Original Article

A confocal laser scanning microscopic analysis of efficacy of different activated irrigants in the removal of calcium hydroxide medicament and subsequent penetrability of Bio-C sealer – An *in vitro* study

Sree Laksmi Bademela, T. B. V. G. Raju, Krishna Prasad Parvathaneni, Abitha Seshadri, Gowtam Dev Dondapati

Department of Conservative Dentistry and Endodontics, Sree Sai Dental College and Research Institute, Srikakulam, Andhra Pradesh, India

Abstract

Aim: The aim of the study was to investigate the efficacy of different irrigants activated by pro-agitator tip system (PATS) Vario on the removal of calcium hydroxide medicament and subsequent penetration depth of Bio-C sealer.

Materials and Methods: Fifty single-rooted mandibular premolars were selected. Access cavities were prepared; biomechanical preparation was done. Metapex that had been combined with rhodamine B dye was used to fill each sample. All the samples were divided into five groups ($n = 10$) – Group I: chitosan–citrate, Group II: intracanal heated sodium hypochlorite (NaOCl), Group III: phytic acid, Group IV: SmearClear, and Group V: saline. All samples were obturated using gutta-percha and Bio-C sealer (combined with fluorescein dye). Later, all samples were sectioned at 3, 6, and 9 mm from the apex and observed under a confocal microscope for residual $Ca(OH)_2$ and sealer penetration into dentinal tubules.

Results: The saline group exhibited the least amount of sealer penetration and high residual $Ca(OH)_2$, both of which were statistically significant ($P \leq 0.05$).

Conclusion: The use of PATS Vario for irrigant activation enhanced the calcium hydroxide removal efficacy and penetration of Bio-C sealer into dentinal tubules. The elimination of calcium hydroxide and sealer penetration, from the apical region of the tooth, can be accelerated by intracanal heating of NaOCl.

Keywords: Chitosan–citrate; intracanal heated sodium hypochlorite; pro-agitator tip system Vario; phytic acid; SmearClear

INTRODUCTION

Microorganisms are the primary etiological agents in pulp and periradicular diseases.[1] Chemomechanical preparation can significantly clean the root canal system but cannot completely free it from microorganisms.[2] Intracanal medicaments are primarily suggested to eliminate the residual bacteria between appointments and also it acts as a physiochemical barrier.[3] One of the most popular and widely accepted intracanal medications is calcium hydroxide. To maximize the effectiveness of the medication, the dressing must be placed for at least 7 days.[4] Calcium hydroxide dressing removal before root canal filling is important.[5] The residual medicament acts as a barrier, impeding the sealer penetration and negatively affecting the adaptation and bonding between the sealer and dentin.[6] Goldberg *et al*. have shown that calcium hydroxide remnants in the root canal affected the sealer penetration into lateral canals.[7] Several irrigants

Address for correspondence:
Dr. Sree Laksmi Bademela,
Sree Sai Dental College and Research Institute,
Srikakulam - 532 001, Andhra Pradesh, India.
E-mail: sreelakshmibadimela31@gmail.com

Date of submission : 18.04.2024
Review completed : 31.05.2024
Date of acceptance : 05.08.2024
Published : 05.10.2024

Access this article online

Quick Response Code:

Website: https://journals.lww.com/jcde

DOI: 10.4103/JCDE.JCDE_216_24

How to cite this article: Bademela SL, Raju TB, Parvathaneni KP, Seshadri A, Dondapati GD. A confocal laser scanning microscopic analysis of efficacy of different activated irrigants in the removal of calcium hydroxide medicament and subsequent penetrability of Bio-C sealer – An *in vitro* study. J Conserv Dent Endod 2024;27:1015-20.

and irrigant activation techniques have been proposed for calcium hydroxide dressing removal.

Earlier investigations in the removal of intracanal calcium hydroxide have been based primarily on two parameters: the action of chelators and other root canal irrigants and the performance of irrigation dynamics.

