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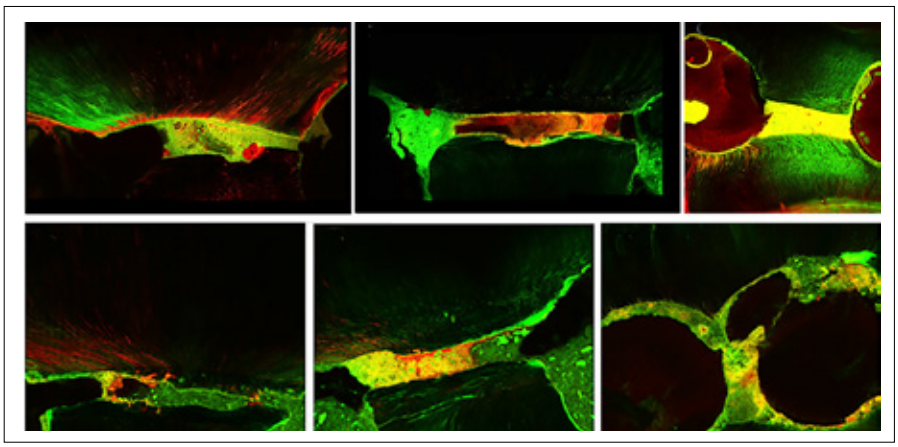

▸ Original articles

Efficacy of passive ultrasonic irrigation on remaining root filling material during retreatment of anatomically complex teeth

Antimicrobial effect of 2% chlorhexidine as a chemical adjuvant in different endodontic protocols: an in vitro study

Applicability of photobiomodulation and antimicrobial photodynamic therapy for pain management after endodontic treatment: a randomized clinical trial

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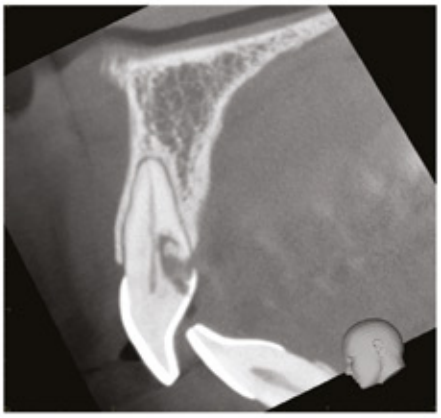

CBCT MORITA , la diagnosi con noi è una certezza !!

MORITA

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REGISTRATION Court of Milan n° 89, 3 March 2009

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Sandro Rengo
Editor-in-Chief
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Editorial

A challenge: the management of pain

One of the main issues of modern endodontics is still represented by the management of pain. Endodontic pain is a controversial aspect since it is considered as a pre-operative diagnostic criterion, a possible consequence during multi-visits therapy as well as an important symptom of success of root canal treatment over time. In addition, the intensity of pain depends on several factors and does not necessarily reflect the pulp inflammation status. Indeed, endodontic flare-up is caused by chemical, microbial and mechanical agents, but there is also a correlation with age, gender, tooth element, number of appointments, use of intracanal medicaments, irrigants, shaping technique and pre-operative pain level. Considering all these variables, correct therapeutical strategies to prevent pain and diagnostic tool able to precisely identify the histopathological pulp status are strongly needed to guide and custom the treatment plan. Even pharmacological therapy and use of specific materials, such as calcium silicate- based cements, would seem to be fundamental to reduce inflammation and decrease pain.

It's also been demonstrated how endodontic therapy may be associated to high level of fear and anxiety, that, on turn, worsen patient's discomfort. The oral health perception is crucial since it may influence the general health status and patient's quality of life. These aspects are of wide interest and are considered very current topics by international scientific community.

In the present issue, different important endodontic themes are developed such as use of irrigants with innovative protocols, advanced endodontic sealers and several treatment approaches with the aim to provide to clinicians various chances to obtain a long-lasting endodontic success.

Have a great endodontics,
Prof Sandro Rengo

Peer review under responsibility of Società Italiana di Endodonzia.

10.32067/GIE.2024.38.01.15

ORIGINAL ARTICLE

Efficacy of passive ultrasonic irrigation in removing residual root filling material during retreatment: *ex vivo* analyses by micro-CT and CLSM

ABSTRACT

Aim: *To evaluate the volume of residual filling material after desobturation and passive ultrasonic irrigation (PUI) with irrigants and an organic solvent using micro-computed tomography (micro-CT), and assess the penetrability of the obturation of retreatment using confocal laser scanning microscope (CLSM).*
Methodology: *Thirty curved mesial roots of mandibular molars were submitted for endodontic treatment. After, the filling material was removed and the root canals were reinstrumented with ProTaper instruments (Stage 1). The specimens were randomly assigned according to the protocol of supplementary cleaning (Stage 2). In the Manual group, orange oil solvent, 2.5% NaOCl, and 17% EDTA were applied in the root canal with syringe and needle. In the PUI group, the same irrigants were submitted to ultrasonic agitation. The volume of residual filling material was measured by micro-CT. After, the specimens were filled and submitted to CLSM. The micro-CT data were analyzed by Mann-Whitney and Friedman tests at 5%. The Wilcoxon and Mann-Whitney tests were used for CLSM data (P<.05).*
Results: *Both supplementary cleaning protocols decreased the amount of residual filling material, when compared with stage 1 (P<.05). The PUI group showed significantly less percentages of residual filling material than did the Manual group, for all of the thirds of the root canal (P<.05). There were no statistically significant difference in the penetrability of the obturation of retreatment in the recessions areas between the Manual and PUI groups (P>.05).*
Conclusions: *The use of the PUI as a supplementary technique in removing residual filling material after desobturation was more effective than manual agitation.*

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KEYWORDS *Confocal laser scanning microscope, micro-computed tomography, retreatment, solvents, ultrasonics.*

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Introduction

Root canal retreatment procedures recommend the complete removal of filling material, because it can cover necrotic material, debris and infected dentine (1, 2). The presence of contaminated root filling material can contribute to the persistence of infection, and consequently, can compromise the final retreatment outcome (3, 4). Furthermore, the removal of the filling material of primary treatment it is important in order to allow for the penetration and the adaptation of the new obturation in the retreatment. Different methods have been proposed to remove filling material, nevertheless, substantial amounts of filling material commonly remain in the canal after retreatment procedures, especially in areas of difficult access (5-8).

The Passive ultrasonic irrigation (PUI) has been used as an effective method in the cleaning and the disinfection of root canals, principally in areas that are inaccessible to instrumentation such as the isthmus, the oval extensions and the irregularities of the root canal walls (9). The PUI potentiates the action of the irrigants, providing an effective removal of the remnants of pulp tissue, dentine debris, intracanal medication (10), microorganisms (planktonic or biofilm) and their products (11, 12) and improves the penetration depth of the endodontic sealers into the dentinal tubules (13).

The PUI also can be used during endodontic retreatment as an auxiliary for removing root filling materials using different irrigants (14-18), including endodontic solvents (19-21). It has been reported that the application of PUI with essential oil can be an effective method in dissolving zinc oxide-eugenol (ZOE) based sealers (21). However, the results of research evaluating PUI in retreatment are inconclusive. Barreto et al. (20) evaluated, by using micro-computed tomography (micro-CT), the effects of PUI with sodium hypochlorite (NaOCl) and orange oil solvent in the endodontic retreatment and they observed that PUI did not improve the filling material removal. The authors also observed a larger volume of filling material in the specimens that contained isthmus.

To the best of our knowledge, no study evaluated the effectiveness of the PUI in the removal of filling material and the adaptation of the obturation during endodontic retreatment in same study. Therefore, the aims of this study were: 1) to evaluate the effect of the PUI with endodontic irrigants and orange oil as a supplementary cleaning technique in removing residual filling material, using micro-CT; 2) to assess the effects of the PUI in the penetrability of the obturation of retreatment in isthmus and irregularities areas of the root canal, using confocal laser scanning microscope (CLSM).

Materials and Methods

This manuscript was written according to the Preferred Reporting Items for Laboratory Studies in Endodontology (PRILE) 2021 guidelines (22). The PRILE 2021 flowchart, figure 3, summarizes the key steps in reporting the present study.

Approval for this study was granted by the research ethics committee of the Federal University of Santa Maria (0698 8212.2.0000.5346).

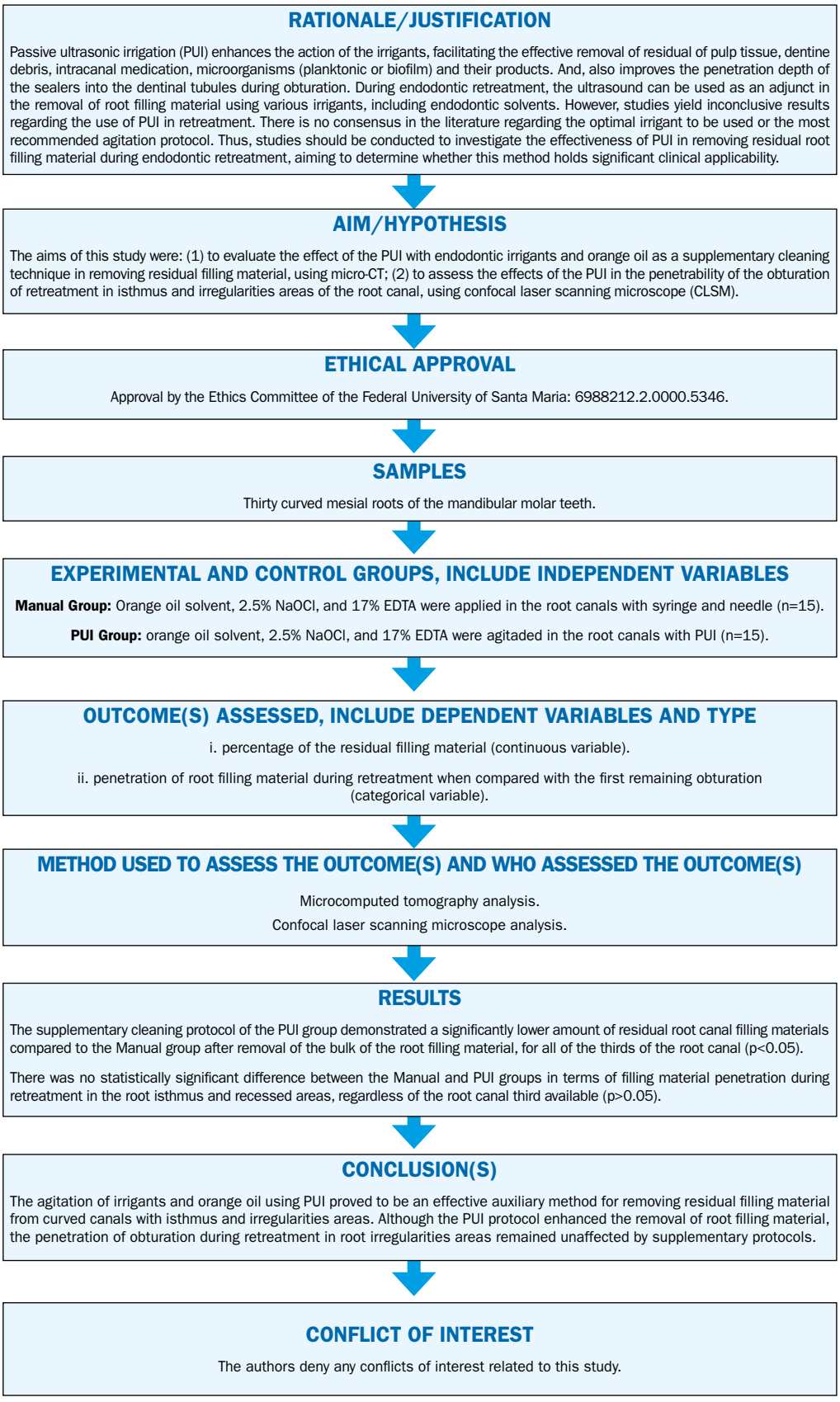
Sample Size

Previous studies (23, 24) have reported differences of nearly 10% for amount of residue when removing the filling material using different instruments and techniques. Assuming a difference of 10% in mean percentage of the volume of residual filling material in the root canal walls, with a standard deviation of 9, the sample size was calculated as 13 specimens per group. To compensate potential losses associated with experimental procedures, 2 samples were included, resulting in a total of 15 specimens per group. The study power was 80% and a significance level was 5%.

Teeth Selection

Thirty mandibular molar teeth with

Figure 3
PRILE 2021 Flowchart



curved mesial roots were collected. Each tooth's mesial and distal roots were sectioned and the distal root was discarded. The selection criteria for the mesial root included a curvature angle of 10°-20º and a curvature radius <12 mm in a bucco-lingual direction, determined through radiographic analysis according with the methodologies described by Schneider (25) and Schäfer et al. (26), respectively. Statistical analysis revealed no significant difference between PUI and Manual groups concerning the curvature angles (P=0.934) and the radii (P=0.575).
Afterward, all specimens were submitted to micro-CT scanning using the SkyScan 1174 scanner (Bruker-Micro CT, Kontich, Belgium) with settings at 50 kV, 800 µA, rotational angle of 180°, and an isotropic resolution of 14.1 µm to confirm the presence of isthmus. Specimens classified as types 2, 3, 5, 6 and 7 according to Vertucci's classification (27) were included in the study.

Cleaning and Shaping
The cavity access of the remaining crown was achieved by using 1014 diamond burs (KG Sorensen, Cotia, São Paulo, Brazil) under water-cooling. The crown of the mesial portion was intentionally maintained to allow a larger irrigant contact with the root canal. The patency of the canals was established using a size-10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) until its tip was visible at the apical foramen. The working length (WL) was determined by subtracting 1 mm from this measurement.
The root canal preparation was performed with ProTaper Universal files (Dentsply Maillefer) using a torque-control motor (X-Smart, Dentsply Maillefer) set at 300 rpm and 2.5 N/cm. S1, SX, S1, S2, F1 and F2 instruments were used in this sequence and between each instrument change, the canals were irrigated with 3 mL of 2.5% NaOCl(Biodinâmica, Ibiporã, Paraná, Brazil) using a syringe with NaviTip irrigation needle (Ultradent, Munich, Bavaria, Germany).
After the instrumentation, all of the root canals were submitted to PUI with 2.5% NaOCl and 17% ethylenediaminetetraacetic acid (EDTA) (Biodinâmica, Ibiporã, Paraná, Brazil). The PUI was performed using a piezoelectric ultrasonic unit (Gnatus, Ribeirão Preto, São Paulo, Brazil) at a high power with a smooth stainless steel wire of size 15 taper .02 (Dentsply Maillefer), 1 mm short of the WL, and oscillating in the toward the isthmus area.

Root Canal Filling
The root canal filling was performed with tapered gutta-percha cones (Dentsply Maillefer) and ZOE based sealer (Endofill, Dentsply Maillefer) using Tagger's hybrid technique (28). The sealer was mixed with 0.1% Rhodamine B dye (CI.45170) (Red) (Synth, São Paulo, Brazil) to allow fluorescence analysis by the CLSM (29). The access cavity was filled with a temporary restorative material (Cavit, 3M ESPE, St Paul, Minnesota, USA). The specimens were stored for 30 days at 37 °C with 100% humidity in order to allow the setting of sealer. Subsequently, all roots were scanned using micro-CT to determine the volume of the root filling.

Retreatment Techniques
The temporary restorations were removed, and a single drop of orange oil (Maquira, Maringá, Paraná, Brazil) was applied for 2 min to soften the gutta-percha at the root canal orifice. The bulk of the root filling material was removed using the ProTaper retreatment system (Dentsply Maillefer) according to the manufacturer's instructions (500 rpm and 3 N/cm).
The root canals were reprepared with F1-F4 ProTper rotatory instruments with 300 rpm and 2.5 N/cm using a torque-control motor (X-Smart, Dentsply Maillefer). After each instrument, the irrigation was performed with 3 mL of 2.5% NaOCl using a syringe with a NaviTip needle. Next, the teeth were scanned with Micro-CT, and the volume of the remaining radiopaque residue was determined (Stage 1). The specimens were randomly assigned (Random Allocation software, Microsoft, Redmond, Washington, USA) in a stratified manner when considering the Vertucci Classification (27) into two groups accord-

ing to the protocol of supplementary cleaning (Stage 2), as detailed below.

Manual group (n=15). First, orange oil-based solvent was used. The root canal was filled with the solvent and the solution was agitated for 3 min using 40 K-type instrument since the last instrument used for root canal preparation was F4 ProTaper instrument (Size 40/0.06 taper). The solvent was replaced at every minute. After, each root canal was irrigated with 2.5% NaOCl for 1 min using a syringe with a NaviTip needle positioned 1 mm short of the WL. Lastly, the root canal was filled with 17% EDTA, and the solution was agitated for 1 min using a 40 K-type instrument.

PUI group (n=15). First, each root canal was filled with orange oil and it was activated using PUI for 3 min. The solvent was replaced at every minute (21). Subsequently, the root canal was filled with 2.5% NaOCl and it was activated using PUI for 1 min. The solution was renovated every 20s. Finally, the root canal was filled with 17% EDTA, and the PUI was performed continuously for 1 min. The PUI was performed as previously described in the topic "Cleaning and Shaping".

After, for both of the groups, each root canal was irrigated with 2 mL of saline solution, dried with sterile absorbent paper points, and then stored at 37 °C with 100% humidity until a new 3D scan was conducted.

Preoperative Micro-CT Scanning and Evaluation Procedures

The specimens were positioned in condensation silicone on the scanning unit to avoid any interference during the process. The scanning procedures were performed by using a desktop X-Ray Microfocus CT Scanner (SkyScan 1174, Bruker-Micro CT, Kontich, Belgium) with a 50 kV, 800 µA, rotational angle of 180°, and an isotropic resolution of 14.1 µm, resulting in 700 to 900 slices per root. The images obtained were reconstructed in order to show 2-D slices of the root structures using NRecon 1.6.4.8 software (Bruker-Micro CT, Kontich, Belgium). CTAn 1.11.10 software (Bruker-Micro CT, Kontich, Belgium) was used to measure the volume of filling material (mm^3) in the apical, middle, and coronal levels (3 mm), from the root apex (30). The percentage of the residual filling material for each third of root canal was calculated for all of the specimens at each stage of the retreatment. A single calibrated and blinded observer performed the micro-CT analyses. Intra observer reproducibility was determined using intraclass correlation coefficient (ICC=0.90).

Obturation during Retreatment

The root canals were filled with tapered gutta-percha cones and AH Plus sealer (Dentsply DeTrey, GmbH, Konstanz, Germany) using Tagger's hybrid technique (28). The 0.1% fluorescein dye (CI.45350) (Green) (Synth) was mixed with the sealer to allow for the analysis by the CLSM.

CLSM Analysis

The specimens were transversally sectioned at 3, 6 and 9 mm from the apex by using a low-speed cutting machine (Isomet, Buehler, Illinois, USA) at 200 rpm and with continuous water cooling. Three root slices with each 2 mm thick were obtained, resulting in 30 slices per group. The slices were polished with a sanding granulation of 300, 600, 900 and 1.200. The coronal surfaces of the slices were evaluated using Olympus FluoView Confocal Laser 1000 Microscope (Olympus Corporation, Tokyo, Japan). The wave length for the absorption and the transmission of Rhodamine B and Fluorescein were 540/590 nm and 494/518 nm, respectively.

The penetration of root filling material during retreatment, compared to the initial remaining obturation was categorized into scores as follows: (0)-0%; (1)-0%-25%; (2)-25%-50%; (3)-50%-75%; and (4)-75%-100%. Two calibrated and blinded evaluators at the protocol of supplementary cleaning performed the analysis by using the LSM Image Browser Program (Zeiss Carl, Gottingen, Switzerland). Each evaluator performed two analyses at different moments. In cases of disagreement, a collective reanalysis was conducted until a consensus was reached. The Kappa co-

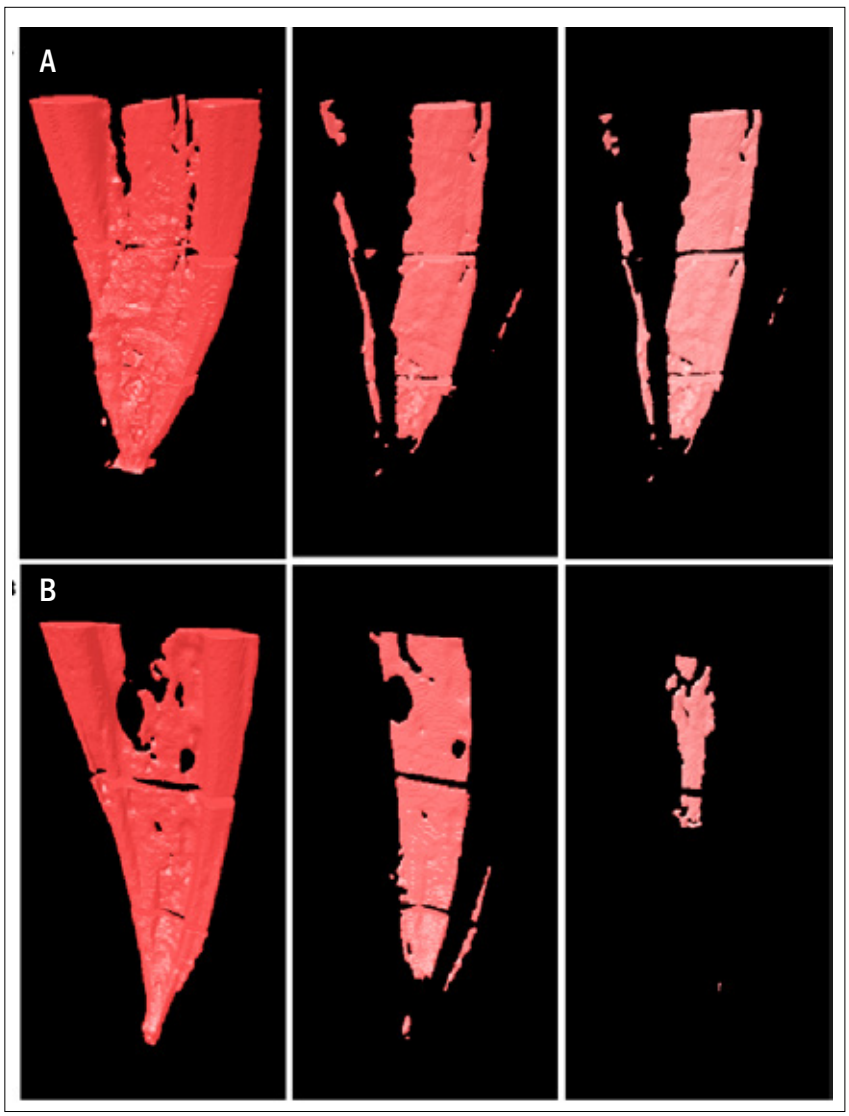
efficient was used to assess interexaminer and intraexaminer agreements. The analyses showed a Kappa coefficients of 0.91 and 0.87 for interexaminer and intraexaminer agreements, respectively.

Statistical Analysis

Data analysis was conducted using Prisma 5.0 software (GraphPad Software Inc., La Jolla, California, USA). The significance level was set at α=0.05. The Shapiro-Wilk normality test was used to assess the data distribution from the micro-CT and CLSM. The data showed non-normal distribution and nonparametric tests were applied.

Micro-CT: The Mann-Whitney test was used to compare the percentages of the residual root canal filling materials between groups at each retreatment step. The Friedman test was used to verify the differences of the residual root canal filling materials in the successive retreatment stages for each canal segment in the same group. The differences amongst the coronal, middle, and apical thirds, in each group and at each stage were also analyzed by using the Friedman test. The Dunn test was performed as the post-hoc multiple comparison method.

CLSM: The prevalence of the root filling material during retreatment was compared with the first one between the experimental groups using the Mann Whitney test. Comparison among the thirds in the same group was verified using the Wilcoxon test.



Results

Micro-CT

The mean percentages of the residual root canal filling materials during retreatment for each group after Stage 1 and Stage 2 are shown in Tables 1 and 2, respectively. The use of the Manual and PUI protocols for supplementary cleaning during retreatment (Stage 2) showed less residual root canal filling material than did Stage 1 (P<0.05). After stage 2, the PUI group showed a significantly less amount of residual root canal filling material compared to the Manual group, for all thirds of the root canal (P<0.05) (Figure 1) (Table 2). It was observed similar amount of residual root canal filling materials among the apical, middle, and cervical thirds in the PUI group (Stage 2) (P>0.05) (Table 2). None

Figure 1
Representative 3D reconstructions of root filling material in the mesial canals of mandibular molars. **(A)** Root filling material in the Manual group. *Left*, root filling material after obturation; *center*, residual root filling material after retreatment with ProTaper; *right,* residual root filling material after suplementary cleaning with Manual protocol. **(B)** Root filling material in the PUI group. *Left,* root filling material after obturation; *center,* residual root filling material after retreatment with ProTaper; *right,* residual root filling material after suplementary cleaning with PUI protocol.

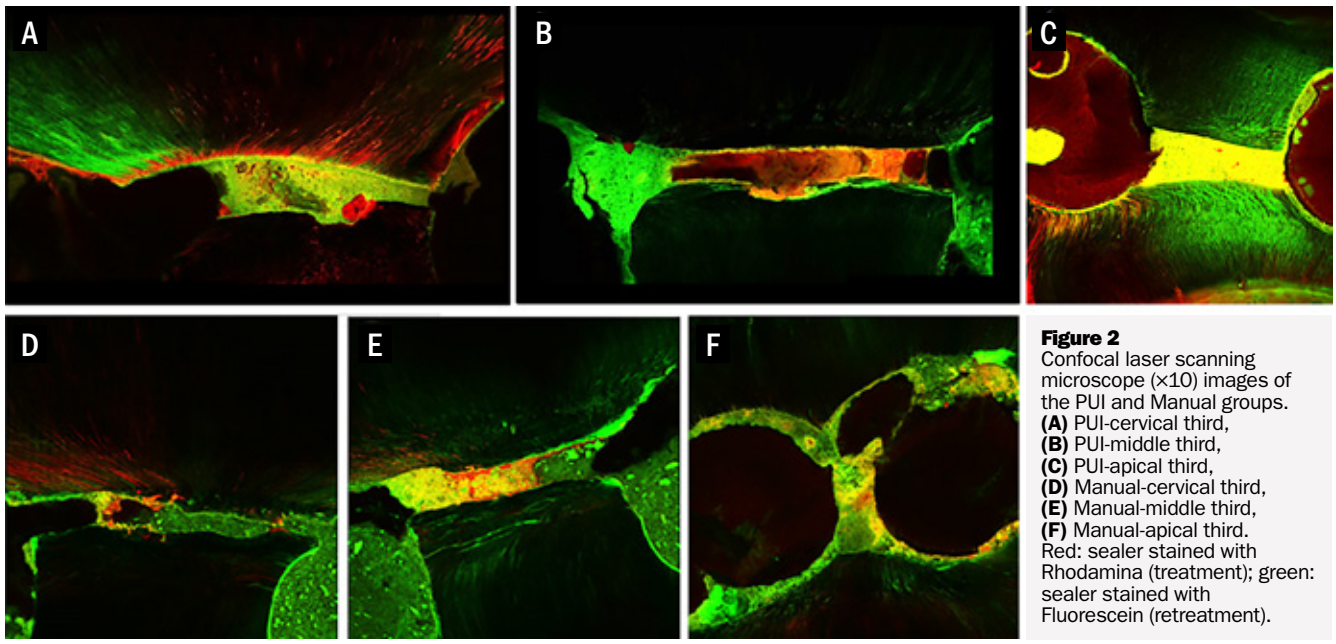


Figure 2
Confocal laser scanning microscope (×10) images of the PUI and Manual groups. **(A)** PUI-cervical third, **(B)** PUI-middle third, **(C)** PUI-apical third, **(D)** Manual-cervical third, **(E)** Manual-middle third, **(F)** Manual-apical third. Red: sealer stained with Rhodamina (treatment); green: sealer stained with Fluorescein (retreatment).

