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Extrusion bond strength and disinfection of *E. faecalis* from canal dentin using synchronized microbubble photodynamic activation and photon-induced photoacoustic streaming

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Abstract. – OBJECTIVE: This study aimed to assess the antimicrobial effectiveness of a new disinfection regimen Curcumin photosensitizer (CP), Synchronized Microbubble Photodynamic Activation (SYMPA), Photon-induced photoacoustic streaming (PIPS), and its impact on the extrusion bond strength (EBS) of root filling material to canal dentin.

MATERIALS AND METHODS: Root canal treatments of sixty mandibular incisors were performed, and an overnight culture of *E. faecalis* was cultivated in the BHI medium. A volume of 1 mL was introduced into twenty root canals. All the samples were randomly allocated into 4 groups based on the irrigation used: –Group 1: 2.5% NaOCl+17% EDTA (Control), Group 2: CP+17% EDTA, Group 3: SYMPA+17% EDTA, and Group 4: PIPS+17% EDTA. The viable bacterial count was estimated, and 15 specimens from each group were obturated, followed by sectioning in 1-mm thick slices. The EBS was measured using a universal testing machine, and failure modes were analyzed using a stereomicroscope at 40x magnification. Means and standard deviations (SD) of the survival rate of *E. faecalis* and EBS of root filling to the dentin were analyzed using ANOVA Tukey multiple comparison t-tests (p=0.05).

RESULTS: Group 1 (2.5% NaOCl+17% EDTA) demonstrated the highest survival rate of *E. faecalis*. Group 3, in which SYMPA+17% EDTA was used to sterilize the canal, unveiled the lowest survival rate (1.55±0.11 CFU/mL). Similarly, the coronal section of Group 3 specimens (8.67±0.43MPa) demonstrated the highest EBS. However, an apical section of Group 1 samples (2.81±0.11MPa) displayed the lowest outcome of bond integrity. Intergroup comparison analysis revealed that Group 4 (PIPS+17% EDTA) and Group 2 (CP+17% EDTA) samples demonstrated comparable values of bond integrity and bacterial survival.

CONCLUSIONS: Canal disinfection using the synchronized microbubble photodynamic activation (SYMPA) technique proved to be a promising alternative technique in decreasing the count of *E. faecalis* and improving extrusion bond strength of gutta percha to canal dentin

Key Words:

Extrusion bond strength, Sodium hypochlorite, Curcumin photosensitizer, Photon-induced photoacoustic streaming, Synchronized Microbubble Photodynamic activation.

Introduction

In the process of endodontic treatment, the thorough removal of pulpal tissues, dentinal debris, and viable microorganisms from the root canal system holds immense importance[1]. Failing to achieve effective elimination can lead to the risk of prolonged inflammation and hindered healing[2]. To address this, proper canal instrumentation and adequate irrigation with sodium hypochlorite (NaOCl) and Ethylenediaminetetraacetic acid (EDTA) have demonstrated their ability to reduce bacterial presence within root canals[3]. However, it is worth noting that even with the incorporation of NaOCl irrigation during the chemo-mechanical debridement phase, root canals may not consistently reach a state entirely free from bacteria[4]. Additionally, the existing data concerning the impact of NaOCl and EDTA on the push-out bond strength (PBS) of root filling materials to the dentin presents uncertainties and requires further clarification[5].

Numerous strategies have been developed to enhance the antibacterial efficacy and PBS of root filling to radicular dentin[6]. One of these techniques is Photon-induced photoacoustic streaming (PIPS), which involves using an Er: YAG laser to activate root canal disinfectant. This approach effectively eliminates the smear layer from the

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canal, leading to improved bond integrity and heightened antibacterial effectiveness[7]. PIPS employs short microsecond pulse rates (50 μs) and low energy levels to achieve power peaks[7,8]. However, there is limited available data in literature on how PIPS affects PBS and germicide activity, necessitating further investigation.

