Pretreatment Materials on the Bond Strength of Resin Composite to Dentin

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Abstract

Aim: To assess the effect of mushrooms, ozone gas, and their combination as cavity disinfectants on the bonding strength of composite to dentin.

Materials and methods: The study was conducted on 40 sound premolar teeth randomly divided into four groups. Group I: control group, Group II: mushroom group, Group III: Ozone group, and Group IV: mushroom + ozone gas (combination) group. After the pretreatment of dentin with the previous material the adhesive bonding agents and composite were applied and polymerized. The shear bond strength was measured using the universal testing machine. A sample from each group was evaluated blindly by scanning electron microscope (SEM) to see changes in dentin morphology after treatment. The data were statistically analyzed using one-way ANOVA for inter-group general comparisons while qualitative data were analyzed using the Chi-squared test.

Results: The mean value of shear bond strength of the control group was 5.44 ± 1.45, the mushroom group was 7.55 ± 3.46, the ozone group was 10.42 ± 6.55 and the mushroom and ozone group was 7.45 ± 5.26. Comparison between the four groups regarding the shear bond strength indicated that there was a non-significant difference between the tested groups, with a *p*-value of 0.52. The SEM result showed a continuous hybrid layer in all groups with no gap formation in the combination group.

Conclusion: It was concluded that ozone and mushrooms could be employed reliably as cavity disinfectants in permanent teeth without affecting bond strength negatively. The ozone group showed the highest bond strength.

Clinical significance: Using antibacterial material before restoration is important to help in the prevention of recurrent caries and increase the longevity of restoration, and this should be performed without affecting bond strength.

Keywords: Antibacterial, Mushroom, Ozone, Scanning electron microscope, Shear bond strength.

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Introduction

During cavity preparation, the success of restorative treatment can be affected by microbial persistence or rehabilitation failure at the dentin-restorative cement interface.[1] Therefore, the inclusive elimination of microorganisms before the insertion of a restoration is decisive for an enduring dentin-restoration bond and for the longevity of restoration. Trying to completely remove deep carious dentin using only mechanical methods can lead to damage to the pulp or significant destruction of the tooth structure and has failed to generate a completely caries-free cavity.[2] Various techniques have been used to disinfect the prepared cavity and potentially to eradicate any microbial remnants that may survive and multiply as a result of microleakage, leading to pulpal irritation, risk of recurrent caries, and ultimately failure of the dental restoration.[3]

The primary objective of adhesive dentistry is to establish a strong, long-lasting bond between tooth structure and the restorative material. The resin-to-dentin adhesion occurs through the infiltration into and polymerization of hydrophilic resins within the collagen matrix exposed through acid decalcification of dentin, thus forming a hybrid layer. For etch-and-rinse adhesives, the adhesive resin monomer must penetrate the collagen fibrils in the dentine exposed to acid etching. However, research has shown that this objective is seldom achieved.[4]

Chlorhexidine (CHX) is one of the major cavity disinfectant synthetic materials with known side effects such as discoloration and taste alteration.[5]

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Recently, scientists have gone into research of natural methods for caries control to avoid side effects of synthetic material. Functional foods like mushrooms have a wide range of bimolecular with nutritional and medicinal substances and with immune-modulators, cardiovascular, liver protective, anti-fibrotic, anti-inflammatory, anti-diabetic, and anti-microbial properties.[6] On the other hand, ozone gas therapy has been suggested as an alternative noninvasive treatment aiming to reduce the levels of caries-associated microorganisms. This form of therapy could serve as an alternative or complementary treatment approach in dentistry. Ozone is an energy-rich and highly unstable form of oxygen that can rapidly and strongly oxidize bacterial cell membranes and is believed to be a strong bactericidal, antiviral, and antifungal factor.[7]

The shear bond strength test is an *in vitro* bond strength test that is useful and essential for predicting the performance of adhesive systems and its possible correlation with clinical issues.[8] This study was performed after a study performed by Alrafee et al.,[9] who found that the antibacterial effect of a combination of ozone gas and Mushroom extract resulted in high efficacy compared with each material alone to assess their effect on bond strength.

