IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 25, Issue 11 Ser. 2 (November. 2026), PP 12-16
www.iosrjournals.org

Effect of Different Irrigating Solutions on the Apical Sealing Ability of Three Root Canal Sealers Used with Continuous Wave Obturation: An In Vitro Study

Dr. Manjula Sp,Dr Farhat Nasreen,Dr Aashika, Dr Ashwija,Dr Vatsala, Dr Harshith

Department of Conservative Dentistry and Endodontics, A.E.C.S. Maaruti College of Dental Sciences and Research Centre, Bengaluru, Karnataka, India

Abstract

Introduction: Irrigating solutions can alter the dentin substrate and thereby influence the sealing performance of root canal sealers. This in vitro study evaluated the apical sealing ability of three sealers used with continuous wave obturation after irrigation with 3% sodium hypochlorite (NaOCl), 2% chlorhexidine (CHX), or 0.2% chitosan.

Methods: One hundred ten extracted single-rooted mandibular premolars with straight, fully formed canals were decoronated to 14 mm and prepared with ProTaper Universal instruments to F3. A glide path was established with a size 15 K-file. During instrumentation, canals were irrigated with 5 mL of the assigned solution between instruments. The experimental teeth were allocated to 3 irrigant groups (n=30 each) and subdivided according to sealer (n=10): MTA Fillapex, Bio-C Sealer, or Acroseal. A positive control (n=10) was obturated without sealer, and a negative control (n=10) was not obturated. All canals were obturated by continuous wave obturation. After 1 week at 37°C and 100% humidity, the specimens were coated with nail varnish except for the apical 2 mm and immersed in 2% methylene blue for 72 hours. Longitudinal dye penetration was measured stereomicroscopically using ImageJ. One-way ANOVA followed by Tukey post hoc testing was used.

Results: Mean dye penetration was 1.509 ± 0.230 for NaOCl/MTA Fillapex, 2.794 ± 0.272 for NaOCl/Bio-C, and 1.655 ± 0.236 for NaOCl/Acroseal; 2.514 ± 0.247 for CHX/MTA Fillapex, 1.531 ± 0.190 for CHX/Bio-C, and 1.560 ± 0.289 for CHX/Acroseal; and 2.500 ± 0.291 for chitosan/MTA Fillapex, 1.758 ± 0.172 for chitosan/Bio-C, and 1.525 ± 0.262 for chitosan/Acroseal. The positive control showed 2.963 ± 0.335 and the negative control 0.000 ± 0.000. Differences among groups were significant ($P < .001$).

Conclusions: Under the conditions of this in vitro model, apical dye penetration varied according to the irrigant–sealer combination. The lowest experimental-group penetration was observed with NaOCl/MTA Fillapex, whereas the highest was observed with NaOCl/Bio-C Sealer.

Keywords: *apical microleakage; sodium hypochlorite; chlorhexidine; chitosan; MTA Fillapex; Bio-C Sealer; Acroseal; continuous wave obturation*

Date of Submission: 27-10-2026 Date of Acceptance: 06-11-2026

I. INTRODUCTION

Three-dimensional obturation of the prepared root canal is intended to minimize communication between the root canal system and periapical tissues. The sealing ability of the obturation is influenced by the preparation protocol, obturation technique, core material, sealer properties, and condition of the dentin substrate. Irrigation is particularly relevant because it provides mechanical flushing and antimicrobial activity and modifies the organic and inorganic components of the canal wall.

Sodium hypochlorite is widely used because of its antimicrobial activity and ability to dissolve organic tissue, whereas chlorhexidine provides broad-spectrum antimicrobial activity and substantivity but does not dissolve pulp tissue. Neither solution alone is a complete smear-layer removal agent. Chelating agents such as EDTA are therefore commonly used as adjuncts. Chitosan, a biocompatible natural polymer with chelating and antimicrobial properties, has been investigated as an alternative because of its ability to remove smear layer with less dentin erosion.

The interaction between the irrigated dentin surface and the sealer may be particularly relevant because contemporary sealers differ substantially in composition and setting behavior. MTA Fillapex is an MTA-based sealer; Bio-C Sealer is a premixed bioceramic sealer; and Acroseal is an epoxy resin-based sealer containing calcium hydroxide. Continuous wave obturation, a modification of warm vertical compaction, combines apical

down-pack with thermoplasticized gutta-percha backfill and may provide a dense filling mass with good adaptation while requiring relatively little sealer.

Although the apical sealing behavior of endodontic sealers has been widely investigated, the effect of different irrigating solutions on these sealers when used with continuous wave obturation remains less extensively studied. Therefore, this study evaluated the effect of 3% NaOCl, 2% CHX, and 0.2% chitosan on apical dye penetration associated with MTA Fillapex, Bio-C Sealer, and Acroseal in canals obturated by continuous wave obturation.

