Current Research in Dentistry

Research Article

Bond Strength of Bioactive vs. Conventional Resin Composites on Dentin Cleaned with Ozonated Water: An In Vitro Study

[1]Fernanda Rafaela Ribeiro, [1]Anna Caroliny Detogni, [2]Julio Katuhide Ueda, [1]Mario Alexandre Coelho Sinhoreti and [2]Veridiana Camilotti

[1]Department of Restorative Dentistry, Dental Materials Division, Piracicaba Dental School, State University of Campinas, Piracicaba, São Paulo, Brazil
[2]Department of Restorative Dentistry, Faculty of Dentistry, State University of Western Paraná, Cascavel, Paraná, Brazil

Article history
Received: 26-02-2025
Revised: 20-04-2024
Accepted: 28-04-2025

Corresponding Author:
Fernanda Rafaela Ribeiro
Department of Restorative Dentistry, Dental Materials Division, Piracicaba Dental School, State University of Campinas, Piracicaba, São Paulo, Brazil
Email: fernandarafaela1377@gmail.com

Abstract: This study aimed to compare the microtensile bond strength (μTBS) of bioactive and conventional resin composite to human dentin after the use of ozonated water (AO) as a cavity cleaning solution. A total of 32 healthy human third molars were randomly assigned to four groups (n = 8) based on the type of composite and dentin cleaning. The first group received Beautifil Flow Plus F00 with ozonated water (BFP AO). The second group received Opus Bulk Fill with ozonated water (OBF AO). The third group received Beautifil Flow Plus F00 with distilled water (BFP AD), and the fourth group received Opus Bulk Fill with distilled water (OBF AD). The samples were stored in distilled water at 37°C for 24 h before being tested in μTBS. Failure modes were examined under a stereomicroscope at 40x magnification and categorized as cohesive-dentin, cohesive-resin, adhesive or mixed. Subsequently, representative specimens from each group were selected for morphological analysis using scanning electron microscopy. Data were initially evaluated for normality using the Shapiro-Wilk test. Given the non-normal distribution, the Kruskal-Wallis test followed by the Dwass-Steel-Critchlow-Fligner post hoc test were employed for statistical analysis. The results showed significantly higher bond strength for the BFP AO group (39.03 MPa; $p \leq 0.05$) than the other groups. It can be concluded that ozonated water, when used as a cavity cleaning solution, followed by the application of a bioactive system, significantly enhances the bond strength to dentin.

Keywords: Resin Composite, Bond Strength, Ozone, Giomer

Introduction

Resin composites are considered one of the greatest successes in modern biomaterial development, as they effectively mimic natural tissue in both appearance and function (Boaro *et al.*, 2019). Despite their widespread use, resin composites face significant challenges in adhesion, such as polymerization shrinkage stress and differences in thermal expansion between the resin and tooth structure. These issues can lead to marginal leakage, reduced bond strength and material failure, emphasizing the need for improvements in adhesive bonding (Dias *et al.*, 2018).

Consequently, a huge demand for restorative procedures in dental clinics focuses on replacing resin composite restorations (Noaman and Fattah, 2022). It is estimated that 56% of restorative procedures performed involve the removal and replacement of existing restorations (Wilson *et al.*, 2016). Due to data, there is currently a wide range of clinical trials and literature reviews in the literature on the performance of composite resin in posterior teeth. According to Opdam (Opdam *et al.*, 2014), in his systematic review, 2,816 resin composite restorations were evaluated, of which 2,585 were Class II and 231 were Class I. During the observation period, a total of 569 failures were recorded.