Antimicrobial photodynamic therapy (aPDT) has demonstrated potential effectiveness in laboratory studies and clinical studies for endodontic treatment[9,10]. The technique described involves the utilization of a photosensitizer solution (PS), which was activated using a low-energy light source. The PS generates a highly reactive oxygen species, leading to the denaturation of bacterial cells[11,12]. The aPDT technique has a multifaceted impact on a bacterium, targeting various components such as the cell wall and plasmids[13]. Furthermore, it has been observed to induce DNA damage and inhibit the activity of enzymes.[11] However, the aPDT faces several challenges, i.e., limited ability to reach and penetrate dentinal tubules, difficulties in navigating through complex anatomical structures, reduced production of reactive oxygen in low oxygen environments, and uneven distribution of light energy throughout the root canal system[10,14,15].

To overcome this shortcoming, synchronized microbubble photodynamic activation (SYMPA) using microbubble emulsions are created using an oxygen carrier and oxidizer. These emulsions have been suggested[16,17] as a way to enhance and supplement the effects of APDT. Other advantages of the SYMPA technique include customizable treatment parameters, enhanced therapeutic efficiency, and potential for combination therapies. However, data related to the effect of this technique on *E. faecalis* and PBS of root filling material needs to be determined[18,19].

Based on the indexed literature, the antimicrobial efficacy of contemporary root canal disinfectants against *E. faecalis* appears to be limited and dubious[20,21]. Additionally, the impact of these disinfection regimes on the PBS of root filling to root canal dentin remains undetermined. Hence, it was postulated that there would be no difference in the antimicrobial efficacy against *E. faecalis* when root canals were sterilized using the latest disinfection methods (CP+17% EDTA, SYMPA+17% EDTA, PIPS+17% EDTA) compared to the conventional control (5.25% NaOCl+17% EDTA). Furthermore, it was also predicted that there would be no significant difference in the PBS between root filling material and radicular dentin when root canals were disinfected using contemporary protocols in comparison to a control group. Therefore, this *in vitro* study aims to assess the antimicrobial effectiveness of new disinfection regimens and their impact on the bond integrity of root-filling material to canal dentin.

Materials and Methods

Sample Inclusion

Sixty single-rooted mandibular incisors with single canals without any caries or fractures were included for experimentation, followed by immersion in phosphate-buffered saline (Dogma Pvt Ltd, Pune, India). The teeth were positioned in such a way that the root canal's long axis remained parallel to the sensor and perpendicular to the center X-ray beam. Schneider criteria were applied to measure the root curvature[22,23]. The debris and calculus adhered to the radicular surface of the teeth were eliminated utilizing an ultrasonic scaler. The specimens were subjected to immersion in a 10% formalin solution for 7 days, after which they were then frozen at a temperature of -17°C in a freezer until the time of their intended utilization.

Sample Preparation

A skilled and experienced operator was responsible for the preparation of all specimens. The standardization of root length to 16 mm was achieved by removing the anatomical crown portion of the teeth using a slow-speed handpiece and a double-sided diamond disc (KG Sorensen, Cotia, SP, Brazil) until reaching the cementoenamel junction. The root canal treatment process commenced with the use of a 10 K file (Dentsply Maillefer Ballaigues, Switzerland). Determination of the working length (WL) involved observing the file tip at the root apex, followed by a 1 mm reduction, resulting in a final working length of 15 mm. Pulp extirpation was conducted using a 25k file, along with ample irrigation using a 2.5% Sodium hypochlorite (NaOCl) solution (Natupharma, Passo Fundo, RS, Brazil). Subsequently, the ProTaper universal system (Dentsply Maillefer, Ballaigues, Switzerland) was employed to progressively enlarge the root canals in the cervical-apical direction until reaching the F3 finishing file[24,25].