Apart from several activation systems, different irrigation solutions are used to achieve a more calcium hydroxide-free root canal system. Four different root canal irrigants activated by pro-agitator tip system (PATS) Vario have been used in this study.

Chitosan, a naturally occurring polysaccharide, has been used in dentistry as a barrier membrane for periodontal therapy and as a chlorhexidine delivery agent for the oral mucosa. In addition, under acidic conditions, it has a high capacity for chelating a variety of metal ions.[8]

Citric acid's chelation property is dependent not only on the direct physical chelating action but also on the solution pH.[9] Studies show that in combination with chitosan, it causes better chelation.

Iandolo *et al*. demonstrated that heating sodium hypochlorite (NaOCl) intracanally at 180°C resulted in a considerable reduction in debris as compared to heating extraorally at 50°C. Numerous research have demonstrated how changing the temperature can increase the effectiveness of NaOCl.[10]

A promising natural organic substance that can be commercially produced from rice bran is phytic acid. It has a high density of negative charges, making it a powerful chelator of multivalent cations such as calcium, magnesium, and iron;[11] the binding of calcium with phytic acid is pH-dependent.[12]

A newly introduced chelating agent named SmearClear (Sybronendo, Orange, CA, USA) is a 17% ethylenediaminetetraacetic acid (EDTA)-based endodontic irrigant that also contains cetrimide, polyoxyethylene, and isooctyl cyclohexyl ether as additional surfactants.[13]

Agitation of intracanal irrigants increases their efficacy. Over time, a variety of innovative techniques have emerged, from ultrasonically induced agitation to hand dynamic agitation of gutta-percha cones. PATS, a novel irrigation system that uses an autoclavable polymer tip to ultrasonically stir the irrigant, was recently introduced by InnovationsEndo Ltd. in Nashik, Maharashtra, India (2017).

Bio-C Sealer (Angelus, Londrina, PR, Brazil) is a new, premixed, ready-to-use sealer composed mainly of calcium silicates. Previous studies stated that Bio-C sealer showed greater penetration and better adaptation to dentinal tubules when compared to AH Plus sealer due to their smaller particle size, fluidity, and hydrophilicity.

To date, no studies have compared the efficacy of chitosan–citrate, SmearClear, phytic acid, and intracanal heated NaOCl in the removal of calcium hydroxide medicament. Therefore, the objective of this study is to evaluate and compare the effect of chitosan–citrate, SmearClear, phytic acid, and intracanal heated NaOCl activated by PATS Vario in the removal of calcium hydroxide and the subsequent penetration of Bio-C sealer into dentinal tubules using a confocal microscope.

MATERIALS AND METHODS

An institutional ethical committee approval (SSDCRI/IEC/2021-22 / 5/1) was obtained for research purposes. Fifty freshly collected intact single-rooted mandibular premolars were selected for this study and stored in 0.1% thymol until further use. Teeth were decoronated to standardize them to a length of 15 mm. The working length was established. Root canals were prepared up to F3 with the ProTaper System (Dentsply Maillefer). Irrigation was performed with 2 mL of 5.25% NaOCl at each change of instrument, and a final rinse was performed with 2 mL of 17% EDTA for 1 min. Then, canals were irrigated with 5 mL of normal saline and dried with paper points. The canals were filled with calcium hydroxide (Meta Biomed Metapex) mixed with 0.1% rhodamine B using Lentulo spirals. The coronal openings of the root canals were sealed with a small cotton pellet and temporary filling material and were stored at 37°C in 100% humidity for 2 weeks.

Preparation of chitosan–citrate solution

The pH of 10% citric acid was adjusted with NaOH. 0.04 g chitosan oligosaccharide (Nano Research Lab, Jharkhand, India) was added to 1 mL of 10% citrate buffer solution, and the mixture was stirred until completely dissolved. The pH of the chitosan–citrate solution was adjusted to 3.5.

All samples were randomly divided into five groups, according to the solution to be used for calcium hydroxide removal.