Table 1

Mean and Standard Deviation (SD) of residual filling material (in %) after removing bulk of the root filling material with ProTaper instruments (before randomization and common to both groups)

Canal Segment	ProTaper Instruments (Stage 1)		
	Manual Group (mean ± SD)	PUI Group (mean ± SD)	*P* value*
Apical	35.4±17.3^{A}	30.6±15.7^{A}	.5069
Middle	25.9±12.2^{A}	26.03±7.42^{A}	.7089
Coronal	10.8±6.97^{B}	9.93±4.69^{B}	.9339

Capital Letters: comparison among the thirds in the same group.
Different letters indicate statistically significant differences (P<0.05).
**Statistically significant differences between the groups if P<0.05.*

irrigation protocol completely removed the residual filling materials of root canal in all of the specimens.

CLSM

The distribution of absolute and relative frequency values of the scores of prevalence of the root filling material during retreatment for each experimental group is described in Table 3. Figure 2 showed confocal images for Manual and PUI groups. The penetrability of obturation during retreatment in the root isthmus and recesses was similar among thirds of the root canal when it was available in the same group (P>0.05). No significant statistical difference was observed between the Manual and PUI groups, independently of the third of the root canal (P>0.05).

Discussion

During endodontic retreatment, effective removal of the root filling is essential to facilitate disinfection and adaptation of new filling material (1-4). In this context, the authors aimed to assess the efficacy of the PUI using endodontic irrigants

Table 2

Mean and Standard Deviation (SD) of residual filling material (in %) after supplementary cleaning techniques (Stage 2)

Canal Segment	Manual Group (mean±SD)	PUI Group (mean±SD)	*P* value*
Apical	28.6±15.9^{A}	9.06±9.46^{A}	.0007
Middle	20.5±10.2^{AB}	8.14±8.81^{A}	.0019
Coronal	9.33±6.34^{B}	3.65±3.83^{A}	.0079

Capital Letters: comparison among the thirds in the same group.
Different letters indicate statistically significant differences (P<0.05).
**Statistically significant differences between the groups if P<0.05.*

Table 3

Distribution of absolute and relative frequency values of the scores of prevalence of the root filling material during retreatment for each third of the root canal within each experimental group

Score	Manual Group (n=15)			PUI Group (n=15)		
	Apical	Middle	Cervical	Apical	Middle	Cervical
0	4 (26.7%)	4 (26.7%)	1 (06.7%)	2 (13.3%)	2 (13.3%)	2 (13.3%)
1	3 (20.0%)	5 (33.4%)	8 (53.3%)	2 (13.3%)	5 (33.3%)	4 (26.7%)
2	2 (13.3%)	2 (13.3%)	2 (13.3%)	2 (13.3%)	1 (06.7%)	2 (13.3%)
3	2 (13.3%)	2 (13.3%)	3 (20.0%)	2 (13.3%)	3 (20.0%)	3 (20.0%)
4	4 (26.7%)	2 (13.3%)	1 (06.7%)	7 (46.7%)	4 (26.7%)	4 (26.7%)

and orange oil solvent as supplementary cleaning method to remove residual filling materials. The results of this study indicated that it was not possible to completely remove the existing root filling materials, principally in root isthmuses, recesses and oval extensions, which is in agreement with previous studies (8, 16, 24, 30, 31). However, the micro-CT analysis demonstrated that the use of PUI protocol significantly improved the removal of the residual filling material compared to the Manual group. The authors attribute the superior outcomes in the PUI group to the ultrasonic activation of the solvent. Ultrasound activation of the orange oil led to a temperature increase in the solvent by 2 °C (21), thereby enhancing its ability to dissolve the sealer (32). Furthermore, the replacement of the orange oil solvent prevented its saturation and facilitated extensive dissolution of the ZOE-based sealer, in according with a previous study (21).

During PUI, it is essential that the file moves freely within the root canal in order to allow for the solution to penetrate more easily into the root canal system (33). In this study, measures were taken to ensure free oscillation of the file within the root canal. The enlargement of the canal (Size 40/0.06 taper) during the retreatment allowed that the oscillation amplitude of the file to be minimally affected by contact with the root canal walls.

According to the endodontic literature, PUI has shown excellent results in disinfection and removal of dentinal debris

during endodontic treatment (9, 12). However, the protocol for using PUI during endodontic retreatment is not well established. The literature presents studies exhibiting considerable methodological variation, wherein factors like solution type, agitation duration, and endodontic sealer type can significantly influence outcomes (16-20). Grischke et al. (31) showed that PUI with NaOCl during retreatment was superior to all of the other irrigation techniques (syringe irrigation, CanalBrush, EndoActivator and RinsEndo) concerning the removal of the sealer from the root canal walls. Despite this, the results achieved with PUI were not entirely satisfactory, because although the root canal walls were free of sealer, the artificial grooves were still filled with sealer remnants. Some studies (15, 16) similarly concluded that PUI with NaOCl supplementary in the final irrigation during endodontic retreatment did not improve the removal of filling material within teeth with complex anatomical structures. In addition, Cavenago et al. (14) evaluated, using micro-CT, the percentage of the remaining filling material in the mesial root canals of mandibular molars after retreatment through three sequential procedures (mechanical cleaning, xylene, PUI with NaOCl). Their findings revealed no significant disparities between xylene and PUI with NaOCl. Despite methodological differences, there is a consensus that PUI has shown superior results compared to other irrigation methods such as syringe and needle, corroborating with the results of the present study.

When considering the 3D analyses, several cases in the PUI group could reasonably be called "extremely cleaned" (i.e., having less than 0.5% residue). The PUI group presented the best statistical results and some of the root canals extremely clean. However, the average and the standard deviation of the amount of the remaining filling material were notably higher in comparison to other studies (24, 30, 34). One possible explanation for this difference is that most of these studies utilized single straight root canals, aiming to simplify specimen standardization. In contrast, our study selected curved mesial roots of mandibular molars with several anatomical variations were selected, which were classified according to Vertucci Classification (27). The authors used teeth with more complex anatomy because they represent a common clinical scenario in endodontic practice (35) and investigations of nonsurgical retreatment techniques in these cases are not common.

The average percentages of remaining filling material in the root canals after Stage 1 were consistent with Abramovitz et al.'s findings (36). When these authors only used ProTaper retreatment files in the curved canals of the mesial roots of mandibular molars, they observed substantial residual root canal filling material in the apical third of the canals. In our study, the application of PUI after the use of rotary instruments during endodontic retreatment notably enhanced apical cleaning. PUI, recognized as the 'gold standard' in agitation studies, as acoustic streaming created around the instrument magnifies the performance of the solution agitation and enhances the removal of debris, smear layer, and root canal filling materials (9, 17).

Based on the CLSM results, the present study observed that the obturation during endodontic retreatment penetrated into the isthmuses areas similarly in both groups. The main objective of combining solvents with PUI was to clean those areas of difficult access to endodontic instruments. The action of the PUI with different irrigants proportioned a higher dissolution of the filling materials (micro-CT results), although this remotion was not fulfilled by a filling during retreatment in isthmus and irregularities areas (CLSM results). A possible explanation for this difference in results between the two assessments was the use of the Tagger hybrid technique for root canal filling, since warm compaction tecnhiques would be better for filling canals with isthmus and irregularities areas. Thus, if the obturation performed during root canal retreatment is capable of providing entrapment of the microorganisms that caused the failure of primary treatment, the absence of nutrients would prevent their survival. The viabili-

ty of these microorganisms could potentially contribute to the maintenance and progression of periapical infection (37) leading to subsequent failure of the endodontic retreatment.

The use of solvents during endodontic retreatment softens gutta-percha, facilitating instrument action, and thereby reducing the risk of deviation, perforations, and instrument fracture during material filling removal (38). Additionally, solvents are used to remove waste filling materials in the root canal (39). In this study, orange oil solvent was used as the irrigant for ultrasonic activation due to its capacity to dissolve sealers similarly to other solvents, yet without causing any deleterious effects on periapical tissues (40, 41). However, the association of ultrasound with a solvent may raise concerns regarding potential solvent extrusion of the solvent and subsequent adverse effects on the periapical tissues. Nevertheless, it was demonstrated that PUI does not increase apical extrusion of the irrigant (42).

It is important to emphasize that, in this study, the authors took care in standardizing the experimental stages to avoid potential biases. The angles and radii of curvature were assessed, and no significant differences were observed between the experimental groups. The root canal anatomy was assessed using Micro-CT, categorized following Vertucci's classification (27), and a stratified randomization was performed, considering this classification. Moreover, no statistical differences were observed between the groups after the use of rotary instruments. This suggests that the specimens exhibited similar characteristics concerning root canal anatomy and presence of filling material before allocation for the protocol of supplementary cleaning. Furthermore, one of the strengths of this study was the utilization of micro-CT, which enabled a three-dimensional evaluation of root filling material.

One potential limitation of this study was the use of different sealers in both endodontic treatment and retreatment, as the sealers employed possess distinct fluidity and flow capacity properties. However, our research could serve as a basis for future studies investigating agitation techniques using differents solutions for removing residual filling material. Furthermore, additional investigations should explore the efficacy of PUI with solvents in dissolving resin-based and bioceramic sealers.

Conclusions

None of the supplementary cleaning protocols succeeded in completely removing the root canal filling material from the root canal system. However, the agitation of irrigants and orange oil using PUI proved to be an effective auxiliary method for removing residual filling material from curved canals with isthmus and irregularities areas. Although the PUI protocol enhanced the removal of root filling material, the penetration of obturation during retreatment in root irregularities areas remained unaffected by supplementary protocols.

Clinical Relevance

This study aims to help clinicians choosing the best supplementary cleaning protocol for removing residual filling material during endodontic retreatment.

Conflict of Interest

The authors deny any conflict of interest related to this study.

Acknowledgments

Nothing to declare.

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ORIGINAL ARTICLE

Antimicrobial effect of 2% chlorhexidine as a chemical adjuvant in different endodontic protocols: an in vitro study

ABSTRACT

Aim: *To analyze the antimicrobial effect of different protocols using 2% chlorhexidine as an irrigating substance, and 2.5% sodium hypochlorite to decontaminate lower molars infected with Enterococcus faecalis.*
Methodology*: 72 mesial roots were sectioned and contaminated with E. faecalis. The samples were randomly distributed into 4 groups (n=14) according to the protocols: 2 ml of 2% chlorhexidine gel and 10 ml of 9% saline solution (CHX G+SS); 2 ml of 2% chlorhexidine gel and 10 ml of 2% liquid chlorhexidine (CHX G+CHX L); 12 ml of 2% liquid chlorhexidine (CHX L); 12 ml of 2.5% liquid sodium hypochlorite (HIP L) (positive control). Bacteriological samples were collected before preparation and irrigation (S1), and after instrumentation and irrigation with different protocols (S2), for the ultimate purpose of quantifying the reduction in planktonic bacteria and intracanal biofilm. The samples were evaluated by using scanning electronic microscopy (SEM) to confirm the presence of biofilm. Bacterial quantification was performed using qPCR and the colony forming unit (CFU)/mL count. Statistical analysis was performed using the Kruskal-Wallis and Wilcoxon tests to compare the protocols of use for chlorhexidine as a according to the time points tested. The Student-Newman-Keuls test was used for multiple comparisons, with a significance level of 5%.*
Results: *The SEM analysis allowed visualizing the biofilm structure. At S1, there was a significant difference among the teeth that made up each group (p<0.001) regarding the CFU count. At S2, there was no difference among the HIP L, CHX G+CHX L and only CHX L groups, but the CFU count was significantly higher in the CHX G+SS group (p<0.001). Significantly lower CFU counts were found after S2, for all the groups (p=0.010).*
Conclusion: *The application of different 2% chlorhexidine protocols was effective in reducing bacterial contamination by E. faecalis. The 2% chlorhexidine application protocols proved to be good alternatives to 2.5% sodium hypochlorite, given the excellent antimicrobial efficacy.*

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Introduction

Endodontic treatment has a high success rate (1, 2), but can fail when confronted with anatomical difficulties (3) and microbial contamination (4). Chemical-mechanical preparation (CMP) is performed to decontaminate the root canal system and promote the healing and repair of periapical tissues (5). Endodontic failure is associated with the persistence of viable microorganisms even after endodontic treatment (6, 7). Among the microorganisms commonly found in cases of endodontic failure, *E. faecalis* has been isolated frequently and presents important factors of virulence and microbial resistance (8, 9, 10). The endodontic instruments used in preparing root canals are often incapable of completely disorganizing the biofilm on the walls of the root canal (11, 12), especially in anatomical regions of difficult access, such as isthmuses, lateral canals, deltas, and apical and dentinal tubules (6, 13). In this case, irrigating solutions are recommended to remove the debris, thereby dissolving organic matter, disinfecting the root canal system, and lubricating the instruments during endodontic preparation (14). The most widely indicated and used irrigating substance in an endodontic clinic is sodium hypochlorite. This substance has excellent antimicrobial properties, is capable of disorganizing the biofilm, and has tissue dissolution ability (15, 16, 17). However, its efficacy is dependent on volume, concentration and duration of contact with the organic matter (18, 19, 20); moreover, it displays cytotoxicity when it comes into contact with periapical tissues, mainly at higher concentrations (21, 22, 23). Chlorhexidine digluconate has been proposed as an alternative to Sodium hypochlorite, because it also has favorable antimicrobial properties and substantivity (24). Chlorhexidine is biocompatible and effective against gram positive and gram negative microorganisms (24-26); however, it also has drawbacks, such as not dissolving organic matter, and not neutralizing bacterial liposaccharides (LPS) (24).
Both sodium hypochlorite and chlorhexidine have been reported in several studies as efficient against microorganisms, including *E. faecalis* (27, 28). However, several clinical studies have demonstrated the efficacy of chlorhexidine (24) but point out that gaps still exist regarding an efficient protocol of use. Authors have reported its use as an antimicrobial at a 2% concentration in liquid form (19, 29); however, there are studies proposing its use at 0.2% (30), 0.12% (31), and 2% concentration in gel form (24). The gel form can be used together with saline solution for irrigation, thus taking advantage to lubricate endodontic instruments and benefit from the gel's rheological action. Some authors have used the gel form in a clinical study protocol (12, 24, 26).
Considering the possibilities of different irrigating solutions, and different protocols found in the literature, this study aimed to evaluate the antimicrobial effect of different protocols using 2% chlorhexidine as a irrigating substance to decontaminate lower molars infected with *E. faecalis*. The null hypothesis was that the application of different protocols for using 2% chlorhexidine as an irrigating substance solution would not alter the bacterial contamination in the root canal.

Material and Methods

Tooth Selection
This study was submitted to approval by the institutional ethics committee (CAAE4. 539.304). A total of 72 mandibular and maxillary molars that met the inclusion criteria were selected from 150 mandibular teeth donated to the study. The sample calculation was conducted using the G Power 3.1.9.4 program, adopting the analysis of variance model. An effect size of 0.459 was obtained from the results presented by Dametto et al. (32), using a significance level of 5% and power of 80%. The sample calculation indicated that 14 mesial roots of human lower molars were needed in each of the groups, for a total of 72 mesial roots. A total of 56 samples were distributed into 4 groups (n=14). 8 samples were also selected for SEM evaluation, 4 samples for positive control and 4 samples for negative control.

The teeth were radiographed mesiodistally and buccolingually, and observed under the optical lens of a clinical microscope (ALL 001, Alliance Microscopy, São Carlos, SP, Brazil), at 20x magnification to determine whether they qualified for the inclusion criteria, and which mesial roots of mandibular first and second molars had separate mesiobuccal (MV) and mesiolingual (ML) canals with independent foramina, fully formed apices, and roots with an anatomical foramen diameter compatible with a #15 K hand file (C-Pilot, VDW, Munich, Germany). Only teeth with a maximum degree of curvature of 10° to 20° were selected, according to Schineider's classification (1971, 33), and teeth with similar canal volume, dentin surface area and foraminal diameter. Presence of internal and external resorptions, calcifications, root cracks, fractures or previous endodontic treatment were considered exclusion criteria.

Tomographic analysis

The anatomical characteristics of the researched teeth were analyzed by cone beam computed tomography (CBCT). CBCT images were acquired using a Carestream 9600 unit operating at 85 kVp and 6 mA, with an exposure time of 14 s (Carestream Dental, Atlanta, GA, USA) of these seconds with an 8x8 cm FOV and 0.1 mm voxel size. The analysis was performed using the GALAXIS 3D software (Sirona Galileos, Bensheim, Germany).

Sample Preparation

The teeth were disinfected with 5% sodium hypochlorite in immersion for 1h, and stored in thymol at room temperature (Berutti, 2013). Root surfaces were cleaned with periodontal curettes (Golgran Instrumental Odontológicos, São Caetano do Sul, Brazil). Coronary access was performed with a high-speed drill under refrigeration, and the roots were separated with a diamond disc (KG Sorensen, São Paulo, Brazil). The root length was standardized at 17 mm (34, 35). The working length was standardized exactly to the measure of 0.0, tangent to the apical foramen (real working length=root canal real length - RL).

The root canals were irrigated with 5 ml of 2.5% sodium hypochlorite (Formula e Ação, São Paulo, SP, Brazil) followed by preparation of the canal with a Prodesign Logic file 03/15 (Easy, Belo Horizonte, Minas Gerais) up to the RL. Subsequently, the root canals were filled with 17% ethylenediaminetetraacetic solution (EDTA, Biodinamica São Paulo, Brazil). The solution was stirred with an ultrasound device (Satelec Booster, Brazil), and inserted (PN43807; Satelec Booster; Acteon, Indaiatuba, SP, Brazil) for 1 minute inside the root. Final irrigation was performed with 5 ml of sterile distilled water. Afterwards, the root canals were dried with #15 paper cones (Dentsply Maillefer, Ballaigues, Switzerland). The apical foramen of the roots was sealed with Z 100 composite resin (3M, St. Paul, MN), according to the manufacturer's protocol, and the external surface was waterproofed with nail polish (Colorama, São Paulo, Brazil). The samples were sterilized in an autoclave at 121 °C for 30 minutes (Cristófoli, Campo Mourão, Brazil).

Contamination of samples with *E. faecalis*

E. faecalis (ATCC-29212) was cultured and stored in brain heart infusion (BHI) broth media with 20% glycerol. The inoculum was prepared by transferring 100 µL of the *E. faecalis* stock to 2 mL of BHI broth, and storing it in a lab incubator at 37 °C. The sterilized roots were contaminated with a micropipette (Kasvi, Curitiba, Paraná, Brazil). Twenty µL of the final concentration of the *E. faecalis* suspension was placed inside the root canals. Next, the samples were stored in a lab incubator (Tecnal-Equipamentos para Laboratórios, Piracicaba, SP, Brazil) at 37 °C with 5% CO2 for four weeks. Confirmation of the viability and purity of the microorganisms inside the canals was carried out weekly by randomly collecting two teeth, seeding them in BHI broth, incubating them in a lab incubator at 37 °C with 5% CO2 for 24 hours, and applying the Gram stain.

Root Canal Prepare and Irrigation

Instrumentation in the cervical, middle and apical thirds was performed with a Reciproc Blue 25/08 file (VDW, Munich, Germany) and a Gold Reciproc motor (VDW, Munich, Germany) with penetration and traction movements, following the manufacturer's

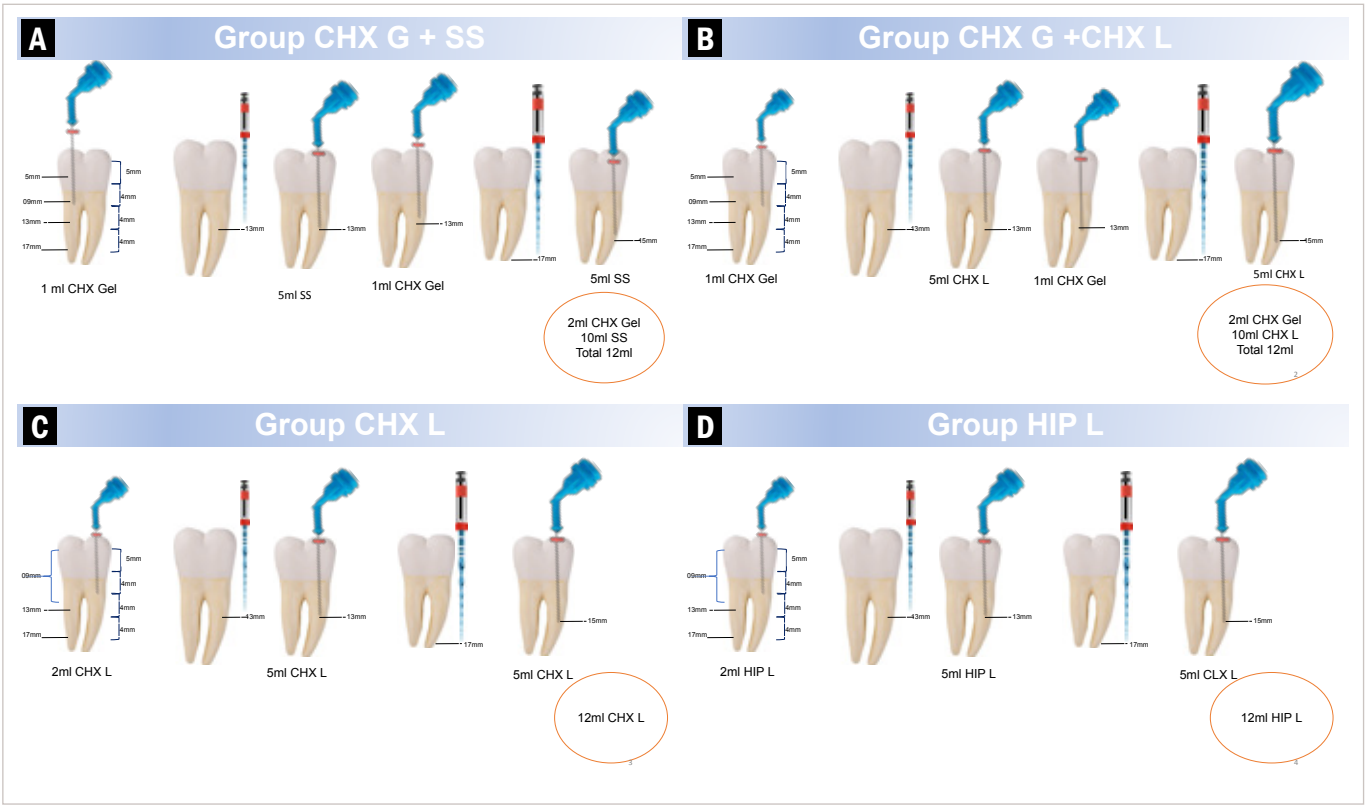

Figure 1
Irrigation protocols: **(A)** 2% chlorhexidine gel and 9% saline solution (CHX G+SS), **(B)** 2% chlorhexidine gel and 2% liquid chlorhexidine (CHX G+CHX L), **(C)** 2% liquid chlorhexidine (CHX L) and **(D)** 2.5% liquid sodium hypochlorite (HIP L).

recommendations. The samples were divided into four experimental groups (n=14), plus a positive control and a negative control. Eight samples were evaluated by scanning electronic microscopy (SEM) to confirm the presence of biofilm. The teeth were divided into groups, according to the irrigation protocol represented in Figure 1:

- Group CHX G+SS: irrigation with 2 ml of 2% chlorhexidine gel and 10 ml of saline solution;
- Group CHX G+CHX L: irrigation with 2 ml of 2% chlorhexidine gel and 10 ml of 2% liquid chlorhexidine;
- Group CHX L: irrigation with 12 ml of 2% liquid chlorhexidine;
- Group HIP L (positive control): irrigation with 12 ml of 2.5% sodium hypochlorite;
- Group CN (negative control): contaminated and non-irrigated samples (no treatment);
- Group CE (sterilization control): samples sterilized and not contaminated.

Single-use Reciproc files were used according to the manufacturer's protocol. Mechanical preparation of root canals was performed by a single operator under sterile conditions in a laminar flow cabinet.

Quantification of E. faecalis contamination

Bacterial collection was performed after four weeks of initial contamination of the samples (S1). Aliquots of 0.1 ml of the suspension, together with each dilution (10^{-2}, 10^{-4}, 10^{-5} and 10^{-6}), were seeded in Petri dishes (CRAL Artigos para Laboratório, Cotia, SP, Brazil) containing BHI agar (KASVI, Curitiba, PR, Brazil), and incubated in a lab incubator with 5% CO2 at 37 °C for 24 hours. Subsequently, the number of colony forming units (CFUs) per plate was counted, the number of CFU/mL was calculated, and quantitative analysis was performed by real time quantitative polymerase chain reaction (qPCR). Immediately after concluding the instrumentation, the final collection (S2) was made with a sterilized #15 Hedstrom file, introduced inside the root canal in the RL. Serial dilutions (10^{-2}, 10^{-4}, 10^{-5} and 10^{-6}) were prepared from the suspension. Aliquots of 0.1 ml of the suspension together with each dilution were plated in Petri dishes (CRAL Artigos para Laboratório, Cotia, SP, Brazil) containing BHI agar (KASVI, Curitiba, PR, Brazil). The seeded plates were incubated in a lab incubator with 5% CO2 at 37 °C for 24 hours.

Subsequently, the number of CFUs per plate was counted, and the number of CFU/ml was calculated.

Quantitative analysis by qPCR
DNA was extracted from half of the sample volume using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. DNA extracts were frozen at 20 °C until qPCR analysis. *E. faecalis* cells in root canal samples were quantified using the qPCR method targeting the 16S rRNA gene, with the Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) on an ABI 7500 real-time PCR instrument (Applied Biosystems) with a total reaction volume of 20 µL. Specific primers for E. faecalis species were used according to a previous study (6). An accumulation of the PCR product was detected at each cycle by monitoring the increased fluorescence of the dye (dsDNA-binding SYBR Green). All measurements were performed in duplicate for samples, and in triplicate for standardization. Data acquisition and analysis were performed using the ABI 7500 v2.0.4 software (Applied Biosystems).
E. faecalis ATCC 29212 was used to create a 10 log standard curve for direct bacterial quantification. DNA was isolated from a pure fresh culture of this strain using the QIAamp DNA Mini Kit (Qiagen), and quantified using a spectrophotometer (BioPhotometer, Eppendorf, Hamburg, Germany). The DNA value measured was converted into target genomic copy levels per microliter, by using the formula

$$m=n\,[1\ \text{mole}/6 \cdot 1023\ (\text{bp})]\,[660\ (\text{g})/\text{mole}] = n\,[1.096 \times 10\text{-}21\ (\text{g})/\text{bp})],$$

where m is the genomic mass of a single cell, and n is the genome size. Genome copying levels were considered numerically equivalent to bacterial cell levels. The standards were then diluted 10 times from 107 to 102 cells in TE buffer, and used to construct the standard curve.

Scanning Electron Microscopy (SEM)
Two roots from each group were randomly chosen and fixed in 10% buffered formalin for one week. Next, the prepared root canal walls were analyzed and topographically evaluated by coronal, medium and apical thirds with SEM (JSM 5600 LV; JEOL, Tokyo, Japan), at a voltage acceleration of 15 kV, with magnifications of 5,000x and 10,000x to confirm bacterial colonization and permanence of the biofilm. The roots were divided longitudinally, mounted on an acrylic stub, and covered by deposition of gold metal ions (sputtering), in a metalizing machine, prior to SEM analysis.

Statistical analysis
Comparisons between the protocols of chlorhexidine used as an irrigating substance-and the evaluation time points were made using Kruskal-Wallis and Wilcoxon tests, following non-normality of the CFU count data to normal distribution and homogeneity of variance. The Student-Newman-Keuls test was used for multiple comparisons. Statistical calculations were performed using the SPSS 23 (SPSS, Chicago, IL, USA), and BioEstat 5.0 (Mamirauá Foundation, Belém, PA, Brazil) programs, at a 5% significance level.

