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Photodynamic therapy on the extrusion bond strength of gutta-percha to radicular dentin sealed with bioceramic and conventional sealers

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Abstract. – **OBJECTIVE:** The aim of this study was to evaluate the influence of photodynamic therapy (PDT) on the extrusion bond strength (EBS) of gutta-percha to radicular dentin sealed with bioceramic sealers.

MATERIALS AND METHODS: Sixty human-extracted mandibular premolars were used in the present study, being decoronated up to the cementoenamel junction, and secured in heat cure acrylic resin for root canal therapy. The specimens were randomly divided into groups based on conventional [2.25% sodium hypochlorite (NaOCl) + 17% EDTA] and disinfection protocols (2.25% NaOCl + PDT + 17% EDTA) (n = 10). Specimens in groups 1, 3, 5: conventional treatment modality (2.25% NaOCl + 17% EDTA). Samples in groups 2, 4, 6: adjunctive PDT treatment modality (2.25% NaOCl+ PDT+ 17% EDTA). Specimens in groups 1 and 2 were sealed with AH Plus sealer (AH). Specimens in groups 3 and 4 were sealed using Endo Sequence BC sealer, and samples in groups 5 and 6 were sealed with MTA Fillapex. All specimens were cut in the coronal and middle segments and positioned in a universal testing machine (UTM) for assessment of extrusion bond strength (EBS). ANOVA and Post-Hoc Tukey multiple comparison tests were applied for performing statistical analysis ($p < 0.05$).

RESULTS: Coronal root samples in group 1 (2.25% NaOCl + 17% EDTA) sealed with AH Plus sealer demonstrated the highest EBS value (9.21 ± 0.62 MPa) whereas the middle-third of specimens in group 6 (2.25% NaOCl + PDT + 17% EDTA) sealed with MTA Fillapex exhibited the lowest EBS value (5.07 ± 0.17 MPa). Intergroup comparison revealed that group 3 (2.25% NaOCl + 17% EDTA) sealed with Endo Sequence BC Sealer and group 5 (2.25% NaOCl + 17% EDTA) sealed with MTA Fillapex disclosed comparable EBS results to group 1 ($p > 0.05$) while group 2 (2.25% NaOCl + PDT + 17% EDTA) sealed with AH Plus sealer and group 4 (2.25% NaOCl + PDT + 17% EDTA) sealed with Endo Sequence BC Sealer unveiled analogous EBS values to group 6 (2.25% NaOCl + PDT + 17% EDTA) MTA Fillapex ($p > 0.05$). The most noticeable failure mode in the coronal and middle thirds of non-PDT subjected groups was cohesive.

CONCLUSIONS: Combination of 2.25% NaOCl with PDT and 17% EDTA for canal disinfection with AH Plus sealer, calcium silicate sealer, and MTA-based bioceramic sealers have an unfavorable effect on the EBS of gutta-percha to the root canal wall.

Key Words:

Photodynamic therapy, Methylene blue, Push-out bond strength, Radicular dentin, Bioceramic sealers.

Introduction

Tooth rehabilitation involves the application of chemo-mechanical debridement for disinfection and canal sealing agent for possible bond formation between canal-bulk fill material and canal wall[1,2]. However past evidence[3] elucidates that due to canal intricacies, microbial remnants may be left in the canal in turn causing periapical periodontitis; therefore, diverse antimicrobial disinfectants have been employed for procuring efficient microbial decontamination, protracted clinical durability, and bonding competence.

Utilization of 'sodium hypochlorite (NaOCl)' as a conventional endodontic irrigant may demonstrate the potent antimicrobial activity with organic matrix solubilizing capability facilitating debris and microbes removal[4]. However, studies[5,6] on NaOCl advocated that the use of NaOCl alone may not exterminate the bacteria proficiently from the canal space, hence, Ethylene diamine tetraacetic acid (EDTA) has been employed for all-embracing disinfection and adhesive results. Substantively, delineation of ethylene diamine tetra acetic acid as a chelator and demineralizing solution, when used in radicular canals may

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function by removing dentinal inorganic debris deposited by the smear layer, in turn, fortifying the collagenous structure by aiding sealer permeation[7,8].