The present study was performed to test the effect of a new natural product (mushroom extract) alone or combined with ozone gas as antibacterial agents on the bond strength of composite to dentin. The null hypothesis for this study was divided into two parts; the first part was that treatment of the adhesive surfaces with ozone gas and mushroom extract before restoration placement would not affect shear bond strength between resin and dentin if compared with the control group and the second part was that combining both agents would boost their effect on shear bond strength (SBS) when compared with each disinfectant individually.

Materials and Methods

This study was performed in the clinic of the Faculty of Dentistry Al-Azhar University for girls- Cairo-Egypt in two weeks starting in April 2024.

The current *in vitro*, comparative study was appraised and inveterate by the Research Ethics Committee (REC) of the Faculty of Dental Medicine for Girls, Al-Azhar University, under Code No. REC-PD-24-11. This study was performed per the ethical standards laid down in the Declaration of Helsinki.

The G*power statistical power analysis program (version 3.1.9.7) was utilized for sample size determination. A total sample size ($n = 40$; subdivided into 10 in each group) was sufficient to detect a large effect size (f) = 0.609, with an actual power ($1 - \beta$ error) of 0.8 (80%) and a significance level (α error) 0.05 (5%) for two-sided hypothesis test.[10]

Twenty anonymous sound first or second premolars (extracted for orthodontic reasons) were collected from the Department of Oral Surgery. Teeth with fractures, enamel malformations, or other defects were excluded from the study. Teeth were meticulously scaled to eliminate calculus and remnants of periodontal tissue then polished with pumice and soft rubber cups rotating at low speed under water coolant. They were stored in distilled water at 37°C for no more than one month before being used in the experiment as described by Alsamolly.[11]

To prepare Mushroom extract, 50 gm of dehydrated Shiitake Mushroom was poached in 500 mL of distilled water until reached 10–20 mL. The remaining solution was filtered and kept in a refrigerator for 24 h until use as described by Devarasanahalli et al.[12]

The ozone was produced using the coaxial dielectric barrier discharge method. The DBD cell was implanted by oxygen gas. The concentration of the generated ozone was controlled using a discharge current and the gas flow rate was corrected to 615 mL/min. ozone analyzer (Model H1-AFX-Instrumentation, USA) was used to measure the ozone concentration inside each tube to be 2100 ppm, ±10% as described by Alrafee et al.[9]

The crowns of the twenty premolars were cut from the roots 1 mm apical to the cemento-enamel junction followed by cutting the crowns into two halves in a buccolingual direction using a 0.3 mm thick diamond disc (Buehler, IL, USA) with water-coolant, so forty samples were obtained. The pulp remnants were removed, and then the dentin surface was ground to be flat and smooth as described by Chaharom et al.[13] Rectangle split Teflon mold was used to mount the specimens in self-cured acrylic resin with their dentin surface upward then the samples were removed and stored in distilled water.

A total of 40 samples were randomly divided into four groups ($n = 10$):

- Group I: control group in which dentine samples received no previous treatment.
- Group II: Aqueous extract of the mushroom group in which 1 mL of the extract was applied on each dentin sample for 30 seconds followed by gentle air drying as described by Goel et al.[14]
- Group III: Ozone gas group in which ozone gas was applied for 30 seconds as described by Johansson et al.[15]
- Group IV: Aqueous extract of mushroom + ozone gas (combination) group in which the ozone gas was applied first for 30 seconds followed by application of aqueous extract of mushroom for 30 seconds then gentle air drying.

After treatment of the samples with previous antibacterial agents, a self-etching adhesive system (Tetric N-Ceram, Ivoclar Vivadent, Schaan, Liechtenstein) was utilized on the dentin surface of all samples by brush with scrubbing motion for 20 seconds followed by gentle air drying for 5 seconds to obtain thin adhesive layer then curing for 20 seconds using LED curing device (Elipar, 3M ESPE, St Paul, Minnesota, USA) as described by Tayal et al.[16] A transparent cylinder (2 mm in diameter and 2 mm in height) was filled with composite resin (Shofu Inc, Kyoto, Japan shade A1), utilized onto the dentin surface then covered with a piece of clear matrix band and squeezed with piece of glass slab of 8 mL thickness (to standardize the weight applied on composite during packing). After removing the excess composite with a sharp instrument (tip of explorer), the glass was removed and the composite was cured for 20 seconds with an LED curing device (Elipar, 3M ESPE, St Paul, Minnesota, USA) where the tip of the light conductor was perpendicular to the surface at zero direction. The specimens were stored in distilled water at 37 °C for 24 h after the removal of transparent cylinders as described by Chaharom et al.[13] All the specimens were then subjected to SBS analysis.