PS and Microbubble Emulsion Preparation

CP was dissolved in a mixture of glycerol, ethanol, and water in a ratio of 30:20:50 to form

PS. The microbubble emulsion was prepared using perfluoro [decahydronaphthalene] as an oxygen carrier, hydrogen peroxide as an oxidizer (Jigs Chemicals, Riyadh, KSA), and Triton-X100 (Triveni Chemicals, Riyadh, KSA) as a nonionic detergent surfactant, as described in a previous study[26].

Bacterial Culture

An overnight culture of *E. faecalis* (ATCC 29212) was cultivated in BHI medium at 37°C, with a concentration of 108 CFU/mL (optical density at 600 nm=1). A volume of 1 mL was introduced into the 20 root canals, and subsequent samples were subjected to centrifugation at varying speeds (1,400 g, 2,000 g, 3,600 g, and 5,600 g) for 5 minutes. The *E. faecalis* solution was replenished after each round. The samples were incubated at a temperature of 37°C for 3 weeks, with the media being replaced every 72 hours[27,28]. Based on different methods of irrigation, samples were randomly allocated into 4 groups (n=20), as follows:

Group 1

Samples in this group were treated using 4.5 mL of 2.5% NaOCl solution.

Group 2

In this group, a 40 M Curcumin photosensitizer (CP) (Sigma, USA) solution was applied to apply on the canals for five minutes. It was then exposed to the LED light with a radiation intensity of 1,200 mW/cm^2 for 60 seconds.

Group 3

In this group, the SYMPA technique was used to disinfect the canal. It consisted of two steps:

1. Photosensitization was performed by applying 0.5 mL of CP and activating it for 15 secs using a reciprocating S-tip to distribute the solution evenly within the canal. Following photosensitization, the solution was activated using both light and a reciprocating S-tip.
2. Microbubble emulsion activation involved the application of 0.5 mL of the emulsion to the canal. Activation was carried out using the S-tip for 1 minute, followed by additional activation using both lights and a reciprocating S-tip for another minute[18,19].

The process concluded with a final disinfection of all the specimens using 1 mL of 17% EDTA as a final rinse and 1 mL of saline for 60 sec.

Group 4

In this group, 4.5 mL of 2.5% sodium hypochlorite (NaOCl) solution was agitated using a 2,940 nm Er: YAG laser (Fotona, Ljubljana, Slovenia) operating at 15 Hz, 20 mJ, and 0.3 W. A 400 µm quartz PIPS tip (Fotona, Ljubljana, Slovenia) was used with the last 3 mm of its polyamide sheath removed. The instrument was positioned coronally and maintained in a stationary position, without being inserted into the root canal system during the irrigation process. The power to the coaxial air-water spray was turned off[29].

Viable Bacterial Count Estimation

A sterile 1.5 mL Eppendorf tube (Levram Life Sciences, Riyadh, KSA) was utilized to hold a bacterial suspension obtained from the canal (StarLab GmbH). To create dilutions, a series of sevenfold dilutions (ranging from undiluted to 10^{-7}) were prepared in separate Eppendorf tubes, using maximum recovery diluent solution (Scharlau Chemie, S.A., Spain). Aliquots measuring 0.1 mL were then subjected to vortexing, spread evenly, and plated on Petri dishes containing plate count agar (Merck Millipore, Germany). The enumeration of colony-forming units (CFUs) was carried out by incubating the plated samples at a temperature of 35±2.0°C for 48 hours. The viable count estimation, based on the dilution factors, allowed for the determination of the actual number of colony-forming units present. Each experiment was repeated twice to ensure reliability. For the calculation of the anticipated survival rate, the following formula was employed:

Survival rates = Colony forming units (CFUs) of each experiment group/CFU count of the positive control

Specimens Obturation

For each group, a total of 15 specimens were subjected to obturation using a modified single-cone technique. In this technique, two additional cones were placed passively alongside the master cone (ProTaper F3, Dentsply, Maillefer, Ballaigues, Switzerland). The root canal sealer AH Plus (AH Plus Jet, Dentsply De Trey, Konstanz, Germany) was employed to perform the obturation process.