Results

The SEM analysis allowed viewing the morphology of the root canals and the biofilm through the section of the roots (Fig. 2A). Fig. 2B was taken from the root canal of the negative control group. It demonstrates the absence of bacteria-like structures, and only the dentinal tubule entrance and amorphous structures that can be identified as debris remains. Fig. 2C also shows the presence of a circular structure similar to *E. faecalis*. Since this genus of bacteria commonly presents ovoid or circular morphology (36), this may represent the presence of bacterial contamination in root canals, and hence biofilm formation.
At S1, there was a statistically significant difference in the CFU count among the teeth that made up each group ($p<0.001$). The group that was to be submitted to the irrigation protocol with 2.5% liquid sodium hypochlorite had a significantly higher

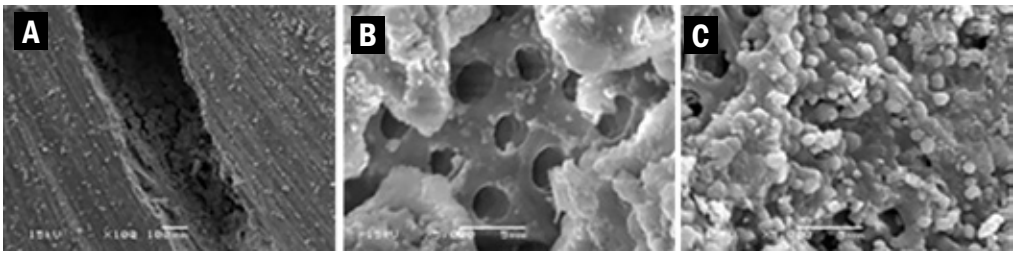

Figure 2
(A) Cutting the root – root canal, distant view (SEM at 100x). **(B)** Root of the control group (SEM at 5000x). **(C)** Bacterial contamination in the root canal (SEM at 5000x).

number of CFUs than the other irrigation groups. In the group whose irrigation was to use 2% liquid chlorhexidine (CHX L), the initial microbiological count did not differ significantly from that found in any of the other three groups of teeth (Table 1).
After instrumentation and irrigation (S2), the protocols showed a statistically significant effect on the microbiological count ($p<0.001$). There was no difference between the groups treated with the irrigation protocols performed with 2.5% liquid sodium hypochlorite, 2% chlorhexidine gel and liquid, and only 2% liquid chlorhexidine, but the CFU count in the group that received 2% chlorhexidine gel and 9% saline solution was significantly higher (Table 1). Significantly lower CFU counts were found in all the groups after instrumentation and irrigation ($p=0.010$), and even in the groups that had no CFUs, but that received 2.5% liquid sodium hypochlorite, 2% chlorhexidine gel and liquid, and only 2% liquid chlorhexidine (Table 1).
A significant difference was also found between the irrigation protocols in regard to the absolute reduction of the CFUs ($p<0.001$). The number of CFUs for the 2.5% liquid sodium hypochlorite group and the 2% liquid chlorhexidine group was significantly lower compared to the group that received 2% chlorhexidine gel and liquid. As for the number of CFUs for 2% chlorhexidine gel and 9% saline solution, there was no significant difference between the group irrigated with 2% liquid chlorhexidine and that receiving 2% chlorhexidine gel and liquid irrigation. However, the protocol using 2% chlorhexidine gel and 9% saline solution promoted a significantly higher number of CFUs, compared with the protocol for 2.5% liquid sodium hypochlorite (Table 1).

Discussion

Endodontic failure is directly related to the perseverance of viable microorganisms after endodontic intervention (4). *E. faecalis* is significantly associated with persisting endodontic infections, and is found in 24% to 77% of teeth with endodontic treatment failures (37). Therefore, this study adopted contamination with *E. faecalis* to simulate a clinical situation that could be used to evaluate the potential of irrigation protocols to resolve these infections. The evaluation of SEM images ensured the methodology chosen for contaminating the samples and revealed the biofilm formation (Fig. 2).
Several irrigation protocols with endodontic solutions have been proposed in the literature to enhance the mechanical cleaning of endodontic instruments (14). Among these solutions, sodium hypochlorite (NaOCl) has been advocated for its high antimicrobial activity, especially against *E. faecalis* (38). Nevertheless, it has limited action, and its efficacy is dependent on volume and concentration (18). Although high concentrations are efficient, they pose the risk of tissue toxicity (23). For this reason, 2% chlorhexidine has been proposed in the literature by several authors, with the aim of exploring its safe biological properties, and antimicrobial ability (24). Therefore, this study sought to evaluate *E. faecalis* decontamination with different chlorhexidine formulations. Furthermore, this study uses irrigating substances at each change in the use of endodontic files, in different groups, simulating clinical use, as well as other studies (4).
Significantly lower results were obtained for reduced CFUs after applying the 2% chlorhexidine protocols ($p=0.010$), absolute reduction was observed in groups 1, 2 and 3. Therefore, the null hypothesis was rejected.

Table 1

Means, standard deviations, medians and mean order of number and reduction of colony forming units (CFU/mL), before and after instrumentation and irrigation with different chlorhexidine use protocols.

Group	Collection time		Absolute reduction	Percent reduction
	S1	S2		
CHX G+SS	554,790 (686,243) Med: 306,000^{Aa}	8,874 (14,465) Med: 2,887^{Bb}	545,916 (685,851) Med: 277,933 Mean ord: 31.6^{BC}	95.9% (4.7%) Med: 98.3%
CHX G+CHX L	165,810 (141,171) Med: 111,333^{Aa}	0 (0) Med: 0^{Ab}	165,810 (141,171) Med: 111,333 Mean ord: 42.4^{C}	100.0% (0.0%) Med: 100.0%
CHX L	584,286 (322.162) Med: 573.333^{Aba}	0 (0) Med: 0^{Ab}	584,286 (322,162) Med: 573,333 Méd. ord: 21,9^{AB}	100.0% (0.0%) Med: 100.0%
HIP L	905,143 (757,758) Med: 606.667^{Ba}	0 (0) Med: 0^{Ab}	905,143 (757,758) Med: 606,667 Méd. ord: 18,2^{A}	100.0% (0.0%) Med: 100.0%

Standard deviation in parentheses. Med=median. Average order=average of the orders. Medians or mean orders followed by distinct capital letters indicate a significant difference between the groups (comparisons within each column). Medians followed by equal lowercase letters indicate no significant difference between the counts before or after instrumentation and irrigation. Chlorhexidine gel 2% and saline 9% (CHX G+SS), chlorhexidine gel 2% and chlorhexidine liquid 2% (CHX G+CHX L), liquid chlorhexidine 2% (CHX L) and sodium hypochlorite 2.5% (HIP L).

All the groups showed a significant reduction in CFUs, with the CHX L and HIP L groups showing the lowest decrease in CFUs ($p<0.001$), but not differing from each other. This corroborates the result of studies that have demonstrated the efficacy of chlorhexidine (CHX) and NaOCl on *E. faecalis* (28). Zand et al. compared the efficacy of CHX with that of NaOCl in reducing endodontic infection and obtained similar results for both solutions (29). In 2020, a systematic review and Meta-analysis by Ruksakiet et al. (27) also compared the effectiveness of CHX and NaOCl as antimicrobials and found no statistical difference between the irrigants. These articles show the good antimicrobial potential of both CHX and NaOCl. As for the difference between the formulations and the protocols, CHX gel (CHX G+SS) and CHX L showed no statistical difference in the absolute reduction of CFUs ($p<0.001$), even though the group with saline solution showed a higher number of remaining CFUs than the other groups ($p<0.0001$). The advantage of using the gel form is that there is less debris extrusion and smear layer reduction, compared with CHX L solutions, because of the properties of viscosity and rheological action (39, 40). These properties can be favorable, despite the non-dissolution of organic tissues, and have substantivity, which causes CHX to have residual effects (24, 29, 40).

The combination of CHX gel and saline solution (CHX G+SS) is proposed as a clinical protocol (41), for the purpose of improv-

ing the fluidity of the material, and was adopted as a protocol for this study. The irrigation dynamics vary according to physical parameters, such as flow velocity, wall shear stress, turbulence and apical pressure, and cause the biofilm to adhere to the root canal, and the debris and smear layer to be detached (42). The irrigant must be fluid when pressed out of the syringe, so that it offers less resistance to the flow, and thus comes into contact with the dentinal tubules in the root canal system to decontaminate them (43). When the irrigant is used as a gel, it cannot penetrate deep enough inside the tubules to promote an antimicrobial effect (29). Therefore it causes a slight lower microbial effect. This suboptimal effect could account for the CHX G+SS having removed less bacteria than the groups in which CXH L had bacterial action (CFU count was reduced by 98.3%, versus 100% for the other groups.

Evaluations of CHX are found in the literature as well as NaOCl gel, Zand et al. (2016) found significantly higher antimicrobial activity against *E. faecalis* for 2.5% hypochlorite in liquid form than gel form (29). The authors mention that the lower antimicrobial ability of NaOCl may be related to the viscosity of the gel, which impairs its penetration into the dentinal tubules. The same may occur with CHX gel. When associated only with saline solution, it showed less CFU removal, but this did not occur when it was combined with liquid CHX irrigation, in which case the liquid CHX penetrated the dentinal tubules and imparted antimicrobial action to the gel form.

A limitation in the methodology of this study was the inability to supply all the groups with the same number of bacteria to compare the difference between contamination prior to instrumentation and to irrigation. A statistical difference in the CFU count was found among the teeth that made up each group ($p<0.001$). However, the difference in the initial contamination among the groups does not interfere with the objective of the study, which was to analyze the antimicrobial effect after different protocols of use of 2% CHX as an irrigating substance. Thus, the decrease in contamination within each group was taken into account ($p<0.001$).

It is noteworthy that this investigation was an ex vivo study, and differs from a clinical situation. The literature has reported on infections consisting of predominantly facultative anaerobes and gram-positive species that resist endodontic treatment; however, the most commonly found microorganism in cases of retreatment is *E. faecalis* (44). Thus, further in vivo studies should evaluate the antimicrobial effect of 2% CHX as an irrigating substance, as well as long-term clinical follow-ups.

Conclusion

Different protocols of 2% chlorhexidine both in gel and liquid formulations, and of sodium hypochlorite, are effective in decontaminating root canals infected with *Enterococcus faecalis*, demonstrating that the reduction of biofilm is significant for all groups tested.

Clinical Relevance

Decontamination of the root canal system is important for the success of endodontic treatment. Irrigating substances are used for this purpose. This study showed that different substances used as a clinical protocol are effective in reducing bacterial biofilm.

Conflict of Interest

The authors deny any conflicts of interest related to this study.

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ORIGINAL ARTICLE

Applicability of photobiomodulation and antimicrobial photodynamic therapy for pain management after endodontic treatment: a randomized clinical trial

ABSTRACT

***Aim**: To evaluate the use of photobiomodulation (PBM), alone or combined with antimicrobial photodynamic therapy (aPDT), for pain management after endodontic treatment.*
***Methodology**: Randomized parallel-group superiority trial. The allocation sequence was generated using an online true random number generator. To ensure blinding, participants were informed about the study and the devices that would be used, but not about group allocation. Ninety mandibular molars diagnosed with symptomatic irreversible pulpitis were selected and randomly divided into three groups (n=30): Group 1, control (CT); Group 2, photobiomodulation (PBM); and Group 3, PBM+aPDT. All canals were instrumented by the Reciproc system in a single visit. The incidence and intensity of pain were evaluated before and 6, 12, 24, and 48 hours after endodontic treatment, using a visual analogue scale (VAS). The results were analysed using the Kruskal-Wallis test followed by Dunn's test.*
***Results**: The combination of PBM and aPDT after conventional endodontic treatment resulted in a significant reduction in pain compared to conventional endodontic treatment alone or followed by PBM alone ($p<0.05$). Preoperative pain was significantly greater than pain at 6, 12, 24, and 48 hours after endodontic treatment ($p<0.05$) in all groups. There were no adverse effects attributable to low-level light therapy in any participants.*
***Conclusion**: Our findings suggest that the combination of photobiomodulation and photodynamic therapy is a promising alternative for this purpose.*

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Introduction

There are two main factors preventing achievement of endodontic treatment goals: the characteristics of the resident microorganisms and the anatomical root configuration, which hinders access to the target region even with recently available technologies (1). Another key issue is postoperative pain (2-4), known as an endodontic flare-up, one of the most common complications and causes of discomfort after endodontic treatment (1). Intracanal laser irradiation is gaining acceptance in endodontic treatment (5) as an adjunct to conventional instrumentation and irrigation protocols, due to its disinfection ability (6, 7). Moreover, studies have reported that the use of laser therapy may result in decreased postoperative pain (8, 9).
Antimicrobial photodynamic therapy (aPDT) can be used as an adjunct to root canal cleaning (10, 11). aPDT combines a visible light source and a photosensitizing agent which, in the presence of oxygen, generates cytotoxic bioproducts such as singlet oxygen, free radicals, and superoxide anions; these damage the microbial cell wall and membrane proteins, leading to cell death (12). Recent publications have shown that aPDT reduces bacterial load, with promising results (13, 14).
Innovative methods have been proposed to reduce postoperative pain and provide greater comfort to patients undergoing endodontic treatment. Various adjuvant therapies are being studied for this purpose (8). Photobiomodulation (PBM) therapy, performed with low-level laser, provides benefits such as analgesia, modulation of the inflammatory process, and cell and tissue regeneration (15, 16). The analgesic and inflammation-modulating effects of PBM are mediated by modulation of prostaglandin synthesis, histamine release, alteration of the pain threshold, increased synthesis of endogenous endorphins, and inhibition of bradykinin synthesis (17).
Given the limited number of published randomized clinical trials in this promising area of research, our study aimed to evaluate whether PBM and aPDT (experimental groups) improved pain management after endodontic treatment compared to a control group not exposed to low-level light therapy. The outcome of interest was control of postoperative pain after endodontic treatment. The null hypothesis was that the PBM and aPDT combined or PBM alone would not influence postoperative pain after endodontic treatment compared to the standard of care.

Materials and Methods

This was a randomized, parallel-group, controlled superiority trial, with an allocation ratio of 1:1.

Eligibility criteria
The study protocol was approved by the relevant institutional ethics committee (Certificate of Submission for Ethical Appraisal: 29404420.6.0000.5374) and was conducted in accordance with the declaration of Helsinki.
The inclusion criteria were as follows:
- Patients diagnosed with irreversible pulpitis (observed as an exaggerated and "persistent" response to cold stimulus) (18) who were not taking any of the following medications: antibiotics, anti-inflammatory agents, analgesics, or immunosuppressants;
- Multirooted mandibular molars (first or second), with moderate curvature (10° to 20°) at mesial canals;
- Crown fit for rubber dam isolation;
- Fully formed roots and foramina;
- Absence of internal/external apical resorptions, dilacerations, and canal calcifications (radiographically confirmed).
The exclusion criteria were:
- Age <18 years, pregnancy, or breastfeeding;
- Periodontal disease on clinical and radiographic examination (changes in bone structure with mineral loss >3 mm) (19);
- Teeth that could not be treated in a single visit (patients who have temporomandibular disorders and/or who cannot endure long treatment period) (20);
- Teeth with extrusion of filling material, as determined on radiographic examination;
- Patients who took any medication after endodontic treatment.

Participants
In total, 90 patients who visited a private dental clinic were invited to participate. All patients were informed of the purpose of the trial and provided written informed consent for participation. Once endodontic treatment was completed, study participants were randomized by an external investigator into 3 groups (n=30). Demographic and dental data, stratified by study groups, are described in Table 1.

Conventional endodontic treatment procedure
All procedures were carried out by a single experienced endodontist. All patients went through a thorough history and clinical and radiographic examination.After radiographs had been obtained (Micro-Image, Indaiatuba, Brazil), thermal pulp testing was performed (Coltene, Cuyahoga Falls, USA). An inferior alveolar nerve block was performed using lidocaine 2% with epinephrine 1:100,000 (Alphacaine™; DFL, Rio de Janeiro, Brazil). The teeth were isolated with a rubber dam (Madeitex, São José dos Campos, Brazil) and a gingival barrier (FGM, Joinville, Brazil). The operative field was disinfected with 2% chlorhexidine (Riohex, São José do Rio Preto, Brazil). The endodontic access cavity preparation was done using carbide burs (#1013, #1015, or FGHL 1016, depending on the tooth; KG Sorensen, Cotia, Brazil). Cervical preflaring was done with FlexMaster Intro rotary files (VDW-Munich, Germany). The working length was determined with an apical locator (VDW-Munich, Germany) and confirmed by radiographic examination. All root canals were instrumented to 0.5 mm short of the root apex with ~21-25 mm C-PILOT hand files (#8, #10 and #15). Reciprocating R-PILOT files (VDW) were used to create the glide path. The canals were shaped with 21-mm or 25-mm VDW RECIPROC R25 files (VDW-Munich, Germany). Each file was used in only three teeth, as recommended elsewhere (21-24).
After shaping, Easy Clean tips (Easy, Belo

Table 1

Demographic and dental data in the study groups

	Randomization			Samples included	
	Groups assessed				
	CT (Conventional endodontic treatment)	**PBM (Photobiomodulation)**	**PBM+aPDT (Antimicrobial photodynamic therapy)**	**Completed**	**Dropouts**
Baseline characteristics	N	N	N	N	N
Total N	30	30	30	90	0
Gender					
Female	22	17	19		
Male	8	13	11		
Type of tooth					
Mandibular first molar	20	19	17		
Mandibular second molar	10	11	13		

Horizonte, Brazil) were used to clean the canal walls through mechanical agitation of the irrigant solution (2.5% sodium hypochlorite (NaOCl) (Asfer, São Paulo, Brazil), 2 mL per canal). Three cycles of agitation (20s each) were performed per canal, with the solution refreshed at each cycle (25). A VDW GOLD motor (Munich, Germany) was used to impart reciprocating motion. Then, 10 mL of 17% EDTA (Fórmula & Ação, São Paulo, Brazil) was placed in each canal for 1 minute. Capillary tips (Ultradent, South Jordan, USA) were used for aspiration, and RECIPROC paper points (VDW, Munich, Germany) were used. Three paper cones, sized according to the reciprocating instrument, were used for each canal.

For obturation, AH Plus filling cement (Dentsply Ltd., Ballaigues, Switzerland) was used, mixed according to the manufacturer's instructions.

The single-cone technique was used, with warm vertical compaction. A single gutta-percha cone (RECIPROC, VDW, Munich, Germany) consistent with the diameter of the file was used. The cone was cut with a plugger, and the cavity was cleaned with a sterile cotton ball soaked in alcohol. Coronal sealing was achieved with a temporary sealer (Coltene, Cuyahoga Falls, USA), and restorative glass ionomer cement (FGM, Joinville, Brazil).

Group 1 (control) – Conventional endodontic treatment (n=30).

Patients received conventional endodontic treatment, without laser therapy.

Group 2 (PBM) – Photobiomodulation (n=30)

After endodontic treatment, photobiomodulation was performed with low-level laser (Therapy EC, DMC, São Carlos, Brazil) in infrared mode (808 nm) with 100 mW power. The energy settings were 2 J per apex, energy density 20.4 J/cm², and a duration of 20 seconds. The laser output spot area is 0.098 cm². Energy was applied in the buccal and lingual regions of the gingival mucosa, close to the root apex (Figure 1).

Group 3 (PBM+aPDT) – Antimicrobial photodynamic therapy and photobiomodulation (n=30).

After instrumentation of the root canal system, aPDT was performed with methylene blue (Chimiolux 0.005%, DMC, São Carlos, Brazil) as photosensitizer and a

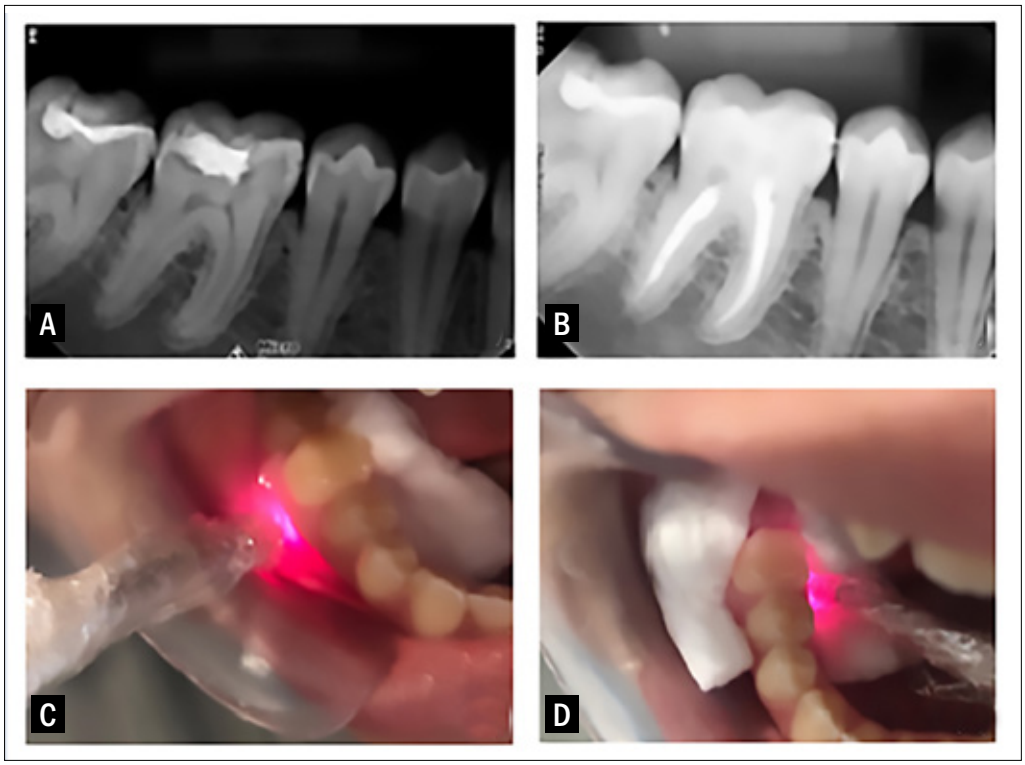


Figure 1
Conventional endodontic treatment combined with photobiomodulation therapy (CT+PBM). **(A)** Initial radiographs; **(B)** after conventional endodontic treatment; **(C)** infrared laser photobiomodulation performed in the buccal region, close to the root apex; **(D)** infrared laser photobiomodulation performed in the lingual region, close to the root apex. Radiographs: Micro-Image, Indaiatuba, Brazil; photographs: Apple iPhone 11 Plus, 2× magnification.

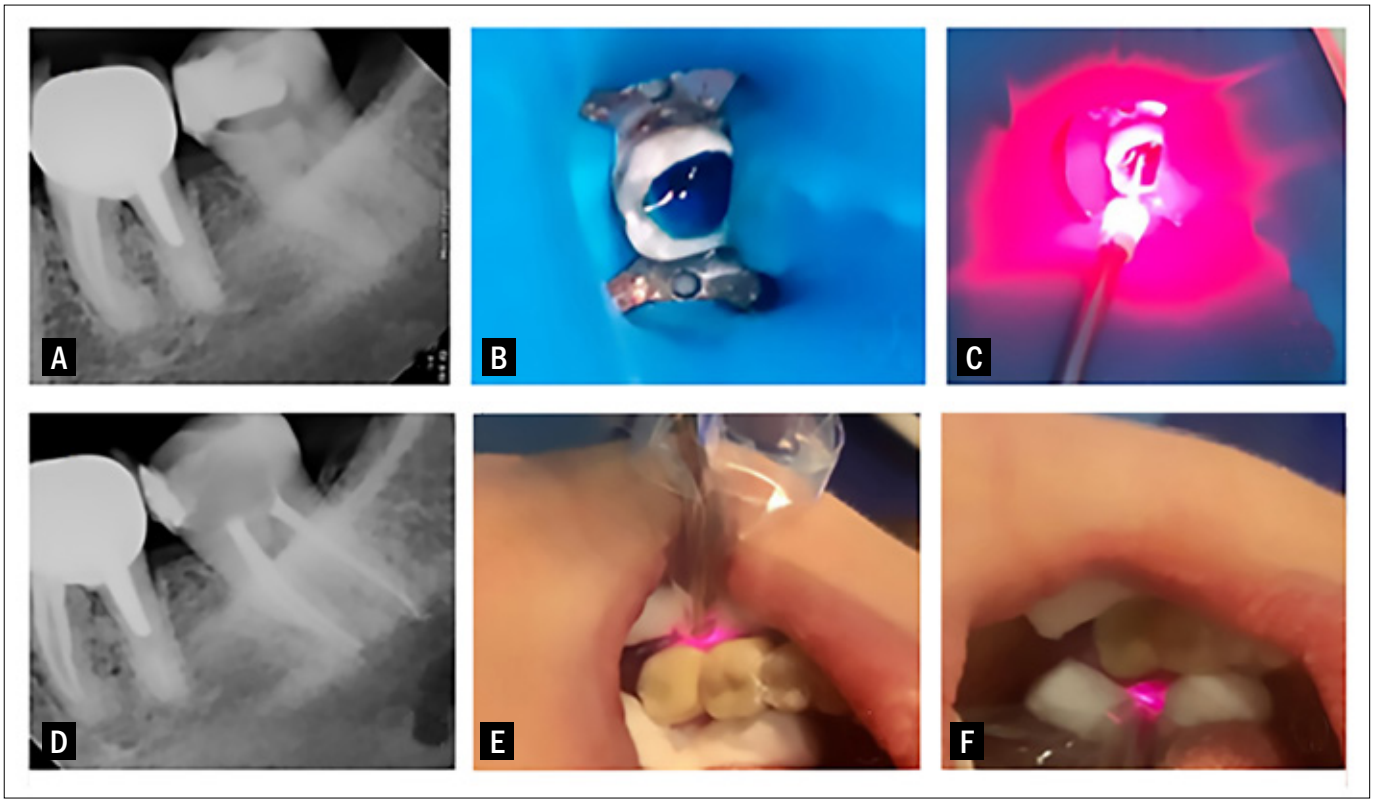


Figure 2
Conventional endodontic treatment combined with antimicrobial photodynamic therapy and photobiomodulation (CT+aPDT+PBM). **(A)** Initial radiographs; **(B)** canal filled with 0.005% methylene blue as photosensitizer; **(C)** antimicrobial photodynamic therapy using a red laser with a coupled fibre optic; **(D)** after endodontic treatment; **(E)** infrared laser photobiomodulation performed in the buccal region, close to the root apex; **(F)** infrared laser photobiomodulation performed in the lingual region, close to the root apex. Radiographs: Micro-Image, Indaiatuba, Brazil; photographs: Apple iPhone 11 Plus, 2× magnification.

pre-irradiation time of 5 minutes. This was followed by irradiation with low-level laser (Therapy EC, DMC, São Carlos, Brazil) in the red (660 nm) wavelength. A fibre optic (DMC, São Carlos, Brazil) was coupled to the laser.

The energy output at the fibre was 5.22 J, with an energy density of 2.899,8 J/cm² and a duration of 90 seconds (Figure 2). Pain assessment was performed at 6 h, 12 h, 24 h, and 48 h after endodontic treatment. Photobiomodulation was performed exactly as in the PBM group. The laser parameters for the PBM and aPDT treatments are described in Table 2.

Outcomes

The outcome of interest was control of postoperative pain after endodontic treatment in the experimental groups (PBM and aPDT).

Sample size

The number of teeth per group was determined through sample size calculation by analysis of variance (ANOVA), with a minimum difference between treatment means=0.10, standard error=0.126, number of treatments=3, statistical power=0.80, and alpha=0.05. The number of teeth per group was thus calculated as 30.

Randomization

A permuted-block randomization strategy with increasing block sizes (2, 4, and 6) was used. Opaque envelopes, each containing the information corresponding to the allocation group, were labelled with sequential numbers. The randomization sequence was generated online (http://www.random.org/).

Blinding

The patients were informed about the study and the devices that would be used, but they were not given information about the group allocation.

Trial registration

The study protocol was registered on the Brazilian Clinical Trials Registry (ReBEC) on January 31, 2022, with access number RBR-7tqy7yw.