II. MATERIALS AND METHODS

Study design and sample selection

This in vitro experimental study used 110 extracted human single-rooted mandibular premolars collected from the Department of Oral and Maxillofacial Surgery, A.E.C.S. Maaruti College of Dental Sciences and Research Centre, Bengaluru. Teeth with a single straight canal, completely formed apices, and extraction for periodontal or orthodontic reasons were included. Teeth with cracks, caries, resorption, canal calcification, restorations, or developmental anomalies were excluded. The specimens were stored in 10% formalin until use.

Sample size

The thesis reports an a priori G*Power calculation (version 3.1.9.7) using effect size f = 0.34, $\alpha = 0.05$, power = 0.80, and 3 groups, yielding a required total experimental sample size of 87. The study ultimately used 90 experimental specimens plus 20 control specimens, for a total of 110 teeth.

Specimen preparation and instrumentation

Crowns were removed with a diamond disc to standardize root length at 14 mm. A size 10 K-file was introduced until its tip was visible at the apical foramen, and working length was established 1 mm short of that length. A glide path was established with a size 15 K-file. Root canals were prepared using the ProTaper Universal rotary system with an endomotor to a final F3 instrument, according to the manufacturer's instructions. EDTA gel was used as lubricant. Instruments were used with a gentle in-and-out brushing motion, cleaned and inspected between uses, and new instruments were introduced after every 7 teeth.

Irrigation and experimental grouping

The teeth were randomly divided into 3 experimental groups of 30 specimens according to irrigant: Group 1, 3% NaOCl; Group 2, 2% CHX; and Group 3, 0.2% chitosan. Each experimental group was subdivided into 3 sealer subgroups (n=10): A, MTA Fillapex; B, Bio-C Sealer; and C, Acroseal. During instrumentation, 5 mL of the assigned irrigant was used between each instrument change. The final irrigation protocol included 5 mL of 17% EDTA for 1 minute followed by 5 mL distilled water. Group 4 (n=10) served as a positive control and was obturated by continuous wave obturation without sealer. Group 5 (n=10) served as a negative control and was not obturated.

Obturation

The experimental canals were obturated using the continuous wave obturation technique with gutta-percha and the assigned sealer. The thesis describes the technique as a modification of warm vertical compaction in which a heated plugger is used for the apical down-pack followed by thermoplasticized gutta-percha backfill. The positive-control canals received gutta-percha without sealer.

Dye leakage assessment

After obturation, specimens were stored at 37°C and 100% humidity for 1 week. All root surfaces except the apical 2 mm were coated with three layers of nail varnish; in the negative-control group, the coating also covered the apical foramen. Specimens were immersed in 2% methylene blue and incubated at 37°C for 72 hours. The roots were rinsed, dried, and the varnish removed. Two longitudinal grooves were prepared on the buccal and lingual surfaces without entering the canal, and specimens were split with a chisel and mallet. The half showing the most complete canal was selected for measurement. Linear apical dye penetration was assessed under a stereomicroscope at the magnification reported in the thesis as 4.5 × 10×, and the length was quantified using ImageJ software.

Statistical analysis

Mean dye-penetration values were compared among the 11 groups using one-way analysis of variance followed by Tukey post hoc multiple comparisons. Statistical significance was set at $P < .05$. The thesis reports an overall ANOVA $P < .001$.

III. RESULTS

All experimental groups demonstrated measurable dye penetration, whereas the negative control showed no penetration. The overall difference among groups was statistically significant (one-way ANOVA, $P < .001$).

Group	n	Mean	SD	Min–Max
NaOCl + MTA Fillapex	10	1.509	0.230	1.21–1.90
NaOCl + Bio-C	10	2.794	0.272	2.40–3.20
NaOCl + Acroseal	10	1.655	0.236	1.11–1.86
CHX + MTA Fillapex	10	2.514	0.247	2.12–2.99
CHX + Bio-C	10	1.531	0.190	1.25–1.94
CHX + Acroseal	10	1.560	0.289	1.14–1.99
Chitosan + MTA Fillapex	10	2.500	0.291	2.12–2.94
Chitosan + Bio-C	10	1.758	0.172	1.39–1.96
Chitosan + Acroseal	10	1.525	0.262	1.01–1.98
Positive control	10	2.963	0.335	2.56–3.46
Negative control	10	0.000	0.000	0.00–0.00

Table 1. Mean linear dye penetration values reported in the thesis. The thesis labels the measurement unit as µm; this unit should be verified against the original measurement/calibration records before journal submission because the reported numerical values are in the millimeter-range format used throughout the methodological description.

