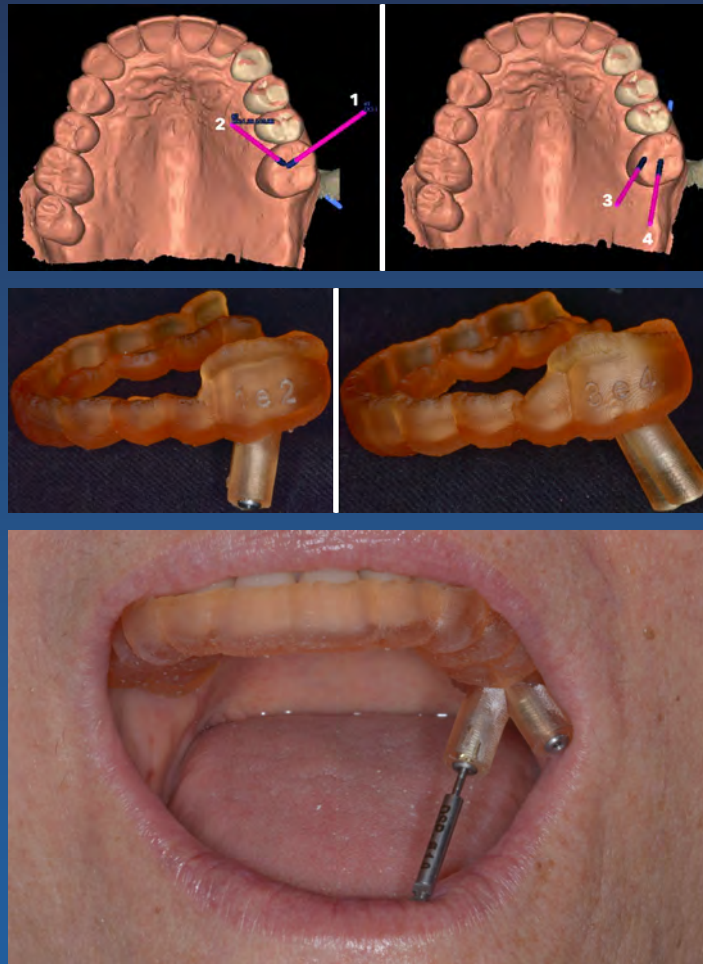




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Authors who used AI technology to conduct the study should describe its use in this section in sufficient detail to enable replication to the approach, including the tool used, version, and prompts where applicable.

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Abstract: An abstract should be submitted with all submissions except for Letters to the Editor. The abstract of Original Articles should be structured with subheadings (Objective, Methods, Results, and Conclusion). Please check Table 1 below for word count specifications.

Keywords: Each submission must be accompanied by a minimum of three to a maximum of six keywords for subject indexing at the end of the abstract. The keywords should be listed in full without abbreviations. The keywords should be selected from the National Library of Medicine, Medical Subject Headings database (<https://www.nlm.nih.gov/mesh/MBrowser.html>).

Manuscript Types

Original Articles: This is the most important type of article since it provides new information based on original research. The main text of original articles should be structured with Introduction, Methods, Results, Discussion, and Conclusion subheadings. Please check Table 1 for the limitations for Original Articles.

Statistical analysis to support conclusions is usually necessary. Statistical analyses must be conducted in accordance with international statistical reporting standards (Altman DG, Gore SM, Gardner MJ, Pocock SJ. Statistical guidelines for contributors to medical journals. *Br Med J* 1983; 7; 1489-93). Information on statistical analyses should be provided with a separate subheading under the Materials and Methods section and the statistical software that was used during the process must be specified.

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TABLE 1. Limitations for each manuscript type

Type of manuscript	Word limit	Abstract word limit	Reference limit	Table limit	Figure limit
Original Article	3500	350 (Structured)	40	6	5 or total of 15 images
Review Article	5000	350	None	6	10 or total of 20 images
Case Report	1000	200	25	No tables	5 or total of 15 images
Case Series	2000	200	25	10 or total of 20 images	10 or total of 20 images
Letter to the Editor	500	No abstract	5	No tables	No media

text should contain Introduction, Clinical and Research Consequences, and Conclusion sections. Please check Table 1 for the limitations for Review Articles.

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Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

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When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)."

All references, tables, and figures should be referred to within the main text, and they should be numbered consecutively in the order they are referred to within the main text.

Limitations, drawbacks, and the shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

Please note that British English spelling and terminology should be used in the manuscripts.

Highlights

Each submission should be accompanied by 3 to 5 "highlight points" which should emphasize the most striking results of the study and highlight the message that is intended to be conveyed to the readers. As these highlights would be targeting endodontics residents, experts and residents of other fields of medicine, as well as endodontics experts, they should be kept as plain and simple as possible. These points should be constructed in a way that provides the readers with a general overview of the article and enables them to have a general idea about the article. The highlights should be listed at the end of the main text, above the reference list.

References

While citing publications, preference should be given to the latest, most up-to-date publications. If an ahead-of-print publication is cited, the DOI number should be provided. Authors are responsible for the accuracy of references. Journal titles should be abbreviated in accordance with the journal abbreviations in Index Medicus/ MEDLINE/PubMed. When there are 6 or fewer authors, all authors should be listed. If there are 7 or more authors, the first 6 authors should be listed followed by "et al." In the main text of the manuscript, references should be cited using Arabic numbers in parentheses. The reference styles for different types of publications are presented in the following examples.

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Book Section:

Author. Title. In: Editor, `editor`.^`editors`.] Book Title]. Edition ed]. Place Published]: Publisher]; Year]. p. Pages].

Suh KN, Keystone JS. Malaria and babesiosis In: Gorbach SL, Barlett JG, Blacklow NR, editors. *Infectious Diseases*. 3rd ed. Philadelphia: Lippincott Williams; 2004. p.2290-308.

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ly Treatment Diabetic Retinopathy Study (ETDRS), Early Treatment Diabetic Retinopathy Study Kidney Int: 2004. Report No: 26.