Table 2

Irradiation parameters for applications

PBM		aPDT (fibre)	
Wavelength	808 nm	Wavelength	660 nm
Output power	100 mW	Output power	58 mW
Working time	20 s	Working time	90 s
Probe/fibre diameter	600µm	Probe/fibre diameter	1 mm
Spot area	0.098 cm^2	Spot area	0.0018 cm^2
Energy	2 J	Energy	5.22 J
Energy density	20.4 J/cm^2	Energy density	2,899.8 J/cm^2

Statistical analysis

The results were analysed in Bioestat 5.0. The Shapiro-Wilk test was used to verify the assumption of normality. The Kruskal-Wallis test followed by Dunn's test was used for comparisons; significance was set at $p<0.05$.

Results

The selection and randomization of participants are described in Figure 3. Participant recruitment took place from May 1, 2020 through August 1, 2023, and the duration of follow-up was 6 months.
There was a significant reduction in postoperative pain with the use of aPDT+PBM after endodontic treatment was observed compared to control or PBM alone ($p<0.05$) (Table 3). In all groups, preoperative pain was significantly greater than pain at 6, 12, and 24 hours post-treatment ($p<0.05$). After 48 hours, there was no significant difference between groups ($p>0.05$) (Table 3). There were no adverse effects attributable to low-level light therapy in any of the participants.

Discussion

Flare-ups remain a challenge for the endodontic practitioner (16) and may have a significant impact on patients' quality of life (25). Therefore, this study was designed to evaluate whether two promising adjunctive modalities, aPDT and PBM could help

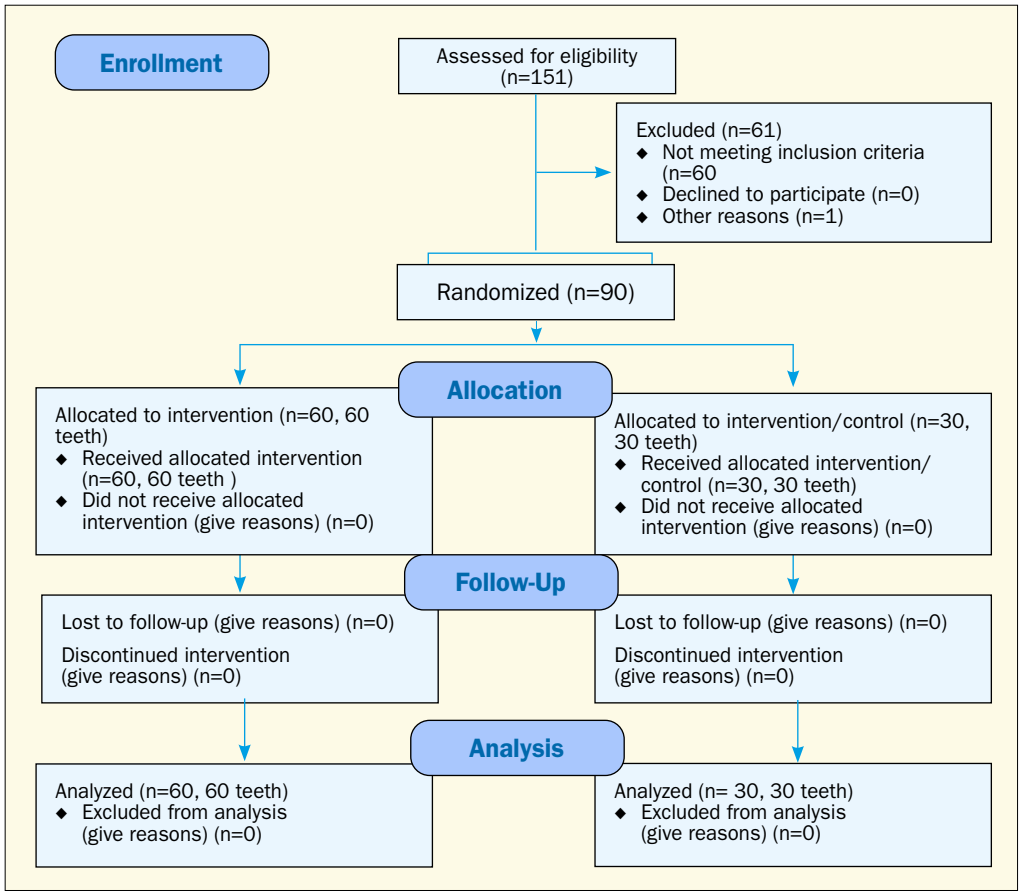
Table 3

Medians, interquartile intervals for VAS scores before and 6, 12, 24, and 48 hours after endodontic treatment in each experimental group (Kruskal-Wallis (Dunn))

	Baseline VAS	VAS 6	VAS 12	VAS 24	VAS 48	(p)
CT	9.00 (3.00)$^{A, a}$	4.00 (4.00)$^{B,1, a}$	3.00 (4.00)$^{B, a}$	2.00 (4.00)$^{B, a}$	0.00 (2.00)$^{B,2, a}$	<0.05
CT + PBM	9.00 (2.00)$^{A, a}$	2.00 (4.00)$^{B,1, ab}$	2.00 (2.00)$^{B,1, ab}$	1.00 (2.00)$^{B, ab}$	0.00 (0.00)$^{B,2, a}$	<0.05
CT + PBM + aPDT	10.00 (2.00)^{A,a}	0.00 (4.00)$^{B, b}$	0.00 (2.00)$^{B, b}$	0.00 (0.25)$^{B, a}$	0.00 (0.00)$^{B, a}$	<0.05
(p)	>0.05	<0.05	<0.05	<0.05	>0.05	

CT: conventional endodontics treatment; CT+PBM: conventional endodontics plus photobiomodulation; CT+PBM + aPDT: conventional endodontics plus photobiomodulation plus antimicrobial photodynamic therapy; VAS: visual analogue scale of pain. VAS 6: VAS score 6 hours after treatment; VAS 12: VAS score 12 hours after treatment; VAS 24: VAS score 24 hours after treatment; VAS 48: VAS score 48 hours after treatment.
Uppercase letters and different numbers in the same row denote statistically significant differences.
Lowercase letters in the same column denote statistically significant differences.

Figure 3
CONSORT flow diagram.



manage postoperative pain after conventional endodontic treatment. An increased reduction in postoperative pain was observed with the combination of PBM and aPDT after endodontic treatment, suggesting that these adjunctive modalities may have helped manage postoperative pain, thus rejecting the null hypothesis. One possible explanation for the outcome of greater reduction in postoperative pain in the combined aPDT+PBM group is that aPDT would have reduced the microbial load present in the extruded debris, thus enhancing the periapical disinfection achieved by instrumentation of the root canal system. The clinical relevance of these findings lies in the observation that PBM performed immediately after aPDT could modulate inflammation and minimize postoperative pain. In the PBM group without aPDT, inflammation modulation alone was not enough for better postoperative pain control. This is consistent with the findings of Coelho et al. (25), who reported that aPDT decreased postoperative pain at 24h and 72h in single-visit treatment of single-rooted teeth with necrotic pulp.

The literature describes several laser settings for PBM (26). Laser-tissue interactions in the infrared wavelength (810-1.064 nm) depend on the target tissue (1). The PBM protocol of this study used a low-power infrared diode laser (2 J energy), as in previous studies (27). Diode laser has proven to be the most promising approach in terms of postoperative pain reduction. This laser can reach periapical tissues and modulate inflammation; one possible explanation for its analgesic effect is this deeper tissue penetration and action (28). As noted above, there was a reduction in postoperative pain in the photobiomodulation (PBM) group compared to control, but less so compared to the PBM+aPDT group. aPDT has been used as an adjunct

to treatment for disinfection of the root canal system in necrotic teeth, eliminating resistant microorganisms (29). Zargar et al. (30) carried out a study of the microbial flora in cases of irreversible pulpitis and primary endodontic infections. Sixteen microbial species were identified in cases of irreversible pulpitis; a significant burden of microorganisms was identified within the root canals (31), highlighting the importance of antimicrobial techniques such as aPDT. Several parameters of this technique can be modulated (32). The present study was carried out according to the protocol described by Moreira et al. (31), using methylene blue 0.005% as the photosensitizer, with a pre-irradiation dwell time of 5 minutes in each canal. A fibre optic was coupled to the laser unit and placed into the root canal for better light diffusion, reaching the apical third (33). In the present study, the combination of aPDT and PBM resulted in a significant reduction in pain after endodontic treatment. This is consistent with the findings of Vilas-Boas et al. (34), who reported a reduction in postoperative pain after laser therapy in patients with symptomatic apical periodontitis.

Some studies have shown that postoperative pain often occurs during the first 24-48 hours after endodontic treatment (1). To assess pain intensity, we used a simple visual analogue scale (VAS) (35) graded from 0 to 10, where 0 means no pain at all and 10 is the worst pain level imaginable by the patient.

The present trial included patients diagnosed with irreversible pulpitis who had multirooted mandibular molars with moderate curvature. The decision to perform single-visit endodontic treatment aimed to reduce cross-contamination. Furthermore, using a reduced number of instruments with a shorter operative time also causes less instrument fatigue (35). Reciprocating instrumentation has been shown to produce effective results in endodontic treatment (36), with studies reporting satisfactory results regarding postoperative pain (37). Rahbani et al. (38) reported no significant difference in postoperative pain when comparing rotary and reciprocating instrumentation for endodontic therapy.

After preparing the root canal system, some agitation method for irrigation is considered important as a way to improve the removal of debris from inside the canals (39). In the present study, it was decided to use Easy clean in reciprocating mode to agitate the sodium hypochlorite (25). However, even though EDTA at a concentration of 17% is the most commonly used protocol for removing smear layers, it was only applied with conventional irrigation, as its potential for demineralization is known (40). The main limitation of the study was the 6-month follow-up, was not sufficient to observe e the potential long-term effects of low-level light therapy after endodontic treatment. Another limitation was individual variability in pain thresholds, which hinders sample homogeneity in any study involving pain management.

Further clinical studies should be conducted to assess the utility of aPDT and PBM for pain management after root canal treatment, adding to the evidence base on laser therapies as adjuvants to conventional endodontic treatment. In this study, the combination of aPDT and PBM improved patient comfort by enhancing postoperative pain control.

Conclusion

A combination of aPDT with a 660-nm diode laser and PBM with an 808-nm laser is a promising alternative for pain management after endodontic treatment.

Clinical Relevance

The combination of photobiomodulation and photodynamic therapy is a promising alternative for pain management after endodontic treatment.

Conflict of Interest

The authors have no conflicts of interest.

Acknowledgements/Financial disclosure

The authors have no financial relationships relevant to this article to disclose.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Ethics approval

This study was approved by the Research Ethics Committees of São Leopoldo Mandic's Faculty (CAAE number: 29404420.6.0000.5374).

Informed consent

All patients were informed of the purpose of the trial and provided written informed consent for participation.

Trial registration

The study protocol was registered on the Brazilian Clinical Trials Registry (ReBEC) on January 31, 2022, with access number RBR-7tqy7yw.

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ORIGINAL ARTICLE

Assessing dentinal tubule penetration of an innovative bioactive glass-based root canal sealer through confocal laser scanning microscopy: an *in vitro* analysis

ABSTRACT

Aim: *To assess and compare the dentinal tubule penetration of zinc oxide eugenol (ZOE)-based, resin-based, bioceramic, and novel bioactive glass-based root canal sealers using a confocal laser 8 scanning microscope (CLSM).*
Methods: *A total of 48 single-rooted permanent teeth were categorized into four groups (n=12) and treated with gutta-percha along with ZOE sealer (Tubli-Seal EWT), resin-based sealer (AH Plus), bioceramic sealer (BioRoot RCS), and bioactive glass (Nishika Canal Sealer-BG). Cross sections of the roots at 3 mm and 6 mm from the apex were examined under a CLSM to evaluate dentinal tubule penetration.*
Results: *Results indicated that bioceramic sealers exhibited the highest depth of dentinal tubule penetration at both levels, followed by bioactive glass and resin-based sealers. ZOE-based sealer demonstrated the least tubule penetration. Bioactive glass displayed the highest percentage of sealer penetration at 3 mm and 6 mm, with no statistically significant difference observed between bioceramic and bioactive glass groups regarding depth and percentage of dentinal tubule penetration at both levels.*
Conclusions: *Of particular significance is the bioactive glass group, demonstrating the most substantial sealer penetration percentage compared to the other groups at both examined depths.*

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Introduction

Root canal treatment (RCT) is a fundamental procedure for preserving natural teeth, involving a series of steps such as shaping, cleaning, and filling the root canal system (1). Core materials and sealers, either used independently or in combination, play a crucial role in achieving comprehensive sealing of the root canal system (2). Grossman's non-staining zinc oxide eugenol (ZOE) sealer has been a long-standing choice (3). The AH Plus, a paste system, delivered through a double barrel syringe, contains Aerosil and radiopaque fillers in the epoxide paste (4, 5). Bioceramics, a subtype of biomaterials, are increasingly recognized for their promising role in endodontic applications, particularly in root canal filling. These materials offer significant advantages, including biocompatibility and the ability to promote periapical tissue regeneration (6). Moreover, there has been a growing interest in the use of "bioactive" materials in restorative and reconstructive dentistry. In restorative dentistry, the term "bioactive" typically refers to a material's capability to stimulate the formation of hydroxyapatite crystals on its surface. Beyond their structural properties, bioactive substances are valued for their ability to foster beneficial interactions with living cells and tissues from a biological standpoint (7-9). In recent years, a more reactive form of calcium-silicate-based bioactive glass, termed "bioactive root canal sealers," has emerged alongside traditional calcium-silicate-based sealers (10). Bioactive root canal sealers are specialized materials used in endodontic procedures to seal and fill the root canal system after it has been cleaned and shaped. Unlike traditional sealers, bioactive sealers possess the unique ability to interact with the surrounding tissues, promoting healing and regeneration. These sealers typically contain biologically active components that can stimulate tissue repair (9, 10). While bioactive glass has traditionally been used for regenerating dental hard tissues, its potential in treating various complex tissues has become evident (11). Specifically, calcium and silicate ions, essential in biological processes, have been found to accelerate both osteoinduction and angiogenesis, which are crucial for supporting periapical healing (12). Given the unique properties of bioactive root canal sealers and their potential to promote periapical healing, it is essential to evaluate their performance in critical aspects such as dentine tubular penetration. This parameter directly influences the effectiveness of the root canal filling by affecting its sealing ability and long-term stability (11, 12). Dentine tubular penetration plays a crucial role in providing a physical barrier against microbial invasion and enhancing the retention of the sealer within the root canal system. Therefore, assessing this aspect is vital, particularly for novel materials aiming to improve endodontic outcomes.

Confocal Laser Scanning Microscopy (CLSM) has emerged as a valuable tool for precisely assessing tubular penetration. Its high-resolution imaging capabilities enable researchers to visualize and quantify the extent of sealer penetration into dentinal tubules accurately. This technique has been widely adopted in numerous published studies across various experimental fields due to its reliability and effectiveness in evaluating material performance (13, 14).

This study aims to assess and compare the penetration of root canal sealers, including ZOE-based, resin-based, bioceramic, and bioactive glass-based sealers, into dentinal tubules. The evaluation will be conducted using CLSM.

Materials and Methods

The study included 48 meticulously chosen intact single-rooted permanent teeth, which were divided into four groups, each comprising 12 teeth, through random allocation. The groups were designated as follows:

- Group 1: Ultrasonic activation of a ZOE-based sealer (Tubli-Seal EWT; Kerr, USA)
- Group 2: Ultrasonic activation of a resin-based sealer (AH Plus; Dentsply Maillefer, USA)
- Group 3: Ultrasonic activation of a bioceramic sealer (BioRoot RCS; Septodont, USA)
- Group 4: Ultrasonic activation of a bio-

active glass sealer (Nishika Canal Sealer-BG; Morita, Japan).
The penetration of dentinal tubules was evaluated and compared using CLSM with the Zeiss LSM 710 system.

Sample Preparation
Each tooth underwent digital radiography in both mesiodistal and buccolingual directions to confirm the presence of a single canal. Subsequently, meticulous cleaning was conducted using an ultrasonic scaler to eliminate calculus and tissue tags.
A disinfection process involving 3% sodium hypochlorite (NaOCl, Prime Dental Products, India) for 48 hours was applied, followed by decoronation to standardize the root canal length at 10 mm. The root canals were enlarged using the Protaper nickel-titanium rotary system up to size F3, while maintaining a distance of 0.5-1 mm from the apical foramen. Continuous irrigation with 3% NaOCl at 2 ml per file was employed during the shaping process. Final irrigation with NaOCl, lasting 1 minute using a U-file attached to an ultrasonic unit handpiece, was performed. To eliminate the smear layer, a 2 ml solution of 17% EDTA (Prime Dental Products, India) was applied for 3 minutes, followed by a final rinse of 2 ml saline. Each root was then dried with paper points before being randomly assigned to one of four groups based on the sealer used.
Sealer Placement
The sealers were prepared according to the manufacturer's instructions and labeled with Rhodamine B (HiMedia, Mumbai, India) at an estimated concentration of 0.1% to facilitate CLSM analysis. Using a tuberculin syringe, 0.05 ml of each sealer was dispensed into the canal. The root canals were then obturated using gutta-percha via the single cone obturation technique, and the orifice was sealed with Cavit (3M ESPE, Germany). Subsequently, all roots were stored at 100% humidity and 37 °C for 14 days to allow the sealer to set.

Sectioning and Image Analysis
The roots were precisely sectioned using a diamond disc at 200 rpm with continuous water cooling to prevent frictional heat. Horizontal sections were made at 3 mm and 6 mm levels from the apical foramen. To remove debris generated by sanding, the surface was polished using sandpapers numbered 400, 600, and 1,200 under running water. Following polishing, dentine segments with a thickness of 2 mm were air-dried and examined under CLSM at 10x magnification. The absorption and emission wavelengths of Rhodamine B dye were set at 540 nm and 590 nm, respectively.
Software analysis was used to measure the depth of sealer penetration, with the canal wall serving as the reference point. Sealer penetration into dentinal tubules was quantified using the built-in ruler, extending to its maximum depth (Figures 1, 2). Additionally, Figure 3 presents a compar-

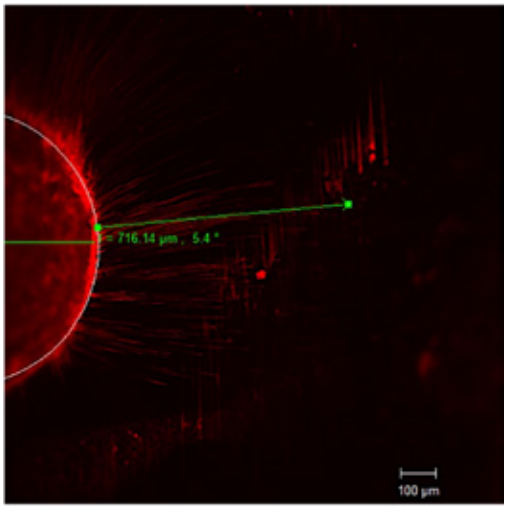

Figure 1
Analysis of sealer penetration depth.

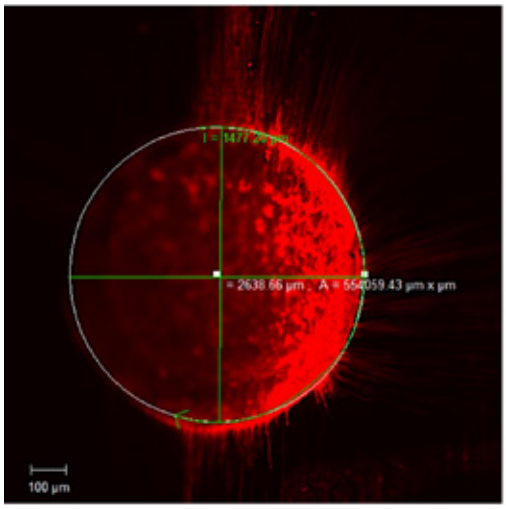

Figure 2
Analysis of sealer penetration percentage.

ative analysis of dentinal tubule penetration at 3 mm and 6 mm levels.
The entire circumference of the root canal was delineated and measured using the built-in ruler. Then, the circumference with visible sealer penetration was marked with a distinct colored line and measured. The percentage of the circumference with sealer penetration was calculated for each sealer type at both 3 mm and 6 mm levels using the formula:

Percentage of Penetration = (y/x)*100

where *y* represents the circumference indicating sealer penetration and *x* denotes the total circumference.

Statistical Analysis

The data were entered and analyzed using the Statistical Package for the Social Sciences (SPSS) for Windows, Version 28.0 (IBM Corp, Armonk, NY). Confidence intervals were set at 95%, and statistical significance was determined at a p-value of ≤0.05. Continuous variables were expressed as Mean±Standard Deviation. Unpaired t-tests were employed to compare the depth of dentinal tubule penetration at 3 mm and 6 mm across all groups. One-way analysis of variance (ANOVA) was used to compare both the percentage and depth of dentinal tubule penetration, followed by Tukey's post hoc test for pairwise comparisons.

Results

Depth of dentinal tubule penetration at 3 mm level, resin-based and bioceramic sealers exhibited notably deeper penetration depths, measuring approximately 403 µm and 679 µm, respectively. Bioactive glass demonstrated a penetration depth of 504 µm. Notably, bioceramic sealers displayed the highest penetration depth overall. Conversely, at the 6 mm level, ZOE-based sealers exhibited the shallowest penetration into dentinal tubules, with an average depth of approximately 227 µm. Resin-based sealers showed improved performance with an average depth of approximately 731.41 µm, while bioceramic bsealers demonstrated the most substantial penetration, averaging approximately 1041.75 µm.
Bioactive glass exhibited an average penetration depth of 885.916 µm. Significant statistical differences ($p<0.05$) were observed between the 3 mm and 6 mm levels for all groups (Table 1). Pairwise comparisons between groups, as presented in Table 2, revealed statistically significant differences between all groups at 3 mm ($p<0.05$). Additionally, in Table 3, pairwise comparisons between different groups demonstrated a significant difference in the average depth of sealer penetration at 6 mm compared to ZOE ($p<0.05$). However, no statistically significant difference was

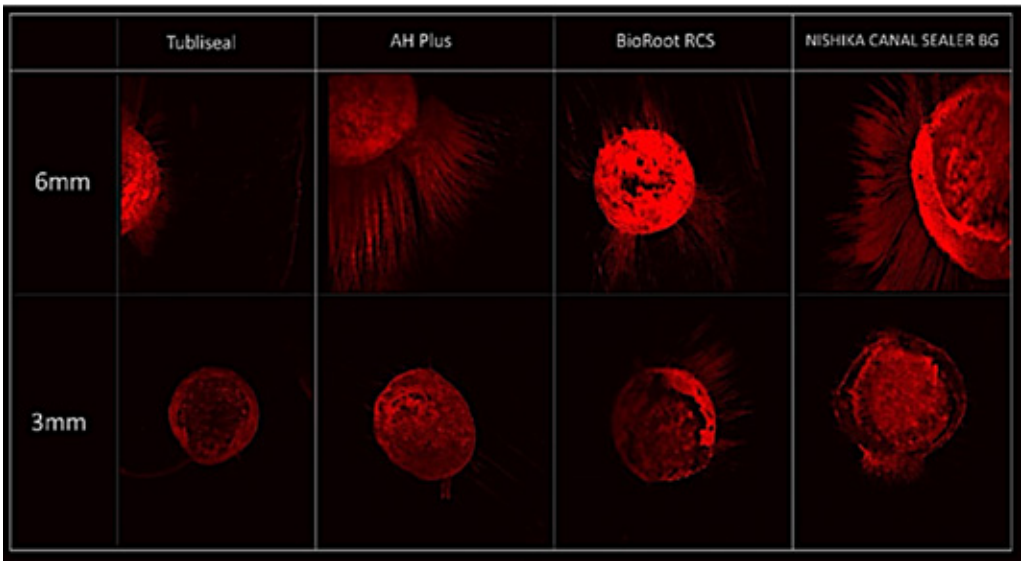

Figure 3
Comparative Analysis of dentinal tubule penetration by ZOE-based, resin-based, bioceramic, and bioactive glass-based root canal sealers using CLSM.

Table 1

Comparison of different sealer types for the depth of dentinal tubule penetration at 3 mm and 6 mm

Sealer Type	Groups		*p-value*
	3 mm	6 mm	
ZOE based	102.5833±8.72	227.5±21.90	0.001*
Resin based	403.6667±44.63	731.4167±109.36	0.05*
Bioceramic based	679±90.08	1041.75±275.92	0.004*
Bioactive glass	504.9167±116.80	885.91±167.44	0.001*

**indicates statistically significant difference (p<0.05)*

Table 2

Pairwise comparison of the average depth of dentinal tubule penetration at the 3 mm level

	Group (I)	Group (J)	Mean difference (I-J)	*p-value*
Depth of dentinal tubule penetration	Group I	Group II	301.08	0.001*
	Group I	Group III	576.42	0.001*
	Group I	Group IV	402.33	0.001*
	Group II	Group III	275.33	0.001*
	Group II	Group IV	101.25	0.001*
	Group III	Group IV	174.08	0.001*

**indicates statistically significant difference (p<0.05)*

Table 3

Pairwise comparison of the average depth of dentinal tubule penetration at the 6 mm level

	Group (I)	Group (J)	Mean difference (I-J)	*p-value*
Depth of dentinal tubule penetration	Group I	Group II	503.92	0.001*
	Group I	Group III	814.25	0.001*
	Group I	Group IV	658.42	0.001*
	Group II	Group III	310.33	0.005*
	Group II	Group IV	154.50	0.16
	Group III	Group IV	155.83	0.15

**indicates statistically significant difference (p<0.05)*

observed between resin-based, bioceramic, and bioactive glass groups (p>0.05).

Percentage of sealer penetration

At the 3 mm level, samples treated with the ZOE-based sealer displayed a mean percentage of sealer penetration along the root canal wall of approximately 44.8%, marking the lowest among all groups. In contrast, samples treated with resin-based and bioceramic sealers demonstrated mean percentages of sealer penetration of approximately 64.41% and 74.12%, respectively. Notably, the bioactive glass group exhibited the highest mean percentage of sealer penetration at 75.7%. At the 6 mm

Table 4

Comparison of sealer penetration percentages at 3 mm and 6 mm levels

Sealer Type	Groups		*p-value*
	3 mm	6 mm	
ZOE based	44.80±8.72	57.51±21.90	0.56
Resin based	64.41±44.63	68.1±109.36	0.63
Bioceramic based	74.12±90.08	77.97±275.92	0.56
Bioactive glass	75.70±116.80	79.09±167.44	0.56

**indicates statistically significant difference (p<0.05)*

Table 5

Inter-group comparisons for sealer penetration percentage at 3 mm depth

	Group (I)	Group (J)	Mean difference (I-J)	*p-value*
Percentage sealer penetration	Group I	Group II	19.61	0.001*
	Group I	Group III	29.32	0.002*
	Group I	Group IV	30.90	0.001*
	Group II	Group III	9.71	0.44
	Group II	Group IV	11.29	0.30
	Group III	Group IV	1.58	0.99

**indicates statistically significant difference (p<0.05)*

Table 6

Inter-group comparisons for sealer penetration percentage at 6 mm depth

	Group (I)	Group (J)	Mean difference (I-J)	*p-value*
Percentage sealer penetration	Group I	Group II	10.58	0.41
	Group I	Group III	20.46	0.002*
	Group I	Group IV	21.58	0.001*
	Group II	Group III	9.88	0.47
	Group II	Group IV	10.99	0.37
	Group III	Group IV	1.12	0.99

**indicates statistically significant difference (p<0.05)*

level, ZOE-based sealer-treated samples showed a mean percentage of sealer penetration along the root canal wall of approximately 57.51%, again representing the lowest among the four groups. In comparison, samples treated with resin-based sealers exhibited a slightly higher extent of penetration along the root canal wall at approximately 68.1%, while bioactive glass showed the highest mean percentage of sealer penetration at approximately 79.09%. No significant difference (p>0.05) was observed between the 3 mm and 6 mm levels for any of the groups, as indicated in Table 4.