- Group I – The root canals were filled with chitosan–citrate solution, and a PATS Vario tip was inserted into the canal at 1 mm short of working length. The tip was moved in a pecking movement in the canal for 1 min, and this process was repeated twice (the activation regimen consisted of four cycles [30 s each], with 1 mL of irrigant used for each cycle)
- Group II – The root canals were filled with NaOCl. The intracanal heating of NaOCl was done for 8 s with a System B heat source XF-tip (40/.03) (Meta Biomed Co. Ltd, Korea), placed 3 mm short of the working length.

After intracanal heating of NaOCl, activation done with a PATS Vario tip was carried on similar to that in Group I

- Group III – The root canals were filled with 1% phytic acid and activation was done similarly to that in Group I
- Group IV – The root canals were filled with SmearClear and activation carried on with a PATS Vario tip similar to that in Group I
- Group V – The root canals were filled with normal saline and activation carried on with a PATS Vario tip similar to that in Group I.

After irrigation, each sample was rinsed with 10 mL of distilled water and dried using paper points. Bio-C sealer was mixed with fluorescein dye and coated on the root canal walls using a Lentulo spiral. Then, the root canal was obturated with gutta-percha cones using the lateral condensation technique. The root canal orifice was sealed with temporary cement.

After final obturation, all samples were stored for 1 week (37°C, 100% relative humidity). Later, all samples were embedded into resin blocks. Then, they were sectioned transversely with a cutting machine (IsoMet 1000; Buehler, Lake Forest, IL), under water coolant at 3, 6, and 9 mm from the apex. The samples were examined under a confocal microscope for residual calcium hydroxide and sealer penetration (epifluorescence with wavelengths of absorption and emission for rhodamine B of 540/590 nm and fluorescein of 536/617 nm). The slices were photographed under a confocal laser scanning microscope (Zeiss LSM 510; Carl Zeiss, Jena, Germany). These Confocal laser scanning microscopic (CLSM) images were analyzed for residual calcium hydroxide, sealer penetration area, and sealer penetration depth. The penetration area of residual calcium hydroxide and sealer was measured using the area calculating tool of the Zeiss Zen software (Carl Zeiss) [Figure 1a and b]. For checking the penetration depth of sealer into the dentinal tubules, the point from the root canal wall to the deepest point where sealer could be observed was measured at four predetermined points (mesial, distal, buccal, and lingual). The mean depth was measured by averaging these four values for each specimen [Figure 1c].

Statistical analysis

Data were analyzed using IBM SPSS version 20 software (IBM SPSS, IBM Corp., Armonk, NY, USA). Descriptive statistics, one-way analysis of variance with Tukey's *post hoc* tests for multiple pairwise comparisons, and independent samples *t*-tests were done to analyze the study data.

RESULTS

Table 1 presents the comparison between residual $Ca(OH)_2$ and sealer penetration, which shows a statistically significant difference between the mean values.

Table 2 shows the comparison of maximum sealer depth (μm^2) between the study groups. In all three regions, Group V demonstrated significantly lesser maximum sealer depth (213.69 ± 38.53, 650.61 ± 151.6, and 693 ± 135.35 in the apical, middle, and coronal thirds, respectively) than the other groups. This difference between the groups in all three-thirds was statistically significant. In the apical region, Group V showed the least penetration depth with significant differences noted in Groups I, II, and IV with Group V in multiple pairwise comparisons using Tukey's *post hoc* tests. In the middle region, Group I showed significantly more sealer penetration than Group V. In the coronal region, Group II showed significantly higher sealer penetration than