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Author. Title]. Type]. Place Published]: Institution]; Year] Date]. Report No.: Report Number]. Kaplan SI. Post-hospital home health care: elderly access and utilization (dissertation). St Louis (MO): Washington Univ; 1995.

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Artificial Intelligence in the Study of Root and Canal Anatomy: A Comprehensive Review on Applications, Advantages, Challenges and Future Directions

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ABSTRACT

A thorough understanding of tooth anatomy is essential for all endodontic therapies. Over the last two decades, technological advances in 3D imaging have revealed the complexities of root and canal anatomy. Recently, artificial intelligence (AI) models have been developed and are being applied to a range of fields within medicine and dentistry. There is an emerging trend for the application of this technology in 2D and 3D imaging tools to study the anatomical features of roots and canals. This narrative review provides a comprehensive analysis of AI applications in the study of root and canal anatomy and their implications for education, research and clinical practice. The analysis reveals that AI applications for the study and teaching of root and canal anatomy are promising; however, more investigations are warranted with larger datasets to provide more accurate deep learning models. Students, researchers and clinicians should understand the inherent limitations of AI data generated from 2D and 3D imaging devices. Future studies are needed to assess what effect deep learning models have on the diagnostic and operative clinical skills of students and dental practitioners when managing teeth with different levels of anatomical complexities.

Keywords: Artificial Intelligence, deep learning, dental pulp cavity, machine learning, neural networks, root and canal anatomy

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HIGHLIGHTS

- This narrative review discusses AI applications in the study of root and canal anatomy and their implication for education, research and clinical practice.
- This review shows that AI applications for the study and teaching of root and canal anatomy are promising; however, more studies are needed with larger datasets.
- Students, researchers and clinicians should understand the inherent limitations of AI data generated from 2D and 3D imaging devices used to study root and canal anatomy.

INTRODUCTION

Artificial intelligence (AI) is the discipline that combines computer science and robust datasets to facilitate problem solving in a range of scenarios (1), and perform tasks that normally require human intelligence (2, 3). Recently, considerable progress has been made toward large-scale applications of AI in health and medicine through various types of Convolutional Neural Networks (CNNs), which are a type of deep learning algorithm for image recognition and processing tasks (4). In dentistry, AI has been used in fields related to restorative dentistry, orthodontics and oral and maxillo-facial sciences (5–10). In endodontics, AI has been used in diagnostic applications, such as identifying periradicular radiolucencies on periapical radiographs, panoramic images, and cone beam computed tomography (CBCT) scans (11, 12), as well as improving the accuracy of diagnosing vertical root fractures (13), external root resorption (14), and fractured instruments (15). This is in addition to applications related to endodontic education such as practical training through simulations and using AI systems to interpret data and plan treatment protocols (16).

Recent years have seen major technological advances in qualitative and quantitative analysis of root, pulp chamber and canal anatomy including the use of 3D imaging techniques such as CBCT (17), and micro-computed tomography (micro-CT) scanning (18, 19). The use of AI with innovative automated algorithms increases segmentation efficiency and consistency, thus providing easier manipulation and more accurate interpretation of the 2D and 3D datasets (20).

It is well-known that 2D radiographs (such as periapical and panoramic radiographs) have drawbacks since they provide only limited information on three-dimensional objects (21). In root and canal anatomy, AI has been used with various 2D imaging tools to aid the labelling/segmenting of the pulp cavity (22), pulp stones (23) and identifying dental anomalies such as Taurodontism and C-shaped canals (24, 25). AI has also been used for the detection of second mesio-buccal canals (MB2) in maxillary molars from CBCT scans (26), as well as canal curvatures on both CBCT and micro-CT scans (27). Given the fundamental importance of understanding root and canal anatomy before providing endodontic therapies, and the rapid evolution of AI to identify and classify roots and canals, this narrative review discusses AI applications in the study of root and canal anatomy and their implications for education and research. In addition, it aims to identify gaps in knowledge and present insights for the direction of future research and its translation to clinical endodontics.

METHODOLOGY

Literature Search Methodology

An electronic search was performed in the PubMed, Scopus and Web of Science databases. The following main keywords were used – “Artificial intelligence” OR “Deep Learning” AND “Root canal” OR “Dental Pulp Cavity” until 31 December 2024. Depending on the search database, free keywords and Medical Subject Headings-MeSH, (<http://www.ncbi.nlm.nih.gov/mesh>) were combined in the search. Boolean operators (AND/OR), truncation, and quotation marks for specific terms were used.

The detailed search strategy is outlined in Supplementary A. No filters except for English language were applied. Citation searching was also performed by reviewing the reference lists and citing articles of the included studies. The citing articles were retrieved using the “cited by” tool in Google Scholar.

Inclusion Criteria

Original research articles that investigated root and/or canal anatomy using artificial intelligence on all 2D or 3D radiographic imaging modalities were included.

Exclusion Criteria

Letters, commentaries, editorials, case reports/series, narrative, scoping or systematic reviews and conference proceedings were excluded, as were studies published in languages other than English.

Study Selection

The study selection process was performed in two phases.

Phase 1: The titles and abstracts of the retrieved studies were assessed independently and in duplicate by two reviewers (HMAA & AA). Papers that did not meet the inclusion criteria were excluded.

Phase 2: The two reviewers independently evaluated the full texts of the included studies with disagreements being resolved by discussion. The reasons for exclusions were reported accordingly.

Data Extraction

Data extraction was performed by two independent reviewers (HMAA & AA). The following details were extracted for each study: name of the first author, year published, study design (imaging system), AI model used, tooth type and the number of teeth included, performance levels, and the main findings reported.

RESULTS

The search resulted in 415 studies. After duplicate removal, the titles and abstracts of 220 studies were screened, with a total of 97 studies subject to further scrutiny. Citation searching resulted in the identification of 20 more papers. Subsequently, the full-text of the 117 studies was assessed for eligibility with 35 studies being included in the review (20, 22, 24–26, 28–57) (Appendix 1-3). The remaining 82 papers were excluded (45 irrelevant papers, 25 review articles and 12 conference proceedings). Figure 1 shows the flow diagram of the search results and the reasons for exclusions.