The post hoc test results in Table 5 high-

light intergroup pair-wise comparisons specifically at the 3 mm level, revealing a significant difference in the percentage of penetration between ZOE and other groups ($p<0.05$). However, no statistically significant difference was observed between resin-based, bioceramic, and bioactive glass groups ($p>0.05$). Table 6 further demonstrates a statistically significant difference ($p<0.05$) between ZOE vs. bioceramic-based sealers and ZOE vs. bioactive glass-based sealers.

Discussion

Bacterial colonization tends to concentrate in the apical region of diseased root canals, posing challenges to effective treatment. Neglecting this area during cleaning may impede the healing of periapical lesions, while inadequate sealing of the apical root canal can create an environment conducive to bacterial proliferation, increasing the risk of endodontic failure (15). Endodontic sealers are indispensable in achieving successful endodontic therapy by completing root canal fillings during obturation procedures. Grossman outlined the characteristics of an ideal sealer, including tackiness for strong adhesion to the canal wall, the ability to create a hermetic seal, and radiopacity for easy radiographic visualization. Additionally, an ideal sealer should blend fine powder seamlessly with liquid, resist contraction upon setting, avoid tooth discoloration, exhibit bacteriostatic properties, remain insoluble in bodily fluids, demonstrate biocompatibility, and be soluble in common solvents (16).

These sealers, classified by composition as ZOE, calcium hydroxide, glass ionomer, silicone, resin, and bioceramic-based, have been extensively studied due to their significant biological and technical implications since their inception in the early twentieth century (2).

Root canal fillings typically involve a robust core material, such as gutta-percha, combined with a sealer to facilitate adaptation and ensure an effective seal of the root canal filling material (17, 18). The interface between the sealer and the root canal wall is pivotal for sealing the entire root canal system (19). The sealer plays a crucial role in filling irregularities on the root canal wall and within dentinal tubules, areas that gutta-percha alone may not reach. Enhanced sealer penetration into the tubules is associated with improved sealability, augmenting the contact surface between the filling material and dentin (20). Additionally, sealer penetration can contribute to an antimicrobial effect within the tubules, particularly when microbes are present nearby (21). Notably, sealer penetration observed in in vitro models closely mimics in vivo conditions (22-26).

The search for the best obturating materials and filling techniques relies heavily on a thorough analysis of the adhesion between the sealer and dentin, as well as its capacity to penetrate deep into dentinal tubules. This meticulous examination is crucial for ensuring successful endodontic therapy. Various microscopic techniques, including stereomicroscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM), and CLSM, are utilized to explore the penetrating capabilities of sealers and the interaction between the sealer and dentin.

While SEM requires the desiccation of root sections, which can result in potential sealer loss and specimen deformation, CLSM offers distinct advantages as it enables evaluation without the need for destructive specimen preparation (27-32). The application of Rhodamine B dye in CLSM facilitates a rapid and detailed assessment of sealer penetration, permitting a closer examination at lower magnifications (33). It is noteworthy that the inclusion of 0.1% Rhodamine B dye into root canal sealers does not affect their flow characteristics (34).

While most root canal sealers do not chemically bond to dentinal walls, their tubular penetration enhances the mechanical retention of root-filling materials within the root canal space (35, 36). This study demonstrates that bioceramic and bioactive glass-based sealers exhibited deeper tubular penetration compared to resin and ZOE groups across all levels examined.

The superior penetration of bioceramic sealer over ZOE sealer may be attributed to its lower film thickness, potentially compensating for its reduced hydrophilic properties (37). Moreover, the deeper tubular penetration observed at the 6-mm section compared to the 3-mm section can be attributed to the greater thickness of dentinal tubules in the middle and coronal parts relative to the apical region of the root (38). The presence of a smear layer occludes tubular ostia and impedes sealer penetration into the tubular space; thus, the removal of the smear layer using 17% EDTA and 5.25% NaOCl enhances the tubular penetration of root canal sealers (39). Research findings indicate that passive ultrasonic irrigation surpasses manual irrigation techniques in eliminating smear layers effectively (40-42).

De-Deus et al. observed that while the vertical compaction technique led to deeper sealer penetration compared to lateral condensation or single-cone techniques, lateral condensation provided better distribution of the sealer, particularly in the middle and coronal thirds (43).

Conversely, Jeong et al. found that the warm vertical compaction technique did not affect the tubular penetration of calcium silicate-based sealers (32). In a separate study examining AH26 sealer, it was noted that sealer penetration significantly increased with the use of 17% EDTA, maleic acid, or citric acid as a final irrigation step following the removal of the smear layer (36).

Additionally, Chandra et al., using a confocal microscope, observed maximum tubular penetration in the RealSeal group, followed by the AH Plus, RoekoSeal, and EndoRez groups, with penetration being greatest in the coronal third, followed by the middle and apical parts (44).

Khader conducted an SEM study, which found comparable levels of tubular penetration between AH Plus and Apexit Plus sealers, while the Tubli-Seal group exhibited less penetration (45).

Conversely, Kuçi et al. observed in a confocal microscopic study that removing the smear layer enhanced the tubular penetration of MTA Fillapex, but not AH26 sealer. They noted deeper tubular penetration in the MTA Fillapex group compared to the AH26 group, suggesting differences in assessment methods and sealer placement techniques as potential factors influencing tubular penetration (46).

Conclusion

The bioactive glass group stood out for its remarkable performance, displaying the highest percentage of sealer penetration compared to the other groups at both depths. Importantly, our statistical analysis found no significant difference between the bioceramic and bioactive glass groups at the 3 mm and 6 mm levels in terms of both depth and percentage of dentinal tubule penetration. Conversely, the ZOE sealer exhibited the least tubule penetration at both levels examined.

Clinical Relevance

This study demonstrates that bioactive glass-based root canal sealers exhibit superior penetration into dentinal tubules compared to traditional sealers like ZOE and resin-based sealers. This enhanced penetration can lead to better sealing of the root canal system, potentially improving treatment outcomes in endodontic therapy. The findings highlight the importance of selecting sealers with optimal penetration capabilities to enhance the success of root canal treatments. Additionally, the study underscores the bioactive properties of novel sealers, which have the potential to promote tissue healing and regeneration, further enhancing their clinical utility.

Conflict of Interest

The authors have no conflicts of interest.

Acknowledgments

Not applicable.

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CASE REPORT

Endodontic management of mesial middle canal in mandibular molars with symptomatic irreversible pulpitis

ABSTRACT

Aim: *This article aims to present three endodontic treatment cases of mandibular molars with three mesial canals and two distal canals.*
Summary: *Three patients were admitted in our clinic with a description of toothache while having hot and cold drinks. Clinical examinations revealed deep decay of teeth, but no pain to percussion. Radiographic images confirmed an intact periapical tissue with deep decay in each tooth, and teeth were diagnosed as symptomatic irreversible pulpitis. Root canal treatments were completed in single appointments. Studies suggest that mandibular molars with MMCs could be recognized more precisely with CBCT. Endodontic treatment of mandibular molars with mesial middle canal (MMC) can cause various challenges for every clinician, thus a proper understanding of root canal anatomical variations is a cornerstone in reaching endodontic success.*
Key Learning Points:

- *To identify the presence of an MMC, the isthmus between the ML and MB canal should be probed with a straight endodontic probe. In case of engagement of the probe at a specific point, it may be legitimate to look for the presence of the MMC.*
- *In root treatments, it is important not only to recognize the shape of the root canals, but also the variations of their course and possible fusion, respectively.*
- *It is of great importance to pay attention to the possible existence of an isthmus between the root canals, which must be completely cleaned and filled.*

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Introduction

Endodontic treatment of mandibular molars with mesial middle canal (MMC) can cause various challenges for every clinician, thus a proper understanding of root canal anatomical variations is a cornerstone in reaching endodontic success. Root canal treatment procedures of mandibular molars with complex anatomical variations is a difficult task, and can lead to complications if not properly conducted (1). The knowledge of the root canal anatomy configuration is essential for the diagnosis and execution of endodontic treatment (2). In most cases, the lower molars have two roots: mesial with two canals in mesial root and distal with one canal in the distal root (3). A high degree of morphological variability, ranging from 20%-46%, can be found in mesial root of lower molars, including the presence of mesial middle canals (MMCs) (4, 5). The description of the MMCs include having a small orifice deep within the isthmus or a developmental groove between the orifices of the mesiobuccal (MB) and mesiolingual (ML) canals (6, 7). Pomeranz has categorized the MMC's into three types - fin, confluent and independent (8). Even though the fin-type lacks a separate orifice, the file is able to move freely between the MB or ML canal and MMC. The confluent type is characterized by a separate orifice which joins apically at the MB or ML canal. The independent type presents with a separate orifice and separate apical foramen (8). Root canal therapy includes localizations of all canal orifices, debriding the root canals chemically and mechanically, and creating the final shape for three-dimensionally obturation and prevent its reinfection (9, 10). The consistent pattern is not necessarily present in the MMCs of lower molars (11). MMC with a generally disclosed orifice, is occasionally situated in the developmental groove in a form of intermediate canal connecting an MB and ML canals (9). The most commonly endodontically treated tooth is the mandibular first molar (12). Several morphological variabilities, can be found in the lower first molar, including: MMC, isthmus, middle distal canal (MDC), radix entomolaris, taurodontism and radix paramolaris. To achieve success in endodontic treatment and therapy, the clinician should have an expanded knowledge of the possible anatomical variations (13). Various authors have concluded that MMC is more common in mandibular first molar with two distal canals (45.4%) than cases that have one distal canal (14). However, Nosrat et al. described no connection between MMCs and the incidence of the second distal canal (5). The most frequent detection of MMC happens in the tooth with an isthmus. The existence of the isthmus in a tooth, can increase the occurrence of MMC. MMCs were found approximately fivefold in mandibular first molar with an isthmus in-between MB and ML canals (5). The study conducted by Azim et al, the MMC was located in 46.2% instances, out of which 6.6% were found following a predictable access preparation and 39.6% following consistent troughing (4). The pervasiveness of the MMCs differs related to ethnicity and varies from 0.26 to 45.8% (15). Age is also related with the occurrence of MMCs and small accessory canals (16). Different literature reported the occurrence of an accessory canal in the mesial root between the MB and ML root canals, with different names such as middle mesial canal (MMC), mesiocentral canal (MCC) or accessory mesial canal (AMC) (4). The mesial middle canal system intricacies present difficulties during shaping and obturation procedures. Usually, they are recognized during the preparation of the access cavity. Adequate knowledge of anatomical variations of root canal (RC), their cavity preparation and 3D obturation, represent an important factor for success in endodontic therapy. The aim of this case report is to present and discuss three clinical cases with mesial middle canal root canal configuration. Thanks to the knowledge of the root canal anatomical variations associated with the use of modern technologies, it is possible to increase the degree of chemico-mechanical debridement and to achieve a successful treatment. RC treatments of lower molars with MMC may represent a difficulty to every clinician, and an understanding of anatomical variations of root canals are essential for achieving successful endodontic treatment outcomes.

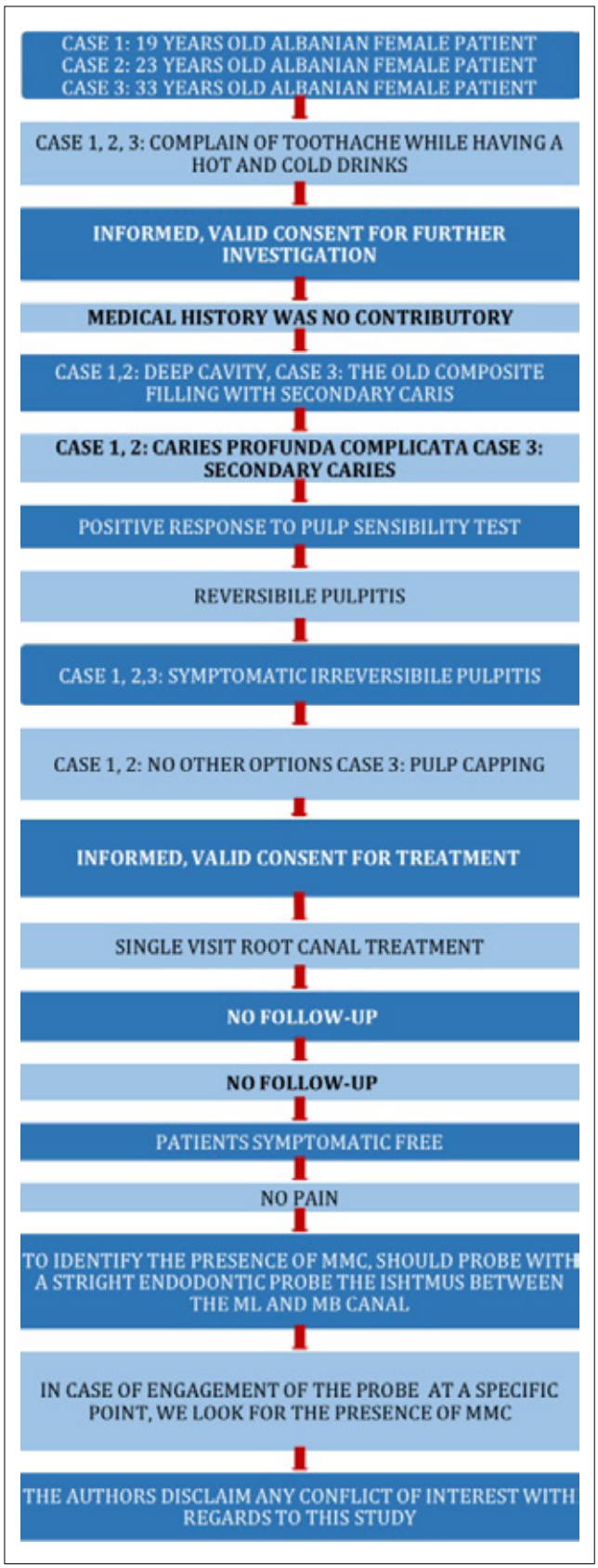

Figure 1 PRICE (Preferred Reporting Items for Case reports in Endodontics) 2020-Flow chart (17).

Case Reports

This case reports have been written according to Preferred Reporting Items for Case reports in Endodontics (PRICE) 2020 guidelines (Figure 1) (17). Prior to intervention, the patients were informed that their radiographic images may be used in a dental magazine, and ensured that their personal identifiable information will be protected. The patients gave signed consent, and signed the informed consent as well.

Case 1
An Albanian female patient, 19 years old, was admitted in our clinic with a description that she has a toothache in lower right side of the face for the last 3 weeks, getting worse when having cold drinks. Clinical examinations revealed deep decay of tooth 47, but no pain to percussion. Positive response to pulp sensibility test. Radiographs confirmed an intact periapical tissue with deep decay in tooth 47 (Figures 2, 3). The determined diagnosis was symptomatic irreversible pulpitis (SIP).

Case 2
An Albanian female patient, 23 years old admitted in our clinic with a description that she had a toothache, and the referred pain frequency increased during the night. Clinical examinations revealed deep decay of tooth 36, but no pain to percussion. Positive response to pulp sensibility test (Figure 4).
The determined diagnosis was symptomatic irreversible pulpitis (SIP).

Case 3
An Albanian female patient, 33 years old admitted in our clinic with a description of toothache while having hot and cold drinks. The patient consumed analgesics to relieve pain. Clinical examinations discovered the old composite filling in tooth 46 with deep decay lesion on the mesial wall of the tooth, but no pain to percussion. Positive response to pulp sensibility test (Figures 5, 6).
The determined diagnosis was symptomatic irreversible pulpitis (SIP).

After administration of nerve block anesthesia Septanest (4% Articaine hydrochloride with 1:100000 Epinephrine), (Septodont, Saint-Maur-des-Fosses Cedex, France) and application of

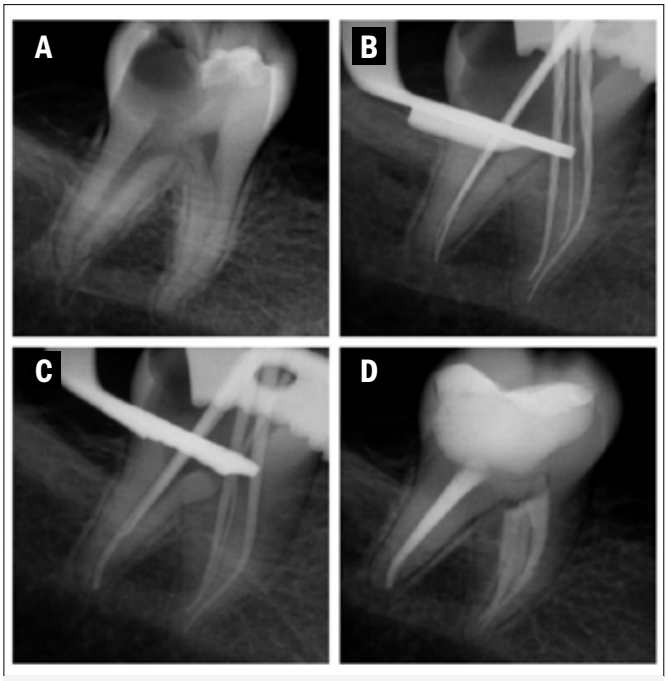

Figure 2
(A) Initial radiographic image; **(B)** measuring the length of canals; **(C)** confirmation length of the canals with gutta-percha cone; **(D)** final radiographic image.

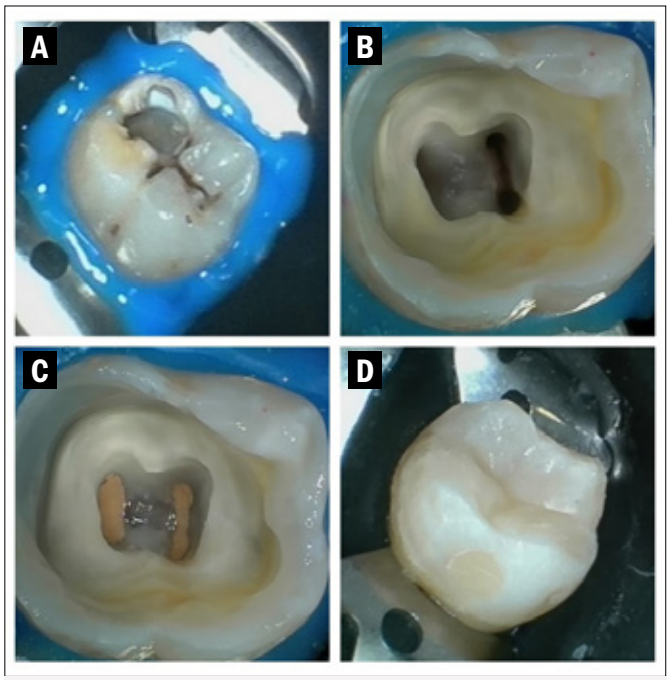

Figure 3
(A) Clinical view before endodontic treatment; **(B)** cavity after shaping and cleaning; **(C)** clinical view of the cavity after obturation; **(D)** clinical view after composite restoration.

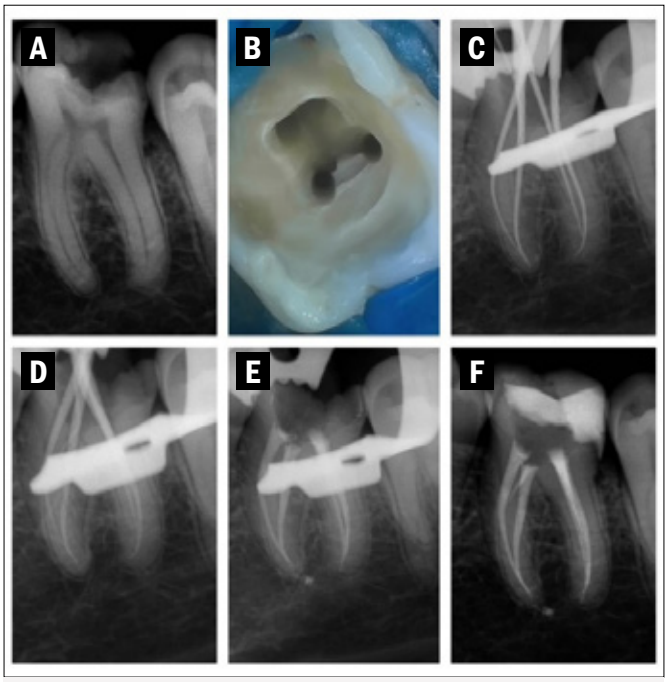

Figure 4
(A) Initial radiographic image; **(B)** clinical view after cleaning and shaping; **(C)** measuring the length of canals; **(D)** confirmation length of the canals with gutta-percha cone; **(E)** obturation-radiographic image; **(F)** final radiographic image.

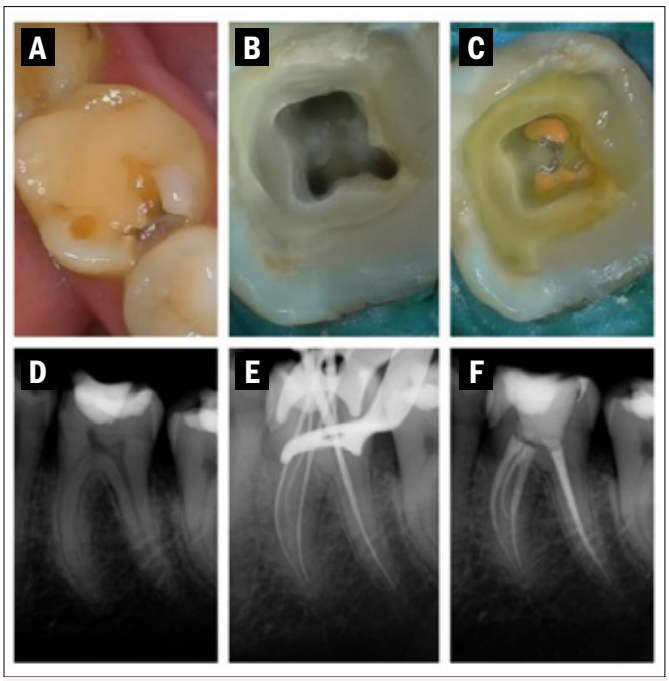

Figure 5
(A) Initial clinical view; **(B)** clinical view after cleaning and shaping; **(C)** clinical view after obturation; **(D)** first diagnostic radiography; **(E)** controlling of working length; **(F)** final radiographic image.

the rubber dam, the access cavity preparation was refined using a round diamond burs and improved with Endo 1 ultrasonic tips (Dentspy Maillefer, Baillagues, Switzerland). The clinician located five RC orifices - three mesial canals and two distal canals.

During the negotiation of canals, the use of magnification (Univet Loupes 3.0 Spa, Rezzato, Italy) proved helpful. MB and ML canals were negotiated and identified with size 06 and 08 K-files (DiaDent Group international, South Korea) and the working length determination was performed with an electronic apex locator FindPex (Changzhou Sifary Medical Technology Co.,Ltd, Eighteeth, China Eighteeth). The presence of MMC was identified with a straight endodontic probe, through the isthmus between the ML and MB canal. After the engagement of the probe at a specific point, we looked for the presence of the MMC. Ultrasonic tips (Endo 1 Dentspy Maillefer, Baillagues, Switzerland) were used for cleaning the area between MB and ML orifices, the coronal isthmus is prepared and use of smaller K-files provided for a careful negotiation of the canal. Irrigation with 5.25% NaOCl was used and the rest of pulpal tissue within the MMC released a stream of bubbles - champagne effect. Irrigation with 5.25% NaOCl and 17% EDTA was used after each file and was activated with an ultrasonic device (Changzhou Sifary Medical Technology Co.,Ltd, Ultrax, Eighteeth, China).

Slow up and down movements have been made during this stage, inserting the needle 2 mm below the working length, and extruding the irrigation solutions drop by drop. Root canal preparation was completed with E flex gold rotary file size 25/04 (Changzhou Sifary Medical Technology Co.,Ltd, Eighteeth, China Eighteeth), using manufacturer recommendations. The canals were cleaned and the length of each root canal was determined using an electronic apex locator FindPex (Changzhou Sifary Medical Technology Co.,Ltd, Eighteeth, China Eighteeth) and confirmed radiographically (Figures 2B, 4B, 5E, 6B). Marcus Haapasalo protocol (18) of irrigation was performed with 5.25% NaOCl and 17% EDTA, by introducing the irrigation solution in each canal and activating it with an ultrasonic device (Changzhou Sifary Medical Technology Co.,Ltd, Ultrax, Eighteeth, China). Irrigation was performed by using Irriflex needle (PD Dental, Vevey, Switzerland). Paper points (DiaDent Group international, South Korea) were used for drying the canals. Gutta-percha cones 25/04 (DiaDent Group international, South Korea) were inserted inside the canals and working length was controlled with radiography (Figures 2C, 4D, 6C). Sealapex sealer (Kerr Endodontics, CA, USA) is inserted inside the canals with gutta-percha cones. Obturation technique was warm vertical condensation (WVC) and thermoplastic gutta-percha injection (Fast fill & Fast pack pro obturation system combo, Changzhou Sifary Medical Technology Co.,Ltd. Eighteeth, China). The hand plugger was used to compact thermoplastic gutta-percha in the canal with the controlled working length. Radiograph was taken with Xios XG Supreme intraoral sensor size 2 (Dentsply Sirona, Charlotte, NC 28277 USA). The postoperative radiograph showed complete obturation of all the five root canals (Figures 2D, 4F, 5F, 6F). Photos of clinical view were taken by an intraoral camera (Figures 3A-D, 4B, 5A-C). Root canal treatments were completed in single appointments. Teeth have been restored by composite filling materials Tetric Prime (Ivoclar Tetric Prime, Ivoclar Vivadent, Liechtenstein). The patients did not report any signs of discomfort or pain after the endodontic treatment.

Discussion

Diagnostic and endodontic therapeutic procedure of MMC due to anatomical and morphological variabilities, require certain knowledge as well as clinical skills. To achieve success, one of the most fundamental steps is the preparation of coronal isthmus. To simplify the endodontic procedure and avoid missing canals, it is important to conduct the radiological examination and establish the clinical diagnosis of an MMC before the endodontic treatment. Practitioners must have a detailed knowledge and understanding of anatomical variations to conduct a successful treatment. To reach the radiological diagnosis, the clinician must perform two preoperative radiographs with using varying horizontal angulations, resulting in defining the roots bucco-lingual width which will display the presence of mesial middle

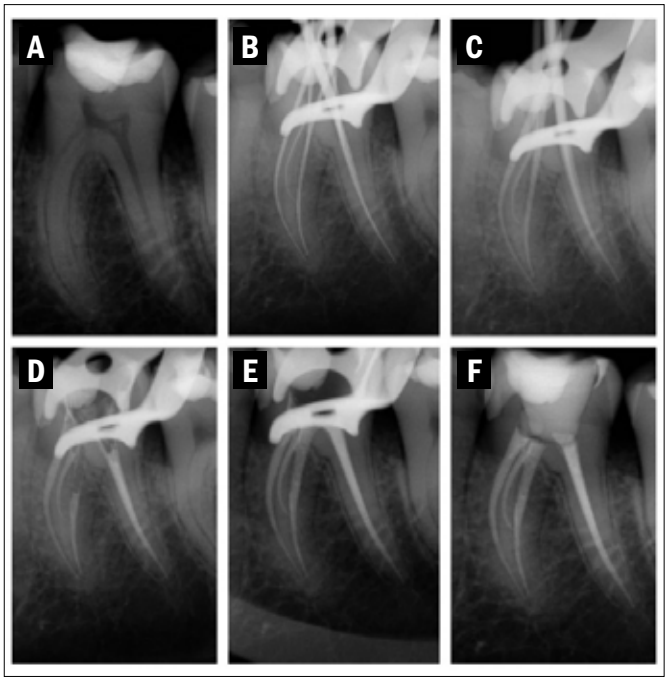

Figure 6 **(A)** Initial radiographic image; **(B)** measuring the length of canals; **(C)** confirmation length of the canals with gutta-percha cone; **(D)** down packing radiographic image; **(E)** obturation-radiographic image; **(F)** final radiographic image.

canal (19). To find the exact position and depth for accessing the obvious portion of canals, the clinician should use CBCT scanned images and conduct a correct treatment.