Giomers were introduced to the dental market to combine the aesthetic and mechanical properties of resin composites with the advantages of glass ionomers. Classified as new hybrid restorative materials, the pre-reacted glass ionomer is immersed in a resin matrix (Burtea *et al.*, 2019; Rusnac *et al.*, 2019) with bioactive particles that release multifunctional ions and are usually activated by acids. It has "Surface Pre-reacted Glass Ionomer" (S-PRG) technology, where the surface is partially reacted and already treated with silane, in addition to unreacted filler particles and a mixture of monomers and camphor Quinone. This system is more

reactive than conventional fluoro alum inosilicate particles from other materials; therefore, there is no need for acid to cause the activation of ionic release, unlike compomers (Francois *et al.*, 2020). Borate, fluoride, silicate and aluminum have antibacterial and bacteriostatic actions and cumulative effects against caries formation, preventing demineralization and favoring mineralization (Miki *et al.*, 2016; Okamoto *et al.*, 2019; Spinola *et al.*, 2020).

Cavity cleaning is an essential technical procedure for disinfection, protecting the tooth structure from post-operative sensitivity and recurring caries disease. In this sense, ozone is a strong antimicrobial agent, considered one of the fastest and most efficient microbicides. It can be used in its most reactive form as gas or diluted in water and/or oil (Dos Santos Vianna Néri, 2022). Coelho *et al.* (2020) proposed the use of ozonated water as a cavity disinfectant before restorative procedures due to its substantivity offering several advantages attributed to its antioxidant, healing and remineralizing properties, which may contribute to enhanced bond strength to the dental substrate (Ihsan Hubbezoglu, 2018; Yeşilöz Gökçen *et al.*, 2019; Dos Santos Vianna Néri, 2022).

Furthermore, it is important to note that studies involving ozonized water as a cavity-cleaning solution, as well as the use of S-PRG particles in the adhesive system and resin composite, are scarce. Therefore, this study aimed to compare the bond strength of a bioactive and a conventional resin composite on dentin treated with ozonized water. The null hypothesis tested was that bioactive resin composite, when bonded after using ozonized water, would not demonstrate greater μTBS to dentin than conventional resin composite.

Materials and Methods

Study Design

The experimental design corresponds to a laboratory study involving human third molars indicated for extraction from patients at the UNIOESTE dental clinic. Before using these teeth, the project was submitted to the UNIOESTE human research ethics committee under number 77767123.6.0000.0107. After approval, participants reviewed and approved the Informed Consent Form (ICF) for this study.

Eligibility Criteria

Previously extracted human third molars were included in the study, which can be included or erupted with a completely healthy surface. Teeth presenting any surface alterations were excluded from this study.

Sample Size Calculation

The sample size calculation was performed based on probability distributions from the t-test family (Wilcoxon and Mann-Whitney tests for comparing two groups). The used effect size of 0.8, type 1 error (α) of 0.05 and analysis power (β error) of 0.8 resulted in a total of 8 teeth per group. The sample size calculation was performed using the GPower software, version 3.1.9.2 (University of Düsseldorf, Germany).

Groups Distribution

The third molars were randomly assigned to 4 groups according to the system used: FL-bond II and Beautifil Flow Plus 00- F03 (Shofu, Kyoto, Japan) (BFP AO; BFP AD) and Ambar Aps and Opus Bulk Fill APS – A3 (FGM, Joinville, SC, Brazil) (OBF AO; OBF AD) together with cavity cleaning mode with ozonated water or distilled water. After extraction, all tissue that remained around the teeth was removed. Then, they were stored in saline and kept at room temperature.

Preparation of Ozonated Water

Ozonized water 4 ppm7 was prepared at room temperature of 25±1.0°C, 5±1.0 min before use and used up to 5±1.0 min after preparation, using a generator device ozone system (Ozone &Life® /O&L3.0RM, São José dos Campos, SP, Brazil) that uses pure oxygen from a cylinder, coupled to a glass tower (1 L/min) The ozone concentration in the water was measured using iodometric titration, as recommended by the International Ozone Association (IOA). This involves adding 50 mL of 1 N potassium iodide (KI) solution to ozonized water, where KI is oxidized by ozone, releasing iodine (I2). To ensure the production of I2, it was necessary to acidify the medium by adding 2.5 mL of sulfuric acid (H2SO4), 1 N, to the KI solution. Subsequently, the titration was carried out with sodium thiosulfate (Na2S2O3), 0.01 N, until the yellowish color of the iodine became barely noticeable. Next, 1 mL of 1% starch indicator solution was added, resuming the titration until the blue color of the solution disappeared. The 4 ppm concentration was chosen based on research by Kumari *et al.* (2023), which showed it improved adhesive bond strength. The ozonated water was used immediately after production, applied to the dentin surface for 30 sec, maintaining its antimicrobial efficacy.