After the canal obturation, the coronal cavity was filled with a temporary filling material (Cavit; 3M ESPE, Seefeld, Germany). The specimens were then placed within an incubator set to maintain 100% humidity and a temperature of 37°C for 24 hours. This allowed for the complete set-

Table I. Survival rates following application of various cavity disinfectants.

Groups	Survival rate CFU/mL
Group 1: 2.25% NaOCl+17% EDTA (Control)	5.65 ± 0.85^{c}
Group 2: CP+17% EDTA	2.11 ± 0.25^{b}
Group 3: SYMPA+17% EDTA	1.55 ± 0.11^{a}
Group 4: PIPS+17% EDTA	2.15 ± 0.31^{b}

Sodium hypochlorite (NaOCl); Ethylene diamine tetraacetic acid (EDTA); Curcumin Photosensitizer (RFP), microbubble emulsion (MBE); photon-induced photoacoustic streaming (PIPS). Different superscript lower-case alphabets denote statistically significant differences within the same column ($p<0.05$). Data with different upper-case alphabets denote significant differences within each row ($p<0.05$).

ting of the obturation material. Following the incubation period, 1 mm thick slices were obtained from each root specimen. This was accomplished by sectioning the roots using a diamond disc operating at low speed and ensuring continuous cooling with distilled water.

Push-Out Testing and Failure Analysis

The EBS was measured using a universal testing machine (UTM, Instron model 3366, Instron Corp., Norwood, MA, USA) with a crosshead speed of 0.5 mm/min until bond failure was observed. The force was measured in MegaPascal (MPa). A stereomicroscope (DHR Holding Mumbai, India) at 40x magnification was used to identify the type of failure. Different types of material failure, including adhesive, cohesive, and admixed, were distinguished.

Statistical Analysis

Statistical analysis was performed using the Statistical Program of Social Sciences (v.23, SPSS, IBM Corp., Armonk, NY, USA). Means and standard deviations (SD) of the survival rate of *E. faecalis* and PBS of root filling to the dentin were analyzed using one-way analysis of variance (ANOVA). Multiple group comparisons were performed using Tukey multiple comparison *t*-tests ($p=0.05$).

Results

Table I and Figure 1 display the survival rates of *E. faecalis* in colony-forming units per milliliter (CFU/mL) after the application of various canal disinfectants**.** The outcomes established that 2.25% NaOCl+17% EDTA (Control) was

