Original Article

In Vitro evaluation of irrigation activation techniques on bond strength of Bioceramic sealer after AgNp-based intracanal medicament removal

Vaishali Srinivasan, Janani Balachandran, Alagarsamy Venkatesh, J. Pooraninagalakshmi, Venkatachalam Prakash, Ramachandran Tamilselvi

Department of Conservative Dentistry and Endodontics, Sree Balaji Dental College and Hospital, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India

Abstract

Objective: The study aimed to determine the pushout bond strength (PBS) of bioceramic root canal sealer after removal of silver nanoparticle (AgNP) based calcium hydroxide paste using different irrigation activation techniques.

Materials and Methods: To remove the intracanal medicament, 60 single-rooted mandibular premolars were separated into four groups based on the irrigation activation used: ultrasonic irrigation (UI), laser-activated irrigation, manual dynamic activation (MDA), and conventional syringe irrigation. Obturation was done with bioceramic root canal sealer and single-c cone. After 1 week, PBS testing was done. Statistical analysis was done by ANOVA followed by *post hoc* Tukey test.

Results: The highest bond strength among the tested groups was shown by UI. *Post hoc* tests revealed significantly higher PBS values for UI ($P = 0.001$), closely followed by MDA ($P < 0.05$).

Conclusions: When compared with other irrigation activation methods, higher PBS values for bioceramic sealer were obtained when UI was used for removal of AgNP based calcium hydroxide intracanal medicament.

Keywords: Bioceramic sealer; laser-activated irrigation; manual dynamic agitation; silver nanoparticle; ultrasonic irrigation

INTRODUCTION

Meticulous chemomechanical preparation is fundamental to successful root canal therapy. Intracanal medicaments enhance disinfection and play a key role in managing periapical infection, reducing inflammation and pain, removing apical exudate, preventing interappointment contamination, limiting inflammatory resorption, and supporting hard tissue repair.[1] This by virtue of their antimicrobial action, excellent biocompatibility, tissue-dissolving properties, ability to reduce inflammation and osteoclastic activity, and their capacity to promote the formation of mineralized tissue.[2] However, in persistent infections, $Ca(OH)_2$ is often ineffective against mature *Enterococcus faecalis* biofilms.[3]

Nanotechnology offers novel solutions across biomedical applications, including antimicrobial therapy. Since Feynman's early concept in 1959 and Freitas' introduction of "nanodentistry" in 2000, nanoparticle-based materials have gained prominence due to their enhanced reactivity and superior

Address for correspondence:
Dr. Janani Balachandran,
Department of Conservative Dentistry and Endodontics,
Sree Balaji Dental College and Hospital, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India.
E-mail: janani.balachandran@gmail.com

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antimicrobial properties. Various nanoparticles – including TiO_2, ZnO, Cu, and Au – show significant antibacterial activity,[4] although silver nanoparticles (AgNPs) demonstrate superior efficacy relative to these alternatives and exert strong antibacterial action against multiple species.[5] Combination approaches may further enhance antimicrobial performance and minimize drawbacks.

$Ca(OH)_2$ combined with AgNPs has shown substantially improved antibacterial activity against *E. faecalis* compared to $Ca(OH)_2$ alone.[2,6] without significant adverse effects such as discoloration.[7] A recent scoping review reinforces AgNPs as a promising interappointment medicament due to its antimicrobial capability and potential for reducing postoperative symptoms.[8]

Residual intracanal medicament compromises sealer penetration within dentinal tubules,[9] adversely affecting sealing, increasing leakage, and interfering with complete material setting. Goldberg *et al*. demonstrated that $Ca(OH)_2$ remnants can obstruct dentinal tubules and impair obturation, with only 13 of 60 simulated lateral canals filled following its use.[10] Prolonged Ca $(OH)_2$ exposure due to incomplete removal may weaken dentin structure[11] and negatively affect sealer bond strength, as verified by Ghabraei *et al*.[11]

Various strategies – including syringe irrigation,[12] ultrasonic or sonic agitation, negative pressure techniques (EndoVac), and mechanical agitation systems (EndoActivator, SAF) – have been examined for medicament retrieval.[13-15] Recent reports indicate that laser-activated irrigation (LAI), particularly SWEEPS, achieves the greatest removal efficiency, whereas needle irrigation is least effective. Reviews concur that LAI and passive ultrasonic irrigation (PUI) represent the most effective modalities.[16] Accordingly, the present study employs these two activation methods.