Preparation of Distilled Water

Distilled water was applied using a 120 mL syringe to the prepared teeth.

Cavity Preparation

After removing the dental enamel from the occlusal surface of the third molars, cavities were prepared in exposed dentin for the subsequent addition of resin composite blocks (5mm in height) covering the entire tooth surface. To prepare the cavity, a spherical diamond bur (#1014, KG Sorensen; Cotia, SP, Brazil) was used, followed by a cylindrical diamond bur (#2094, KG Sorensen; Cotia, SP, Brazil) and finishing with a superfine diamond bur (#1093FF, KG Sorensen; Cotia,

DOI: 10.3844/crdsp.2025.1.9

SP, Brazil). Diamond tips are replaced after five preparations to maintain effective cutting. The depth of the cavity was measured with a millimeter periodontal probe (Golgran; São Caetano do Sul, SP, Brazil).

Table 1: Description of materials used

Material	Composition	Manufacturer	Mode of use
FL- Bond II	Primer: Carboxylic acid monomer, phosphinic acid monomer, 6-MHPA, water, solvent and photoinitiator. Adhesive: HEMA, UDMA, TEGDMA, 40% S-PRG-filler particles and photoinitiator	Shofu, Kyoto, Japan	Step 1: Apply the Primer to dentin and enamel, rest for 10 sec and dry with oil-free air for 5 sec (no rinsing). Step 2: Apply a uniform layer of the Bonding Agent and light cure for 10 sec with halogen light or 5 sec with LED
Beautifil Flow Plus F00	Base resin: Bis-GMA (15%) / TEGDMA (13%) Fillers: Multifunctional glass and S-PRG filler (fluoroboroaluminosilicate glass) Filler content: 67.3% by weight (47.0 vol%) Particle size: 0.01-4.0 μm (average 0.8 μm) Photoinitiator: DL-Camphorquinone	Shofu, Kyoto, Japan	For deep cavities, apply and cure in layers up to 4 mm, light-curing each layer for 10 sec LED light
Ambar APS	Active: MDP, methacrylate monomers, photoinitiator (APS), co-initiators, stabilizers Inactive: Silica particles (filler), ethanol (vehicle).	FGM, Joinville, SC, Brazil.	Apply two adhesive layers: First layer: Rub for 10 sec. Second layer: Apply for 10 sec, then dry with air for 10 sec. Light cure for 10 sec
Opus Bulk Fill APS A3	Active ingredients: urethane dimethacrylate monomers, stabilizers, photoinitiator (APS) and co-initiators. Inactive ingredients: silanized silica filler, stabilizers and pigments.	FGM, Joinville, SC, Brazil	Apply OPUS BULK FILL APS in increments of up to 5mm. Light cure each increment for 40 sec with a light cure power of 450-1000 mW/cm², or for 30 sec with a power of 1000-2000 mW/cm²

Bis-GMA: Bisphenol-A-diglycidyl methacrylate; TEGDMA: triethylene glycol dimethacrylate; 6-MHPA: 6-methacryloxyhexyl 3-phosphonoacetate; HEMA: 2-hydroxyethyl methacrylate; UDMA: urethane dimethacrylate; S-PRG filler: Pre-reacted glass ionomer filler on the surface.
(Shofu Dental Corporation, Japan) (FGM Dental Group, Brazil).