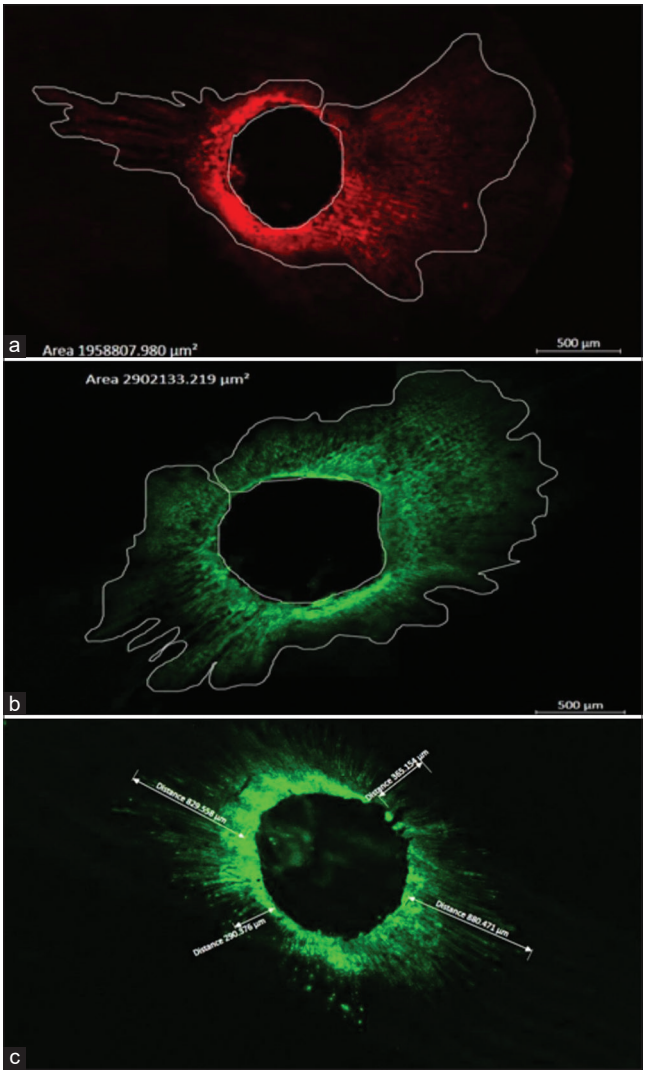

Figure 1: (a) Penetration area of residual calcium hydroxide, (b) Penetration area of Bio-C sealer, (c) Penetration depth of Bio-C sealer (measured at four predetermined points)

Group V in multiple pairwise comparisons using Tukey's *post hoc* tests.

DISCUSSION

Treatment of teeth with apical periodontitis has two microbiological goals: reducing the microbial bioburden to levels compatible with periradicular tissue repair and preventing microbial recolonization of the treated canal.[14] Long-lasting antibacterial intracanal treatment should be used in between endodontic sessions until the final canal sealing.[15] The most commonly used intracanal medicament is calcium hydroxide because it is effective against the majority of endodontic pathogens and is a biocompatible material.[16]

In this study, Metapex (iodoform in oil vehicle) was used as a medicament because it claims to have good antimicrobial activity against *Enterococcus faecalis*. Cwikla *et al*. compared calcium hydroxide mixed with water, $Ca(OH)_2$ mixed with iodine–potassium iodide, and $Ca(OH)_2$ mixed with iodoform and silicone oil (Metapex). They concluded that Metapex is the most effective dentinal tubule disinfectant.[17] Any intracanal medicament has to be completely removed before obturation. Incomplete removal of $Ca(OH)_2$ may interfere with the sealing, bonding, and dentinal tubule penetration of endodontic sealers, thus negatively affecting the performance of the sealer and possibly impairing the long-term prognosis.[18,19] To increase the removal of calcium hydroxide from the canals, various chelators and other root canal irrigants, in conjunction with irrigation activation systems that use characteristics such as acoustic streaming in passive ultrasonic irrigation, negative pressure in EndoVac, vigorous intracanal fluid agitation in EndoActivator, and finally, manual dynamic agitation, were helpful in removal of intracanal medicament and these activation systems proven to increase the dentinal tubule penetration in apical third in comparison to conventional needle irrigation. In the present study, we compared and evaluated the area of residual calcium hydroxide, area of sealer penetration, and maximum sealer penetration depth into dentinal tubules,

Table 1: Comparison between residual calcium hydroxide and sealer penetration stratified by area and group