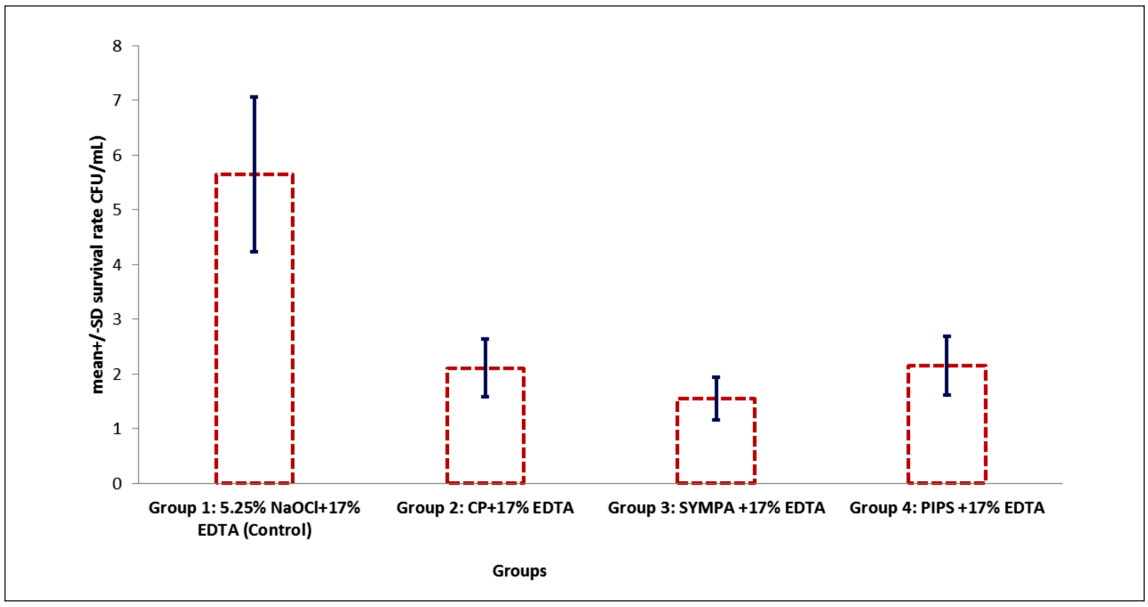

Figure 1. Survival rates following application of various cavity disinfectants.

Table II. Mean and standard deviation (SD) of extrusion bond strength values measured in mpa across experimental groups at the cervical, middle, and apical root levels.

Groups	Cervical	Middle	Apical
Group 1: 2.5% NaOCl+17% EDTA (control)	$4.65 \pm 0.23^{a,A}$	$3.15 \pm 0.14^{c,A}$	$2.81 \pm 0.11^{a,B}$
Group 2: CP+17% EDTA	$6.71 \pm 0.51^{b,A}$	$5.75 \pm 0.31^{b,A}$	$4.01 \pm 0.32^{b,B}$
Group 3: SYMPA+17% EDTA	$8.67 \pm 0.43^{a,A}$	$6.50 \pm 0.35^{a,A}$	$5.33 \pm 0.26^{c,B}$
Group 4: PIPS+17% EDTA	$6.33 \pm 0.45^{a,A}$	$5.26 \pm 0.23^{b,A}$	$3.12 \pm 0.14^{a,B}$

Sodium hypochlorite (NaOCl); Ethylene diamine tetraacetic acid (EDTA); Curcimin Photosensitizer (RFP), microbubble emulsion (MBE); photon-induced photoacoustic streaming (PIPS). Different superscript lower-case alphabets denote statistically significant differences within the same column ($p<0.05$). Data with different upper-case alphabets denote significant differences within each row ($p<0.05$).

found to have the highest survival rate (5.65±0.85 CFU/mL). However, Group 3, in which SYMPA+17% EDTA was used to sterilize the canal, unveiled the lowest survival rate (1.55 ± 0.11 CFU/mL). Furthermore, Group 2 (CP+17% EDTA) (2.11±0.25 CFU/mL) and Group 4 (PIPS +17% EDTA) (2.15±0.31 CFU/mL) displayed comparable outcomes of survival rate of *E. faecalis* ($p>0.05$).

Table II and Figure 2 present the mean and standard deviation (SD) of PBS among different experimental groups at the cervical, middle, and apical levels of the root. The findings suggested that the coronal third of Group 3 specimens treated with SYMPA+17% EDTA (8.67±0.43 MPa) demonstrated the highest EBS of filling to root dentin. However, the apical section of Group 1 samples disinfected with 2.5% NaOCl+17% EDTA (2.81±0.11 MPa) displayed the lowest outcome of EBS. Intergroup comparison analysis unveiled that Group 4 (PIPS+17% EDTA) (Coronal: 6.33±0.45 MPa, Middle: 5.26±0.23 MPa Apical: 3.12±0.14 MPa) and Group 2 (CP+17% EDTA) (Coronal: 6.71±0.51 MPa, Middle: 5.75±0.31 MPa Apical: 4.01±0.32 MPa) samples demonstrated comparable values of bond integrity. However, group 1 (Coronal: 4.65±0.23 MPa, Middle: 3.15±0.14 MPa Apical: 2.81±0.11 MPa) samples exhibited lowest outcomes than all the tested group $p<0.05$.