The bond strength was evaluated using a circular interface shear test was designed to evaluate. All samples were individually mounted on a computer-controlled universal testing machine (Model 3345; Instron Industrial Products, Norwood, USA) with a load cell of 5 KN then data were recorded using computer software (Bluehill Lite; Instron Instruments). Samples were fixed to a specially designed sample holder secured to the lower fixed compartment of the testing machine by tightening screws. The shearing test was done by compressive mode of load applied at the tooth-resin interface using a mono-beveled chisel-shaped metallic rod attached to the upper movable compartment of the testing machine traveling at a cross-head speed of 0.5 mm/min. The force needed to lead to de-bonding was measured in Newton.

Shear Bond Strength Calculation

The load at failure was divided by bonding area to express the bond strength in MPa: $\tau = P/\pi r^2$, where τ = shear bond strength (MPa),

Table 1: Represents the mean ± standard deviation (SD) of the shear bond strength (MPa) between different tested groups

			95% Confidence interval for mean					
Groups	*Mean*	*Std. deviation*	*Lower bound*	*Upper bound*	*Minimum*	*Maximum*	*F*	*p-value*
Control	5.44	1.45	3.12	7.76	3.36	6.48	0.794	0.52ns
Mushroom	7.55	3.46	2.03	13.07	2.85	10.83		
Ozone	10.42	6.55	0.001	20.85	5.10	19.71		
Mushroom and ozone	7.45	5.26	–0.9231	15.823	2.16	14.57		

ns, non-significant ($p > 0.05$)

P = load at failure (N), $\pi = 3.14$, and r = radius of resin disc (mm) specimens in each test groups were viewed using a USB digital-microscope (U500x Digital Microscope, Guangdong, China), magnification × 25, and the images were captured and transferred to a personal computer equipped with the Image-tool software (ImageJ 1.43U, National Institute of Health, USA) to determine failure mode patterns according to the following criteria; adhesive, cohesive or mixed.

Technique; the images were taken with the following image acquisition system:

- Digital camera with 3 mega pixels of resolution, placed vertically at a distance of 2.5 cm from the samples. The angle between the axis of the lens and the sources of illumination is approximately 90°.
- Illumination was achieved with eight LED lamps (adjustable by control wheel), with a color index close to 95%.

  The images were taken at maximum resolution (2272 × 1704 pixels) and connected with a compatible personal computer using a fixed magnification of 25×. The images were recorded with a resolution of 1280 × 1024 pixels per image.

One sample from each group was examined by scanning electron microscope (SEM) to confirm microscopic imaging. The four samples were treated with an antibacterial agent according to their group then the adhesive resin and the composite were applied as mentioned previously. The specimens were cut cervico-occlusal through the center of the composite core into two halves using a diamond disk and copious amounts of water. The cut surfaces were acid etched with 35% phosphoric acid for 15 seconds, rinsed for 20 seconds, and then, gently air dried. The specimens were immersed in 5% sodium hypochlorite for 120 seconds and washed under running water for 5 minutes. The specimens were dehydrated in ascending concentrations of ethanol, 50, 70, and 90% for 20 minutes each, and then immersed in 100% ethanol for 1 hour. The specimens were left to dry on absorbent paper in a closed container overnight as described by El-Askary and Nassif.[17] The specimens were gold coated via sputtering with a thin layer of gold under vacuum to render the specimen's surfaces electrically conductive. The specimens were scanned using SEM Model Quanta 250 FEG (Field Emission Gun) attached with EDX Unit (Energy Dispersive X-ray Analyses), with accelerating voltage 20 kV, magnification 14× up to 1,000,000 and resolution for Gun.1n. The examined surface was viewed at magnification 1000×.