Group	Area	*n*	Residual $Ca(OH)_2$, mean±SD	Sealer penetration, mean±SD	*P*
I	Apical third	10	450,374.05±262,603.69	525,119.3±392,960.27	0.114
	Middle third	10	2,077,869.52±1,225,442.74	2,761,399.6±1,621,546.7	0.005*
	Coronal third	10	2,421,965.00±1,533,789.61	3,048,303.1±1,850,084.3	0.005*
II	Apical third	10	380,962.44±143,884.46	438,179.25±177,807.18	0.037*
	Middle third	10	1,732,771.3±596,433.92	2,409,677.3±859,471.44	0.005*
	Coronal third	10	2,833,595.49±939,998.32	4,026,294.1±1,098,336.8	0.005*
III	Apical third	10	361,448.3±94,263.7	348,904.4±97,135.9	0.285
	Middle third	10	1,187,533.7±379,175.28	1,373,709.5±498,584.8	0.007*
	Coronal third	10	3,085,943.5±749,388.02	3,261,959.1±875,620.98	0.059
IV	Apical third	10	440,646.1±108,863.1	591,521.79±193,339.06	0.005*
	Middle third	10	2,056,684.4±817,836.95	2,396,701.3±1,038,632.6	0.047*
	Coronal third	10	3,176,671.6±616,837.82	4,014,079.5±1,008,695.2	0.005*
V	Apical third	10	599,444.4±170,021.4	372,268.8±126,157.1	0.005*
	Middle third	10	1,571,027.02±637,172.25	1,592,401.1±789,733.45	0.959
	Coronal third	10	3,292,767.8±795,672.90	3,239,152.1±8,96,428.80	0.878

*Significance (Group I: Chitosan–citrate, Group II: Intracanal heated NaOCl, Group III: Phytic acid, Group IV: SmearClear, Group V: Saline). Independent samples *t*-test, $P \leq 0.05$ considered statistically significant. $Ca(OH)_2$: Calcium hydroxide, SD: Standard deviation

Table 2: Comparison of maximum sealer depth between the study groups

Area	Group	*n*	Mean	SD	SE	95% CI lower bound	95% CI upper bound	*F*	*P*
Apical third	I	10	302.47[a]	102.49	32.41	229.15	375.79	5.12	0.002*
	II	10	314.8[b]	59.88	18.936	271.96	357.63		
	III	10	263.95	37.25	11.78	237.29	290.6		
	IV	10	341.84[c]	85.11	26.91	280.96	402.73		
	V	10	213.69[a,b,c]	38.53	12.18631	186.1290	241.26		
Middle third	I	10	839.30[a]	232.13	73.40	673.24	1005.36	3.09	0.025*
	II	10	664.64	114.271	36.13	582.9	746.39		
	III	10	688.69	128.947	40.776	596.44	780.93		
	IV	10	806.87	123.084	38.922	718.83	894.92		
	V	10	650.61[a]	151.601	47.94	542.17	759.06		
Coronal third	I	10	805.84	134.96	42.67	709.3	902.39	3.92	0.008*
	II	10	928.09[a]	166.95	52.79	808.65	1047.52		
	III	10	812.05	104.32	32.98	737.42	886.67		
	IV	10	862.77	141.63	44.78	761.45	964.09		
	V	10	693.003[a]	135.35	42.80	596.17	789.82		

*Significance. One-way ANOVA; $P \leq 0.05$ is considered statistically significant. Mean values with the same superscripts within the group depict significant differences in multiple pairwise comparisons with Tukey's *post hoc* tests (Group I: Chitosan–citrate, Group II: Intracanal heated NaOCl, Group III: Phytic acid, Group IV: SmearClear, Group V: Saline). ANOVA: Analysis of variance, NaOCl: Sodium hypochlorite, SD: Standard deviation, SE: Standard error, CI: Confidence interval