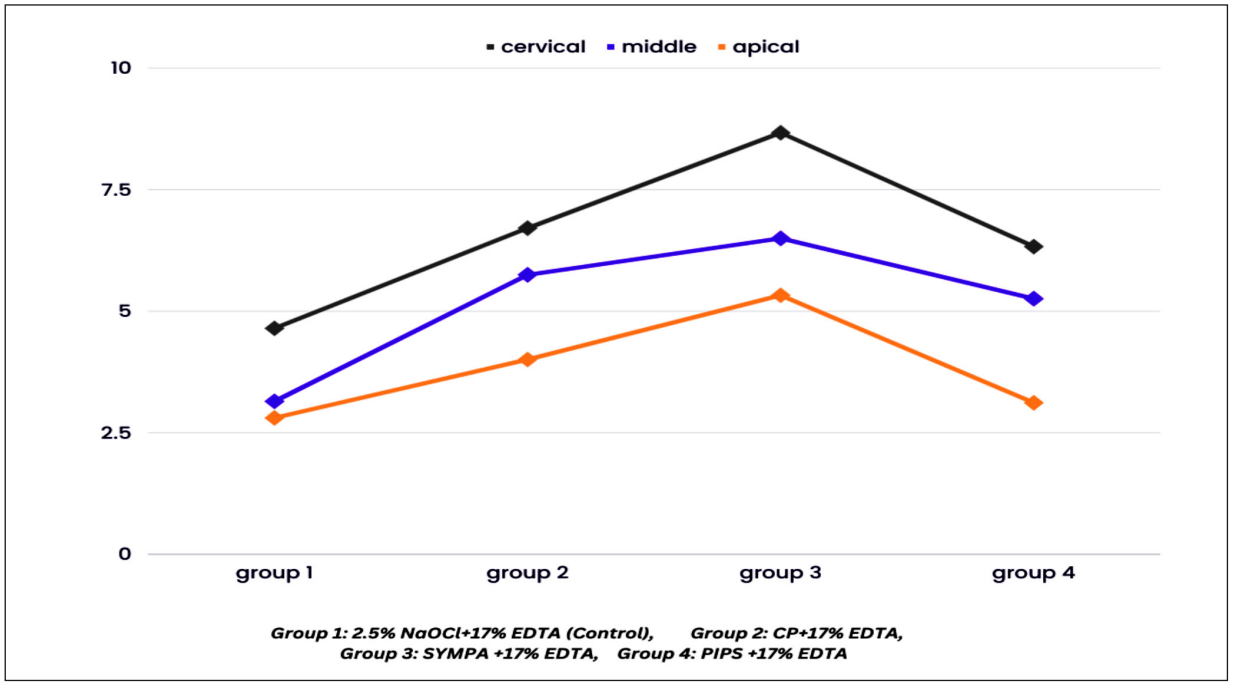

Figure 2. Mean and standard deviation (SD) of extrusion bond strength values measured in mpa across experimental groups at the cervical, middle, and apical root levels.

Figure 3 presents the distribution of failure modes by percentage for each group. Groups 2, 3, and 4 predominantly experienced cohesive failures. However, Group 1 exhibited the highest occurrence of adhesive failures.

Discussion

The contemporary research investigated the hypothesis that there would be no significant difference in the antimicrobial efficacy against *E. faecalis* when root canals were sterilized using various contemporary disinfection methods (CP+17% EDTA, SYMPA+17% EDTA, PIPS+17% EDTA) compared to the conventional control (5.25% NaOCl+17% EDTA). Additionally, it was predicted that there would be no significant difference in EBS between the root filling material and radicular dentin when root canals were disinfected using contemporary protocols, in comparison to a control group. However, the postulated hypotheses were completely rejected, as the latest regimes demonstrated improved antibacterial efficiency against *E. faecalis* and enhanced bond scores compared to the conventional control. In this research, culture-based methods, specifically quantifying bacteria using colony-forming units (CFUs), were commonly employed to evaluate antibiofilm activity.