Rejuvenation in dental decontamination protocol may deem the introduction of photodynamic therapy (PDT) as an innovative, reassuring, and enthralling antimicrobial approach that consumes different photosensitizers (PS) when activated by light under aerobic conditions[9,10]. Amongst the various PS, methylene blue (MB) 'a hydrophilic phenothiazine derivative' has been contemplated[11,12] as an efficient, inexpensive, and expansively used PS in various dental aspects owing to its persistent antimicrobial efficacy. MB when activated releases reactive oxygen species (ROS) resulting in bacterial lysis. The photosensitizer has a light absorption of 660 nm[12]. However, the bond strength of different sealers with the radicular dentin by implementing varied PS needs to be reconnoitered comprehensively for a doable adhesive outcome.

Nevertheless, obturation of endodontic canals is a pivotal aspect for practicable prognosis necessitating sealer and its adhesive aptitude, possessing antimicrobial property, biocompatibility, radiopacity, heightened flow rate, low solubility, and enhanced canal-wall adherence ability[13,14]. Sealers are based on different chemical compositions and are classified as conventional 'epoxy-resin-based' and newly introduced 'calcium silicate-based and MTA-based bioceramic' sealers.

AH Plus is a resin-based sealer that has been widely used in dentistry for obturation purposes owing to its highly acceptable physical and adhesive properties[15]. Likewise, MTA Fillapex 'an MTA hydrophilic calcium-silicate resin-based sealer' has been introduced for acquiring improved biological properties, biocompatible and bactericidal sealer with the merit of eradicating smear layer due to high flow rate. However, the sealer displays shortcomings of prolonged setting time, staining possibility, and difficult application[16-18]. Alternatively, a newly premixed calcium silicate-based bioceramic sealer 'Endo Sequence BC Sealer' has been established for overcoming MTA drawbacks by exhibiting bioactive binding capacity with boosted mechanical properties[18-20]. Markedly, all sealers authenticated acceptable sealing capacity; however, their adhesive proficiency with canal walls after different disinfection modalities should be probed further[21].

Nonetheless, within the restraints of the study, systematic confirmation of the interaction of different disinfectants as influential endodontic canal disinfectants and their effects on the extrusion bond strength (EBS) of varied canal sealers remains uncharted and unparalleled obliging further *in vitro* consideration. It is hypothesized that adjunctive use of MB activated by PDT with various sealers will demonstrate EBS comparable to conventional disinfection methods. The present study aimed to measure the effect of adjunctive photodynamic therapy (PDT) on EBS of gutta-percha (GP) to radicular dentin using calcium silicate and MTA-based bioceramic sealers.

Materials and Methods

The current study was carried out following the checklist for reporting *in vitro* study (CRIS) guidelines and was approved by the Ethics and Research Committee of the Specialist Dental Practice and Research Center, in Riyadh, Saudi Arabia.

Specimen Preparation

Sixty human-extracted mandibular premolars (n = 60) were collected for endodontic treatment execution and bond strength analysis. As per the inclusion criteria, non-traumatized and non-carious teeth with a single straight canal having matured apex were validated to steer the study. Specimens were assessed by using a stereomicroscope (MSL, Guangdong, China) at a 20x magnification for any intact tissues, and later the ultrasonic scaler (OEM, Ultrasonic Scaler, Jiangsu, China) was employed for the eradication of attached periodontal tissues, debris, calculi, and plaque. Afterward, all debrided specimens were stowed in a 0.1% thymol solution (Brisben Chemicals, Mumbai, India) at 4°C for 48 hours.

To attain homogeneity and standardization, all examined samples were decoronated up to the cementoenamel junction under constant refrigeration *via* a slow-speed diamond saw (IsoMet 5000; Buehler, Lake Bluff, IL, USA) and a radicular segment of approximately 15 mm was retained and positioned perpendicularly in heat cure acrylic resin (Astra Chemtech Private Limited, Mumbai, India) using a Teflon mould (3 mm) for ensuing endodontic therapy.