after using chitosan–citrate, intracanal heated NaOCl, phytic acid, and SmearClear irrigants, combined with sonic activation using PATS Vario, for removal of calcium hydroxide, in the three radicular thirds, using confocal laser scanning microscope. Two fluorescent dyes were utilized to distinguish between the two materials: rhodamine B (red) with $Ca(OH)_2$ and fluorescein (green) with Bio-C sealer. Residual calcium hydroxide can enter dentinal tubules, reducing dentin permeability and, ultimately, decreasing sealer penetration.[20] Consistent with earlier research conducted by Gokturk *et al*., 2017,[21] the observed data demonstrated that the apical third of the root canals had greater levels of residual calcium hydroxide in comparison to the coronal and middle thirds. The coronal and middle thirds of prepared canals have greater surface areas than the apical third, allowing for better contact between chelating molecules and calcium in the root canal walls. In addition, further research has shown that coronally lost solution causes a drop in hydrostatic pressure toward the apical third.[22] The results of this study showed that the samples in the saline group (Group V) have a lower residual calcium hydroxide in the middle third compared to Groups I, II, and IV without any significant difference but a significant increase in residual calcium hydroxide and a decrease in sealer penetration in the coronal and apical third. This may be explained as saline being a working solution rather than an irrigant neither having tissue dissolving nor chelating properties. The mechanical agitation with polyamide tip of PATS Vario favors three dimensional movement of saline, thereby flushing out the intracanal medicament from the root canal. The inferior retrievability of $Ca(OH)_2$ in the apical thirds may be due to the "vapor lock effect" of the air trapped which limits the irrigant flow. These results are in agreement with studies by McMichael *et al*.(2016) and Wang *et al*.(2018) who reported less sealer tubule penetration in the apical third compared to the middle and cervical root canal thirds[23,24]. The antibacterial and smear-clearing properties would be enhanced by the chitosan and citric acid combination. Praveen *et al*. (2017) suggest that employing citric acid-incorporated chitosan as a final rinse solution during biomechanical preparation seems promising.[25] The residual $Ca(OH)_2$ in the chitosan–citrate group is significaznty less when compared with the saline which is in accordance with a research paper by Nandini *et al*. (2006)[26] and Vineeta *et al*.,[8] stating that 0.2% chitosan was more effective than 17% EDTA solution in removing oil-based $Ca(OH)_2$. In a study done by Raghu *et al*, where metapex or calcium hydroxide with distilled water were eliminated from the canal using either EDTA, citric acid, or chitosan in combination with ultrasonic agitation; they concluded that 0.2% Chitosan with ultrasonic agitation results in a smaller amount of $Ca(OH)_2$ residue.[27] In line with Abidin *et al*., 2022, the sealer penetration depth in the middle third of chitosan–citrate group samples in this study was the highest among all the groups. In their investigation, they observed that using a 2% chitosan solution increased the penetration of bioceramic sealers compared to resin-based sealers.[28] The maximum penetration depth of Bio-C sealer in the coronal third is seen in the intracanal heated NaOCl group which is statistically significant when compared to the saline group. Comparing the experimental groups, phytic acid showed a reduced sealer penetration. These results are in line with research conducted by Eskander *et al.*, where 17% EDTA led to deeper sealer penetration as compared to 1% phytic acid.[29] Reducing the surface tension of endodontic solutions improves their dentin wetting ability and improves their flow into narrow root canals[30] which might be the probable reason for the better calcium hydroxide removal and sealer penetration from the apical area in the SmearClear group in our study. The depth of sealer penetration is also influenced by the chemical and physical properties of the sealer. Previous studies stated that smaller particle size, hydrophilicity, and low contact angle of bioceramic sealer increase the penetration. In this study, we used Bio-C sealer which contains nanometric particles and is premixed. Previous studies[31,32] show that bioceramic sealers have an increased penetration depth when compared to AH Plus sealer.

Decoronation of the samples removes the irrigant's coronal reservoir. This could provide a limitation to the ongoing research. Another limitation concerns the subjective interpretation of the images, which could lead to prejudice among examiners. Although the results are satisfactory, many of the samples showed remnants of $Ca(OH)_2$, thus further research is necessary to optimize irrigation protocols for the removal of intracanal medicament and also to establish its effectiveness in complex root canal anatomies and impact on the surrounding tissues.