Outcomes of the existing study revealed that SYMPA+17% EDTA disinfected samples exhibited the lowest viable count of *E. faecalis* and highest PBS among all the investigated groups. This is in agreement with the findings of the study conducted by Teed et al[26]. They reported that when microbubble emulsion was added to the PS disinfection process it boosts oxygen levels within the canal and makes it more exposed to light. Further, it was stated that there is more oxygen present in the microbubble emulsion, leading to more singlet oxygen production[26]. The emulsion was created by adding Triton-X (Triveni Chemicals, Riyadh, KSA) to the microbubble solution at a very low concentration[19]. CP photo-oxidation capability was much enhanced when combined with microbubbles and activated with light and mechanical agitation at the same time[30]. George and Kishen[31] found that when methylene blue was added to a solution of 30 parts of glycerol to 20 parts of ethanol to 50 parts of water, singlet oxygen generation increased.

In the present study, the conventional method of canal disinfection 2.25% NaOCl+17% EDTA displayed the lowest bond integrity and highest

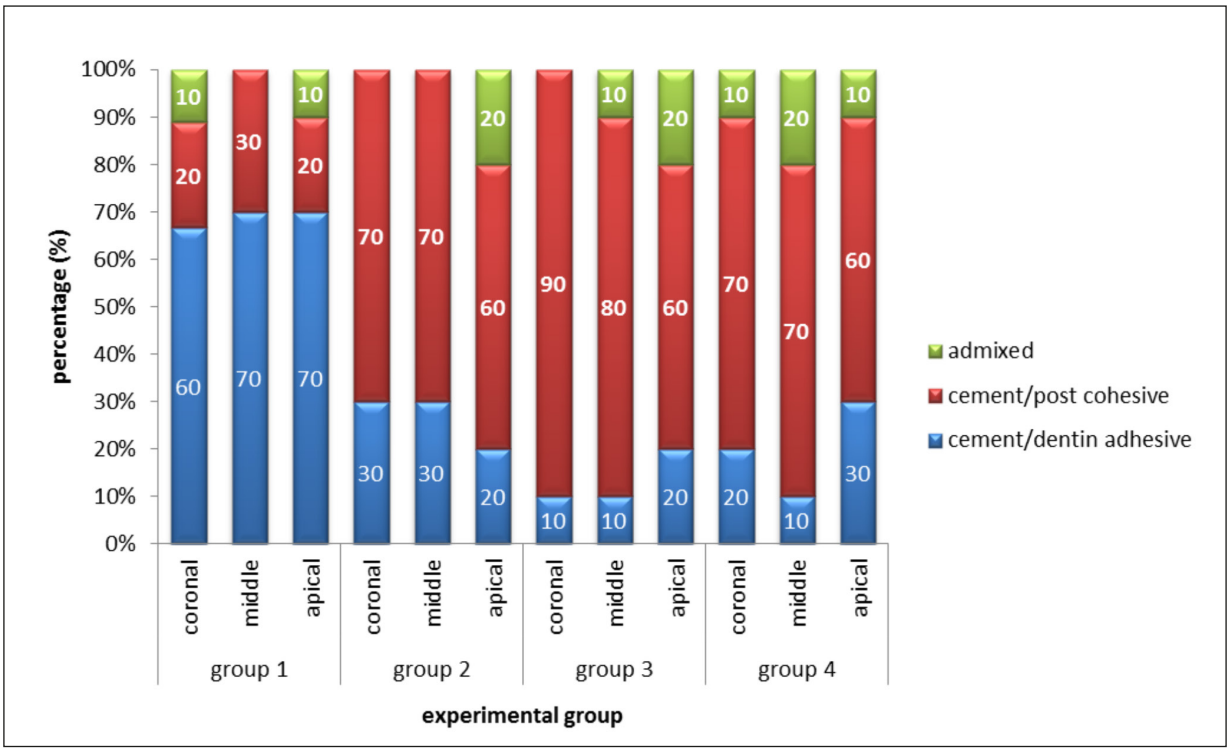

Figure 3. Adhesive, cohesive, and admixed failure among various investigated groups.