Endodontic Treatment Execution

Sequentially, the determination of working length was done using a #15K file (R&D Impex International, Ludhiana, India) till apical constriction, keeping it 1 mm short of the root apex and further broadened up to the #25K files. Further mechanical preparation and broadening of the canals were implemented by utilizing the crown down technique with the NiTi pro taper universal system (Dentsply Maillefer, Charlotte, NC, USA). Subsequently, after progressive cleaning and shaping, the specimen's radicular canals were meticulously cleansed with saline, and samples were arbitrarily divided into groups for further exploration.

Experimental Groups

The specimens were indiscriminately alienated into groups based on conventional (2.25% NaOCl + 17% EDTA) and (2.25% NaOCl + PDT + 17% EDTA) disinfection protocols, comprising 10 samples each.

Groups 1, 3, 5: Conventional Treatment Modality (2.25% NaOCl + 17% EDTA)

Thirty radicular specimens (n = 30) were disinfected with 2.25% NaOCl (Acura Organics Limited, New Delhi, India) for 60 seconds then treated with 3 ml of 17% EDTA (Acura Organics Limited, New Delhi, India) for a further 1 minute for efficient removal of smear layer.

Groups 2, 4, 6: Adjunctive PDT Treatment Modality (2.25% NaOCl + PDT + 17% EDTA)

After initial disinfection with 2.25% NaOCl, specimens in groups 2, 4, and 6 were subjected to an adjunctive PDT sanitization process by injecting MB dye solution into the radicular canal of each specimen using a 27 gauge endodontic micro-needle and then the solution was agitated for about 1 minute by the use of a #30 K file (Dentsply Maillefer, Charlotte, NC, USA). Later, photoirradiation of canals was performed for 40 sec using an LED curing unit (Leomed, Chongqing, China) by introducing an emitter in the canal and placing 3 mm above the apical root canal portion. 3 ml of 17% EDTA (Acura Organics Limited, New Delhi, India) was introduced in the canal for 1 minute for efficient removal of the smear layer.

Grouping of Specimens Based on the Application of Sealer

Specimens from all groups were dried with paper points (Dentex International, Ludhiana, India) and then gutta-percha (Dentex International, Ludhiana, India) was obturated with different sealers using the lateral condensation technique. Specimens in groups 1 and 2 were sealed with AH Plus sealer (AH) (Dentsply Maillefer, Charlotte, NC, USA). Similarly, specimens in groups 3 and 4 were sealed using Endo Sequence BC sealer (Brasseler, Savannah, GA, USA), and samples in groups 5 and 6 were sealed with MTA Fillapex (Angelus Dental, Londrina-PR - Brazil). All obturated samples were preserved at 37°C for about 48 hours until further experimentation.

Extrusion Bond Strength (EBS)

The root's coronal and middle thirds were segmented into 1 mm thick horizontal slices using an Isomet slow-speed saw (IsoMet 5000; Buehler, Lake Bluff, IL, USA) held perpendicular to the tooth's long axis and kept under constant water cooling. A universal testing machine (UTM, OEM, Jinan Focus test, Shandong, China) was used for the evaluation of EBS. All of the sections were compressed using a cylindrical stainless steel plunger at a crosshead speed of 0.5 mm/min until debonding when placed in UTM and the EBS scores were assessed and deliberated in Megapascal (MPa).

EBS (MPa) = Fracture force (N) / bonding surface area (mm^2).

Statistical Analysis

The modes of failure were identified as an adhesive (between radicular dentin-sealer interface), cohesive (within the root dentin or sealer), and admixed (combination of both) by using a stereomicroscope at 40x magnification (MSL, Guangdong, China). The means and standard deviations of EBS were appraised using a one-way analysis of variance (ANOVA) and for the comparison of EBS means, Post Hoc Tukey multiple comparison tests were used at a statistically significant level of $p < 0.05$.

Results

EBS Analysis

The normal data distribution was validated by the Kolmogorov-Smirnov test. The mean EBS of two different bioceramic sealers and a conventional sealer for all inspected groups are presented in Table I and Figure 1. In the present study's discussion, it is pronounced that the coronal root samples in group 1 (2.25% NaOCl + 17% EDTA) sealed with AH Plus sealer demonstrated the highest EBS value (9.21 ± 0.62 MPa) whereas

Table I. Mean extrusion bond strength of two different bioceramic sealers and a conventional sealer.