Prior to further analysis, the included studies were divided into two categories – AI studies involving 2D radiographic imaging (periapical, bitewing and panoramic radiographic views) and AI studies involving 3D radiographic imaging (CBCT and micro-CT).

AI Studies Analysing Root Canal Anatomy on 2D Radiographic Images

Periapical and bitewing radiographic views

A number of studies reported the successful application of various CNN models for detecting a range of root and canal anatomical features on 2D periapical radiographic images including the presence of teeth with extra roots (such as radix

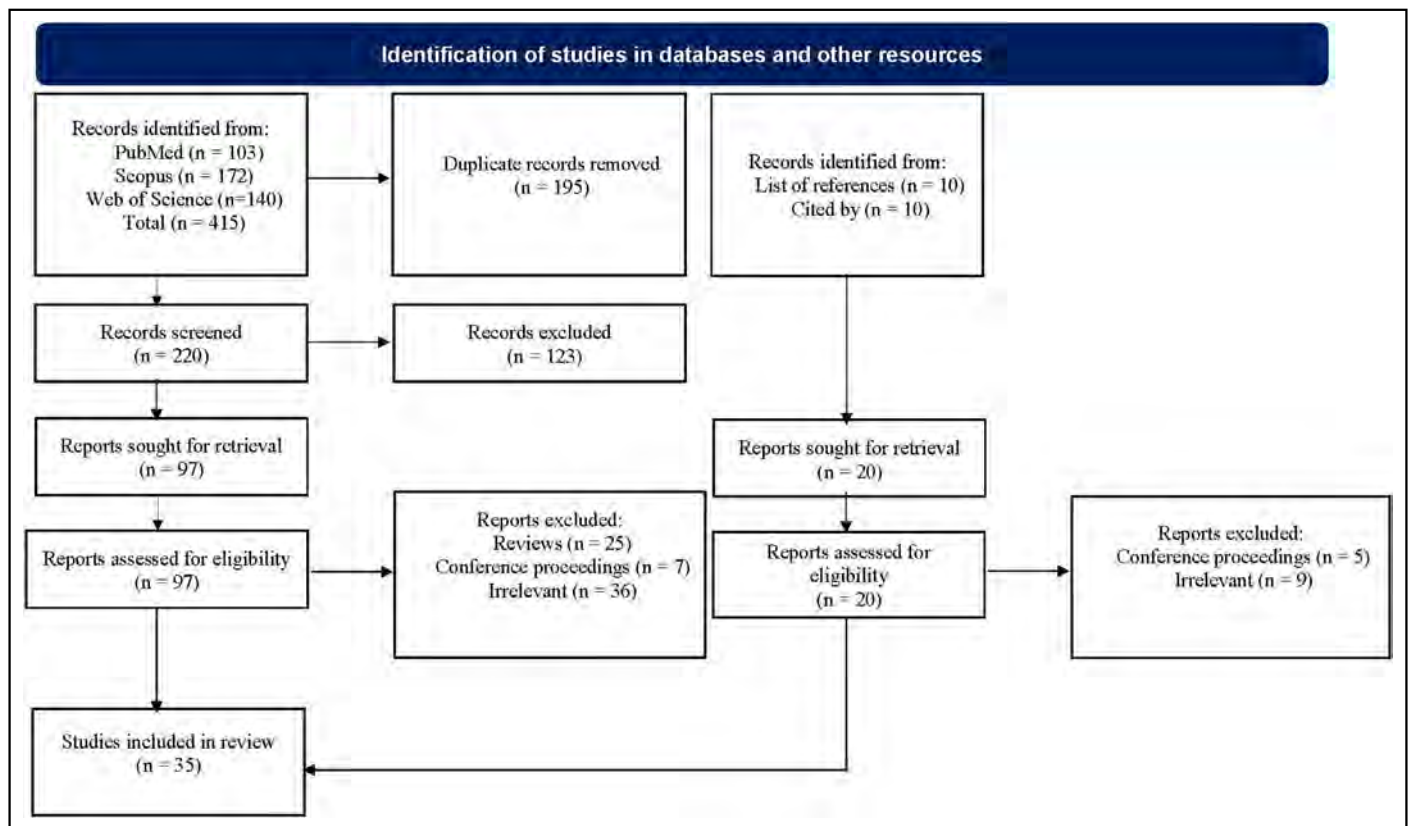


Figure 1. Flow diagram of the review.

entomolaris), canal bifurcations, narrow and curved canals (29, 30) (Appendix 1). Other studies examined the ability of AI models to identify complex anatomical variations such as C-shaped canals in comparison with other 3D diagnostic models, including CBCT (33, 36). One study found that the EfficientNet deep learning architecture had similar levels of accuracy for the detection of C-shaped canals as those of specialists (36). Another study found that a specific AI model was more accurate in identifying C-shaped canals compared with endodontic residents (28).

Two studies examined an approach for locating the minor apical foramen using feature-extracting procedures on 2D peri-apical radiographs and then processing data using an artificial neural network (ANN) (37, 38). It was concluded that the ANN model was an accurate method for determining the working length to the minor apical foramen. However, it was unclear whether this method is accurate in determining locations of the minor apical foramen (such as bucco-lingual) that cannot be identified on 2D radiographic images. Two studies trained a number of AI models with bitewing radiographic images to identify the components of the teeth including the coronal pulp cavity (22, 31) (Appendix 1).

Panoramic Radiographic Imaging

Despite the fact that panoramic radiographs are not used as a routine diagnostic tool for the detailed analysis of roots and canals, a number of studies examined the ability of AI models to detect tooth anomalies including mandibular molars with C-shaped canals, root dilacerations, Taurodontism and accessory roots (Appendix 2). The results revealed that

deep learning models can be used effectively to automatically identify dental anomalies on panoramic radiographs (Appendix 2), close to the expert level (24).