With increasing adoption of single-cone obturation, bioceramic sealers – owing to their bioactive and antimicrobial characteristics – are widely recommended.[17] However, treatment success remains influenced by the pushout bond strength (PBS) of the sealer-dentin interface,[18] which may be compromised by residual medicament that obstructs tubule penetration.[19]

Existing literature primarily addresses techniques for eliminating conventional medicaments,[9] with limited attention to nanoparticle formulations. Nanoparticle-based intracanal medicaments have superior dentinal penetration, which in turn can pose as a potential challenge for their retrieval. Remnant AgNP-based medicament can also potentially stain the tooth due its silver component.[8] Thus, standardized, evidence-based protocols for their removal are essential. The present study aims to address this gap in the literature and furthermore, their effect on sealer bond strengths.

Hence, the current study intended to determine and analyze the PBS of bioceramic sealer following removal of $Ca(OH)_2$ combined with AgNPs using different irrigant activation techniques.

MATERIALS AND METHODS

The study obtained approval from the Institutional Ethical Committee (SBDCH-IEC/23-08/46). The Preferred Reporting Items for Laboratory studies in Endodontology 2021 guidelines have been followed for the conduct of this laboratory study. Sample size was calculated using G*Power software, version 3.1.4 (Heinrich Heine University Düsseldorf, Düsseldorf, Germany) as $n = 60$ based on the study with a power of 95%, effect size of 0.45, and alpha error of 5%. Subsequent to obtaining informed consent, 60 freshly extracted adult human permanent mandibular premolars with completely formed single root and straight single canal, with no signs of resorption of root and/or cracks, were included for the current study. The exclusion criteria were the presence of caries, restoration, noncarious defects, developmental defects, previous endodontic therapy, or dental anomalies. Angulated view radiographs were taken for all the collected teeth samples. The samples were stored in distilled water immediately after extraction and maintained at room temperature for 37°C in 100% humidity.

Preparation of nanoparticle based intracanal medicament

100 mL of water was used to dissolve 0.017 g of silver nitrate, and distilled until fully dissolved. Ammonium hydroxide was then added to the solution. This silver ion solution was combined with glucose solutions at molar ratios of glucose to Ag^+ of 1:4, 1:8, and 1:12. Polyvinyl alcohol was added in concentrations of 1%, 3%, and 5%, and the mixture was heated to 60°C in an Erlenmeyer flask while being stirred with a magnetic stirrer until it turned brown. Heating was stopped, but stirring continued until the mixture cooled to room temperature [Figure 1c]. The resulting colloidal AgNPs were analyzed using UV-Vis spectroscopy up to 14 days postsynthesis and characterized by transmission electron microscopy and X-ray diffraction. Finally, 2 g of AgNPs were mixed manually with 1.8 g of calcium hydroxide to produce a homogeneous paste.[20]

Sample preparation

The samples were prepared by sectioning the crowns below the cementoenamel junction to standardize root length at approximately 16 mm [Figure 1b]. Mechanical shaping of the root canals was performed using ProTaper files (Dentsply Tulsa Dental, Tulsa, OK, USA) up to size F3, with 5.25% sodium hypochlorite (NaOCl) irrigation between each file size. Canal irrigation comprised 5 mL of NaOCl for 5 min for removal of organic tissue debris, followed by 5 mL of 17% ethylenediaminetetraacetic acid (EDTA) for another 5 min