Type of irrigation	Canal sealer	Extrusion bond strength (MPa ± SD)
Group 1 2.25% NaOCl + 17% EDTA	AH Plus sealer	Coronal: 9.21 ± 0.62^{A} Middle: 8.54 ± 0.55^{A}
Group 2 2.25% NaOCl+ PDT (MB) + 17% EDTA	AH Plus sealer	Coronal: 7.45 ± 1.78^{B} Middle: 6.30 ± 0.37^{B}
Group 3 2.25% NaOCl + 17% EDTA	Endo Sequence BC Sealer	Coronal: 8.11 ± 0.63^{A} Middle: 8.36 ± 0.73^{A}
Group 4 2.25% NaOCl+ PDT(MB) + 17% EDTA	Endo Sequence BC Sealer	Coronal: 6.39 ± 0.72^{B} Middle: 5.46 ± 0.74^{B}
Group 5 2.25% NaOCl + 17% EDTA	MTA Fillapex	Coronal: 9.04 ± 0.13^{A} Middle: 8.14 ± 0.91^{A}
Group 6 2.25% NaOCl+ PDT(MB) + 17% EDTA	MTA Fillapex	Coronal: 6.67 ± 0.12^{B} Middle: 5.07 ±0.17^{B}

Different superscript upper case alphabets ($^{A, B}$) denote statistically significant differences ($p < 0.05$).

the middle-third of specimens in group 6 [2.25% NaOCl + PDT (MB) + 17% EDTA] sealed with MTA Fillapex exhibited the lowest EBS (5.07 ± 0.17 MPa).

Intergroup comparison revealed that group 3 (2.25% NaOCl + 17% EDTA) sealed with Endo Sequence BC Sealer (Coronal: 8.11 ± 0.63 MPa, Middle: 8.36 ± 0.73 MPa) and group 5 (2.25%