AI Studies That Analysed Root Canal Anatomy Using 3D Radiographic Imaging

Cone beam computed tomography/micro-computed tomography

Several studies examined various AI models (such as U-Net, V-Net and other models in development) to segment the roots and canals from 3D images (mainly CBCT) with the aim of detecting fine details of the canals more accurately and more rapidly (Appendix 3). One study examined the application of AI models to detect MB2 canals in maxillary molars on CBCT scans (26). The MB2 canals were first detected in axial and coronal sections by two operators and then labelled with deep learning being undertaken using You Only Look Once v5 (YOLOv5). The AI model successfully segmented the MB2 canals with a high level of accuracy (26) (Appendix 3). Another study examined the ability of one U-Net architecture to detect missed MB2 canals in root filled maxillary molars (49). Despite the high accuracy for MB2 detection, the AI algorithm was adversely affected by metallic artefacts, canal calcifications and different configurations of the MB2 in relation to the MB1 canal (49).

One study examined the ability of three AI models (U-Net, Residual U-Net, Xception U-Net) to provide a fully automated segmentation and classification of C-shaped canals in mandibular second molars (20). Even though Xception U-Net and Residual U-Net performed significantly better than

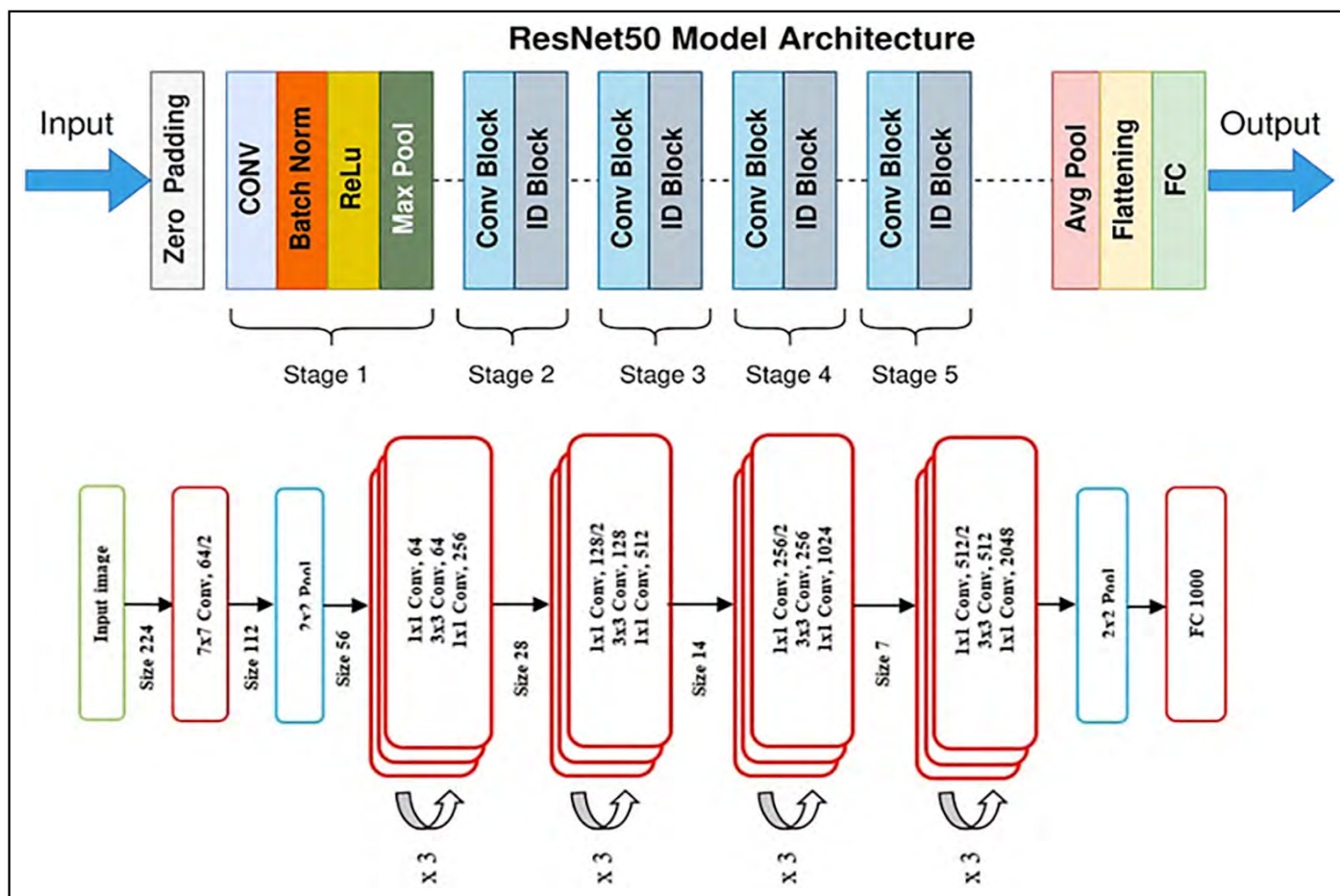


Figure 2. An example for one of the deep learning models. ResNet50 is a convolutional neural network architecture that belongs to the family of Residual Networks (ResNets) established by He et al. (58). The “50” in the name refers to the number of layers in the network, which is 50 layers deep. One of the important innovations of ResNet50 is the use of residual connections, which allow the network to learn a set of residual connections, that enable the network to learn a set of residual functions that map the input to the desired output. The residual connections are able to network to learn deeper architectures without facing problems from vanishing gradients (skip connections).

U-Net, it was concluded that the application of deep learning models had the potential to classify automatically C-shaped canal anatomy on CBCT images.

A recent study validated an AI-driven tool for automated segmentation of the pulp cavity system of mandibular molars on CBCT images (45) (Appendix 3). AI-driven segmentation proved to be accurate and time-efficient in segmenting the pulp cavity system in mandibular molars, in which the pulp segmentation took approx. 4.3 seconds compared to around 39 minutes for the manual segmentation method. However, this study used relatively small datasets (66 CBCT scans).

DISCUSSION

Recent years have seen the application of a wide range of deep learning models in the field of dentistry and endodontics with the main aim being to train and analyse large volumes of data in a short time using specific algorithms to identify diagnostic features that might be missed by the human eye (16) (Fig. 2). In radiographic imaging, convolutional neural networks (CNNs) are used to analyse the object of interest on images, particularly for minor changes such as small carious lesions, early periodontal bone loss and minor changes in the pattern of bone around the root apex evident on periapical radiographs (60).