Restorative Procedure

After the dentin was exposed and completely plain, the bonding protocol was carried out, using FL- Bond (SHOFU, Kyoto, Japan) for the Beautifil Flow Plus resin composite and Ambar Universal - APS (FGM, Joinville, SC, Brazil) for the Opus Bulk Fill resin, following the manufacturer's recommendations for application (Table 1). The resin composite was inserted in such a way as to build a block 5 mm high. The photoactivation procedures used the Valo light-curing unit (Ultradent Products, South Jordan, UT, USA; radiant emittance of 1400 mW/cm^2). The increments of the Beautifil Flow Plus -00 resin groups were light-cured for 10 sec and those of the Opus Bulk fill - APS groups for 30 sec, following the manufacturer's guidelines.

Microtensile Bond Strength Test

The teeth were then positioned in a cutting machine containing a diamond blade (Isomet 1000, Buehler; Lake Bluff, IL, USA) and sectioned in the mesiodistal and buccolingual directions to obtain sticks with a cross-sectional area of 0.75±0.1 mm 2 and the long axis perpendicular to the pulp wall.

The section area of the sticks was measured with a digital caliper to transform kgf into MPa. The sticks were then attached to a microtensile device (SG01, Geraldeli's Jig) with cyanoacrylate adhesive (Henkel; Itapevi, SP, Brazil) and loaded to tensile stress using a universal testing machine (Instron 4411; Canton, MA, USA) at a cross-head speed of 1.0 mm/min^2 until failure. The fractured sticks were examined with an optical microscope (Leica Microsystems; Wetzlar, Germany) at 40X magnification by a single-blinded and calibrated operator to determine the failure mode.

Fracture Type Analysis

The fractured composite-dentin interfaces were analyzed under a stereoscopic loupe at 40x magnification (Olympus SZ40, Japan). The fracture types were classified as: Adhesive (A): failure at the resin composite-dentin interface; Mixed (M): failure at the adhesive/dentin/resin composite interface, including cohesive and adhesive failure; Cohesive in resin composite (CC): failure exclusively in the resin composite; Cohesive in dentin (CD): failure exclusively in dentin.

Scanning Electron Microscopy (SEM)

Common procedures for preparation of the specimens for examination by Scanning Electron Microscopy (SEM) for failure analysis were undertaken, including fixation with 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.3 (Karnovisky's fixative), dehydration in ascending concentrations of ethanol (25, 50, 75, 95 and 100%) and immersion in hexamethyldisilazane (HMDS) for 10 min to chemical drying (12). After mounting on aluminum stubs, the specimens were sputtered coated with gold/palladium (SCD 050; Balzers, Schaan, Liechtenstein) and examined using a scanning electron microscope (JSM 5600LV; JEOL, Tokyo, Japan) operating at 15 kV in different magnifications (x500, x1000 and x2000).

DOI: 10.3844/crdsp.2025.1.9

Statistical Analysis

Statistical analysis was done using Jamovi software (Jamovi, Version 2.3, Computer Software, https://www.jamovi.org). Initially, the Shapiro-Wilk test was applied to assess the normality of the data for the quantitative dependent variable (bond strength). Since the data did not follow a normal distribution, data transformation was considered. However, none of the tested transformations achieved a satisfactory normal distribution. Therefore, the non-parametric Kruskal-Wallis test was used to compare the groups ($p<0.001$). This analysis was complemented with the Dwass-Steel-Critchlow-Fligner (DSCF) test for multiple comparisons.

The fractured specimens were then evaluated using a stereoscopic magnifying glass at 40X magnification. These fractures were categorized as adhesive, cohesive in resin composite, cohesive in dentin, or mixed. The results of this classification were then subjected to a detailed analysis using descriptive statistics. Spearman's correlation test was also used to assess possible correlations between adhesive strength and fracture type.

Results

To understand the distribution of the data, the median values and interquartile range of the microtensile bond strength of the groups are shown in (Table 2). The Shapiro-Wilk test revealed that the samples from all the groups do not follow a normal distribution, as the p-value was less than 0.05, indicating an asymmetrical distribution.