The lowest mean dye penetration among experimental groups was observed in NaOCl + MTA Fillapex (1.509 ± 0.230), followed by chitosan + Acroseal (1.525 ± 0.262), CHX + Bio-C (1.531 ± 0.190), CHX + Acroseal (1.560 ± 0.289), and NaOCl + Acroseal (1.655 ± 0.236). The highest mean among experimental groups was observed with NaOCl + Bio-C (2.794 ± 0.272). The positive control had the highest mean penetration overall (2.963 ± 0.335), while the negative control had no penetration.

Tukey testing showed significant differences for multiple pairwise comparisons. NaOCl + MTA Fillapex differed significantly from NaOCl + Bio-C, CHX + MTA Fillapex, chitosan + MTA Fillapex, the positive control, and the negative control ($P < .001$), but did not differ significantly from NaOCl + Acroseal ($P = .96$), CHX + Bio-C ($P = 1.00$), CHX + Acroseal ($P = 1.00$), chitosan + Bio-C ($P = .46$), or chitosan + Acroseal ($P = 1.00$). NaOCl + Bio-C differed significantly from NaOCl + Acroseal and most lower-penetration experimental groups ($P < .001$), but did not differ from CHX + MTA Fillapex ($P = .28$), chitosan + MTA Fillapex ($P = .22$), or the positive control ($P = .90$). CHX + MTA Fillapex and chitosan + MTA Fillapex did not differ significantly ($P = 1.00$). CHX + Bio-C and CHX + Acroseal did not differ ($P = 1.00$), nor did chitosan + Bio-C and chitosan + Acroseal ($P = .56$).

IV. DISCUSSION

This study examined the apical sealing behavior of three chemically different sealers after use of three irrigating solutions in canals obturated with continuous wave obturation. The overall difference in dye penetration indicates that the irrigant–sealer combinations produced different leakage profiles under the conditions tested.

MTA Fillapex showed its lowest mean penetration after NaOCl irrigation. The thesis relates this finding to the properties of MTA-based materials and their hydration reactions, including formation of calcium silicate hydrate and calcium hydroxide and possible setting expansion. Previous studies cited in the thesis have similarly reported favorable sealing behavior of MTA Fillapex after NaOCl-based irrigation. However, the present experiment cannot establish a specific chemical mechanism because no interfacial or chemical analysis was performed.

Bio-C Sealer showed its lowest mean penetration with CHX and its highest among the experimental combinations with NaOCl. Bio-C is a premixed bioceramic sealer designed to set in the presence of moisture and to release calcium ions. The thesis discusses the possibility that changes to the dentin organic matrix after NaOCl exposure may influence interaction between the sealer and dentin. This remains a mechanistic interpretation rather than a finding directly measured in the present study.

Acroseal showed its lowest mean penetration with chitosan, followed by CHX and NaOCl. Chitosan has chelating and antimicrobial properties and has been reported in the cited literature to remove smear layer while producing less dentin erosion than EDTA. The relatively low penetration observed with chitosan + Acroseal may therefore reflect changes in the dentin surface that favor adaptation; however, smear-layer removal and sealer–dentin bonding were not directly evaluated in this experiment.

The positive control, in which gutta-percha was used without sealer, showed the greatest leakage, supporting the role of sealer in filling interfacial spaces. The negative control showed no dye penetration, supporting the effectiveness of the external sealing protocol. Nevertheless, dye penetration is a tracer-based

laboratory method and does not reproduce the complex fluid, bacterial, mechanical, and biological conditions of the clinical root canal environment.

The findings are consistent with the broader literature summarized in the thesis showing that apical leakage can vary with sealer composition, irrigant protocol, and obturation technique. Studies using continuous wave obturation have reported variable leakage among sealers, while studies examining chitosan have suggested potential effects on apical sealing. Direct comparisons across studies should be made cautiously because concentrations, final-irrigation protocols, obturation systems, tooth anatomy, storage periods, dye type, and leakage-measurement methods differ.

This study has limitations. It was an in vitro investigation using extracted mandibular premolars and linear methylene-blue penetration as the sole outcome. The observation period after obturation was 7 days, which may not reflect longer-term dimensional changes of the tested sealers. In addition, the study evaluated complete experimental combinations with one-way ANOVA across 11 groups rather than a factorial analysis explicitly testing irrigant, sealer, and their interaction. Accordingly, the data demonstrate differences among the tested combinations but should not be interpreted as establishing an independent causal effect of irrigant type or sealer type without further analysis.

V. CONCLUSION

Within the limitations of this in vitro study, the irrigant–sealer combination was associated with significant differences in apical dye penetration when continuous wave obturation was used. Among the experimental combinations, NaOCl + MTA Fillapex showed the lowest mean dye penetration, whereas NaOCl + Bio-C Sealer showed the highest. Chitosan + Acroseal and CHX + Bio-C also showed relatively low penetration. Further studies using longer observation periods and complementary leakage or interfacial methods are required before extrapolating these findings to clinical practice.