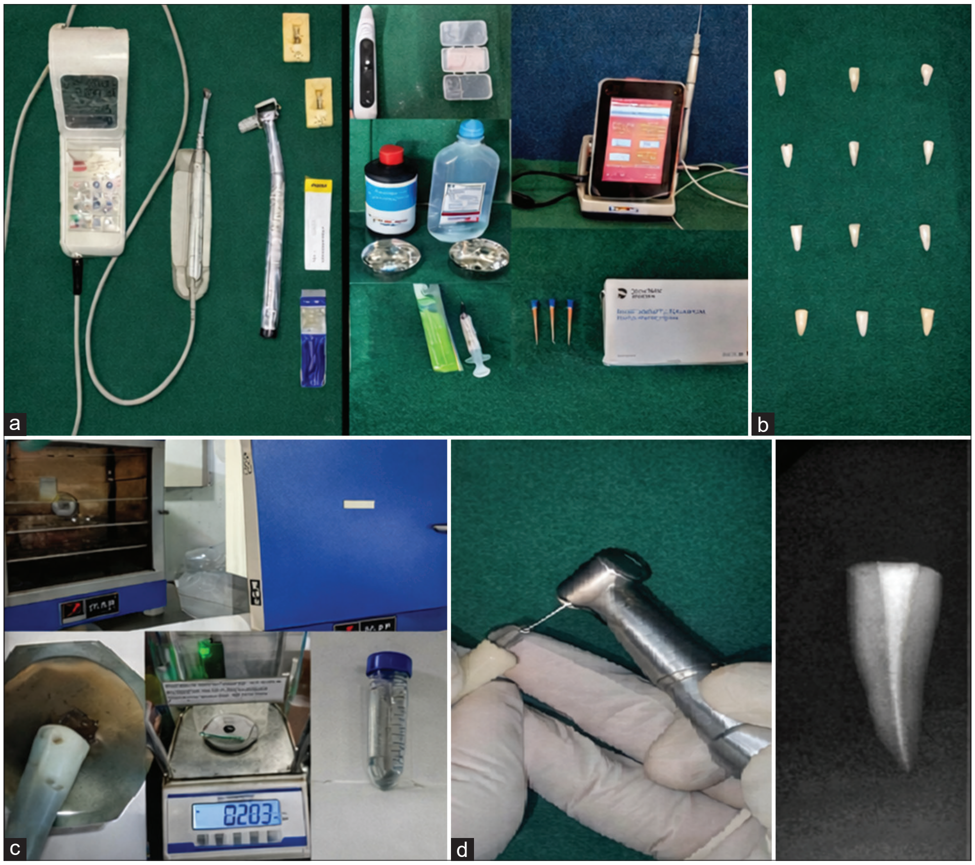

Figure 1: (a) Armamentarium, (b) Decoronated tooth samples, (c) Preparation of Silver Nanoparticles, (d) Placement of Intracanal Medicament

to eliminate the smear layer. Specimens were then rinsed with 10 mL of distilled water to neutralize the effects of EDTA and NaOCl [Figure 1a]. Following drying with paper points, the prepared intracanal medicament was introduced in all specimens using a lentulospiral [Figure 1d], and all samples were incubated at 37°C and 100% relative humidity for 2 weeks.[17]

Specimens were then divided into four groups according to different irrigation activation methods for removal of intracanal medicament. All canals were further irrigated with 2.5% NaoCl for optimum reduction of bacterial load while aiding flushing of intracanal medicament without added cytotoxic effects due to the use of a higher concentration of NaOCl.[18] Group 1 (n = 15) was subjected to ultrasonic irrigation (UI) with 5 mL of 2.5% NaOCl and Ultra X (Eighteeth, Changzhou Sifary Medical Technology Co., Ltd, Changzhou City, China) with a flexible X Silver tip (#25, 0.02) according to the manufacturer's guidelines [Figure 2a]. Group 2 (n = 15) was subjected to LAI with a 980 nm Diode laser (Intense Medical and Dental System Limited, Delhi, India) and a radial firing tip of diameter 415 µm and 5 mL of 2.5% NaOCl [Figure 2b]. The panel settings were 2 W, 30 Hz, 60 µs pulse duration, 30% water, and 20% air. Group 3 (n = 15) was subjected to manual dynamic agitation (MDA) with 5 mL of 2.5% NaOCl, agitated manually with a size F3 Gutta Percha point in 3 mm up and down motion, 100 strokes per 30 s [Figure 2c]. Group 4 (n = 15), subjected to conventional syringe irrigation (CSI), was designated as the control group, as it represents the routine, nonactivated irrigation method commonly used in clinical practice. The irrigants were replenished periodically to simulate clinical conditions. This enabled comparison of the experimental activation techniques against standard irrigation. 5 mL of 2.5% NaOCl was then used to irrigate the root canals [Figure 2d].