Due to the unequal size of the samples - BFP AO group (n = 18), BFP AD group (n = 85), OBF AO group (n = 77) and OBF AD group (n = 69) - and the lack of normality in the data, the Kruskal-Wallis test was used to compare the groups. The result ($p<0.001$) indicated a statistically significant difference among the groups, leading to the rejection of the null hypothesis (Figure 1). This suggests that at least one of the groups differed significantly from the others in terms of bond strength.

Table 2: The median bond strength (MPa) and Interquartile Range (IQR) of the groups evaluated; Different letters in the column show a statistically significant difference ($p<0.001$); *Significant difference

Group	Median	Interquartile range (IQR)
BFP AO	39.03^{a*}	21.06
BFP AD	9.89^{b}	20.27
OBF AO	10.02^{b}	8.34
OBF AD	10.85^{b}	9.91

To identify which groups differed significantly from each other, the Dwass-Steel-Critchlow-Fligner (DSCF) test, a non-parametric multiple comparison test, was performed. The results showed statistically significant differences, with the following p-values: BFP AO vs. BFP AD ($p < 0.001$), BFP AO vs. OBF AO ($p<0.001$), BFP AO vs. OBF AD ($p<0.001$), BFP AD vs. OBF AO ($p = 0.753$), BFP AD vs. OBF AD ($p = 1.000$) and OBF AO vs. OBF AD ($p = 0.521$). As shown in Table (2), the BFP AO group exhibited significantly higher microtensile bond strength compared to the other groups (BFP AD, OBF AO and OBF AD).

Figure (1) shows a box plot of bond strength results. The box represents the dispersion of the data between the first and third quartiles. The central horizontal line represents the median. In each box, vertical lines extend to the minimum and maximum values obtained and different letters indicate a statistically significant difference.

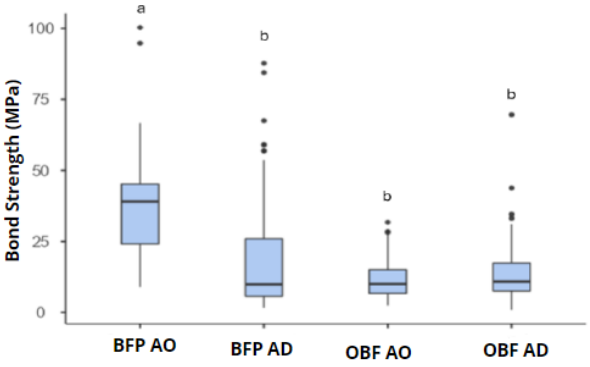

Fig. 1: The "BFP AO" group shows a significant difference compared to the other groups, represented by the letter "a"

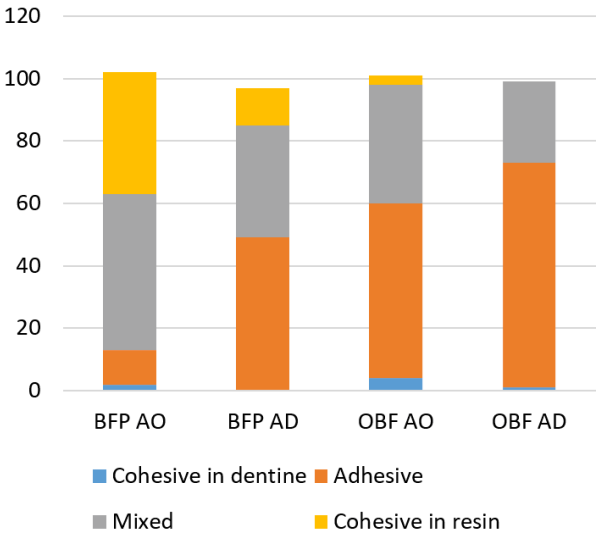

Fig. 2: Percentage of fracture mode in the groups

After the microtensile test, the specimens were assessed for fracture mode using a stereoscopic magnifying glass at 40x magnification. It was observed that most fractures in the BFP AO group were classified as mixed, representing 50% of this group. In the other groups, fractures were predominantly adhesive (49% in the BFP AD group, 56% in the OBF AO group and 72% in the OBF AD group) (Figure 2).