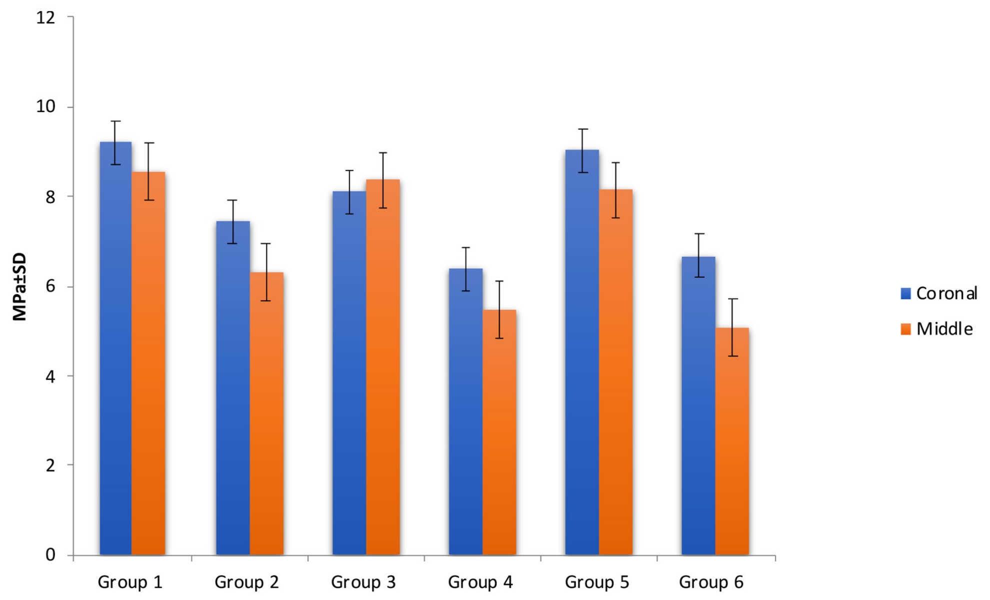

Figure 1. Mean extrusion bond strength of two different bioceramic sealers and a conventional sealer. Group 1: NaOCl/ EDTA+ AH Plus sealer (Na-E-AH), Group 2: NaOCl+PDT+EDTA+AH Plus sealer (Na-PDT-E-AH) Group 3: NaOCl/17% EDTA+ Endo Sequence BC Sealer (Na-E-ESBCS), Group 4: NaOCl+PDT+EDTA + Endo Sequence BC Sealer (Na-PDT-E- ESBCS), Group 5: NaOCl/17% EDTA+ MTA Fillapex (Na-E-MF), Group 6: NaOCl+PDT +EDTA+ MTA Fillapex (Na-PDT-E-MF).

NaOCl+17% EDTA) sealed with MTA Fillapex (Coronal: 9.04 ± 0.13 MPa, Middle: 8.14 ± 0.91 MPa) disclosed comparable EBS results to samples in group 1 (Coronal: 9.21 ± 0.62 MPa, Middle: 8.54 ± 0.55 MPa) ($p > 0.05$) while group 2 (2.25% NaOCl + PDT + 17% EDTA) sealed with AH Plus sealer (Coronal: 7.45 ± 1.78 MPa, Middle: 6.30 ± 0.37 MPa) and group 4 2.25% NaOCl + PDT + 17% EDTA (Coronal: 6.39 ± 0.72 MPa, Middle: 5.46 ± 0.74 MPa) sealed with Endo Sequence BC Sealer unveiled analogous EBS values to group 6 (Coronal: 6.67 ± 0.12 MPa, Middle: 5.07 ± 0.17 MPa) ($p > 0.05$), respectively.

It was instituted that the effect of adjunctive use of PDT in AH Plus, MTA Fillapex, and Endo Sequence BC sealer groups i.e. group 2, group 4, and group 6 represented the lowest and comparable EBS values in contrast to non-PDT treatment groups i.e. group 1, group 3 and group 5. The intragroup comparison indicated that all scrutinized groups demonstrated a declining EBS from the root coronal third to the middle third, except for group 3 (2.25% NaOCl + 17% EDTA) sealed with Endo Sequence BC Sealer where the mid-third root section (8.36 ± 0.73 MPa) presented a higher value of EBS than the coronal third (8.11 ± 0.63 MPa), respectively.

Failure Mode Analysis

The percentage of modes of failure in each group is illustrated in Figure 2. As per the verdict, it was perceived that the most noticeable failure mode in coronal and middle thirds of non-PDT subjected groups: group 1 (60%) (50%), group 3 (80%) (60%) and group 5 (90%) (70%) was a cohesive failure whereas PDT treatment groups i.e., group (60%) (80%), group 4 (50%) (70%) and group 6 (60%) (50%) suffered adhesive failure mode in predominance. However, admixed failure was the least acquired failure mode in all examined groups.

Discussion

This current *in vitro* study was established to assess the effect of adjunctive photodynamic therapy utilizing MB on EBS of radicular dentin to calcium silicate and MTA-based bioceramic sealers. Unambiguously, the existing data was accredited on the postulation that MB driven by PDT disinfection with various sealers applications will demonstrate EBS comparable to conventional disinfection methods. However, this intuition was implausibly rejected as per the study's verdict, as PDT-treated groups displayed the least EBS. Therefore, it was elucidated that