Analysis of AI Applications In 2D Radiographic Imaging

The application of AI for the detection of anatomical structures and restorations on 2D periapical radiographs involves several steps starting from image enhancement, through labelling, classification, object detection and segmentation (Figs. 3, 4). This review has identified applications of deep learning models for the detection of a range of root and canal anatomical structures on 2D radiographs with high accuracy (Appendix 1). This is an important advance since the assessment of case complexity for teeth indicated for root canal treatment is a challenge that requires comprehensive evaluation through detailed clinical examination and radiographic analysis (29). Such deep learning models have also been applied to predict the difficulty levels for other critical clinical procedures such as extraction of third molar teeth (61). This paves the way for standardization and consistency in radiographic interpretation, which is particularly beneficial for dental students, inexperienced general dental practitioners and those in training.

It is important to emphasise that AI technology has to function within the inherent limitation of 2D radiographs in which a number of anatomical landmarks are difficult or impossible



Figure 3. An example of the enhancement of a periapical radiograph using OpenCV library. (a) original image; (b) enhanced image.

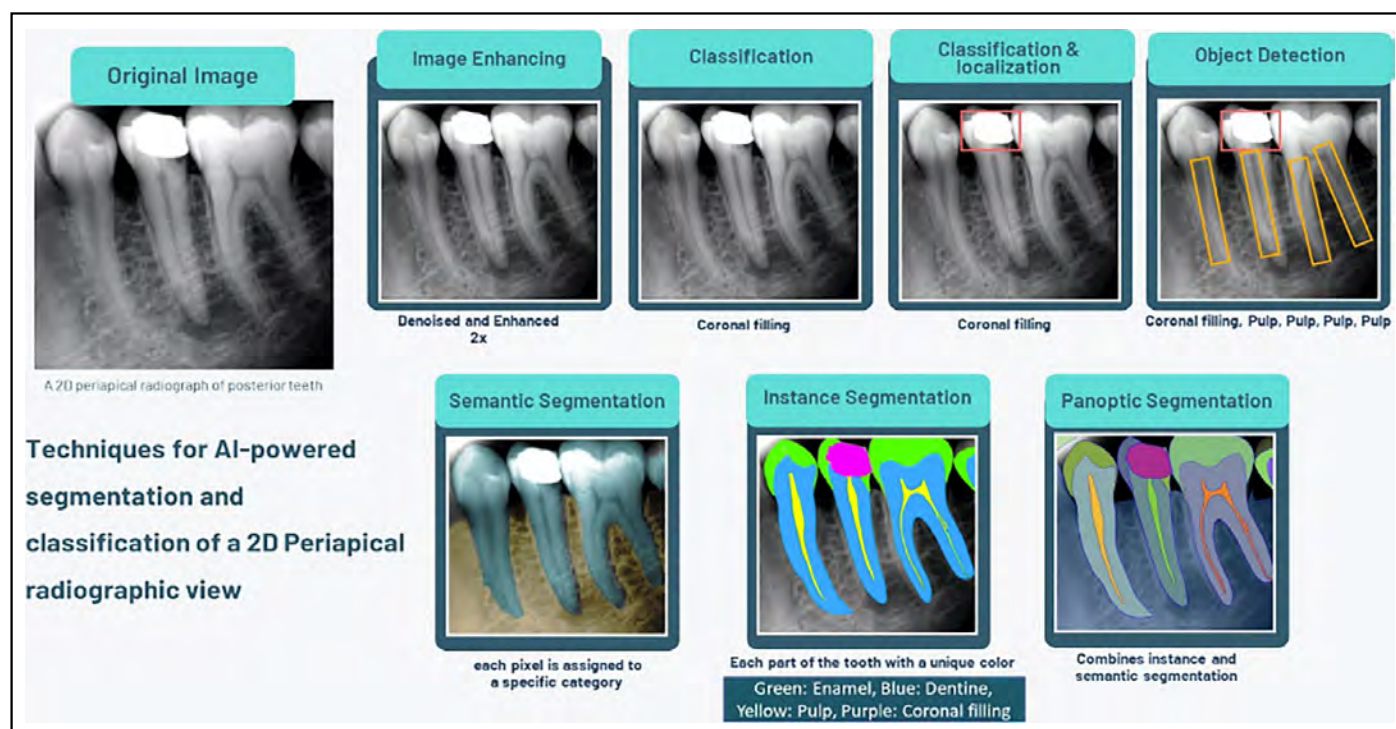


Figure 4. A summary of evolving techniques involved in AI processes for 2D radiographic imaging.

to identify, such as bucco-lingual canal curvatures, narrow canals, canal shapes and bucco-lingual bifurcations (29, 32). Studies have revealed that the majority of errors associated with AI models occurred in cases with reduced canal visibility (mainly in the apical third of roots), which is a common concern among students and dental practitioners (29, 32, 33). This error also increases when the AI is challenged to predict morphological types of root canals, such as symmetrical, asymmetrical and merging types of C-shaped canals (28), compared to only the absence/presence of C-shaped canals (33).

The potential to miss anatomical features using AI-generated detection also occurs during clinical root canal treatment when the operator may identify additional root canal orifices after careful exploration of the pulp chamber floor under mag-

nification or after troughing using ultrasonic tips. For example, the middle mesial canals in mandibular molars that cannot be identified on preoperative radiographs (Fig. 5a-c), or the detection of root canal bifurcations after negotiation using pre-curved K-files along the canal walls (Fig. 5d-g). The use of AI models to detect the endodontic map on the floor of the pulp chamber and potential locations of the root canal orifices can also be a useful tool for clinical practice and education (62).