DOI: 10.3844/crdsp.2025.1.9

Spearman's correlation analysis revealed a weak positive correlation ($\rho = 0.283$, $p<0.001$) between bond strength (MPa) and fracture type in a sample of 249 observations, with a 95% confidence interval. This result suggests a gradual increase in the incidence of fractures, from cohesive in dentin to adhesive, mixed and finally to cohesive fractures in resin composite, as the bond strength increases.

Scanning Electron Microscope (SEM)

Fracture patterns were initially classified under 40× stereomicroscopy to determine the predominant failure mode. Subsequently, representative specimens from each fracture type were selected for detailed morphological characterization using Scanning Electron Microscopy (SEM), allowing for a more comprehensive evaluation of the adhesive interface.

In Figure (3), image A, a predominantly adhesive failure is observed, characterized by the detachment at the resin–dentin interface with minimal resin composite remnants on the dentin surface. Image B demonstrates a cohesive failure within the dentin, evidenced by the presence of an intact resin composite layer adhering to the fractured dentin substrate. Image C reveals a mixed failure pattern, with the coexistence of adhesive failure zones and areas exhibiting cohesive fracture within both the dentin and resin composite, indicating partial interaction and bonding. Image D shows a cohesive failure within the resin composite, with visible striations and fracture lines confined to the composite structure, suggesting adequate adhesion to the underlying substrate.

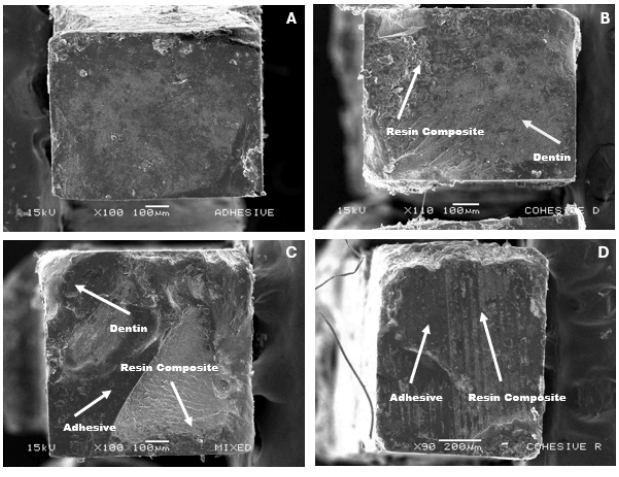

Fig. 3: SEM analysis

Scanning electron microscopy (SEM) images of representative specimens from each fracture type; (A) Adhesive failure at the resin–dentin interface, with minimal composite remnants on the dentin surface (×100); (B) Cohesive failure within dentin, with the composite layer intact and adhering to the dentin substrate (×110); (C) Mixed fracture pattern, showing both adhesive failure zones and cohesive fracture areas in both dentin and resin composite (×100).(D) Cohesive failure within the resin composite, with visible striations and fracture lines confined to the composite material (×90).

Discussion

The new generation of hybrid materials containing S-PRG particles has emerged as one of the latest innovations in the field of fluoride-releasing restorative materials (Rusnac *et al.*, 2019).

This study explored the relationship between this new class of hybrids and ozonated water as a cavity-cleaning solution. Considering that disinfectant solutions aim to clean the cavity and optimize the action of the adhesive system, ozone therapy has been highlighted as an option. The study's hypothesis was confirmed when it was observed that the group submitted to the bioactive system, cleaned with ozonized water, showed significantly better results in the microtensile test on third molars.