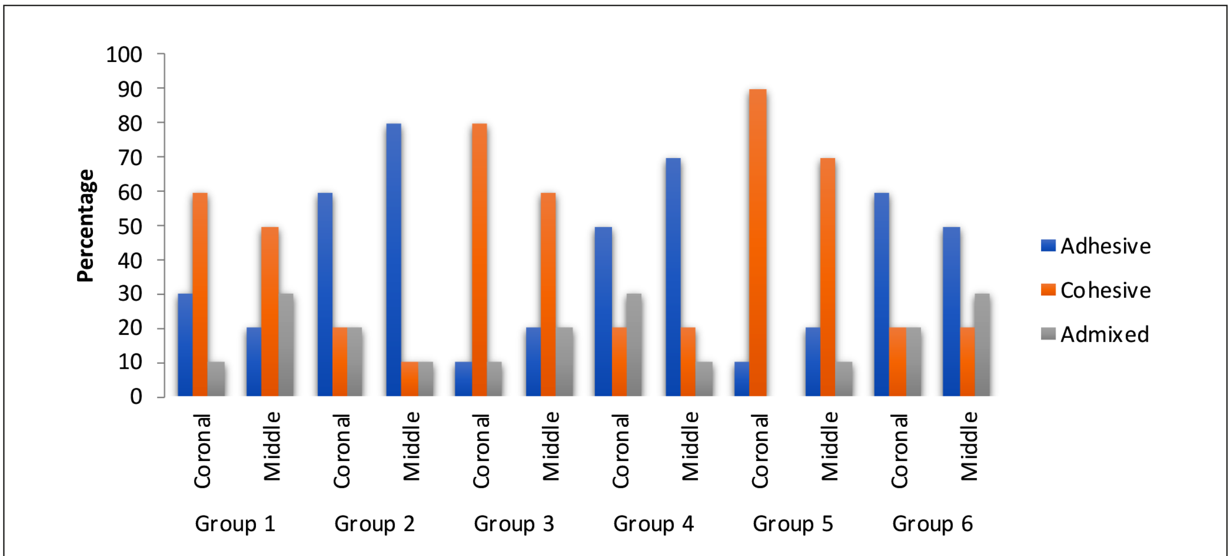

Figure 2. Percentage of modes of failure in each group. Group 1: NaOCl/ EDTA+ AH Plus sealer (Na-E-AH), Group 2: NaOCl+PDT+EDTA+AH Plus sealer (Na-PDT-E-AH) Group 3: NaOCl/17% EDTA+ Endo Sequence BC Sealer (Na-E-ESBCS), Group 4: NaOCl+PDT+EDTA + Endo Sequence BC Sealer (Na-PDT-E- ESBCS), Group 5: NaOCl/17% EDTA+ MTA Fillapex (Na-E-MF), Group 6: NaOCl+PDT +EDTA+ MTA Fillapex (Na-PDT-E-MF).

non-PDT treated groups when obturated with examined sealers demonstrated better EBS results in contrast to PDT used adjunctively.

Endodontic treatment can achieve biological and mechanical goals through meticulous canal debridement, exclusion of pathogenic organisms, and proper three-dimensional obturation with gutta-percha and endodontic sealer to create a fluid-tight seal, restricting bacterial intrusion from the oral milieu and propagation to the periapical tissues[1]. Profoundly, it was perceived in work by Yamashita et al[22] that NaOCl and EDTA irrigating solutions have been commended for chemomechanical root canal preparation due to their enhanced tissue-dissolving, sealer-permeation, and antimicrobial properties. This is in line with our results. On the contrary, PDT is an innovative disinfection technique and its influence on the bond strength of different sealers has not been documented comprehensively due to limited experimentation.

Adhesion is one of the fundamental characteristics of endodontic sealers, and it is critical for maintaining the integrity of the sealer-dentin interface during mechanical stresses induced by masticatory forces and restorative processes of post-space preparation, however, any breach in the adhesive capacity may lead to reinfection and endodontic treatment failure[23,24]. Furthermore, the push-out test was performed in this study to assess sealer adhesion to dentin owing to its effectiveness, reliability, and oral-milieu simulation ability with reduced premature failure rates as reported by Goracci et al[25]

According to the outcome of the current study, it was acknowledged that the conventional endodontic disinfection procedure (2.25% NaOCl + 17% EDTA) with the application of AH Plus sealer (AH) exhibited the highest EBS to radicular dentin. The conceivable reason could be the noteworthy antimicrobial efficacy of both irrigants, smear layer dissolution capacity of NaOCl for the organic portion of dentin with the combined use of chelating agent 'EDTA' that causes inorganic dentinal content solubilization and formation of a softened dentinal matrix that in turn lead to sealer penetration aiding in strengthened bond formation[26-29]. This is in harmony with the study executed by Guo et al[30]. Considering AH, it has been deemed to be the gold standard among different sealers[31]. AH is a paste-paste formulation entailing the blend of epoxy resin and amines. The open-epoxy resin ring in this sealer undergoes a reaction with an exposed amino group of dentinal collagen that results in covalent bond formation augmenting EBS[32]. Enhanced dimensional stability, low polymerization stress, high resorption resistance, hydrophobicity, and heightened infiltration rate result in improved mechanical retention of sealer to the radicular dentin[6,33,34]. Stelzer et al[35] corroborated the significant correlation.