Statistical Analysis

Study data were analyzed using SPSS version 26 as mean ± SD for numerical data. p-values ≤ 0.05 were regarded as statistically significant. Numerical Values were explored for normality by checking the data distribution and using Kolmogorov–Smirnov and Shapiro–Wilk tests. They were normally distributed in all groups.

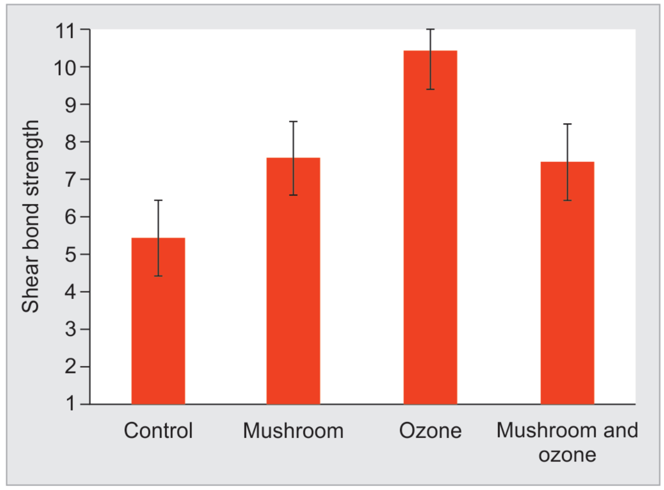


Fig. 1: Bar chart represents SBS of different tested groups

Statistical analysis was done for numerical data by using one-way ANOVA analysis for inter-group general comparisons. Qualitative data were analyzed using the Chi-squared test.

Results

Shear Bond Strength Results

The shear bond strength was expressed in MPa. The mean and standard deviation (SD) of the shear bond strength of the control group were (5.44 ± 1.45), the mushroom group was (7.55 ± 3.46), the ozone group was (10.42 ± 6.55) and the mushroom and ozone group were (7.45 ± 5.26). Comparison between the four groups regarding the shear bond strength using one-way ANOVA expressed that there was non-significant difference among the tested groups with a p-value of 0.52). The highest mean and standard deviation (SD) values of bond strength (MPa) were recorded in the ozone group while the lowest Mean and standard deviation (SD) values of bond strength were recorded in the control group (Table 1 and Fig. 1).

Failure Pattern Results

The elevated percentage of cohesive patterns was listed in the ozone group (100%), while the lowest percentage of mixed patterns was recorded in the ozone group (0%). Also, the lowest percentage of adhesive patterns was recorded in the ozone group and control group (0%). The mixed and cohesive pattern in the control group was (75 and 25%), respectively.

However, both the mushroom group and the mushroom and ozone group) recorded a combination of the three patterns of failure (mixed, cohesive, and adhesive failure) with the following

Table 2: Represents the failure pattern between different tested group

	Group					
Failure pattern	*Control (%)*	*Mushroom (%)*	*Ozone (%)*	*Mushroom and ozone (%)*	χ^2	*p-value*
Mixed	75	50	0	25		
Cohesive	25	25	100	50	8.33	0.21 ns
Adhesive	0	25	0	25		

ns, non-significant ($p > 0.05$)

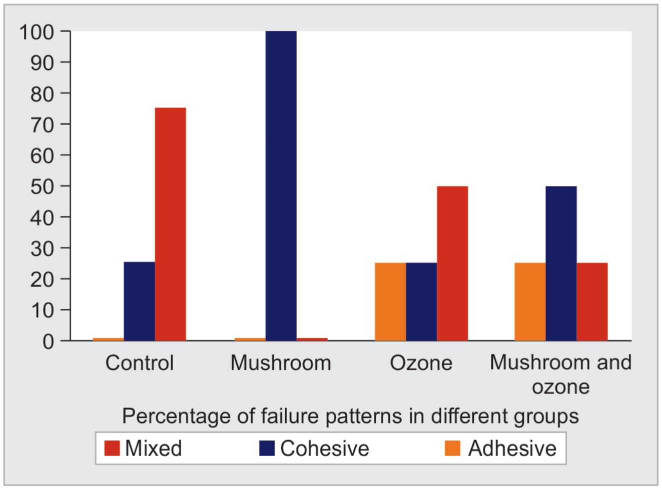


Fig. 2: Bar chart represents failure pattern between different tested groups

percentage in the Mushroom group (50, 25, and 25%) respectively and (25, 50, and 25%) for the mushroom and ozone group, respectively. A Chi-squared test revealed that there was a non-significant difference between failure patterns in the different tested groups with a *p*-value of 0.21 (Table 2 and Fig. 2).