It should be noted that case difficulty assessment tools [such as the American Association of Endodontists (AAE) case difficulty assessment form and guidelines], usually categorize the difficulty of clinical cases based on a combination of patient-related factors, tooth-related factors in addition to other factors including trauma (63). Therefore, the operator has to con-

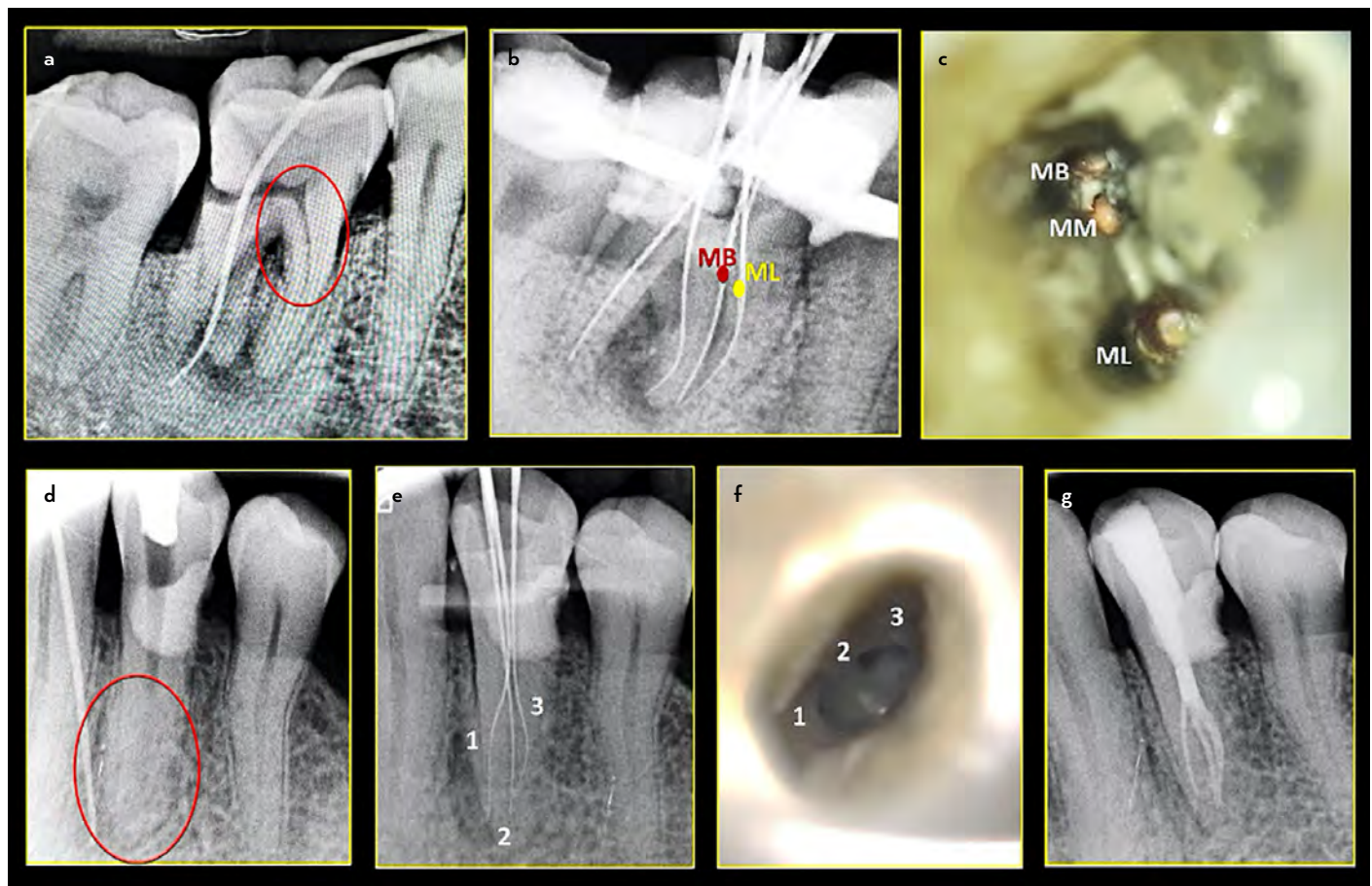


Figure 5. Challenges presented during interpretation of periapical radiographs. Upper row: Root canal treatment of a three-rooted mandibular first molar (radix entomolaris). (a) The exact root canal configuration of the mesial root is not obvious on the periapical radiograph (red circle). (b) After access cavity preparation and inserting the files for working length determination (mesial shift radiograph), it is obvious that the mesial root has two separate canals (MB and ML canals). (c) After further exploration and troughing, a middle mesial canal (MM) was identified using direct visualisation and magnification. Lower row: Root canal treatment of a mandibular first premolar with complex canal anatomy. (d) The periapical radiograph does not provide clear information about the canal configuration. (e) More information was gained after access cavity preparation and canal exploration using pre-curved K-files. (f) Three canals apically with a common coronal canal were identified by direct visualization under magnification. (g) After root canal filling the 3 canals apically are confirmed.

MB canal: mesio-buccal canal, ML canal: Mesio-lingual canal.

sider the data generated from AI models as one component of the case difficulty assessment. It is obvious that there is a need to develop AI-optimized rating guidelines, better suited to algorithmic analysis that can be augmented with the findings from the patient history and clinical examination (29).

Several studies have included the segmentation of the pulp as one of the objectives for AI models on bitewing radiographs (22, 31). Indeed, such an imaging modality does not provide adequate information for the study of root and canal anatomy since it is mainly used for the detection of proximal caries; however, it provides some information on pulp chamber anatomy such as the height of pulp horns, pulp chamber width and height, which have important clinical implications for access cavity preparations and age estimation in forensic dentistry (64). Similar to periapical radiographs, bitewings provide a 2D image of a 3D object, therefore, AI applications on this imaging method will face the challenge of attempting to provide bucco-lingual details such as locations of the pulp horns under the cusps of multi-rooted teeth.

It is well-known that dental panoramic radiographic views provide limited information on roots and canals, however, a number of studies examined the ability of AI models to detect major dental anomalies such as root dilacerations, accessory roots, Taurodontism and C-shaped canals (Appendix 2), which can aid in clinical interpretation. However, further radiographic analysis (including CBCT) is usually required if such teeth with complicated dental anomalies are scheduled for root canal treatment in order to provide more detailed information on the roots and canals (65).