CONCLUSION

All the irrigants used in this study displayed remnants of calcium hydroxide in all thirds of tooth sections. The chelators employed in this study, specifically chitosan–citrate, intracanal heated NaOCl, and SmearClear, can improve the removal of calcium hydroxide and increase the sealer penetration in the coronal, middle, and apical thirds of the tooth when used in conjunction with PATS Vario.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Himadri P, Arpita S, Lopamoodra D, Subrahata H, Subir S. Application of intracanal medicaments: A review. J Dent Med Sci 2019;18:14-21.
2. Almyroudi A, Mackenzie D, McHugh S, Saunders WP. The effectiveness of various disinfectants used as endodontic intracanal medications: An *in vitro* study. J Endod 2002;28:163-7.

3. Chawla A, Kumar V. Evaluating the efficacy of different techniques and irrigation solutions for removal of calcium hydroxide from the root canal system: A scanning electron microscope study. J Conserv Dent 2018;21:394-400.
4. Reddy S, Prakash V, Subbiya A, Mitthra S. 100 years of calcium hydroxide in dentistry: A review of literature. Indian J Forensic Med Toxicol 2020;14:1203-19.
5. Machado ME, Veintimilla Lozada VN, Carrillo Rengifo KJ, Guillén RE, Caballero-Flores H, Nabeshima CK. Confocal laser scanning microscopic analysis of the penetration of an epoxy resin-based sealer into dentinal tubules after calcium hydroxide dressing. Aust Endod J 2021;47:480-6.
6. Calt S, Serper A. Dentinal tubule penetration of root canal sealers after root canal dressing with calcium hydroxide. J Endod 1999;25:431-3.
7. Goldberg F, Artaza LP, De S. Influence of calcium hydroxide dressing on the obturation of simulated lateral canals. J Endod 2002;28:99-101.
8. Vineeta N, Gupta S, Chandra A. Retrievabilty of calcium hydroxide intracanal medicament with chitosan from root canals: An *in vitro* CBCT volumetric analysis. J Conserv Dent 2014;17:454-7.
9. Chockattu SJ, Deepak BS, Goud KM. Comparison of efficiency of ethylenediaminetetraacetic acid, citric acid, and etidronate in the removal of calcium hydroxide intracanal medicament using scanning electron microscopic analysis: An *in-vitro* study. J Conserv Dent 2017;20:6-11.
10. Iandolo A, Amato M, Dagna A, Poggio C, Abdellatif D, Franco V, *et al.* Intracanal heating of sodium hypochlorite: Scanning electron microscope evaluation of root canal walls. J Conserv Dent 2018;21:569-73.
11. Eymirli A, Nagas E, Uyanik MO, Cehreli ZC. Effect of laser-activated irrigation with ethylene diaminetetraacetic acid and phytic acid on the removal of calcium hydroxide and triple antibiotic paste from root dentin. Photomed Laser Surg 2017;35:43-8.
12. Nikhil V, Jaiswal S, Bansal P, Arora R, Raj S, Malhotra P. Effect of phytic acid, ethylenediaminetetraacetic acid, and chitosan solutions on microhardness of the human radicular dentin. J Conserv Dent 2016;19:179-83.
13. Afkhami F, Sadegh M, Sooratgar A, Montazeri E. Effect of smear clear and conventional root canal irrigants on push-out bond strength of resilon/epiphany system. Iran Endod J 2018;13:481-5.
14. Vera J, Siqueira JF Jr., Ricucci D, Loghin S, Fernández N, Flores B, *et al.* One- versus two-visit endodontic treatment of teeth with apical periodontitis: A histobacteriologic study. J Endod 2012;38:1040-52.
15. Singh M, Kasat VO. Efficacy of various combinations of irrigants and medicaments on *Candida albican*: An *in vitro* study. J Int Soc Prev Community Dent 2015;5:157-62.