Scanning Electron Microscopic Results

The SEM pictures are shown in Figure 3. A SEM of the control group revealed that there was a uniform hybrid layer with nearly no gap and thin resin tags extended to a short distance with no lateral branches (Fig. 3A). The mushroom group showed thick uniform hybrid layer with slightly gap formation and thin resin tags (Fig. 3B). Ozone group showed thin uniform hybrid layer with no gap formation (Fig. 3C). The mushroom and ozone group revealed a thin uniform hybrid layer with obvious gap formation and thin short resin tags (Fig. 3D).

The tested materials can be used effectively without affecting bond strength negatively. The ozone group showed the highest bond strength while the control group showed lower bond strength.

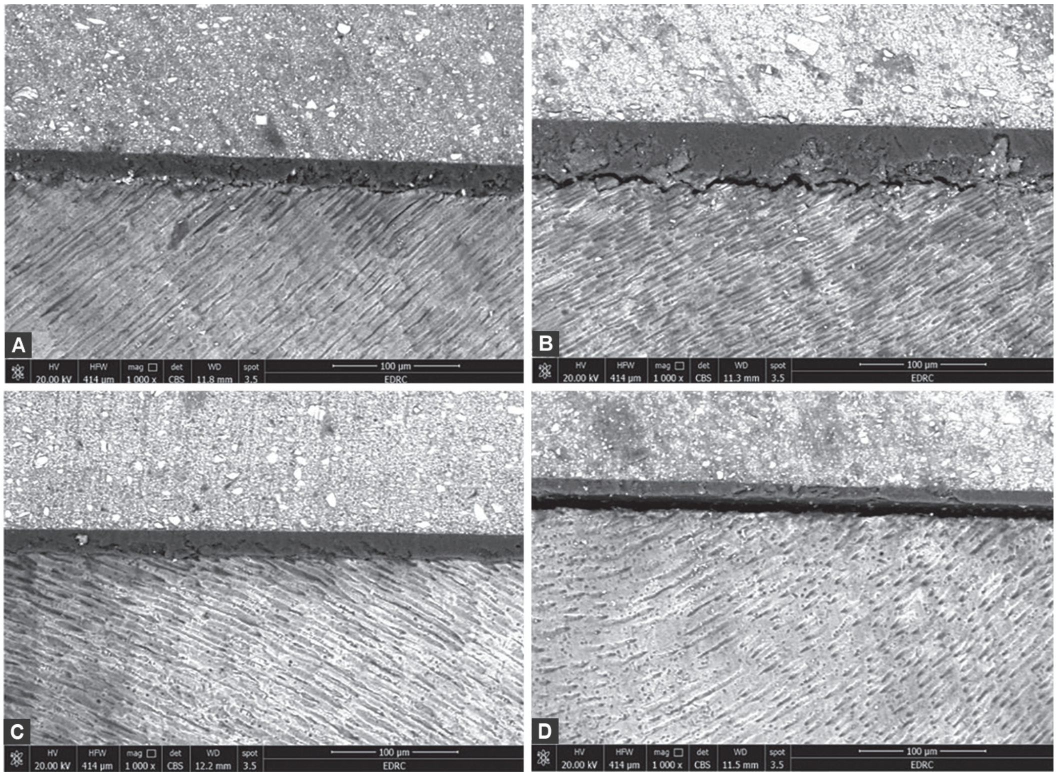
Discussion

The effectiveness of adhesion between resin and dentin surface depends on the surface quality of the adhering site and its surface energy. Chemical preparation of the adhering site can positively change the surface quality by changing its area, surface energy, and adhesive potential, so enhances the micromechanical retention of the resin.[18] Additionally, research has shown that even for one year the microorganisms that remain in the cavity may be fertile and are capable of causing recurrent caries in the presence of micro-leakage, hence leading to failure of restoration.[19]