There are three main ways of applying ozone: Gaseous ozone (produced by an ozone generator that converts oxygen into ozone), ozonated water (ozone diluted in water) and ozonated oils (ozone diluted in oils) (Khan *et al.*, 2019). According to Gallo and Scribante (2021), ozone is increasingly showing its potential, with positive effects in various specialties due to its analgesic, antimicrobial, detoxifying and immunostimulant properties. Rinsing the cavity with ozonated water can reduce the accumulation of biofilm bacteria on the surface of the teeth. This hypothesis is endorsed by Nisar's study (Nisar *et al.*, 2022), which indicated that ozonized water could be used reliably as a cavity disinfectant, as it showed better shear strength without compromising the resins' ability to bond.

However, the study by Bilgili *et al.* (2022) contradicts the results of Nisar *et al.* (2022) and the present study by revealing a statistically significant decrease in bond strength values after applying ozone compared to the control group. Disparity in results can be attributed to methodological differences among the studies, including the use of deciduous teeth and the application of ozone in gaseous form and at different concentrations.

Studies on hybrid materials with S-PRG particles, as shown by Tsujimoto *et al.* (2017)**,** demonstrate mechanical properties similar to resin composites. The particles are produced through an acid-base reaction between polyacrylic acid and a mixture of alumina, silica, aluminum fluoride and calcium fluoride glass ionomer, resulting in reactive S-PRG particles. These particles are treated with γ-methacryloxypropyl trimethoxysilane to improve surface hydrogel protection and adhesion to the resin matrix (NSW, 2016). The porous silicon dioxide layer formed allows ion diffusion

and the particles are then dispersed in a BisGMA and TEGDMA matrix, providing suitable viscosity for clinical handling and stability in the oral environment (Hodisan *et al.*, 2018). According to the manufacturer, the ions in the particles offer several advantages. Sodium acts as a catalyst and helps with the buffer effect, released when oral pH drops. Borate reduces bacterial adhesion and enhances remineralization. Aluminum forms crystals in dentinal tubules, improving radiopacity. Silicate promotes dentin remineralization, bone formation and hydroxyapatite binding. Strontium accelerates calcification, reduces dentin sensitivity and stimulates bone formation. Fluoride increases acid resistance by forming fluorapatite, which is more resistant than hydroxyapatite and has antibacterial effects, enhances remineralization and prevents caries (Kaga *et al.*, 2014; Amaechi *et al.*, 2018).

Concerning the mechanism of action of FL-Bond II adhesive (Shofu, Kyoto, Japan), Fujimura *et al.* (2018) highlighted the use of Methacryloyl Oxy Dodecyl Pyridinium Bromide (MDPB), a monomer with a functional group capable of destroying bacterial cell membranes and inhibiting enzymatic activity. Li *et al.* (2019) demonstrated that incorporating Dimethylaminohexadecyl Methacrylate (DMAHDM) and amorphous calcium phosphate particles, enhanced the antibacterial properties and improved the interfacial durability of adhesive systems. The FL Bond II primer contains low molecular weight acid monomers such as Methacryloxy Ethyl Trimellitate Anhydride (4-META) and (Methacryloyl Isohexyl Phosphonoacetate (6-MHPA). The second bottle, with the bonding agent, is filled with S-PRG filler. When the FL Bond II self-etching primer is applied, acid monomers infiltrate, causing partial demineralization of the dentin and creating microporosities for micromechanical locking (Van Meerbeek *et al.*, 2011). The thin hybrid layer is formed by the infiltration of the monomers into the collagen fibril coated by the hydroxyapatite network. Additional chemical interactions between the acidic monomers (4-META and 6-MHPA) and calcium ions from residual hydroxyapatite occur, strengthening the adhesion of the material (Ikemura *et al.*, 2008).