16. Athanassiadis B, Abbott PV, Walsh LJ. The use of calcium hydroxide, antibiotics and biocides as antimicrobial medicaments in endodontics. Aust Dent J 2007;52:S64-82.
17. Cwikla SJ, Bélanger M, Giguère S, Fox AP. Dentinal Tubule Disinfection Using Three Calcium Hydroxide Formulations. J Endod 2005;31:50-2.
18. Ghabraei S, Bolhari B, Yaghoobnejad F, Meraji N. Effect of intra-canal calcium hydroxide remnants on the push-out bond strength of two endodontic sealers. Iran Endod J 2017;12:168-72.
19. Böttcher DE, Hirai VH, Da Silva Neto UX, Grecca FS. Effect of calcium hydroxide dressing on the long-term sealing ability of two different endodontic sealers: An *in vitro* study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;110:386-9.
20. Porkaew P, Retief DH, Barfield RD, Lacefield WR, Soong SJ. Effects of calcium hydroxide paste as an intracanal medicament on apical seal. J Endod 1990;16:369-74.
21. Gokturk H, Ozkocak I, Buyukgebiz F, Demir O. Effectiveness of various irrigation protocols for the removal of calcium hydroxide from artificial standardized grooves. J Appl Oral Sci 2017;25:290-8.
22. Bitter K, Paris S, Mueller J, Neumann K, Kielbassa AM. Correlation of scanning electron and confocal laser scanning microscopic analyses for visualization of dentin/adhesive interfaces in the root canal. J Adhes Dent 2009;11:7-14.
23. McMichael GE, Primus CM, Opperman LA. Dentinal Tubule Penetration of Tricalcium Silicate Sealers. J Endod 2016;42:632–6.
24. Wang Y, Liu S, Dong Y. In vitro study of dentinal tubule penetration and filling quality of bioceramic sealer. PLoS One 2018;13:e0192248.
25. Praveen M, Aarthi G, Meenapriya PK, Kumar SS, Kumar NSM, Karunakaran JV. A Comparative Evaluation of Intraradicular Smear Removal Efficacy of 2% Chitosan (Low Molecular Weight), 4% Chitosan Citrate, and 10% Citric Acid when Used as Final Rinse in Irrigation Protocols: A Field Emission Scanning Electron Microscopic Study. J Pharm Bioallied Sci 2017;9:S73-8.
26. Nandini S, Velmurugan N, Kandaswamy D. Removal efficiency of calcium hydroxide intracanal medicament with two calcium chelators: volumetric analysis using spiral CT, an in vitro study. Journal of Endodontics. 2006;32:1097-101.
27. Raghu R, Pradeep G, Shetty A, Gautham PM, Puneetha PG, Reddy TV. Retrievability of calcium hydroxide intracanal medicament with three calcium chelators, ethylenediaminetetraacetic acid, citric acid, and chitosan from root canals: An in vitro cone beam computed tomography volumetric analysis. J Conserv Dent 2017;20:25-9.
28. Abidin T, Darmawan GL, Dalimunthe FD. The effect of oligosaccharide chitosan irrigating agent on penetration of two type endodontic sealers into the dentinal tubules: References 85 a confocal laser scanning microscopy study. Padjadjaran J Dent 2022;34:284-92.
29. Eskander M, Genena S, Zaazou A, Moussa S. Effect of phytic acid and ethylenediaminetetraacetic acid on penetration depth of bioceramic and resin sealers. Australian Endodontic Journal 2021;47:506-11.
30. Dua A, Dua D, Uppin VM. Evaluation of the effect of duration of application of smear clear in removing intracanal smear layer: SEM study. Saudi Endod J 2015;5:26-32.
31. Uzunoglu-Özyürek E, Erdoğan Ö, Aktemur Türker S. Effect of calcium hydroxide dressing on the dentinal tubule penetration of 2 different root canal sealers: A confocal laser scanning microscopic study. J Endod 2018;44:1018-23.
32. Cruz AT, Grecca FS, Piasecki L, Wichnieski C, Westphalen VP, Carneiro E, et al. Influence of the calcium hydroxide intracanal dressing on dentinal tubule penetration of two root canal sealers. Eur Endod J 2017;2:1-6.