A better visualization of the cavity, enhanced diagnostic and straight-line access to the orifice, is made possible by the proper access cavity preparation (20). In various cases the orifice of MMC can be differently positioned – adjacent to ML or MB canal or halfway from MB and ML canals. It is noticeable from the available literature, that the orifice of the MMC presents itself closer to the ML canal than to the MB canal, followed by the frequency of the orifice in-between the two main canals, even though it is quite unusual to find the existence of the orifice close the MB canal (5, 8, 14). When the mesial middle canal is adjacent to MB or ML, the reach in-between them is around 3.21 mm, while the tooth lacking MMC presented an inter-orifice medium distance of around 3.7 mm (21). Several authors described an inter-orifice distance of about 3.6 mm in roots with MMC and 3.8 mm in roots without MMC (21, 22). To locate the canal orifice the most commonly used dye is the 1% methylene blue. The dye is directly applied into the cavity and visualized after rinsing with water and drying. The absorption of the dye into the orifices, aids the identification of the RT orifices and is called a Dye test (23). Further, the prepared cavity was rinsed with 5.25% NaOCl to check for the orifice of MMC. If the MMC is present, the sodium hypochlorite will react with the rest of pulpal tissue within the MMC and release a stream of bubbles creating the champagne effect. The presence of the canal orifice is indicated by the bubble originating area (24). To detect the originating place of the bubbles, the clinician needs to observe the access cavity under a dental operative microscope (DOM).

Using the higher magnification of the DOM allows us to identify the very thin MMCs which can present themselves deeply into the isthmus (16). Based on the overall observations, it can be concluded that the dimensions of MMCs are 2-3 times smaller than the main canals (23). Considering that most MMCs are thin, twisted, curved, or joined to ML or MB canals, the clinician ought to meticulously clean and shape MMCs so to avoid practical mistakes (25). A more efficient canal preparation can be achieved with rotary glide files than with K-files. However, in cases when the practitioner choses to prepare a glide path using K-files, the usage of intermediate files, such as 08 and 10, is much more advantageous in reaching a flatter conversion between the files and easing the penetration through canal constrictions (26).

The chosen instrumentation technique for cleaning and shaping the MMCs is carefully applied crown-down technique (27). Avoiding the procedural errors and efficiently cleaning and shaping the MMCs can be achieved by an appropriate glide path preparation, the correct choice of rotary files (28). It is suitable to use ultrasonic tips when cleaning the area between MB and ML orifices and use of smaller files provides for a careful negotiation of the canal (29). Syringe irrigation is effective when cleaning the main root canals but not so effective when used for RC abbreviations and anatomic irregularities which are quite common in MMCs (28). To obtain clean canals, it is recommended to use ultrasonic and negative apical pressure supplementary irrigation techniques, since these techniques proved more successful than conventional techniques in debris removal (30). Choosing the right instrument to clean and shape the root canal space is as important as

any other step (31). Chosen obturation technique depends on the skills of the clinician, configuration of the root canal, as well as available materials and equipment. It is important to always choose the best technique for each individual case in order to achieve an optimal canal obturation.

Conclusion

Detection of MMC, followed by careful and proper root canal treatment and finished by exact obturation, improves the outcome of demanding therapeutic procedures. For proper detection, it is of utmost importance to know the morphology of the canal, have a radiological finding and perform scouting with a manual k-file. When processing the canal manually, it is necessary to use a certain technique with adequate irrigation and removal of debris from the canal (rinsing, ultrasonic activation). For the final filling of the canal, it is of great importance to use the appropriate technique, with which an adequate three-dimensional filling of a certain canal can be carried out. The clinician needs to be aware of the existence of this root canal anatomical variations in order to reach the best possible results.

Clinical Relevance

Root canals treatments of lower molars with MMC may represent a difficulty to every clinician, and an understanding of anatomical variations of root canals are essential for achieving successful endodontic treatment outcomes.

Conflict of Interest

The author disclaims any conflict of interest in regard to this study.

Acknowledgements

The authors deny any financial affiliation.

Patients Consent Statement

Written informed consent was obtained from the patients when the patient registered at the private dental clinic for future publication of the case report and any accompanying images.

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SYSTEMATIC REVIEW

Novel non-obturation based concept of regeneration

ABSTRACT

***Aim**: This systematic review aimed to evaluate the healing achieved through a novel SealBio technique and assess the inherent complications or failures caused during treatment of endodontic diseases.*
***Methodology**: The present review was conducted according to the PRISMA guidelines and was registered in PROSPERO (Registration number – CRD42020201943). The research question was formulated based on the PICO strategy. A comprehensive electronic literature search was conducted across PubMed/Medline, Google Scholar and Cochrane Database independently by two reviewers. Articles published on SealBio up to May 2024 were included. Based on the specified inclusion and exclusion criteria, the selected articles were subjected to quality assessment, and the risk of bias was conducted using the Cochrane risk of the bias assessment tool.*
***Results**: A total of 4 studies were included in the present systematic review and reported success rates with the SealBio technique around 97-100%. However, all included papers demonstrated a high overall risk of bias and some limitations.*
***Conclusion**: Based on the present study, SealBio technique did not furnished concrete evidence to replace the standard endodontic protocol. However, additional evidences provided by standardized and well conducted clinical trials with low risk of bias are needed.*

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Introduction

The endodontic procedure can be defined as a process where the complex root canal anatomy of teeth is treated in a simplified way. Endodontic success is attributed to three major factors: the complete disinfection of the root canal system, proper instrumentation and three-dimensional obturation (1). Ideally, an endodontic therapy aims at achieving the three-dimensional seal both coronally and apically after optimal shaping, cleaning, and disinfection of root canal space (2). Especially in cases of infected root canals, the combating strategies target microbial biofilm dislodgement which ultimately reduces the microbial colonies, although complete eradication of microbes is never achievable (3).

Endodontic literature has shown that outcome-based studies mainly categorize healing in terms of clinical resolution of symptoms and radiographic reduction of apical periodontitis if previously reported (4-6). The overestimation by periapical radiography and the questionable validity of peri-apical index (PAI) in detecting periapical lesions were considered serious limitations. A recent review discussed the fact that future studies should re-evaluate the outcomes through long-term longitudinal studies using CBCT-based criteria (7). Although endodontic therapy attempts to resolve the above-mentioned criteria by achieving adequate apical seal using sealer and gutta-percha, the ultimate goal would be to obtain a fibrous or cemental barrier at the root apex (8). Conventional endodontic treatment replaces the tissues with artificial materials resulting in a reparative process; however, endodontics' progression is towards regeneration rather than repair.

Considering the event of revascularisation conceptualized by Ostby in 1961 (9), few years later Rule & Winter (10) demonstrated the root development and apical barrier formation after pulpal necrosis. This concept of neurogenesis following a revascularization procedure gained significant interest in endodontics' regenerative aspect. It's seen that in immature permanent teeth, the periapex seems to harbour a variety of progenitor cells, which actively participate in the regeneration of tissues (11). Therefore, only an empty disinfected canal would not aid in tissues' growth from the periapex (12). Moreover, an intentional bleeding induced into the root canal space promotes a granulation tissue formation, creating a more favourable environment to allow self-renewal of stem cells and progeny, thereby providing a niche for future dental progeny (11).

Taking this scientific literature into consideration, a novel, non-obturation, regenerative treatment protocol "Seal Bio" was developed and a patent filed (US patent no: US, 9, 180, 072B2, Australian patent no: 2010355508) by N.Shah, A.Logani in 2009. It's based on the concept of revascularisation and depends on the claimed regenerative potential of the periarticular area near root apex. The release stimulated cells after bleeding induction should lead to a biological barrier at the root apex (2).

The basic technique of SealBio (2) includes the complete disinfection of root canal space following an optimal enlargement of root canals. Once the adequate disinfection is achieved, "Apical Clearing" is initiated, which includes the intentional over enlargement of apical diameter. Specifically, the apical third is enlarged with 2-4 file sizes larger than master apical file until the radiographic terminus without transporting the original canal path. Then, foramen widening is obtained using #25 or #30 k files. This process is claimed to clean the cemental part of the canal. Once the infection control is achieved and clinically symptom-free, intentional over instrumentation is done beyond the apex using the #20 K file to induce bleeding near the apical foramen. When a clot stabilizes the bleeding, an adequate coronal seal is given using a calcium sulfate-based material (Cavit). The benefit of this material was the achievement of good sealing (13) and more accessible removal during retreatment as compared to glass ionomer cement (GIC) or mineral trioxide aggregate (MTA) (2).

Although this new concept is gaining popularity and might change the endodontic treatment protocol, evidences have shown that outcome data are still lacking. Hence, this systematic review mainly aimed to evaluate the healing achieved through this technique and assess the inherent complications or failures caused during treatment of endodontic diseases with the following research question: "Is there any variation in the healing, failure or complications by employing the Seal Bio technique as an alternative to routine endodontic therapy?"

Methodology

The present review was conducted according to the PRISMA guidelines and was registered in PROSPERO (Centre for Reviews and Dissemination University of York; http://www.crd.york.ac.uk/PROSPERO) database with registration number CRD42020201943.
The PICOS was taken as
Population: teeth needing endodontic treatment;
ntervention/Comparison: teeth undergoing SealBio technique instead of conventional endodontic therapy;
Outcome: primary outcome was to assess the healing in terms of clinical and/or radiographic success, and the secondary outcome was to assess the inherent complications or failures.

Search Strategy
A comprehensive literature search was conducted on electronic databases as PubMed/Medline, Google Scholar, and Cochrane Database.
Articles published on SealBio up to May 2024 were included in the present review. Search was conducted with a combination of various Boolean operators which included key terms as the following; "Mature teeth", "Human teeth", "Irreversible pulpitis", "Apical periodontitis", "Conventional endodontic therapy", "SealBio", "Non-obturation technique", "Regeneration", "Healing", "Complications", "Failure". In addition, hand searching was done in the following journals; International Endodontic Journal, Journal of Endodontics, Restorative Dentistry and Endodontics, and European Endodontic Journal.

Inclusion criteria
The following inclusion criteria were used to select the studies:
- studies published in Peer-reviewed Journals;
- studies published in English language;
- studies reporting clinical trials: randomized clinical trials, comparative clinical trials, prospective clinical trial;
- studies reporting on use of SealBio technique assessing healing and complications/failures.

Exclusion criteria
The following exclusion criteria were used to select the studies:
- laboratory-based studies, case reports, case series or ex vivo studies;
- studies on animal samples;
- grey literature.

Study Selection
Screening and selection of studies was performed by two independent calibrated examiners (T.K.V & V.K.A) following the inclusion/exclusion criteria. After duplicates removal, papers were evaluated by title and abstract. Then, full texts of potentially relevant articles were obtained and data extracted. Reasons for studies exclusion were also reported. In case of disagreement, a consensus was reached after discussion with a third reviewer (M.M).

Data extraction and analysis
The qualitative assessments of included articles were undertaken independently during the data extraction process. For each studies the following information were reported: authors, year, study design, treatment groups and samples, patients age, gender, selected teeth, preoperative condition, sample size calculation, used anaesthetic and technique, isolation method, number of treatment visits, instrumentation system used (technique, taper, apical preparation size); type, concentration and volume of irrigants used and agitation systems; type of intracanal medicament;

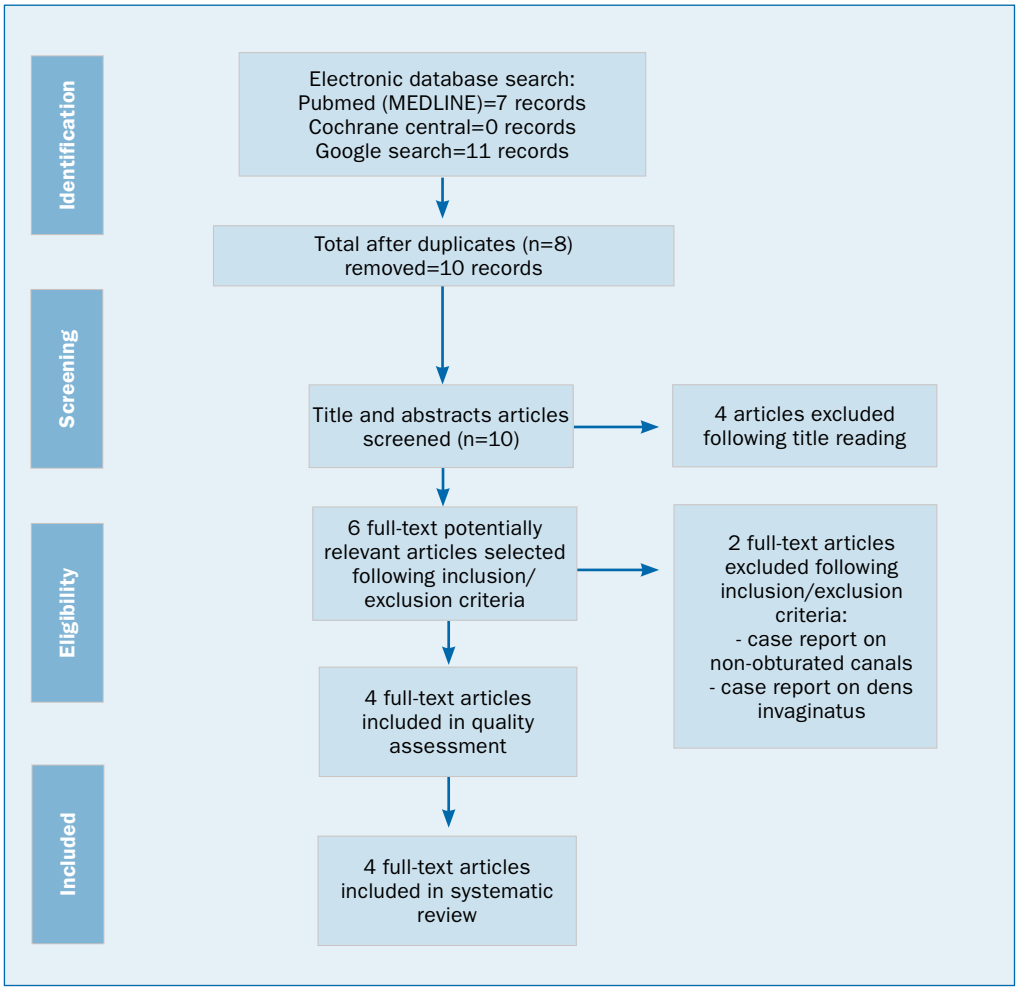

Figure 1
PRISMA flowchart study selection.

apical clearing protocol; foramen widening protocol, over instrumentation protocol; coronal seal, outcomes variables and their assessment, follow-up, drop-outs, success, failures/complications, conclusion. The risk of bias of included studies was assessed using the Cochrane Risk of Bias assessment tool based on major and minor assessment criteria. No additional analysis was performed, and only qualitative review was possible due to the heterogeneity of included studies.

Results

The search identified ten articles after the removal of duplicates. Then, four papers were excluded after title and abstract evaluation. The remaining six papers underwent full-text reading and according to eligibility criteria, four articles were included in the present systematic review and processed for quality assessment and data extraction (Figure 1).

Summary of included studies

Among the 4 included papers, (2, 16-18) three were prospective studies (2, 17, 18) and one was a retrospective based study (16). Three evaluated the SealBio technique in nonsurgical root canal treatment (2, 16, 18) whereas the remaining one assessed the success of SealBio technique in large periapical cysts managed by surgical fenestration followed by application of non-obturation technique for healing of surgical lesion (17).

Included studies have various heterogeneities ranging from study designs to the operator-based variants, such as instrumentation technique, choice of instrument, irrigation protocol, and selection of intra-

Figure 2
Risk of bias summary.

canal medicament and final irrigation activation. Although various factors were not similar, the actual SealBio technique, which included apical clearing protocol, foramen widening protocol, over instrumentation protocol for inducing bleeding, was almost identical in all included articles (Table 1). Coronal seal was obtained with only calcium sulfate-based cement (CAVIT) in almost all studies due to the easier removal during retreatment (if required) compared to MTA. On the other hand, the paper that involved a surgical design, used a double seal concept (17), namely a glass ionomer restoration was used on the top of the calcium sulfate. None of the included articles discussed data on the type of neither the irrigating needle used nor the gauge or depth of placement volume of irrigants (Table 1). The primary important factor missed in 3 of the included studies was the comparative group or control group (2 16, 17). Ideally, success or failure could not be evaluated using a single group. Only one study (18) included a comparative obturation and reported no differences between analysed groups.

When clinical success and failure rates among the included studies was analysed, a high heterogeneity with the assessed data and interpretation was observed. Only one study by Shah (16), had represented the data in terms of success and failure rates. Moreover, none of the selected articles discussed complications associated with the novel technique. Reasons of failure was only reported by Shah in 2016 (16).

Concerning clinical and radiographical evaluation criteria, they varied within different included studies. Most clinical criteria were more or less similar considering asymptomatic functional tooth with a resolution of sinus tract if reported before the treatment procedure as clinical success. With regard to radiographic criteria, 2 studies used PAI for radiographic evaluation (2, 18) and in few cases (n=3) CBCT was employed to measure the resolution of lesions (2). The study conducted by Shah in 2017 (17) reported no specification on the clinical or radiographic criteria used for assessments during follow-up.

The follow-up periods also varied among different included studies. Maximum follow-up period was up to 6 years in the study by Shah 2016 (16).

Figure 3
Risk of bias graph.

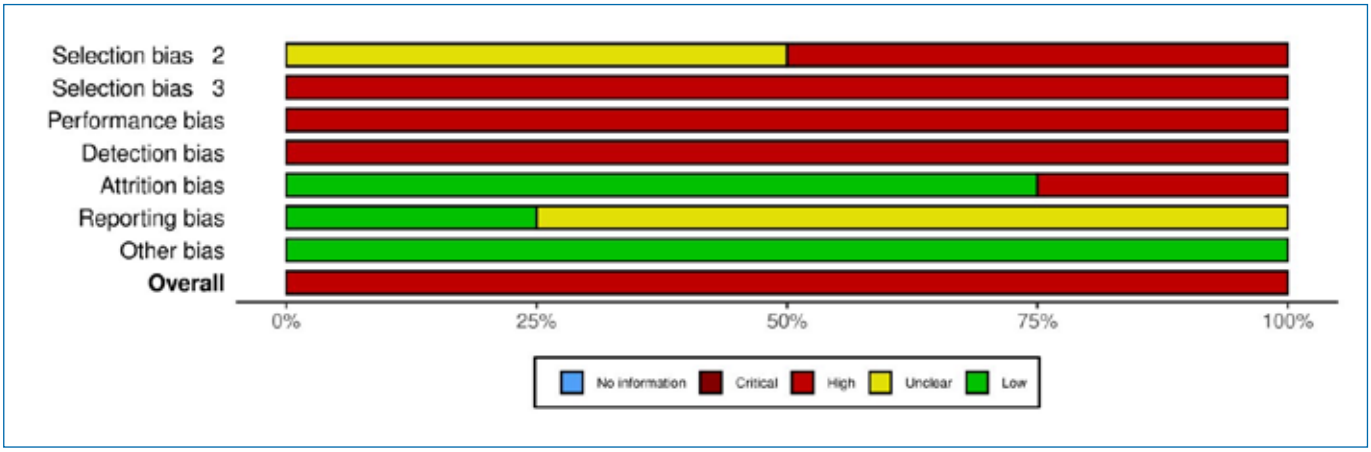

Table 1

Characteristics of included studies

	Shah & Logani 2012 (2), Prospective study	Shah N. 2016 (16), Retrospective study	Shah N. 2017 (17), Prospective study	Preeti Jha et al., 2019 (18), Prospective study
Treatment Groups and sample size	SealBio group (18 patients); No comparative or control group.	SealBio group (116 patients and 134 teeth); no comparative or control group.	Surgical curettage and SealBio group (5 patients); no comparative or control group.	SealBio group and obturation group.
Inclusion criteria: Patients age/gender, selected teeth, arch, preoperative condition, sample size calculation	15-76 years; both male and female; no specification on selected teeth and arch; teeth with pulp and periapical infection; sample size calculation not specified.	Age limit and gender not specified; no specification on selected teeth; either irreversible pulptis, acute or chronic apical periodontitis; sample size calculation not specified.	14-38 years; 4 males and 1 female patient; maxillary and mandibular anterior teeth were selected; radicular cyst cases; sample size calculation not specified.	Aged 9-15 years were selected; both male and female patients; no specification on teeth selected, arch; teeth with apical periodontitis with Ostravik's PAI score>=3 were included.
Treatment protocol (anaesthetic and technique; isolation method; number of treatment visits; instrumentation system (technique, taper, apical preparation size); type, concentration and volume of irrigants and agitation systems; intracanal medicament; apical clearing protocol; foramen widening protocol, over instrumentation protocol; coronal seal	Not specified the type of anaesthetic and method of administration; isolation; number of visits (one or two depending on infection); crown down technique: not specified used instrumentation system, taper and preparation size; irrigants: 2.5% sodium hypochlorite (no specification on volume, other irrigants and agitation technique); triple antibiotic paste as intracanal medicament; apical clearing: 2-4 file sizes larger than master apical file; foramen widening using 25-30 k files; over instrumentation using 20 k files beyond the apex; coronal restoration calcium sulphate based cement (Cavit) packed until cervical third of root canal.	Not specified the type of anaesthetic and method of administration; isolation; number of visits (one or two depending on infection); crown down technique: not specified used instrumentation system, taper and preparation size; irrigants: 2.5% sodium hypochlorite (no specification on volume, other irrigants and agitation technique); both triple antibiotic paste and calcium hydroxide intracanal medicament were used; apical clearing was done with 2-4 file sizes larger than master apical file; foramen widening using 25-30 k files; over instrumentation using 20 k files beyond the apex; coronal restoration calcium sulphate based cement (Cavit) packed until cervical third of root canal.	2% lignocaine hydrochloride (technique of administration varied depending on the arch and tooth); rubber dam isolation; no specification on number of treatment visits; hand k files, not specified instrumentation technique; 2% tapered preparations with no specifications on preparation sizes (it varied with cases); 1% sodium hypochlorite (no specification on other irrigants, volume of irrigant used, agitation method); calcium hydroxide paste as intracanal medicament; prior to the surgical fenestration, apical clearing was done with 2-4 file sizes larger than master apical file; foramen widening using 25-30 k files and access cavity was closed with cotton; after surgical curettage and closure of the site, cotton pellet was removed from the access cavity, canals were dried and over instrumentation using 20 k files beyond the apex; coronal restoration calcium sulphate based cement (Cavit) packed until cervical third of root canal followed by glass ionomer permanent restoration.	3% mepivacaine without adrenaline; rubber dam isolation; no specification on number of treatment visits; dentsply protaper universal files used for root canal preparation with no specified preparation technique; no specification on taper and apical preparation sizes; 2.5% sodium hypochlorite was used for irrigation using negative pressure EndoVac technique, 17% EDTA liquid was used as final irrigation solution; no specification on agitation systems used; triple antibiotic paste was used as an intracanal medicament; apical clearing was done with 2-4 file sizes larger than master apical file; foramen widening using 25-30 k files; over instrumentation using 20 k files beyond the apex; coronal restoration calcium sulphate based cement (Cavit) packed until cervical third of root canal.
Outcome variables and outcome assessments criteria (clinical and radiographic criteria).	Clinical and radiographic evaluations; no note on clinical methods; radiographic evaluation included both CBCT and IOPA based assessments; no specification on criteria used for IOPA based assessments; pre and post treatment CBCT was done for 3 cases (6 teeth) using single iCAT machine at 120kvp, 5mA, exposure time of 7 seconds and voxel size of 0.25; parameters evaluated included the lesion size, bone and cementum density in HU and assessment criteria followed was (CBCT-PAI index scoring).	Clinical and radiographic evaluations; clinical criteria included- asymptomatic and functional teeth, healed intraoral sinus or swelling if any presented prior to the treatment; radiographic evaluation included the complete healing or decrease in the size of radiolucency; in cases with normal periapex no lesion should develop subsequent to the treatment. No specification on index used nor the devices used for radiographic evaluation.	Clinical and radiographic evaluations; no specification on criteria used for clinical and radiographic assessments.	Clinical and radiographic evaluations; clinical criteria included: absence of clinical signs and symptoms (spontaneous pain, presence of sinus tract, swelling, mobility, periodontal probing depths greater than baseline measurement, sensitivity to percussion or palpation); radiographic evaluation included: assessments of change in apical bone densities at subsequent follow-up's and PAI index.
Follow-up period and drop-outs	6 months to 5 years; no drop-outs.	6 months to 6 years; 16 drop-outs.	No specification on follow-up periods. Only mentioned short follow ups (6-12 weeks).	6, 12 and 18 months of follow-up.
Success and failure of treated teeth (percentages); reasons for failures if specified; complications associated with the studied treatment (if specified).	All cases showed good healing response :success rate was 100% and failure rate was 0%. No the complications were reported.	Success rate: 97%, failure rate: 3%; reasons for failure: unsuccessful technique, endodontic cause, coronal leakage. No complications were reported.	No failures or complications were reported; success rates: 100%, failure rate: 0% within short follow-up period.	13 out of 15 cases completely healed and 2 out of 15 cases were healing with no persistent disease (success rates 100% and failure rate 0%); no complications were reported.
Conclusions	The novel treatment protocol showed to be favourable in resolving periapical infection, both clinically and radiographically.	SealBio was found to be successful non obturation and regeneration based endodontic treatment protocol.	SealBio combined with Surgical fenestration was found to be highly effective in healing large periapical cystic lesions.	Both groups showed favourable outcomes at the end of 18 months without any statistical significant difference.

CBCT: cone-beam computed tomography
HU: Hounsfield units
IOPA: Intra-Oral Periapical Radiograph
PAI. Peri-apical index

Table 2
Evidence level of selected articles

	Author & Year	Study Design	Level of Evidence
1	Shah & Logani 2012 (2)	Non-randomised clinical trial	Level 3
2	Shah 2016 (16)	Retrospective cohort study	Level 4
3	Shah 2017 (17)	Case control study	Level 4
4	Preeti Jha et al. 2019 (18)	Randomized clinical trial	Level 2

Qualitative analysis
The overall risk of bias was assessed and all the included studies showed high risk (Figure 2 and 3). There are many variables, which were unclear in the performed studies. The evidence level of included studies was also low except for Preethi Jha et al 2019 (18) namely a randomized clinical trial that included a comparative group of conventional root canal obturation and followed-up the groups for almost 18 months (Table 2).

Discussion

Most of the failures after endodontic treatment can be attributed to improper debridement (19, 20). In 1953, Grossman stated that the optimal concentration of the necrotic debris or toxic load is necessary to sustain or increase the periapical infection (21). Fabricius et al., on the other hand, stated that the permanent root canal filling has limited effect on endodontic treatment outcome unless and until the bacterial load was controlled, especially at the time of obturation (22). The concept of bacterial threshold was put forth by Siqueira & Rôças (23). They specified that any disease-causing species should reach a population density or load to cause direct tissue damage or modulate the host tissue response to infection. So, for a clinical successful procedure, the bacterial threshold has to be reduced below the cultivable levels (103-104 cells). Considering all these aspects, a novel regenerative non-obturation-based SealBio technique was introduced based on the concept of revascularisation.