The superior results of the bioactive system conditioned with ozonated water can be explained by the efficient cavity cleaning promoted by the cleaning agent. In addition to its antimicrobial effect, ozonated water also exhibits immunostimulatory potential. The interaction of ozone with the collagen fibers of dentin intensifies collagen production, which may have influenced the action of the 4-META monomer (de Souza *et al.*, 2024). When dentin is properly cleaned with ozonated water, calcium ions are assumed to remain on the surface, ready to bind to monomers such as 6-MHPA, providing a chemical bond between the substrate and the material. The 4-META monomer also plays an active role in this process, as it bonds to the collagen in the collagen fibers, generating micromechanical adhesion. The involvement of these ions and molecules contributes to both micromechanical retention and chemical adhesion of the material. In this way, both the adhesive and the resin material applied achieved better adhesion to the dentin surface when compared to conventional systems. Thus, the use of FL-Bond II associated with a resin composite with the same technology (Beautifil Flow Plus F00) (Shofu, Kyoto, Japan) form a single system reinforced by the ions present in both the adhesive and the composite resin (Condò *et al.*, 2017).

Most of the fractures in the BFP AO group were classified as mixed, representing 50% of the fractures in this group. In the other groups, the fractures were predominantly adhesive (49% in the BFP AD group, 56% in the OBF AO group and 72% in the OBF AD group). Stereomicroscopic observation and descriptive analysis in this study revealed the presence of adhesive fractures in all groups. This finding validates the study, as the recorded values reflect both the overall and actual bond strength at the adhesive interface (Rodrigues *et al.*, 2011). Another relevant observation found during the analysis of the fracture type was the gradual increase in the prevalence of mixed and cohesive in resin composite failures as the mean values of microtensile bond strength increased. The transition from predominantly cohesive fractures in dentin to predominantly adhesive fractures and then to mixed and cohesive fractures in resin reflects the interaction between the strength of the adhesive material and the structural characteristics of the tooth and the resin composite (Ahlholm *et al.*, 2023). As adhesive strength increases, it is expected that there will be a greater capacity for the material to bond to the tooth structure, leading to a higher incidence of adhesive and mixed fractures, where there is a separation at the adhesive-substrate interface. This pattern suggests an improvement in the effectiveness of the adhesive system in promoting a stronger bond between the restorative material and the tooth structure, resulting in a more balanced and varied distribution of fractures. However, variations in the failure mode can be caused by various factors, including the storage time, the test used to analyze the bond strength and the different adhesive and restorative materials tested.

The results of the present study indicate a promising potential for the materials and techniques evaluated, contributing to the advancement of scientific knowledge and offering clinical relevance. The research explores innovative therapeutic approaches, providing valuable information for future research and improving dental practice. However, the study is restricted to the in vitro environment, which limits its direct applicability in clinical practice, which also raises the question of the feasibility of using ozonated water in this environment. In addition, the lack of clinical evaluation and long-term studies restricts the generalizability of the results. Additional in vitro experiments and clinical studies are therefore required to validate the findings obtained in

this research, aiming at the establishment of protocols for better clinical performance to make it a clinically viable option.

Conclusion

According to the results, it can be concluded that ozonated water significantly increased bond strength in dentin when used as a cavity-cleaning solution for subsequent use of a bioactive system.

Acknowledgements

The authors would like to express their sincere gratitude to the State University of Western Paraná - UNIOESTE and the State University of Campinas – FOP UNICAMP, for their support of this project, as well as to the laboratories utilized for conducting the tests.

Funding Information

The authors declare that this research did not receive any financial support.

Author's Contributions

Fernanda Rafaela Ribeiro: Data curation, investigation, project administration, resources, writing, review and editing.

Anna Caroliny Detogni: Data curation, investigation, visualization, and reviewing and editing the manuscript.

Julio Katuhide Ueda: Formal analysis, software development, supervision, writing the original draft, and reviewing and editing the manuscript.

Mario Alexandre Coelho Sinhoreti: Formal analysis, methodology, supervision, validation, writing – review & editing.

Veridiana Camilotti: Conceptualization, formal analysis, methodology, resources, supervision, validation, writing the original draft, and reviewing and editing the manuscript.

Ethics

All the authors declare that all the experiments were examined and approved by the appropriate ethics committee and were, therefore, carried out in accordance with established ethical standards. This project was submitted to and approved by UNIOESTE's human research ethics committee under CAAE number 77767123.6.0000.0107.

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