All 60 samples were subject to irrigation with 5 mL of 17% EDTA for 1 min and a final flush of 5 mL of saline for 3 min. Obturation was done with bioceramic root canal sealer and matched the single cone technique. The prepared samples were sealed with Cavit and incubated at 37°C under 100% relative humidity for 1 week.

Pushout bond strength test

2-mm thick cross-sections were prepared from the middle third of the roots perpendicular to the root surface using a disk (Buehler, Lake Bluff, NY). Each test group had fifteen slices prepared [Figure 2e]. A 1 mm-diameter custom stainless steel cylindrical plunger mounted on a universal testing machine was used to load the root filling material (Instron, Canton, MA, USA).

A pushout force was applied with a cylindrical piston measuring 0.8 mm in diameter at a crosshead speed of 1 mm/min, in a coronal to apical direction until bond failure happened [Figure 2f]. The bond strength at failure was measured in megapascals (MPa) by dividing the load in newton (N) by the area of the bonded interface. The area in each section was calculated using the following formula: Area = 2 πr × h (where π = constant value of 3.14, *r* = radius of the intraradicular space, and *h* = height in mm).[19]

Statistical analysis

The presentation of numerical data was conducted as values of mean and standard deviation (SD). Since the data exhibited a parametric distribution, intergroup analysis (i.e., between the four groups) was conducted using Kruskal–Wallis test followed by Tukey's *post hoc* test, which was used for intragroup (i.e., within each single group) comparisons. The level of significance was determined at $P \leq 0.05$. Statistical analysis was performed using SPSS software (version 4.1.2 for Windows; IBM Corp., Armonk, New York, USA) for Windows was used to perform statistical analysis.

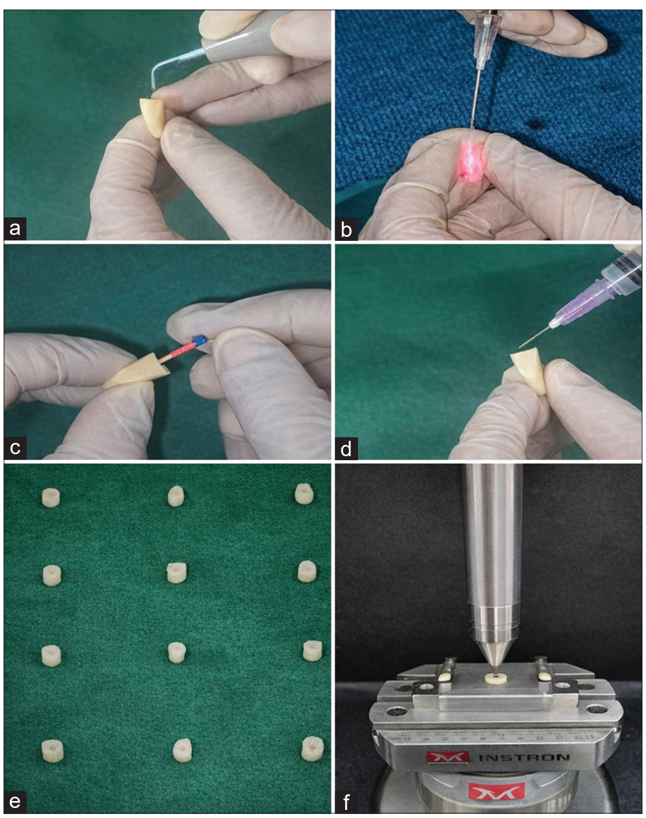

Figure 2: Removal of Intracanal Medicament using different Irrigation Activation Techniques and Push-out bond strength (PBS) testing (a) Ultrasonic Activation, (b) Laser Activation, (c) Manual Dynamic Agitation, (d) Conventional Needle Irrigation, (e) Transverse sectioning of tooth samples into 2 mm thick discs for PBS testing, (f) PBS Testing using Universal Testing Machine