survival rate of *E. faecalis*. Cecchin et al[32], in a study, revealed that a combination of NaOCl and 17% EDTA produced similar bond strength to that of untreated dentin. Previous SEM analysis[32,33] revealed that the use of NaOCl and EDTA together effectively eliminated the smear layer in the coronal third. However, their effectiveness in removing the smear layer in the middle and apical third was limited[32,33]. The middle and apical third of the canal exhibited a high presence of a thick smear layer and a smaller number of completely open dentin tubules, leading to reduced bond strength[34,35].

Results also indicated that CP and PIPS disinfected groups demonstrated comparable outcomes of antimicrobial efficiency and bond strength. However, their germicidal effect and EBS were significantly lower than the SYMPA-treated group. Current evidence suggests that CP has inhibitory effects on *E. faecalis*. One possible explanation for the increased bond integrity of root-filling material to canal dentin is the anionic nature of CP, which allows it to bind with Calcium (Ca++) ions, leading to a change in the dentin substrate[36,37]. This change promotes micromechanical retention of the resin cement, thus improving bond integrity. Additionally, the hydrophobic and polyphenolic properties of CP may have contributed to the enhancement of EBS values[38]. Recent work by Al Shahrani et al[39] unveiled that PIPS using NaOCl solution has demonstrated the highest efficacy in eliminating bacterial biofilm. PIPS, laser-activated irrigation (LAI), possesses the potential to improve canal system disinfection. Similarly, the improved bond strength achieved by PIPS+EDTA disinfected samples follows the findings of the study conducted by Wan et al[29]. They stated that the PIPS technique effectively removes most of the smear layer and opens the majority of open dentinal tubules. The emission wavelength coincides with the peak absorption of water and utilizes a short pulse duration to generate high peak power, resulting in a photomechanical phenomenon[40].

In the context of failure analysis, the present research observed that groups with high bond integrity demonstrated a higher occurrence of cohesive failures as part of the failure mode. In cohesive failure, the material itself fractures or separates internally rather than at its interface with another material. This type of failure occurs due to weaknesses or defects within the material, such as voids, inclusions, or structural inconsistencies. Cohesive failures are indicative of the material's inability to withstand the applied stress or load, resulting in its internal breakdown[41-43]. On the other hand, specimens treated with NaOCl+17% EDTA exhibited a predominant adhesive type of failure.

Limitations

It is important to note that there are certain limitations associated with the current *in-vitro* investigation. The study's outcomes are primarily constrained by the concentration of the chemical agents used and the specific laser parameters employed. To gain a comprehensive understanding of dentinal changes following canal disinfection, it is recommended to assess the debonded dentin surface topography using techniques like scanning electron microscopy (SEM) and atomic force microscopy. Likewise, a note of caution is warranted when attempting to generalize the findings of the current study. This caution arises from the fact that the study was conducted in a controlled laboratory environment and may not fully mirror the complex conditions encountered in clinical settings.

Conclusions

Canal disinfection through the synchronized microbubble photodynamic activation (SYMPA) technique has demonstrated promise as an alternative method when compared to conventional approaches. It has shown effectiveness in reducing the count of *E. faecalis* bacteria and improving the extrusion bond strength of gutta-percha to canal dentin. This suggests that SYMPA could potentially offer improved outcomes in root canal disinfection and obturation procedures.

Conflict of Interest

The authors declare that they have no conflict of interests.

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Ethics Approval

Not applicable.

Authors' Contribution

Conceptualization.; Methodology; Software, Validation, Formal analysis, Investigation data curation, writing—original draft preparation, and writing—review and editing, visualization, supervision, and project administration, funding acquisition performed by KHA and MFA All authors have read and agreed to the published version of the manuscript.

Informed Consent

Not applicable.

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