According to Kapdan and Öztaş,[7] "The bond strength is the potential of the unit area in the tooth restoration interface." This strength is often evaluated using shear and adhesion tests. SBS testing was used in this study because the restoration in oral cavity conditions is normally subjected to shear stresses during mastication. An efficient and low-cost SBS approach has been used to provide a relative statistical analysis by simulating oral ambiance and dispersing interfacial stress sustaining other variables.[1] Failure mode analysis provides valuable information for better interpretation of bond strength results.[19] Despite the limitations of the shear test, the ease of sample preparation, minimal laboratory equipment needed, lower incidence of pretest failure, ease of specimen alignment with the loading device, and overall non-technique sensitivity make it an extremely used technique for the evaluation of dental adhesives.[20]

Ozone gas is one of the famous pharmaceutically least invasive techniques for caries prevention and healing. It can be used separately or combined with other techniques to manage decay which offers a conservative approach and shorter periods of mouth opening.[9] Both gaseous ozone and ozonated water have been recently introduced as an alternative way for cavity disinfection due to their known antimicrobial and strong antioxidant properties. Polydorou et al.[21] reported that gaseous ozone eliminated 99.9% of the microorganisms in carious lesions in 20 seconds. Besides its strong antimicrobial effects, particularly against *Streptococcus mutans*, ozone also possesses antifungal and antiviral properties. Authors analyzing the effect of either ozonated water or gaseous ozone on adhesion reported positive results, which may be justified by the opening of the dentinal tubules caused by oxygen.[21] Although there is limited information about the use of ozone as a cavity disinfectant in primary teeth, it looks like a promising alternative.[22]

On the other hand, regarding the mushroom's chemical structure, it was revealed that it contains erythritol which is classified as a non-cariogenic sweetener. It has been reported that *S. mutans* and *Streptococcus sobrinus* showed no adherence to glass in the presence of erythritol, which suggests that erythritol is not consumed by these bacteria so the byproduct lactic acid is not produced.[23] Shiitake mushrooms have proved their bacteriostatic action by inhibiting the synthesis of DNA and by the low molecular mass (LMM) fractions which induce bacteria elongation with interrupted septa. The morphogenetic result performed by the mushroom is the same as that observed in streptococcal thermo-sensitive temperature or exposure to inhibitory doses of B-lactam antibiotics.[24] Moreover; An *in-vitro* study previously performed by Alrafee et al.,[9] compared the effect of Ozone and mushroom extract on Streptococcus mutans bacterial count, and the results showed higher bacterial sensitivity to mushroom extract than ozone.



Figs 3A to D: Scanning electron microscope of different samples with 1000 × magnification (A) Control group; (B) Mushroom group; (C) Ozone group; (D) Mushroom and ozone group

The same study has proved that combining mushroom extract and ozone caused synergistic antibacterial action on *S. mutans* and indicated a statistically significant difference when compared with the results of each material alone.

There is a gap in the literature correlating the effect of mushrooms as a cavity disinfectant with the adhesion bond strength of dentin to resin. Therefore; In the present *in-vitro* study, when ozone gas and Aqueous extract of mushroom were used individually and in combination for cavity disinfection (as per manufacturer's instructions), the results showed that before using a self-etch dentin bonding agent, there was a non-significant increase in shear bond strength in contrast with that of control group. While the ozone group indicated a greater increase in shear bond strength than other groups.

The results of the current study were similar to the observation by Nisar et al.,[1] who found that ozonated water disinfection unveiled the highest SBS for the adhesive bond to the caries-affected dentin surface of deciduous teeth. This is probably due to the high oxidative potential of oxygen which helps in de-occluding the dentinal tubules by cleaning out organic debris and assisting in adhesive penetration forming resin tags (hybrid layer), therefore boosting SBS with higher antimicrobial efficacy. In addition, the Magni et al.[25] study confirmed a similar outcome to the current study in which ozone treatment resulted in non-significant differences in the mechanical properties of the adhesive systems.