RESULTS

After removal of intracanal medicament using different irrigation activation methods, mean, SD values of PBS (MPa) of bioceramic root canal sealer in the middle third of the root canal were measured and demonstrated in Table 1 and Figure 3. The experimental groups showed a significant difference compared to the control group ($P < 0.001$). The highest mean value was observed in the ultrasonic activation group (1.36 ± 0.39), followed by manual dynamic agitation (1.13 ± 0.53), Laser activation (0.986 ± 0.49), and least was observed in the control group (0.61 ± 0.41). Table 2 shows the statistical comparison of groups. Intergroup comparison revealed the ultrasonic activation group showed statistically significant results ($P = 0.000$), closely followed by the manual dynamic agitation group, which also showed statistically significant values ($P = 0.018$).

DISCUSSION

The study was aimed at evaluating the effectiveness of different irrigation activation methods on the removal of calcium hydroxide paste with AgNPs and to analyze their effect on PBS of bioceramic root canal sealers.

The strength of the bond between dentin and obturation materials is crucial for the success of endodontic procedures, as enhanced adhesive properties to dentin can improve resistance to root fractures, decrease the risk of microleakage, and increase the long-term survival of a tooth after endodontic treatment.[20]

Bioceramic sealers are hydraulic materials which are gaining popularity due to their biocompatibility and superior bond strength. These premixed cements, derived from tricalcium silicate, offer excellent bioactivity, enabling them to bond with pulp or periodontal tissue and stimulate hydroxyapatite production. This makes them both osteoconductive and antibacterial. Their favorable chemical characteristics, such as hydrophilicity, radiopacity, slight expansion over time, and good flowability, make them ideal for a variety of endodontic applications. The advent of bioceramic sealers has transformed obturation techniques, with a shift toward using a higher ratio of sealer to gutta-percha, improving the biological and antibacterial efficacy.[21] In the past study done by Ferriera *et al*., total fill demonstrated the highest

Table 1: Mean, standard deviation values of pushout bond strength (MPa) of bioceramic root canal sealer for different types of irrigation activation methods

Groups	*n*	Mean±SD	Median	*P*
Ultrasonic activation	15	1.3613±0.39805	1.4500	0.001
Laser activation	15	0.9887±0.49209	0.7800	
MDA	15	1.1327±0.53872	1.2400	
Control	15	0.6140±0.41672	0.3700	
Total	60	1.0242±0.52926	0.8500	

SD: Standard deviation, MDA: Manual dynamic agitation

PBS values, after which was Biodentine followed lastly by mineral trioxide aggregate. Thus, regardless of which hydraulic cement was tested, blood contamination did not impact the resistance to dislodgement.[22,23]

In the current study, different irrigation activation methods were used to evaluate PBS (MPa) of bioceramic root canal sealer in the middle third of the root canal after removal of intracanal medicaments. The experimental groups showed a significant difference compared to the control group ($P = 0.001$). Mean PBS values were highest in ultrasonic activation group (1.36 ± 0.39), followed by manual dynamic agitation (1.13 ± 0.53) and laser activation (0.986 ± 0.49). The least values were observed in the control group (0.61 ± 0.41) [Table 1]. Table 2 shows the statistical comparison of groups. In intergroup comparison, the ultrasonic activation group ($P = 0.000$) and manual dynamic agitation group, when compared with the control group ($P = 0.018$), showed a higher PBS.

Evidence shows that intracanal medications be used in between endodontic treatment sessions to maintain disinfection. The features shown by root dentin at the dentin/obturation material interface can be influenced by chemical irrigants and intracanal medications, as shown by several studies. Intracanal medications affect the bond strength between the sealer and dentin, which appears to be significant. Residual intracanal medicament can serve as an impediment that prevents the process of development of the mechanical bonds and midst the sealers and dentin, which has a negative effect on the adaptation with dentinal walls, resulting in decreased PBS.