Analysis of AI Applications in 3D Radiographic Imaging

A number of studies have investigated the ability of AI models to detect and classify roots and canals from data derived from CBCT scans (Appendix 3). The MB2 canal in the mesio-buccal root of maxillary molars is one of the most challenging canals for detection and negotiation (66). One study found that a deep-learning algorithm (YOLOv5x) was highly successful in detecting MB2 canals from axial CBCT sections (26). Another study examined the ability of AI models to detect missed MB2

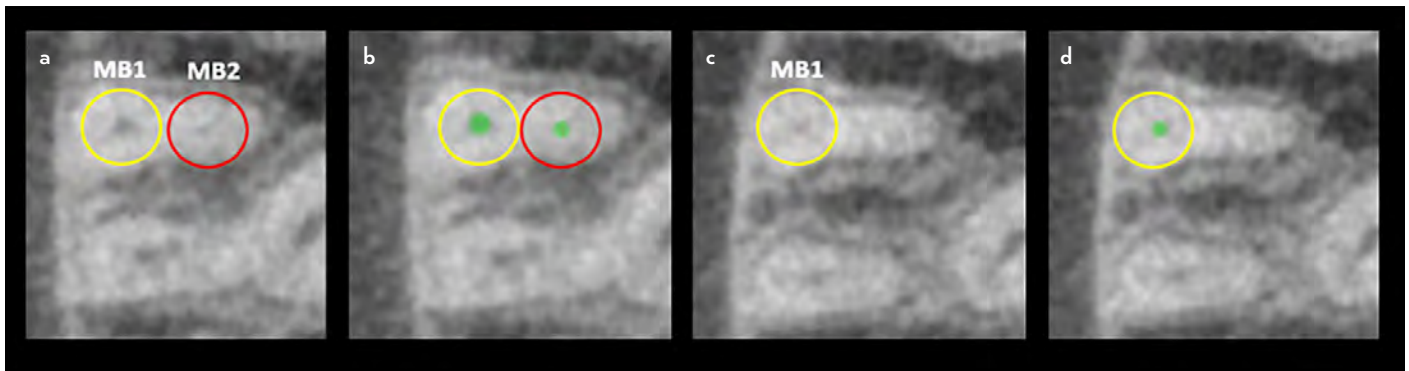


Figure 6. Challenges for detecting MB2 canals in maxillary first molars. (a, b) In some clinical situations, the operator can identify and segment both MB1 and MB2 canals (manual segmentation was done using Mimics software), (c, d) In other situations, only the MB1 can be detected and segmented, however, the operator can still predict the presence of a narrow MB2 when the MB1 is located buccally in the MB root.

MB canal: mesio-buccal canal.

canals in root filled maxillary molars (49) and concluded that in some cases, the AI model (U-Net) was able to detect missed MB2 canals while a human observer could not, mainly because of low resolution and contrast on the images. In addition, the operator found it difficult to segment the canal on all slices as it was challenging to distinguish the MB2 from the background due to beam hardening artefacts and the presence of calcifications along different areas of the canal (49).

Indeed, the beam hardening artefacts associated with the presence of root filling materials add more challenges and increase the likelihood of false-positive diagnosis both for the clinician and AI models (49). However, it should be noted that even if the operator is not able to identify the presence of an MB2 canal on one or more of the CBCT slices, the operator can still predict the presence of an MB2 by observing the path of the MB1 in which its eccentric location buccally in the MB root increases the likely presence of an MB2 in a more palatal location (Fig. 6), which can be confirmed by careful clinical exploration under magnification.

Because of the inherent limitations of CBCT images mentioned above, one CBCT study on extracted teeth examined a modified deep learning model (Basicvrs ++) to design a CBCT inter-layer correlation-based network architecture (super-resolution processing) to better utilize information from previous and subsequent slices simultaneously (46). This model improved the detection of MB2 canals (92%) compared to the CBCT group (72%) and was close to the micro-CT group (the gold standard). The use of this method for refining CBCT images in extracted teeth is promising (Fig. 7); however, this model has to be validated clinically, especially since it was not able to provide accurate predictions when dealing with very narrow root canals, which is a common limitation in current clinical imaging modalities (46).

The classification of C-shaped canals has been examined in a study using three deep learning models (20); it concluded the AI models had the potential to classify automatically C-shaped canal anatomy on CBCT images. Notably, the use of CBCT imaging in that study allowed the classification of Fan et al. (67) to be used in order to define the anatomy of C-shaped canals on axial sections (C1, C2, C3a/b, C4, and C5), which was impossible to apply in studies that examined deep learning

models on 2D radiographs. It is obvious that the inherent limitations of a given diagnostic modality (2D or 3D) play an important role in the ability of how AI models should classify specific anatomical features.

One study proposed a fully-automatic deep learning-based method to accurately identify the tooth and its root canal from CBCT data (48). The full automated segmentation was performed in a short time (2 mins), which is more convenient to the operator compared to the tedious efforts and longer time needed with manual segmentations, and would allow rapid analysis of large datasets. The researchers also presented 3D printed resin models of the 3D reconstructed teeth, which can be used for training, research and preoperative treatment planning (48).

It should be highlighted that AI models cannot “learn/identify” complex anatomical features from CBCT data derived from a limited number of labelled samples (52). Using data from axial, coronal and sagittal sections facilitate better and more accurate segmentation of roots and canals (52). The use of CBCT scans with limited field of view and small voxel sizes produce high-resolution images, which can also assist AI models to better detect canals (26). However, one study demonstrated improvement in the analysis of CBCT images using interpolation, which is a common computational process used in digital image processing to estimate unknown pixel values when an image is resized. This process refined the voxel size of the original CBCT images, and allowed the detection of small root canals that could not be recognized because of the large voxels on the original CBCT images (51). However, this interpolation process also requires training and validation using large datasets of images to provide accurate results.