The technique basically claims the benefit of utilizing the blood clots innate response formed at the apical area to allow the occurrence of healing. The present systematic review proves that the success rates using the SealBio technique were estimated to be around 97-100%. However, there are many limitations among the included studies, which hinder the generalized population's reliability. When these techniques have to be critically appraised, there were no data on the type of repaired tissue, both intra, and extra-radicular. Systematic reviews on histological assessments of failed revascularisation cases showed that the necrotized spaces were either replaced by the cementum or bone-like tissue or fibrous connective tissue (24). So, the hypothesis formulated as regeneration of the pulp-like tissue is not yet proven. Revascularization procedures are based on the utilization of various stem cells which reside in the periapical region, including the dental pulp stem cells (DPSC), periodontal ligament stem cells (SCPDL), bone marrow mesenchymal stem cells (BMMSC), and finally, the stem cells of the apical papilla (SCAP), that play a role in neurogenesis and the revascularization procedure (25, 26). None of the laboratory studies, either histological or molecular, has shown the presence of any of these cells at the terminus of the mature root apex. So, the concept of utilizing the native stem cells of inflamed periapex is not documented or proved yet.
Concerning the maintenance of results over time, one of main limitations of SealBio technique included the impossibility to predict its prognosis. The estimated success of conventional primary endodontic treatment ranges from 68-85% (4) and secondary endodontic treatment about 77% (5). On the contrary, the same success rate cannot be expected with SealBio technique as proved by varied reported outcomes. The other important factor that has to be discussed is the generation or propagation of cracks (incomplete or complete) caused by the enlargement of the apical foramen (27), even though a recent study by Pradeep et al. (28) showed no evident crack formed in vivo. However,

this aspect has to be clarified. Moreover, although some authors discussed that post and core restoration could be performed after a Bio Seal procedure (2) there are many concerns about blindly restoring an open space with a post and guarantee a long-term success. Ideally, performing post and core restorations in the teeth that underwent SealBio technique is still a controversial topic, which has to be critically analysed.

The major disadvantage of the abovementioned technique is tooth status estimation. Ideally, there is no vital pulp nor tissue that may respond to vitality test. Therefore, the prediction of pulpal status is not quantifiable. Furthermore, deposition of calcific material in the open root canal spaces after revascularization procedures have been reported (29-31), and it should be considered that deposition rate or the type of formed deposition are not predictable due to unexpected response to treatment procedure.

In conclusion, the limitations are more as compared to benefits. Therefore, when risks have to be addressed, the limited data are insufficient to prove that SealBio treatment strategy is superior or similar when compared with the present high standard of endodontic therapy.

Conclusion

Prospective research has to be performed with the aim to analyze various molecular insights of healing response after SealBio technique. In addition, histological studies have to confirm the type and rate of tissue deposition. The role of stem cell within disinfected root canal space should also be analysed and investigated at a vast level to modify the present regenerative treatment protocol. Although the present review concludes the success rates of 97-100% with SealBio technique in surgical and nonsurgical endodontics, the literature is scarce with the low evidence-based studies. Therefore, the present technique did not furnish concrete evidence to replace the standard endodontic protocol.

Clinical Relevance

The present review provides an insight into the success of the SealBio technique for endodontic treatment and its potential to be considered as a valid alternative to traditional endodontic treatment.

Conflict of Interest

None.

Acknowledgements

None.

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CASE SERIES

Vital pulp therapy of permanent mature teeth

ABSTRACT

Aim: *Vital pulp therapy (VPT) has been recently suggested as an alternative clinical procedure to treat symptomatic mature permanent teeth presenting deep caries lesions, to maintain the pulp vitality over time and to avoid or post-pone root canal therapy. Therefore, the aim of the present study was to report cases of pulpotomy in mature permanent teeth with reversible pulpitis and to propose this technique as a viable alternative to traditional endodontic treatment.*

Summary: *The present case series was reported following Preferred Reporting items for Observational studies in Endodontics (PROBE) guidelines. Eight systemically healthy subjects presenting deep caries lesions approximating/involving the pulp of mature permanent teeth and with signs and symptoms of reversible pulpitis, underwent full pulpotomy using hydraulic calcium-silicate based cement. After final restorations, dental elements were clinically and radiographically followed-up for different time intervals (6 to 12 months). An overall clinical and radiographical success rate of 100% was reported up to 12 months.*

Key learning points:

- *Full pulpotomy should be considered as a valid non-invasive treatment in mature permanent teeth with signs and symptoms of pulpitis.*
- *VPT allows to maintain pulp vitality in the middle term.*
- *VPT may be regard as a viable alternative to traditional endodontic treatment.*
- *A larger sample size and a longer follow-up period are needed to confirm the preliminary obtained results.*

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KEYWORDS *Hydraulic cements, mature permanent teeth, pulp inflammation, root canal treatment, vital pulp therapy.*

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Vital pulp therapy (VPT) includes various treatment modalities aimed at preserving the integrity and vitality of dental pulp in cases of deep caries lesions approximating/involving the pulp tissue, or pulp exposure due to trauma or mechanical injury (1). VPT encompasses direct and indirect pulp capping procedures, as well as pulpotomy techniques - partial and complete pulpotomy - which are routinely applied in the management of primary teeth to enhance their longevity, and in immature permanent teeth to promote root development (2). Recently, the application of VPT may include symptomatic mature permanent teeth with deep caries lesions, providing an alternative approach to maintain pulp vitality and potentially avoid non-surgical root canal therapy (NSRCT) (3, 4).

This minimally invasive approach is supported by the reparative potential of dental pulp, that, as an inflamed tissue, has the possibility to heal. However, the effectiveness and success of VPT in permanent dental elements are closely associated with the severity of pulp inflammation and the extent of histopathological involvement of pulp tissue (5). In this light, it should be considered a lack of precise correlation between clinical symptoms and the histopathological state of the pulp, particularly evident in cases of irreversible pulpitis, which can lead to diagnostic inaccuracies (5-7); in addition, there is an absence of specific diagnostic tools able to provide a precise diagnosis based on histological status (8).

The chance to perform VPT on mature teeth not only has the advantage to preserve the tooth structure, tooth vitality and immune functions but also eases treatment procedures, decreasing economic and biological costs (9).

Therefore, the aim of the present study was to report cases of pulpotomy in mature permanent teeth with reversible pulpitis and to propose this technique as a viable alternative to traditional endodontic treatment.

Case Series

The present case series was reported following Preferred Reporting items for Observational studies in Endodontics (PROBE) guidelines (10) (Table 1). Eight systemically healthy subjects (M:F=3:5; aged between 9 and 32 years) referred to Dental Clinic of University of Naples Federico II, presenting deep carious lesions approximating/involving the pulp of mature permanent teeth.

Since data collection for observational analysis was not considered a clinical study, the study was notified to the ethical committee of the University of Naples Federico II. The study was conducted in accordance with the Declaration of Helsinki.

To provide a precise diagnosis of reversible pulpitis (8), clinical examination was performed recording the following symptoms: spontaneous pain, pain subsequent to cold or percussion, pain relief few seconds after stimulus removal, sensitiveness to ethyl chloride. In addition, intraoral radiographic examination was performed to verify the absence of periapical reaction (Table 2).

Before therapy, subjects were informed about the potential subsequent need of traditional endodontic therapy in case of pulp necrosis, irreversible pulpitis or untreatable suffering. Then, treatment procedures were explained to patients and signed informed consents were obtained.

All included teeth underwent full pulpotomy treatment following a standardized technique performed by a single operator (L.E.). Specifically, following local periapical anesthesia administration (mepivacaine without epinephrine), teeth were isolated using rubber dam, and cavities were cleaned using round burs at low speed under copious irrigation. After caries removal and exposure of pulp tissue, the roof of the pulp chamber was removed, and coronal pulp was amputated using a high-speed diamond bur under copious irrigation. Hemostasis was achieved using sterile cotton pellets moistened with saline solution up to 5 minutes.

Biodentine (Septodont, Saint-Maur-des-

Table 1

PROBE 2023 Checklist

Section/ Topic	Item Number	Checklist items	Reported on page number
Title	1a	The specific area(s) of interest must be provided using words and phrases that identify the clinical problem(s) and focus of the study	1
	1b	The study design must be included in the Title, e.g., cross-sectional, cohort, case-control, case-series etc.	1
Keywords	2a	Keywords indicating the specific area(s) of interest using MeSH terms or other more applicable terms must be included	1
Abstract	3a	The Introduction/Background must briefly explain the rationale or justification for the study	1
	3b	The aim(s)/objective(s) of the study must be provided	1
	3c	The Methodology must provide (where relevant) essential information on the nature of the study design (retrospective, cross-sectional, prospective, etc.), setting, location(s), and relevant dates, including periods of recruitment, exposure, follow-up, outcome(s) assessed and statistical analysis	1
	3d	The Results must describe the number of subjects that were included and analysed as well as the most significant results for all experimental and control groups. The results of statistical analysis must be reported in terms of unadjusted and confounder-adjusted outcomes (if relevant). Adverse events or side-effects must also be reported if present or confirmed as absent	1
	3e	The Conclusion must interpret and summarise the primary aim/objective and main findings as well as emphasise the clinical implications	1
	3f	The source(s) of funding must be provided	N/A
Introduction	4a	The clinical problem/question, scientific background and rationale for the study must be provided, including the gap(s) or inconsistencies in the existing knowledge base	2
	4b	The primary and, if applicable, any additional/secondary aim(s) and objective(s) of the study must be provided, including any pre-specified hypotheses	2
Methods Ethics	5a	The details (name, reference number, date) of the approval or exemption granted by an ethics committee, such as an Institutional Review Board, must be provided	2
	5b	The process used for obtaining and storing informed consent must be provided	2
Study design	5c	The key elements of the study design must be described early in the Methods section	2
Setting	5d	The details of setting(s), location(s), socioeconomic status of participants (if available) and relevant dates, including periods of recruitment, exposure, follow-up, and data collection must be provided	N/A
Sample size	5e	Information on how the sample size was determined *a priori* must be provided as well as the rationale for sample size calculation, preferably with reference to the published literature or a pilot study with additional detail as to why the defined sample size makes the study worthwhile	N/A
Participants – unmatched studies	5f	All studies should include inclusion/exclusion criteria as well as the sources and methods of participant selection. Methods of follow-up must also be provided in cohort studies and the rationale for the choice of 'cases' and 'controls' in case-control studies	N/A
Participants – matched studies	5g	For matched studies (e.g., cohort, case-control) the matching criteria and the numbers of participants in each group must be provided	N/A
Variables	5h	All outcomes, exposures, predictors, potential confounders, and effect modifiers must be defined clearly	3
Data sources/ measurement	5i	Sources of data and details of the methods of assessment (measurement) for each variable of interest must be provided	3
Bias	5j	Efforts taken to identify and address potential sources of bias must be provided	N/A
Quantitative variables	5k	The handling of quantitative variables in the analyses must be explained. Decisions on how groupings were made and/or how category boundaries were defined for continuous variables must be described	N/A
Statistical methods	5l	All statistical methods, including those used to control of confounding factors in the study and in the analysis of the data, must be described	N/A
	5m	The methods used to examine subgroups and interactions must be described, if applicable	N/A
	5n	Missing data (e.g. drop-outs, data not reported) must be addressed and described	N/A
	5o	The analytical methods that take account of the sampling strategy (if applicable) in *Cross-sectional studies* must be described	N/A
	5p	Sensitivity analyses, must be described when used	N/A

Continued on the next page

Results *Participants*	6a	The number of participants in each stage of the study (i.e., eligibility, recruitment, available at follow-up and included in analyses for relevant outcome(s)) must be described	3
	6b	Reasons for non-participation (e.g., not eligible, losses/drop-outs) must be described	N/A
Dates	6c	Changes in baseline dates of recruitment, follow-up, and study duration reported in the Methodology must be described, if applicable	3
Descriptive data	6d	The baseline demographic and clinical characteristics of study participants as well as information on exposures and potential confounders must be provided	N/A
	6e	The number of participants with missing data must be provided for each variable. If relevant, follow-up times should be summarised clearly and accurately (e.g., average or total time)	N/A
Outcome data	6f	Information on number of outcomes or summary measures over time must be described	3
	6g	For multivariable analyses developing risk profiles or reducing the effect of confounders, the effect of all included independent variables may be reported, as well as their effects on the prediction model (if applicable)	N/A
Main results	6h	Unadjusted (or uncorrected or crude) estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence intervals) must be described. Which confounders were adjusted for and why they were included must also be described	N/A
	6i	Results in terms of relative risk should also be translated to absolute risk for a meaningful time period, if relevant	N/A
Additional analyses	6j	The results from any other analyses (e.g., sensitivity, subgroup analyses) must be described, if applicable, as well as adjusted analyses, distinguishing pre-specified from exploratory	N/A
Discussion *Key results*	7a	The main findings must be summarized with reference to the study aim(s)/objective(s)	3
Rationale	7b	The rationale for inclusion/exclusion criteria, exposure, and duration must be provided	3
Clinical relevance	7c	An explanation of the clinical relevance of the primary and any additional/secondary outcome(s) must be provided	3
Strength	7d	The strength(s) of the study must be provided	3
Limitations	7e	The limitations of the study must be provided - addressing the sources of potential bias, imprecision, study design, study size and potentially important but missing confounding variables. Both direction and magnitude of any potential bias must be discussed	3
Summary and validity	7f	The discussion of the strength and limitations should be summarized in an overall assessment of the internal validity of the study	3
Interpretation	7g	A detailed interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence must be provided	3
Generalisability	7h	The generalizability (external validity, applicability, real-world relevance etc.) of the study findings must be discussed	4
Future directions	7i	Implication for future research and clinical practice must be described	4
Conclusion(s)	8a	Explicit conclusion(s) from the study must be provided and address all the aims/objectives	4
Funding details	9a	All sources of funding and other support (such as supply of drugs, equipment etc.) as well as the role of funders must be acknowledged and described	N/A
Conflict of interest	10a	An explicit statement on conflicts of interest must be provided, together with full affiliations of every author(s)	1
Quality of images (if applicable)	11a	Details of the equipment, software and settings used to acquire the image(s) must be described in the text or legend (if applicable)	N/A
	11b	The reason why the image(s) was acquired and the rationale for its inclusion in the manuscript must be provided in the manuscript. A justification for all images that involve ionising radiation must be included	3
	11c	The circumstances (conditions) under which the image(s) were viewed and evaluated by the author(s) must be provided in the text	3
	11d	The resolution, any magnification of the image(s) or modifications/enhancements (e.g., adjustments for brightness, colour balance, magnification, image smoothing, staining, etc.) that were carried out must be described in the text or figure legend	N/A
	11e	Patient(s) identifiers (names, patient numbers) must be removed for General Data Protection Regulation (GDPR) and to ensure they are anonymized or de-identified in all images	3
	11f	An interpretation of the findings (meaning and implications) from the image(s) must be provided in the text	3
	11g	The figure legend associated with each image must describe clearly what the subject is and what specific feature(s) is illustrated. If cases are offered to illustrate descriptions of a cohort, then the age, gender, ethnicity, and other specific attributes that are relevant to the cohort should be provided	5
	11h	Markers/labels must be used to identify the key information in the image(s) and defined in the figure legend	N/A
	11i	The figure legend of each image must include an explanation on whether it is pre-, intra- or post-treatment and follow-up and, if relevant, how images were standardised over time	5

N/A: NOT APPLICABLE

Table 2

Clinical signs and symptoms reported by patients at baseline

	Spontaneous Pain	Pain - after application of cold or percussion stimulus	Pain - relief after few seconds following removal of stimulus	Periapical Reaction	Sensitivity test with ethyl chloride
Patient 1	NO	YES	YES	NO	+
Patient 2	NO	YES	YES	NO	+
Patient 3	NO	YES	YES	NO	+
Patient 4	NO	YES	YES	NO	+
Patient 5	NO	NO	YES	NO	+
Patient 6	NO	YES	YES	NO	+
Patient 7	NO	YES	YES	NO	+
Patient 8	NO	YES	YES	NO	+

Fossés, France) was used to seal the pulp chamber. The cement was prepared according to the manufacturer's instructions and placed on root pulp stumps with a thickness of 2-4 mm. Then, teeth were temporarily restored with polymer-reinforced zinc oxide-eugenol cement (IRM, Dentsply International Inc., Milford, DE, USA). Final composite resin restoration was performed after 3 days, following the clinical evaluation of cement hardness with a dental explorer. Treated teeth underwent clinical and radiographic evaluations by a single calibrated and blinded examiner at 7 days, 1 month, 3, 6 and 12 months.

All subjects referred slight sensitivity 1- or 2-days post-treatment, that was controlled by the administration of analgesic, if needed.

The clinical cases were followed for different time intervals (6 to 12 months); specifically, 5 subjects out 8 were followed up to 6 months (Figure 1), while the remaining 3 cases were evaluated up to 12 months (Figure 2). An overall clinical and radiographical success rate of 100% was reported.

Discussion

Permanent teeth with signs and symptoms of pulpitis routinely undergo root canal therapy in order to remove the inflamed tissue. However, if the removal would interest only the inflamed/infected pulp, the repair potential of the healthy tissue should be preserved and the tooth vitality is partially maintained (11). In this light, the present study observed mature permanent teeth presenting signs and symptoms of reversible pulpitis that were treated with full pulpotomy rather than conventional endodontic treatment. An overall 100% of clinical and radiographical success rate was reported within a follow-up period up to 12 months. Based on the obtained outcomes, the amputated pulp was subjected to a healing process - thanks to its reparative potential (11, 12) – that resulted in the resolution of clinical signs and symptoms.

To achieve and maintain the clinical success, the coronal sealing plays a fundamental role in preventing bacterial microleakage (13, 14). In the present study, Biodentine was used as hydraulic cements, thanks to its biocompatibility, bioactivity and sealing property (15, 16). In agreement to previous studies, the same materials showed 100% clinical and 98.4% radiographic success after one year (17) and 100% after two years of follow-up (18), when applied after VPT in permanent teeth with irreversible pulpitis and apical periodontitis. Accordingly, Taha et al. (19) reported comparable success rates (93%) between pulpotomy with Biodentine and root canal therapy; moreover, the pulpotomy group showed significantly lower pain levels 1 day after pulpotomy than traditional endodontic, as well as shorter pain

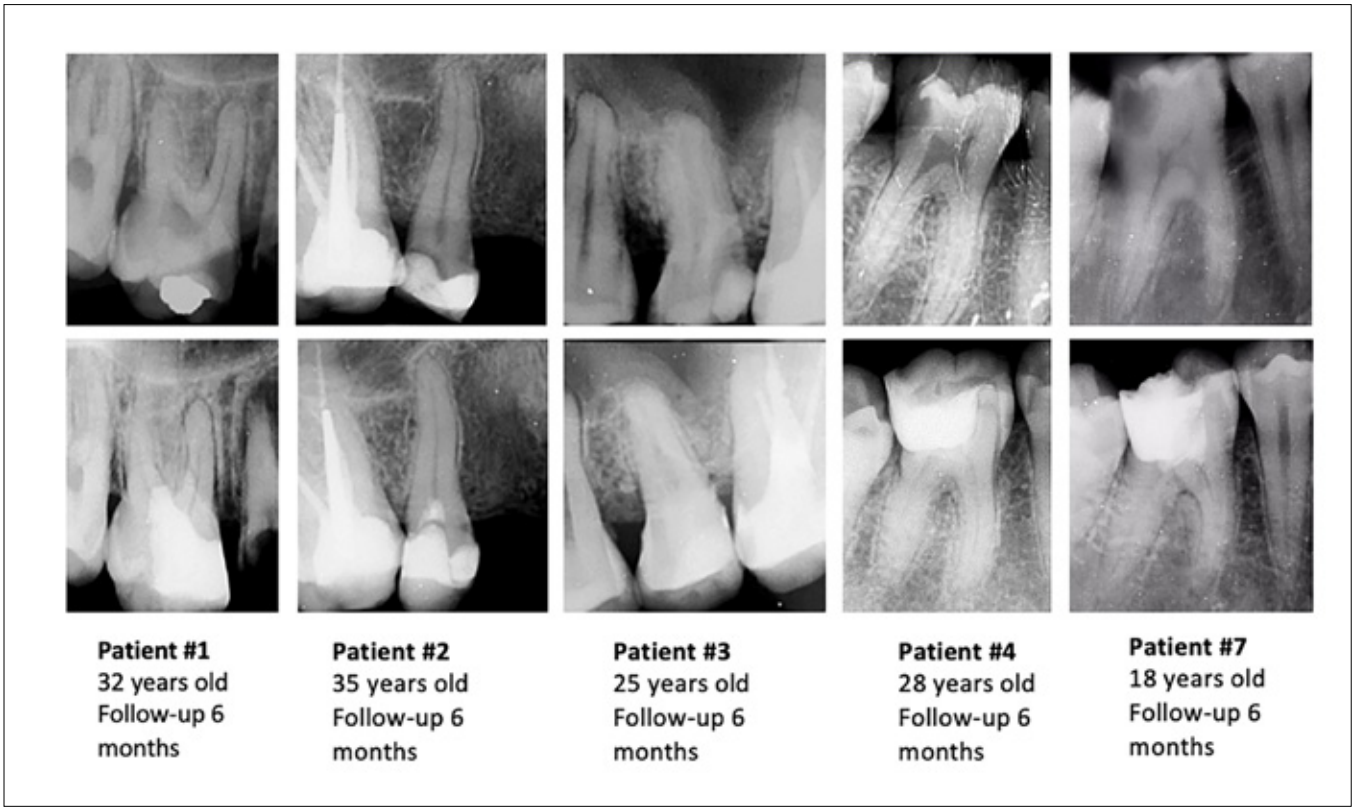

Figure 1
Periapical radiographs of baseline and 6 months follow-up of five patients.

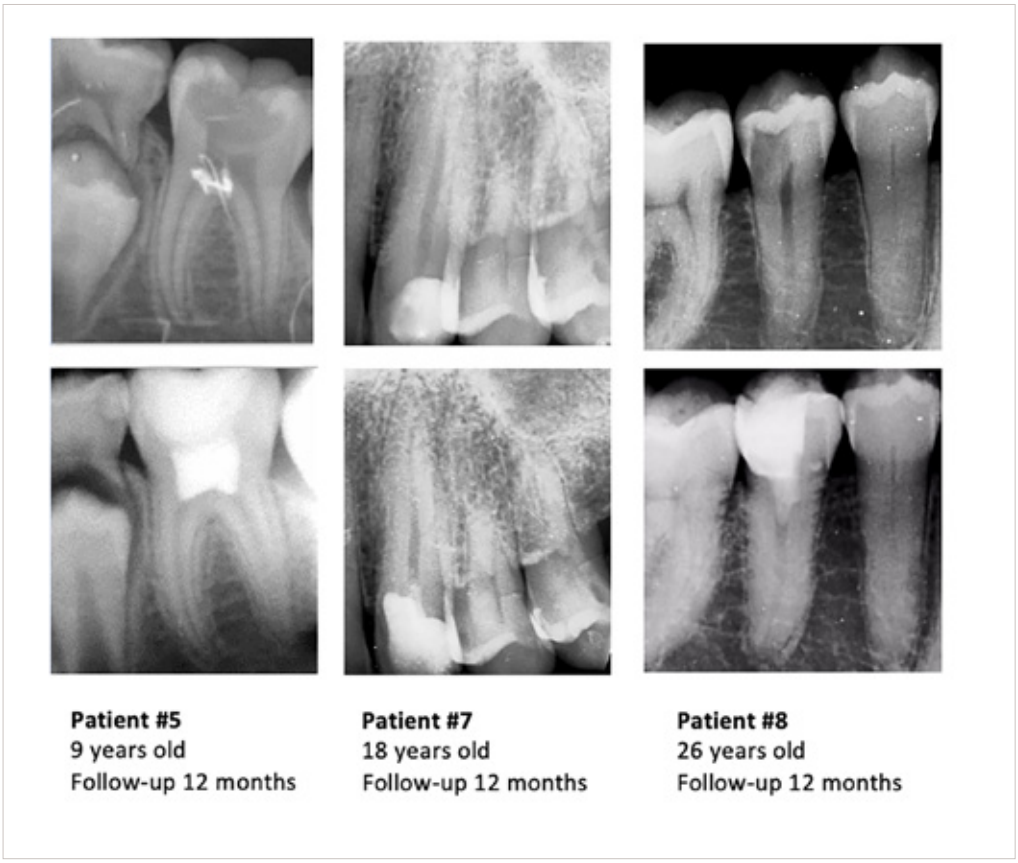

Figure 2
Periapical radiographs of baseline and 12 months follow-up of three patients.

relief and less requirement of analgesics (19). In this light, it should be stressed that, although the use of calcium silicate-based hydraulic cements in VPT has demonstrated promising outcomes in reducing pulp tissue inflammation and enhancing pulp healing, their role in reducing postoperative pain (8, 20, 21,) or benefit on pain management remains controversial and needs to be studied in depth.

Nowadays, VPT performed on mature symptomatic teeth is gaining a wide clinical interest and is becoming a valid alternative to traditional endodontic treatment (3,4, 22, 23). However, the major limitation of this technique is that current diagnosis of pulp diseases depends on various parameters such as subjective pain perception by the patient, objective/subjective clinical examination, and radiographic findings, without considering the histopathological state of the pulp tissue and its reparative potential (8). Indeed, during pulpotomy, tissue health is only appreciated by the time interval for hemostasis after pulp amputation (2-5 minutes), that is indicative of absence of acute inflammation. However, additional diagnostic criteria are needed to make this technique as predictable as possible.

Clinicians possess a wide range of therapeutic choices to individualize treatment based on patient symptoms, age, and dental element involvement, with the aim to preserve teeth function over time (24); nevertheless, the use of minimally invasive treatments is strictly related to several factors related to biology, subjects and clinical operator that might influence the prognosis (25, 26).

Therefore, these aspects should be prospectively evaluated with well-designed clinical trials supporting VPT as a strong clinical alternative to pulpectomy of vital dental elements.

Conclusions

Within the limitations of the present study, it can be concluded that a success rate of 100% was achieved, and appropriate diagnosis of pulp inflammation and use of proper materials, as hydraulic cements, are key factors in maintaining dental vitality over time. Therefore, VPT can be considered a valid minimally invasive treatment with medium success when applied to mature permanent teeth diagnosed with reversible pulpitis, ensuring greater reliability in terms of function, tooth weakening, and fracture risk compared to elements treated with traditional therapy. However, a larger sample size and longer follow-up period are necessary to confirm the obtained preliminary results, supporting VPT as a valid alternative to conventional endodontic treatment.

Clinical Relevance

VPT seems to be an alternative approach even in symptomatic mature permanent teeth with deep caries lesions to maintain pulp vitality over time and to post-pone or avoid non- surgical root canal therapy.

Conflict of Interest

None.

Acknowledgements

None.

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WEBINAR

Dopo il successo dei webinar primaverili, la proposta culturale della Società Italiana di Endodonzia prosegue: oltre ai meeting "in presenza" propone approfondimenti monotematici online, definiti **Endo Tuesday**, programmati **martedì sera dalle 20:30 alle 22**, aperti a tutti i dentisti.
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Lettera DEL PRESIDENTE

Cari Amici, Soci e Colleghi,
come certamente sapete, la nostra rivista, il GIE Giornale Italiano di Endodonzia, l'anno scorso ha ottenuto l'Impact Factor e da quest'anno è passato a tre numeri annuali procedendo nel progetto di sviluppo frutto del lavoro portato avanti con determinazione in tanti anni di impegno da parte di tutti noi Soci. Sono certo che questo sforzo continuerà nei prossimi anni con la condivisione dei vostri lavori scientifici e clinici e, soprattutto, con la citazione, negli articoli che scriverete, di lavori pubblicati sul GIE, come, da sempre, ci raccomanda il nostro Editor Sandro Rengo, che ringrazio, a nome di tutti noi, per l'impegno suo e del suo team.