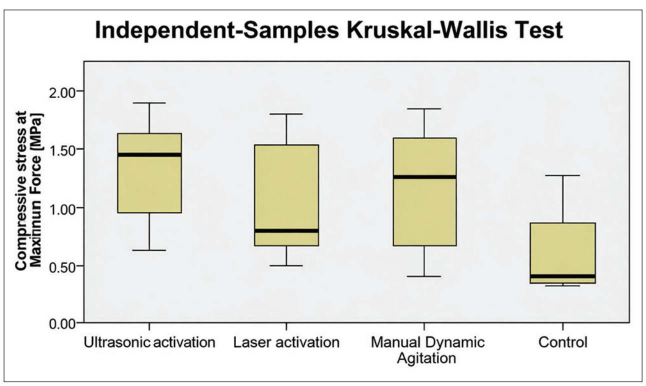

Figure 3: Independent-samples Kruskal–Wallis test showing comparison of push-out bond strength among the study groups

In multiple investigations, including Rödig *et al*.[24] and Uzunoglu-Özyürek *et al*.,[25] it was discovered that the application of calcium hydroxide reduced dentinal permeability by physically blocking the tubules within the dentin. This could provide an explanation for the PBS drop that is seen after applying calcium hydroxide. Factors that determine sealing efficacy are a sealer's solubility and ability to adhere to root canal filling cones and dentin. The push-out test is usually used to evaluate the adhesion of endodontic sealers to root canal dentin. Bond strength is the force per unit area needed to dislodge the adhesive substance from the dentin. There are limited sources in the literature citing the influence of the combined effects of Calcium hydroxide with nanosilver particles as intracanal medication on the bond strength of Bioceramic sealer to root dentin. Hence, in this study PBS of bioceramic sealer after removal of AgNP based intracanal medicament with various irrigation activation methods was assessed.

The ultrasonic activation group showed the highest PBS for bioceramic root canal sealer. This could be due to better removal of intracanal medicament using ultrasonic activation, which resulted in better penetration of sealer into the dentinal tubules, resulting in higher PBS. Similar results were obtained by Ghabraei *et al*., where PBS of AH plus and bioceramic sealers were significantly higher in specimens in which calcium hydroxide removal was performed by UI.[2] The core concept of UI is the idea that energy is transferred from an ultrasonic device to the irrigating solution inside the root canal.[11] The elimination of both organic and inorganic debris from the root canal walls was directly linked to the use of an irrigant solution and ultrasonic vibration. The majority of the studies show that when it comes to removing intracanal medication, UI works better than conventional syringe and needle irrigation.[26] This might be caused by the fact that ultrasound, as opposed to manual irrigation, produces a higher speed and flow volume of the irrigant in the canal during irrigation, which leads to the removal of more debris, less apical packing, improved irrigant solution access to accessory canals, and even a flush effect.[27] The current results were in contrast with the study by Swathi *et al*.,[28] where LAI was more efficient in removing $Ca(OH)_2$

Table 2: Intergroup comparison regarding pushout bond strength of bioceramic root canal sealer at the middle third of root canal

Groups	Test statistic	SE	Standard test statistic	Significant	Adjusted significant
Control - laser activation	15.500	6.373	2.432	0.015	0.090
Control - MDA	18.933	6.373	2.971	0.003	0.018
Control - ultrasonic activation	27.700	6.373	4.346	0.000	0.000
Laser activation - MDA	−3.433	6.373	−0.539	0.590	1.000
Laser activation - ultrasonic activation	12.200	6.373	1.914	0.056	0.334
MDA - ultrasonic activation	8.767	6.373	1.376	0.169	1.000

MDA: Manual dynamic agitation, SE: Standard error and MTAP than UI. LAI is based on the idea of energy transmission, which results in temporary cavitation in the liquid as a result of optical breakdown.

Manual dynamic agitation showed the second-highest PBS after ultrasonic activation. The manual dynamic agitation technique can help prevent the entrapment of apical gas within 0–2 mm of the apical seat by repeatedly inserting gutta-percha. According to Susin *et al*.,[29] where evaluation of the effectiveness of different irrigation activation methods in irrigant penetration was done, it was found that the penetration of the irrigant in the MDA group was inferior to PUI group. This may be due to the fact that the push-pull motion of the gutta-percha point at 100 strokes/min produces currents at a lower frequency (3.3 Hz), whereas PUI generates higher frequencies of 40–45 kHz through secondary acoustic streaming. Consequently, the lower frequency effectively breaks the vapor lock more efficiently, resulting in increased irrigant flow in the PUI group. Hence, since the irrigant penetration is less in MDA when compared to ultrasonic activation, it leads to lesser removal of intracanal medicament from the tubules, which results in lesser penetration of sealer, and thus reduction in bond strength of sealer is seen. However, the push-pull motion of the gutta-percha point within the canal creates physical stretching, folding, and cutting of fluid layers, which increases intracanal pressure and yields results comparable to those achieved with ultrasonic activation.