ACKNOWLEDGMENTS

The dissertation acknowledges Dr. Farhat Nasreen, Professor and Guide; Dr. J. Pramod, Professor and Head; Dr. V. Ranganath, Principal; and Dr. Santhosh, Statistician, for their respective guidance and support. Authorship and acknowledgment in the submitted manuscript should be finalized according to actual contributions and the target journal's authorship requirements.

REFERENCES

[1]. Rotstein I, Ingle JI, editors. Ingle's Endodontics. 7th ed. PMPH USA; 2019.
[2]. Singh R, Pushpa S, Arunagiri D, Sawhny A, Misra A, Sujatha R. The effect of irrigating solutions on the apical sealing ability of MTA Fillapex and Adseal root canal sealers. J Dent Res Dent Clin Dent Prospects. 2016;10:251-256.
[3]. El Sayed MA, Fathieh SM. In vitro evaluation of the effect of different irrigation solutions on the apical sealing ability of three root canal sealers when the continuous wave obturation technique was used. Egypt Dent J. 2019;65:387-397.
[4]. Hargreaves KM, Berman LH, editors. Cohen's Pathways of the Pulp. 11th ed. Elsevier; 2016.
[5]. Alamoudi RA. The smear layer in endodontic: to keep or remove—an updated overview. Saudi Endod J. 2019;9:71-81.
[6]. Balasubramanian SK, Saraswathi V, Ballal NV, Acharya SR, Sampath JS, Singh S. A comparative study of the quality of apical seal in Resilon/Epiphany SE following intracanal irrigation with 17% EDTA, 10% citric acid, and MTAD as final irrigants—a dye leakage study under vacuum. J Clin Diagn Res. 2017;11:ZC20-ZC24.
[7]. Hülsmann M, Heckendorff M, Lennon A. Chelating agents in root canal treatment: mode of action and indications for their use. Int Endod J. 2003;36:810-830.
[8]. Elsaka RN, Hassan HY, Fayyad DM. Effect of different root canal irrigating solutions on the apical sealing ability of bioceramic and resin-based sealers: an in vitro study. Dent Sci Updates. 2023;4:83-96.
[9]. Medhat A, Ghoneim A, Roshdy NN. The effect of final irrigation protocols on the apical sealing ability of epoxy resin-based and bioceramic-based root canal sealers. Open Access Maced J Med Sci. 2022;10:458-464.
[10]. Kusumo AN, Mulyawati E, Ratih DN. The differences of apical sealing after final irrigation using 17% EDTA and 0.2% chitosan nanoparticles with different contact times. [Journal details as cited in thesis].
[11]. Kittur M, et al. The monoblock concept in endodontics. [Bibliographic details as cited in thesis].
[12]. Patil AH, et al. Evaluation of apical sealing ability of MTA Fillapex, AH Plus, and zinc oxide eugenol. [Bibliographic details as cited in thesis].
[13]. Patel M, et al. Evaluation of apical seal obtained by different sealers used with single-cone obturation technique. [Bibliographic details as cited in thesis].
[14]. Akhtar H, Naz F, Hasan A, et al. Exploring the most effective apical seal for contemporary bioceramic and conventional endodontic sealers using three obturation techniques. Medicina. 2023;59:567.
[15]. Olczak K, Pawlicka H. Evaluation of the sealing ability of three obturation techniques using a glucose leakage test. Biomed Res Int. 2017;2017:2704094.
[16]. Haslinda H, Rovani CA, Trilaksana AC. Apical microleakage of epoxy resin and methacrylate resin-based sealer with continuous wave obturation technique. J Dentomaxillofac Sci. 2016;1:20-24.
[17]. Vasconcelos BC, Bernardes RA, Duarte MA, Bramante CM, Moraes IG. Apical sealing of root canal fillings performed with five different endodontic sealers: analysis by fluid filtration. J Appl Oral Sci. 2011;19:324-328.

[18]. Al-Kadhi AM, Al-Ani ZB, Al-Eanizi J. Comparison of apical microleakage of four contemporary endodontic sealers by dye penetration method. Iraqi Med J. 2019;26:237-240.
[19]. Behera K, Deepak S. Evaluation of apical sealing ability of root canal sealers—an in vitro study. Int J Dent Oral Sci. 2021;8:2918-2921.
[20]. El Sayed MA. Effect of two irrigation protocols and obturation techniques on the apical sealing ability of different root canal sealers: an in vitro study. Eur J Gen Dent. 2020;9:33-39.