Likewise, when employing the same conventional endodontic sanitization protocol as AH, Endo sequence Bioceamic sealer (ESBCS), and MTA Filapex (MF) exhibited comparable EBS results. The antimicrobial and dissolution capacity of NaOCL and EDTA, which cause de-occlusion of dentinal tubules and thus assist in the penetration of ESBCS and MF sealers, are reasons cited for improved EBS[26-28,36]. The analogous results were authenticated by Shokouhinejad et al[24] and Cakici et al[37]. Moreover, ESBCS and MF sealers' notable sealing ability, low solubility, biocompatibility, bactericidal effect, low setting expansion, dimensional stability, and grander flow ability may all contribute to EBS advancement[21,38,39].

The effect of the adjunctive use of PDT in AH, MF, and ESBCS sealer groups depicted the lowest and comparable EBS values in comparison to non-PDT treatment groups. The noteworthy EBS drop was witnessed in the middle root segment obturated with MF and in the coronal radicular portion sealed with ESBCS. The palpable elucidations for the low EBS of MB treated groups can be explicated by the cationic affinity of PS employed that binds with the dentinal anionic Calcium (Ca^+) and phosphate (P) ensuing in ion ratio modification in dentinal structure and causes calcio-phosphate precipitation on canal surface forming a physical barrier for sealers to penetrate and interact with dentinal collagen[40-43]. Furthermore, MB's and sealer's (MF and ESBCS) hydrophilicity may also advocate low EBS by acting as an adhesion impediment, causing volumetric expansion and solubility increment[44]. Sayhon et al[45] and Alonaizan et al[46] deduced similar EBS results to our study's outcome.

As per the results, it was also evinced that all inspected groups unveiled a declining EBS trend from the coronal third to the middle third, except for group 3 (Na-E-ESBCS). The ascription is given to the dentinal tubules' dense nature that decreases from coronal to middle direction occasioning less sealer penetration and exhibiting an EBS diminution. An equivalent trend was prominent in a study directed by Hashimoto et al[47].

Regarding ESBCS, it demonstrated the opposite trend which may be due to its high wettability to intraarticular dentin compared to the other sealers may owe to its high hydrophilicity[48].

In failure analysis, it was stated that cohesive failure was prevalent in the conventional disinfection groups and adhesive failure was predominance in PDT treatment groups. Admixed failure suffered the least in all examined groups. The author speculated that the manifestation of MB in the PDT group might have interacted with the sealer diffusion and wettability causing bond weakening.

Within the restraints of the current study, progressive explorations should be acclaimed for scrutinizing the conventional and PDT disinfection outcome utilizing various PS on different sealers and their impact on radicular dentin by acknowledging structural dentin variability at different root levels, odontoblastic processes, and root length with smear layer manifestation. Additional mechanical testing of radicular dentin should be executed by imitating the oral milieu in *in-vivo* clinical trials. Dispersive spectroscopy should be operated for enhanced qualitative analysis of failure modes. Also, for the demonstration of the physical influence of PDT, scanning electron microscopy (SEM) and atomic force microscopy (AFM) of radicular dentin before and after photosensitization should be assessed systematically.

Conclusions

Combination of 2.25% NaOCl with PDT and 17% EDTA for canal disinfection with AH Plus sealer, calcium silicate sealer, and MTA-based bioceramic sealers have an unfavorable effect on the EBS of gutta-percha to the root canal wall.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Informed Consent

Not applicable.

Ethics Approval

The study protocol was reviewed and approved by the Research and Ethics Committee of Specialist Dental Practice and Research Center.

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