In the present study, LAI had lower bond strength when compared to ultrasonic activation and manual dynamic agitation. The reason for this could be because that the laser tip is kept immobile centrally in the root canal without contacting the walls during emission of laser radiation, without agitation of irrigant unlike other activation methods. This could result in lesser removal of intracanal medicament, thus interfering with the sealer penetration, leading to reduced bond strength. The results of our study were in contrast with the study by Gorduysus *et al*.[30] where The effectiveness of the LAI protocol was superior to that of UI, likely because LAI relies on energy transmission to induce transient cavitation in the liquid through optical breakdown.

The concept behind LAI is the use of photothermal and photomechanical processes to produce particular cavitation phenomena and acoustic streaming in intracanal fluids. Significant vaporization and the development of large elliptical vapor bubbles are caused by the significant absorption of laser energy in water and NaOCl. An irrigant with high intracanal pressure experiences a volumetric expansion up to 1600 times its initial volume due to the vapour bubbles, forcing the fluid out of the canal. After 100–200 μs, the bubbles burst, generating pressure that forces fluid back into the canal and causing a secondary cavitation effect.[31] It has been shown that this method works well for removing the smear layer and intracanal dentine debris. Furthermore, diode lasers have been used in our study, which have low penetration depth in dentin and bactericidal effects when compared to Nd: YAG lasers, however morphological changes in the dentine walls after diode laser irradiation are similar to those obtained with a Nd: YAG laser (disruption and melting of the smear layer, closed, and opened dentinal tubules). This supports the use of diode lasers in our study.[32,33]

In the control group where we used CSI to remove intracanal medicament, the bond strength of bioceramic sealer was the least. It can be attributed to the fact that NaOCl, alone with no activation, has a limited ability to dissolve inorganic substances such as calcium. When the intracanal medicament is not removed effectively, it interferes with sealer penetration, resulting in lower values of sealer bond strength.[34]

A limitation of this study is that it did not assess the effectiveness of various irrigation activation methods for removing AgNP-based intracanal medicaments from dentinal tubules. Future research should focus on evaluating the removal of these medicaments and examining sealer penetration postremoval using advanced imaging techniques.[35]

Although nanoparticle-based intracanal medicaments are being explored for their disinfecting and antimicrobial properties,[36] there is a lack of research on their removal efficiency and its impact on sealer penetration, which could ultimately affect treatment success rates. The current study is the first of its kind to evaluate bond strength of bioceramic sealer with the root canal dentin after removal of AgNP-based intracanal medicament using different irrigation activation methods.

Ultrasonic method of irrigant activation provided the best technique for intracanal medicament removal as it showed the highest the PBS values for bioceramic sealer ($P = 0.000$). However, further studies are warranted to record the amount of intracanal medicament removal from dentinal tubules after irrigant activation methods. When using calcium hydroxide with nano silver particles as an intracanal medicament, it is crucial to utilize the UI activation method to ensure a strong seal between the root canal sealer and dentinal wall.

CONCLUSIONS

The PBS of the bioceramic root canal sealer was significantly higher in the ultrasonic activation group ($P = 0.000$) and slightly lower but still notable in the manual dynamic agitation group ($P = 0.018$).

The popularity of utilizing AgNP-based antibacterials as intracanal medicament is increasing.[37] UI provides an excellent method for effectively removing nano silver particle-based medicaments, thus promoting an optimum sealer bond. Manual dynamic agitation presents itself as a viable and cost-effective alternative for intracanal medicament removal, ranking closely behind UI also ensuring predictable sealer bond strength. With the advent of nanoparticle-based medicaments revolutionizing the market and our practice, development of an evidence-based protocol is essential for ensiring successful endodontic outcomes.

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Conflicts of interest

There are no conflicts of interest.

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