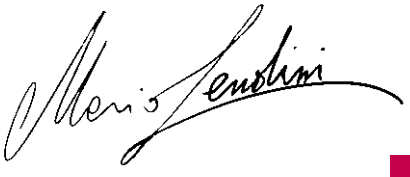
Il nostro Closed Meeting si è svolto a Torino nel mese di giugno, eravamo in tanti e sarei stato felice se fossimo stati ancora più numerosi. Considero questo appuntamento fondamentale per la vita della Società perché rappresenta la conferma della vitalità della SIE e la fucina dei progetti che ci caratterizzeranno nel futuro. La sessione societaria del venerdì ha visto i lavori del Consiglio Direttivo e delle Commissioni, nel pomeriggio l'assemblea dei Soci Attivi è stata vivace e produttiva di tanti spunti che certamente vedranno il loro naturale sviluppo nell'Assemblea annuale dei Soci Attivi in occasione del nostro congresso di Roma. La visita privata serale al Museo Egizio è stata un evento veramente interessante e la cena svoltasi nelle sale museali ha avuto un fascino esotico per tutti i fortunati partecipanti. La sessione societaria, per la prima volta aperta a tutti, si è svolta il sabato mattina nella particolare cornice della Nuvola del Museo Lavazza. Relazioni di alto livello sia per i contenuti clinici sia per la qualità delle esposizioni da parte di relatori prestigiosi, concluse da una tavola rotonda vivace ed estremamente interessante.

Il 2024 è un anno importante per la SIE che festeggia i suoi **primi cinquant'anni**! La SIE nasce infatti nel 1974, trasformandosi dal Gruppo di Studio di Endodonzia, nato nel 1970, nella attuale Società Italiana di Endodonzia. La nostra SIE ha attraversato momenti brillanti e momenti difficili, ma ha sempre mantenuto una rotta certa nell'evoluzione della professione in generale e dell'endodonzia in particolare, analizzando, certificando e, in definitiva guidando i cambiamenti nella clinica e nella chirurgia. Abbiamo discusso, a volte litigato, su tutti i protocolli, gli strumenti, i materiali e le tecniche, ma sempre con il fine di offrire il nostro onesto parere e migliorare la predicibilità e la sicurezza della nostra specialità. Tutti noi ci siamo formati a questa scuola e tutti la amiamo, ognuno a suo modo. Sono sicuro che, come me, tutti i Soci possono essere orgogliosi dei risultati che come SIE abbiamo ottenuto, determinati a proseguire nel cammino e affermare i nostri valori nel futuro.

Festeggeremo **inSIEme** il cinquantennale durante il 39° Congresso Nazionale di Roma, dal 14 al 16 novembre. Il titolo del congresso **"Le fondamenta della multidisciplinarietà. 5 discipline, 18 relatori: da dove partire?"** è una sintesi delle sfide e dei progetti che la Sie affronta da sempre e rappresentano la sua mission per il futuro. Il nostro congresso non è solo la sala principale, ci sono tantissime occasioni di approfondimento e confronto tra i Soci e tutti i partecipanti che è impossibile citare qui. Su endodonzia.it, il nostro sito che prestissimo avrà una nuova veste completamente rinnovata, troverete il programma completo e le modalità di iscrizione.

Arrivederci a Roma, saremo tantissimi, impareremo cose nuove, troveremo delle conferme e festeggeremo inSIEme i primi 50 anni della nostra SIE!

Mario Lendini



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COME DIVENTARE SOCIO ATTIVO/AGGREGATO

Scaricabile dal sito **www.endodonzia.it**

SOCIO AGGREGATO

Per avere lo status di Socio Aggregato si dovrà presentare la documentazione descritta nel sito www.endodonzia.it che sarà valutata dalla Commissione Accettazione Soci. La documentazione che verrà presentata dovrà mostrare con rigore, attraverso casi clinici, l'interessamento del candidato alla disciplina endodontica.
Un meccanismo a punti è stato introdotto per valutare l'ammissibilità del candidato allo "status" di Socio Aggregato: i punti saranno attribuiti in base al tipo di documentazione presentata. Possono accedere alla qualifica di Socio Aggregato tutti i Soci Ordinari della SIE, in regola con le quote associative degli ultimi tre anni, che completino e forniscano la documentazione alla Segreteria Nazionale (Via Pietro Custodi 3, 20136 Milano) entro i termini che verranno indicati all'indirizzo web: www.endodonzia.it.
La domanda dovrà essere firmata da un Socio Attivo, in regola con la quota associativa per l'anno in corso, il quale è responsabile della correttezza clinica e formale della documentazione presentata.

DOCUMENTAZIONE NECESSARIA PER DIVENTARE SOCIO AGGREGATO

Qualsiasi Socio Ordinario, con i requisiti necessari, può presentare la documentazione per ottenere la qualifica di Socio Aggregato. Un meccanismo a punti è stato introdotto per valutare il candidato: un minimo di 80 punti è richiesto per divenire Socio Aggregato.
La documentazione clinica per ottenere la qualifica di Socio Aggregato dovrà presentare almeno sei casi, di cui non più di tre senza lesione visibile nella radiografia preoperatoria e non più di uno di Endodonzia Chirurgica Retrograda.
Nella domanda non potranno essere presentati casi la cui somma superi i 120 punti per la qualifica di Socio Aggregato.
L'aspirante Socio Aggregato potrà presentare la documentazione clinica in più volte, con un minimo di 40 punti per presentazione, in un arco massimo di tre anni. Il mancato rinnovo della quota associativa, anche per un solo anno, annulla l'iter di presentazione dei casi.

SOCIO ATTIVO

Per avere lo status di Socio Attivo si dovrà presentare la documentazione descritta nel sito www.endodonzia.it che sarà valutata dalla Commissione Accettazione Soci. La documentazione che verrà presentata dovrà mostrare con rigore, attraverso documentazione scientifica e casi clinici, l'interessamento del candidato alla disciplina endodontica.
Un meccanismo a punti è stato introdotto per valutare l'ammissibilità del candidato allo status di Socio Attivo: i punti saranno attribuiti in base al tipo di documentazione clinica e scientifica presentata. Possono accedere alla qualifica di Socio Attivo tutti i Soci Ordinari della SIE, in regola con le quote associative degli ultimi tre anni, che completino e forniscano la documentazione alla Segreteria Nazionale (Via Pietro Custodi 3, 20136 Milano) entro i termini che verranno indicati all'indirizzo web: www.endodonzia.it.
La domanda di ammissione allo status di Socio Attivo rivolta al Presidente della SIE dovrà essere firmata da un Socio Attivo in regola con la quota associativa per l'anno in corso, il quale dovrà aver esaminato e approvato la documentazione. Quest'ultimo è responsabile della correttezza clinica e formale della documentazione presentata.

DOCUMENTAZIONE NECESSARIA PER DIVENTARE SOCIO ATTIVO

Qualsiasi Socio Ordinario, con i requisiti necessari, può presentare la documentazione per ottenere la qualifica di Socio Attivo. Il Socio Aggregato che volesse presentare la documentazione scientifica e clinica a integrazione di quella clinica già approvata dalla CAS per lo status di socio Aggregato, potrà farlo già dall'anno successivo all'ottenimento della sua qualifica.
Un meccanismo a punti è stato introdotto per valutare il candidato a Socio Attivo. Un minimo di 200 punti è richiesto per divenire Socio Attivo.
Nella domanda non potranno essere presentati casi la cui somma superi i 240 punti per la qualifica di Socio Attivo.
La documentazione scientifica potrà essere presentata, a completamento della documentazione clinica, solo per la domanda per divenire Socio Attivo e non potrà superare i 80 punti.
La documentazione clinica dovrà presentare un minimo di sei casi, di cui almeno 4 di molari pluriradicolati con delle precise tipologie: tra questi casi almeno uno deve essere un ritrattamento con lesione visibile nella radiografia preoperatoria e dei restanti tre almeno due devono avere una lesione visibile nella radiografia preoperatoria.
La documentazione clinica non deve presentare più di un caso di Endodonzia Chirurgica Retrograda con immagini e non più di uno senza immagini.
La documentazione scientifica non potrà presentare più di due articoli come coautore.

MODALITÀ DI DOCUMENTAZIONE DEI CASI CLINICI

Criteri e modalità per la valutazione dei casi clinici idonei ad accedere alle qualifiche di Socio Aggregato e di Socio Attivo sono espressi nell'apposita sezione del Regolamento

della Società Italiana di Endodonzia (SIE) all'indirizzo web: www.endodonzia.it.

CRITERI DI VALUTAZIONE

I casi clinici verranno valutati nel loro complesso, coerentemente con gli scopi e fini della SIE, e devono essere presentati dai Candidati considerando non solo l'aspetto clinico, ma anche quello formale della documentazione presentata.
La documentazione scientifica verrà valutata considerando la classificazione ANVUR delle Riviste Scientifiche, i documenti scientifici dovranno essere tutti di pertinenza endodontica.

ADEMPIMENTI DEL CANDIDATO

La domanda di ammissione allo status di Socio Aggregato/Attivo, rivolta al Presidente della SIE, dovrà pervenire, insieme alla documentazione di seguito elencata, alla Segretaria della SIE con un anticipo di 20 giorni sulle date di riunione della CAS, sufficiente per poter organizzare il materiale dei candidati. Le date di scadenza saranno rese note sul sito. La domanda dovrà essere firmata da un Socio Attivo in regola con la quota associativa per l'anno in corso, il quale dovrà aver esaminato e approvato la documentazione. Quest'ultimo è responsabile della correttezza clinica e formale della documentazione presentata.

PRESENTAZIONE DEI CASI ALLA COMMISSIONE

La presenza del Candidato è obbligatoria durante la riunione della CAS; è altresì consigliabile la presenza del Socio presentatore.

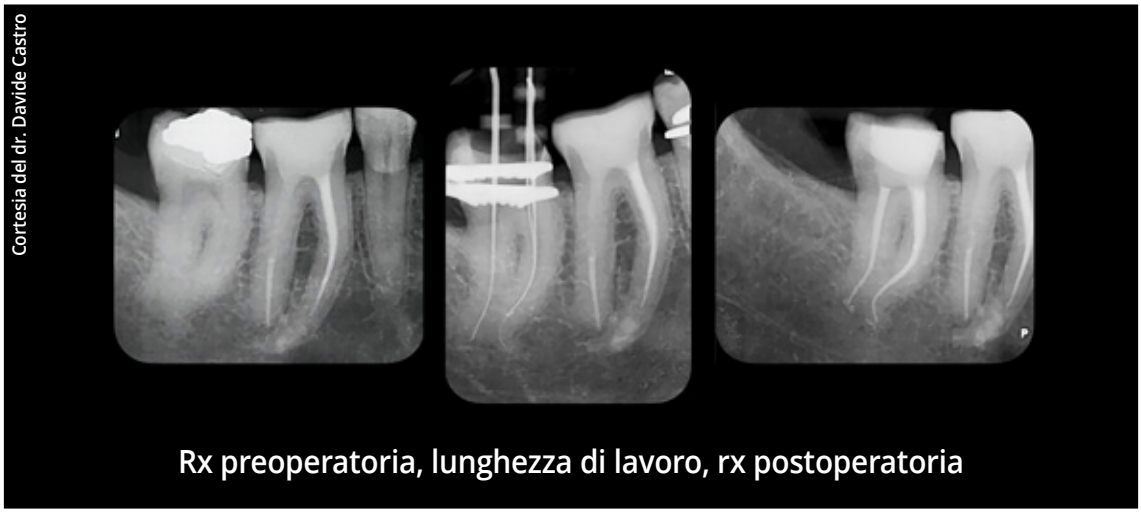
LA COMMISSIONE ACCETTAZIONE SOCI

La CAS (Commissione Accettazione Soci) è formata cinque Membri di indiscussa esperienza clinica, quattro Soci Attivi con almeno cinque anni di anzianità in questo ruolo eletti a ogni scadenza elettorale dall'Assemblea dei Soci Attivi e Onorari e uno dei Past President della Società incaricato dal CD a ogni riunione. Compito della CAS è quello di esaminare e valutare la documentazione presentata dagli aspiranti Soci Aggregati e Soci Attivi. Per rispetto del lavoro dei Candidati e per omogeneità di giudizio, in ogni riunione CAS verranno valutati non più di 12 candidati a Socio Attivo; resta libero, invece, il numero dei candidati a Socio Aggregato valutabile in una singola riunione. Il Consiglio Direttivo (CD) incaricando la Commissione Accettazione Soci (CAS) la rende responsabile dell'applicazione delle regole descritte nell'articolo 2 del regolamento. Il giudizio della CAS è insindacabile.

MEMBRI DELLA COMMISSIONE ACCETTAZIONE SOCI BIENNIO 2023-24

Francesco Riccitiello
Maurizio Boschi
Marco Colla
Claudia Dettori
Giuseppe Multari

Endodonzia mini-invasiva



Cortesia del dr. Davide Castro

Rx preoperatoria, lunghezza di lavoro, rx postoperatoria

La sistematica B4U sviluppa una conicità 05 e si avvantaggia di strumenti con lega Niti trattati termicamente a conicità progressiva unitamente a una sezione romboidale e due angoli di taglio che consentono di sfruttare una elevata elasticità e resistenza alla fatica ciclica. Lo Starter, previo scouting con K-file 010, esegue il glide path meccanico con preventivo svuotamento del contenuto pulpare ed elimina il rischio di taper lock creando i presupposti clinici per sfruttare in massima sicurezza la performance dello Starter L e Finisher. Si creano così i presupposti per l'utilizzo delle tecniche di otturazione 3d più' efficaci: cono singolo e bioceramico, carrier based e warm gp condensation.

Caso clinico

L'elemento 4.7 presenta un quadro clinico di pulpite acuta con sintomatologia spontanea esacerbata alla masticazione. Si rende necessario il trattamento endodontico. Utilizziamo la sistematica B4U. La radice distale è caratterizzata da una lieve curvatura. Dopo aver sondato il canale con un K-FIle 10 e misurata la lunghezza di lavoro elettronica si irriga con ipoclorito di sodio e si attua il glide path meccanico con lo Starter ottenendo un sentiero di scorrimento adeguato al passaggio degli strumenti di finalizzazione. Viene riconfermata la lunghezza di lavoro e si termina la sagomatura con Starter L e Finisher.

Le due radici mesiali presentano una elevata difficoltà operativa a fronte di una doppia curvatura e di una spiccata atresia canalare che rendono difficoltoso il sondaggio preliminare. Oltre all'abbondante irrigazione con ipoclorito di sodio ed edta è stato necessario l'utilizzo alternato e ripetuto di K-File 06-08-10-12-15 per guadagnare progressivamente la pervietà fino al termine della prima curvatura. Il passaggio dello Starter e poi, a scalare rispettivamente di 1 e 2 mm, dello Starter L e del Finisher fino a questa lunghezza di lavoro provvisoria ha consentito di ridurre le interferenze del terzo coronale e del terzo medio ottimizzando l'azione degli irriganti nella porzione canalare più profonda e la successiva discesa progressiva dei file manuali fino al termine della seconda curva determinando la lunghezza di lavoro elettronica definitiva. Il passaggio alla lunghezza di lavoro finale dello Starter, Starter L e Finisher ha permesso di creare una conicità 05 ideale per ottenere una otturazione tridimensionale tramite tecnica Carrier based.

MORITA

New Tri Auto ZX2+

Il nuovo Tri Auto ZX2+ ridefinisce ancora una volta la preparazione endodontica e porta il movimento reciprocante ad un livello superiore. Con la nuova modalità OGP2 e la modalità OTR migliorata, potrete sfruttare appieno il potenziale delle vostre lime preferite, sia reciprocanti che rotanti. La collaudata tecnologia Morita contribuisce a ridurre la rottura e il bloccaggio delle lime, per una preparazione sicura.

Inoltre, la funzione OGP2 semplifica il trattamento: sondaggio, glide path e sagomatura in un unica modalità, che consente di ottenere un flusso di lavoro ottimale senza perdite di tempo. Tutto, in combinazione con il localizzatore apicale di Morita, leader mondiale per precisione e affidabilità. Il Tri Auto ZX2+ renderà ogni dentista un fan del trattamento endodontico.

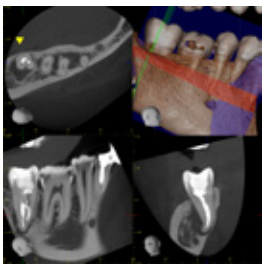
www.jmoritaitalia.com

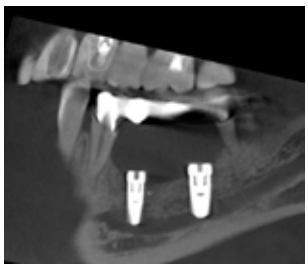
Veraview X800

la nuova tecnologia radiologica mette in evidenza ogni minimo dettaglio con una risoluzione incredibili- mente nitida. Per la sua altissima risoluzione (voxel di 80 µm/2.5 LP mm) e per le sue funzioni innovative, questa apparecchiatura fornisce la base perfetta per diagnosi certe Veraview X800 combina la qualità d'immagine ottimale con una bassa dose di radiazioni. combinazione tra, Panoramico, cefalometrico e sistema imaging 3D. Inoltre, con undici FOV disponibili, garantisce la dose più bassa con la migliore qualità di immagine.

11 FOV disponibili, da 4x4 fino a 15x14

Morita è riconosciuta in tutto il mondo per la sua grandissima qualità delle immagini e tu, sei il prossimo ad averla ?





GUIDELINES FOR AUTHORS

Giornale Italiano di Endodonzia (GIE) was founded in 1987 and is the official journal of Società Italiana di Endodonzia, SIE (Italian Society of Endodontics) https://www.endodonzia.it/

It is a peer-reviewed journal, only available in electonic format and publishes original scientific articles, reviews, clinical articles and case reports in the field of Endodontology. Scientific contributions dealing with health, injuries to and diseases of the pulp and periradicular region, and their relationship with systemic well-being and health. Original scientific articles are published in the areas of biomedical science, applied materials science, bioengineering, epidemiology and social science relevant to endodontic disease and its management, and to the restoration of root-treated teeth. In addition, review articles, reports of clinical cases, book reviews, summaries and abstracts of scientific meetings and news items are accepted. **Please read the instructions below carefully for details on the submission of manuscripts, the journal's requirements and standards as well as information concerning the procedure after a manuscript has been accepted for publication in Giornale Italiano di Endodonzia**.

Giornale Italiano di Endodonzia is indexed in Scopus, Science Direct, Embase and published online by Ariesdue, Milan, Italy and hosted by PAGEPress, Pavia, Italy. All articles are available on www.giornaleitalianoendodonzia.it.

We publish new articles in the Early Access section while the full Journal is issued three times a year, in March, July and November.

Authors are encouraged to visit www.giornaleitalianoendodonzia.it for further information on the preparation and submission of articles and figures.

Ethical guidelines

Giornale Italiano di Endodonzia adheres to the below ethical guidelines for publication and research.

Authorship and Acknowledgements

Authors submitting a paper do so on the understanding that the manuscript has been read and approved by all authors and that all authors agree to the submission of the manuscript to the Giornale Italiano di Endodonzia. Giornale Italiano di Endodonzia adheres to the definition of authorship set up by The International Committee of Medical Journal Editors (ICMJE). According to the ICMJE, authorship criteria should be based on 1) substantial contributions to conception and design of, or acquisiation of data or analysis and interpretation of data, 2) drafting the article or revising it critically for important intellectual content and 3) final approval of the version to be published. Authors should meet conditions 1, 2 and 3. It is a requirement that all authors have been accredited as appropriate upon submission of the manuscript. Contributors who do not qualify as authors should be mentioned under Acknowledgements.

Manuscript preparation

Manuscripts should be uploaded as Word (.doc) or Rich Text Format (.rtf) files (not write-protected) plus separate figure files: TIF, EPS, JPEG files are acceptable for submission.

The text file must contain the **abstract, main text, references, tables and figure legends**, but no embedded figures or title page. The title page should be provided as a separate file. In the main text, please reference figures as for instance **figure 1, figure 2** etc to match the tag name you choose for the individual figure files uploaded.

Please note that **manuscripts must be written in English**. Authors whose native language is not English are strongly advised to have their manuscript checked by a language editing service or by a native English speaker prior to submission.

Manuscript Types Accepted

Original Scientific Articles must describe significant and original experimental observations and provide sufficient detail so that the observations can be critically evaluated and, if necessary, repeated. Original Scientific Articles must conform to the highest international standards in the field.

Systematic Review Articles reconsider and bring previously published systematic reviews up to date. This allows authors to present changes to the review while avoiding unwarranted duplication in the literature. A guiding principle for an update is that it is an event that is discrete and distinct from the conduct and reporting of the original systematic review (or previously updated review). This means that at a minimum the search for studies will have been brought up to date and that any changes to the results and conclusions of the original review (or a previously updated review) are described. Systematic review updates will not usually warrant publication of a new full-length article. However, any published update will be an independent publication. It will not be part of the original review publication (or previously updated review).

We encourage authors to be innovative in how they report and present systematic review updates. Systematic review updates are not appropriate for corrections/errata. Authors must clearly acknowledge and reference any previously-published work they are updating.

Review Articles are accepted for their broad general interest; all are refereed by experts in the field who are asked to comment on issues such as timeliness, general interest and balanced treatment of controversies, as well as on scientific accuracy. Reviews should generally include a clearly defined search strategy and take a broad view of the field rather than merely summarizing the authors´ own previous work. Extensive or unbalanced citation of the authors´ own publications is discouraged.

Mini Review Articles are accepted to address current evidence on well-defined clinical, research or methodological topics. All are refereed by experts in the field who are asked to comment on timeliness, general interest, balanced treatment of controversies, and scientific rigor. A clear research question, search strategy and balanced synthesis of the evidence is expected. Manuscripts are limited in terms of word-length and number of figures.

Case Reports or **Case Series** illustrating unusual and clinically relevant observations are acceptable, but they must be of sufficiently high quality to be considered worthy of publication in the Journal. On rare occasions, completed cases displaying nonobvious solutions to significant clinical challenges will be considered. Illustrative material must be of the highest quality and healing outcomes, if appropriate, should be demonstrated.

Case reports should be written using the **Preferred Reporting Items for Case reports in Endodontics (PRICE) 2020 guidelines.** A PRICE checklist and flowchart (as a Figure) should also be completed and included in the submission material. The PRICE 2020 checklist and flowchart can be downloaded from: http://pride-endodonticguidelines.org/price/. It is recommended that authors consult the following papers, which explains the rationale for the PRICE 2020 guidelines and their importance when writing manuscripts:

- Nagendrababu V, Chong BS, McCabe P, Shah PK, Priya E, Jayaraman J, Pulikkotil SJ, Setzer FC, Sunde PT, Dummer PMH. *PRICE 2020 guidelines for reporting case reports in Endodontics: a consensus-based development.* Int Endod J. 2020 Feb 23. Doi: 10.1111/iej.13285. https://onlinelibrary.wiley.com/doi/10.1111/iej.13285.
- Nagendrababu V, Chong BS, McCabe P, Shah PK, Priya E, Jayaraman J, Pulikkotil SJ, Dummer PMH. *PRICE 2020 guidelines for reporting case reports in Endodontics: Explanation and elaboration.* Int Endod J. 2020 Mar 28. Doi: 10.1111/iej.13300. https://onlinelibrary.wiley.com/doi/abs/10.1111/iej.13300.

Manuscript Format

The **official language** of the publication is **English**. It is preferred that manuscript is professionally edited. All services are paid for and arranged by the author and use of one of these services does not guarantee acceptance or preference for publication.Authors should pay special attention to the **presentation** of their research findings or clinical reports so that they may be communicated clearly.

Technical **jargon** should be avoided as much as possible and clearly explained where its use is unavoidable. **Abbreviations** should also be

kept to a minimum, particularly those that are not standard. Giornale Italiano di Endodonzia adheres to the conventions outlined in *Units, Symbols and Abbreviations: A Guide for Medical and Scientific Editors and Authors.* If abbreviations are used in the text, authors are required to write full name+abbreviation in brackets [e.g. Multiple Myeloma (MM)] the first time they are used, then only abbreviations can be written (apart from titles; in this case authors have to write always the full name). If names of equipments or substances are mentioned in the text, brand, company names and locations (city and state) for equipment and substances should be included in parentheses within the text.
The **background** and **hypotheses** underlying the study, as well as its main conclusions, should be clearly explained.
Titles and abstracts especially should be written in language that will be readily intelligible to any scientist.

Structure
All manuscripts submitted to Giornale Italiano di Endodonzia should include Title Page, Abstract, Main Text, References, Clinical Relevance, Conflict of Interest, Acknowledgements, Tables, Figures and Figure Legends as appropriate.
Title Page should bear:

I. Title, which should be concise as well as descriptive (no more than 150 letters and spaces);
II. Initial(s) and last (family) name of each author;
III. Name and address of department, hospital or institution to which the work should be attributed;
IV. Running title (no more than 30 letters and spaces);
V. Three to five key words (in alphabetical order);
VI. Name, full postal address, telephone, fax number and e-mail address of author responsible for correspondence (Corresponding Author).

Abstracts should be no more than 250 words giving details of what was done.

Abstract for Original Scientific Articles should be no more than 250 words giving details of what was done using the following structure.
Aim: give a clear statement of the main aim of the study and the main hypothesis tested, if any.
Methodology: describe the methods adopted including, as appropriate, the design of the study, the setting, entry requirements for subjects, use of materials, outcome measures and statistical tests.
Results: give the main results of the study, including the outcome of any statistical analysis.
Conclusions: state the primary conclusions of the study and their implications. Suggest areas for further research, if appropriate.

Abstract for Systematic Review Articles should be divided into Aim, Methodology, Result, Conclusion.
Aim: Provide an explicit statement of the main objective(s) or question(s) the review addresses.
Methodology: Specify the inclusion and exclusion criteria for the review, the information sources (e.g. databases, registers) used to identify studies and the date when each was last searched. Specify the methods used to assess risk of bias in the included studies and the methods used to present and synthesis of studies.
Results: Give the total number of included studies and participants and summarise relevant characteristics of studies. Present results for main outcomes, preferably indicating the number of included studies and participants for each. If meta-analysis was done, report the summary estimate and confidence/credible interval. If comparing groups, indicate the direction of the effect (i.e. which group is favoured).
Conclusion: Provide a brief summary of the limitations of the evidence included in the review (e.g. study risk of bias, inconsistency and imprecision) and a general interpretation of the results and important implications.

Abstract for Review Articles should be non-structured, no more than 250 words giving details of what was done including the literature search strategy.

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Abstract for Case Reports and **Case Series** should be no more than 250 words using the following structure.
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Conclusions should contain a summary of the findings.

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Standard journal article
(1) Somma F, Cammarota G, Plotino G, Grande NM, Pameijer CH. The effectiveness of manual and mechanical instrumentation for the retreatment of three different root canal filling materials. J Endod 2008;34:466-9.

Corporate author
British Endodontic Society - Guidelines for root canal treatment. Giornale Italiano di Endodonzia 1979;16:192-5.

Journal supplement
Frumin AM, Nussbaum J, Esposito M. Functional asplenia: demonstration of splenic activity by bone marrow scan (Abstract). Blood 1979;54 (Suppl. 1):26a.

Books and other monographs
Personal author(s)
Gutmann J, Harrison JW. Surgical Endodontics, 1st edn Boston, MA, USA: Blackwell Scientific Publications, 1991.

Chapter in a book
Wesselink P. Conventional rootcanal therapy III: root filling. In: Harty FJ, ed. Endodontics in Clinical Practice, (1990), 3rd edn; pp. 186-223. London, UK: Butterworth.

Published proceedings paper
DuPont B. Bone marrow transplantation in severe combined immunodeficiency with an unrelated MLC compatible donor. In: White HJ, Smith R, eds. Proceedings of the Third Annual Meeting of the International Society for Experimental Rematology; (1974), pp. 44-46. Houston, TX, USA: International Society for Experimental Hematology.
Agency publication
Ranofsky AL Surgical Operations in Short-Stay Hospitals: United States-1975 (1978). DHEW publication no. (PHS) 78-1785 (Vital and Health Statistics; Series 13; no. 34.) Hyattsville, MD, USA: National Centre for Health Statistics.

Dissertation or thesis
Saunders EM. In vitro and in vivo investigations into root-canal obturation using thermally softened gutta-percha techniques (PhD Thesis) (1988). Dundee, UK: University of Dundee.

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