Conversely, few researchers negate this finding, the differences in the fallouts of other studies could be accredited to the use of different sorts of adhesives, ozone application duration, dosages, and ozone equipment variations.[26] The present results revealed that the lowest SBS was detected in the control group. The combination group showed lower SBS than that of the mushroom group alone which contradicts the hypothesis of synergistic effect of the use of both agents together. According to a previous study by Cangul et al.,[27] the mean value bonding strength of the adhesive resins to dentin after the application of ozone as a cavity disinfectant in three subgroups, ranged from 3.43 ± 1.03, 7.79 ± 3.97, 5.18 ± 1.8. Based on Cangul's study, ozone adhesive systems can be safely currently used as a cavity disinfectant because they have been shown to increase bonding strength and eliminate bacteria at the same time.

Regarding failure patterns, there was no significant difference between groups in the current study. Adhesive fractures occurred only in mushroom and combination groups (25%) each; however, cohesive fractures were the common type of fracture in all groups, mostly recorded in the ozone group (100%) followed by the combination group (50%). No mixed-type failure was found in the ozone group while the control group showed the highest percentage of mixed fractures (75%). According to Candan et al.,[28] it is important to establish ideal adhesion between the dentin and the restoration to achieve optimal clinical restoration. In general, the bond is acceptable when a fracture occurs within dental restorative material rather than at the bonded interface (i.e., cohesive instead of adhesive), so prevailing of the cohesive fractures is an indication of clinically successful bond strength. Similarly, cohesive patterns of fractures were frequently detected among the groups evaluated in the present study. Based on the above-mentioned results, the first part of the null hypothesis of this study was accepted while the second part was rejected.

The development of a hybrid layer is a determinant of the clinical outcome of restorations bonded to dentin.[29] Therefore, the presence and thickness of the hybrid layer were examined with SEM in an attempt to assess the interfacial seal between composite and dentine and to determine the quality of this seal. According to the

SEM results of the current study, all sample teeth showed a visible uniform hybrid layer between composite and dentin. Both the ozone group and control group revealed no interfacial gap between restoration and dentin with thin resin tags. On the other hand, the mushroom group showed a thick hybrid layer with slight gap formation and thin resin tags while the combination group revealed a thin hybrid layer with obvious gap formation and thin short resin tags. This may be due to the chemical residue that comes from mushroom extract having a decrease in the wettability of dentin bonding resins and causing a decrease in its ability to penetrate the dentin surface.[30] Türkün et al.[30] suggested that a cavity sterilizer could enhance the sealing effectiveness of dentin bonding agents by rehydrating the cavity before applying the bonding material that adheres to moist tooth surfaces. However, this depends on the chemical structure of the cavity disinfectant and its interaction with the tooth substrate and the adhesive bonding agents. Venugopal[31] noted that the sealing ability of certain adhesive systems appears to be inhibited by residual disinfecting agents. Contrarily, Pradhan et al.,[32] found that Chitosan as a cavity disinfectant improved the sealing ability of the self-etch adhesive when compared with CHX, octenidine dihydrochloride, and the control groups.

The findings of the present study suggested that the pretreatment of the prepared cavity with antimicrobial agents would not significantly affect the shear bond strength of composite to dentin. However, ozone as a cavity disinfectant showed better interfacial sealing than the mushroom and mushroom-ozone combination.

The limitation of this study was performing it *in vitro*. The strength of this study was that a combination of ozone gas and mushroom extract can be used safely in cavity disinfection without affecting bond strength. However, further long-term *in vitro* and clinical studies are required to estimate the effectiveness of these cavity disinfectant agents on bond strength and interfacial seal.

Conclusion

It was concluded that ozone and mushrooms could be employed reliably as cavity disinfectants in permanent teeth as they demonstrated acceptable shear bond strength without jeopardizing the adhesive binding capacity of composite to dentin. The pretreatment of dentin with the Ozone alone resulted in a better-sealed interface similar to that of the control group.

Author Contributions

Menna-Allah Salem Ali contributed to the conception and design, data acquisition, analysis, and interpretation, and drafted and critically revised the manuscript. Shaimaa Ahmed Alrafee contributed to the conception and design, data analysis and interpretation, and critically revised the manuscript. Noha I. Metwally and Aytallah Salem contributed to the conception and design, data acquisition, and critically revised the manuscript. Sherine Badawy and Shahenda Ahmed Abdallah contributed to the conception, design, data acquisition, analysis, and interpretation and critically revised the manuscript. All authors gave their final approval and agreed to be accountable for all aspects of the work.

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