Implications of AI Applications on Radiographic Imaging for Root and Canal Anatomy Education

Dental education encompasses a combination of theoretical, preclinical, and clinical training, during both didactic and clinical skills instructions (68). AI is being utilized to manage the educational process, support instructors and teaching methods, and enhance student learning assessment in theoretical education (68). In human anatomy, a number of innovative technologies have been introduced through the development

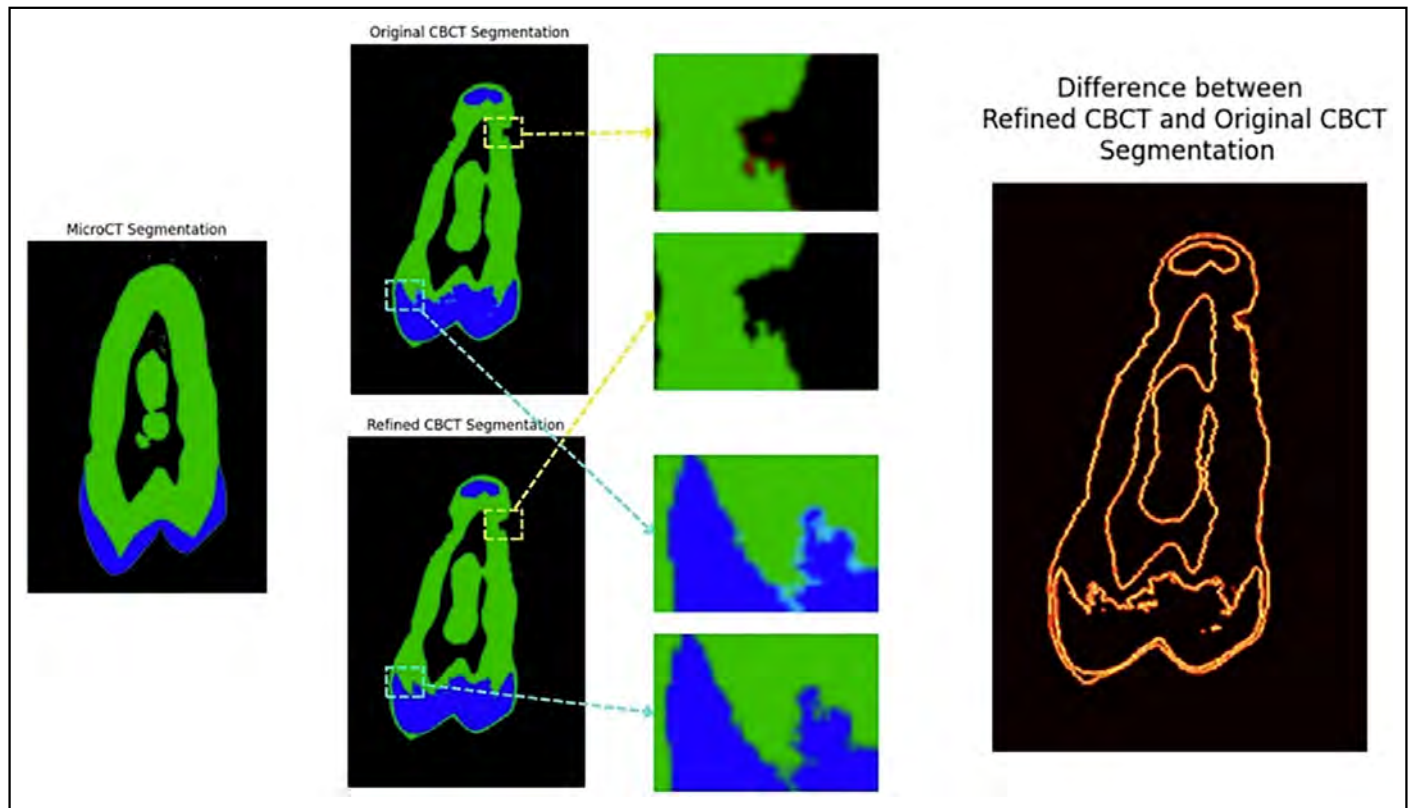


Figure 7. The process of refining of a segmented CBCT image (coronal section) of a scanned maxillary premolar tooth with reference to micro-CT imaging of the same tooth. The experimental framework was implemented using Python in Visual Studio Code (VSCode), combining image processing and visualization components. For morphometric analysis, OpenCV (v4.5) was employed. The system was equipped with an Intel Core i9-13980HX processor for high-speed computations, complemented by 16GB of DDR5 RAM (4800MHz) to handle large datasets and complex analytical operations. For accelerated graphics processing and deep learning tasks, an NVIDIA GeForce RTX 4070 GPU with 8GB GDDR6 memory was employed. This hardware configuration provided the necessary computational power for seamless execution of both image processing and visualization algorithms.

CBCT: cone beam computed tomography.

and incorporation of a range of AI tools across the spectrum of healthcare professional education (69).

The 2D radiographic imaging modalities (i.e. periapical, bitewing and panoramic views) are important teaching tools involved in the curriculum of a range of disciplines such as oral radiology, oral pathology and endodontics. This review reveals that the application of AI in the analysis of 2D imaging is a promising platform for root canal anatomy education. Deep learning models that guide students to focus on certain landmarks on enhanced 2D radiographic images may help improve their skills. Such landmarks can be either anatomical including the size of the pulp canal space, curvature of the roots and canals, canal bifurcations, calcifications of the pulp chamber (pulp stones), proximity of roots to certain structures (such as the mental foramen, mandibular alveolar canal and maxillary sinus) and dental anomalies (23, 70) (Appendix 2, 3), or pathological such as root resorption defects (14).

In both preclinical and clinical education, dental students and inexperienced early career dentists usually need help in radiographic interpretation, diagnosis and treatment planning (70). The use of real-time AI-assisted analysis as students interpret 2D radiographs could improve their diagnostic abilities (16).

This would also improve their ability during case difficulty assessments, especially in molars. Figure 8 shows an example of a manually segmented mesial and distal pulp canal space of a mandibular first molar in a 2D periapical radiographic image that demonstrates complex apical trifurcations in the distal canal and an accessory canal in the mesial root. Such AI-assisted visualizations could enhance educational outcomes by providing immediate, accurate anatomical references during preclinical training. AI also can provide automated versions for current trends and updates to classifications of root and canal anatomy of both normal and unusual variations, as well as guidelines to help students navigate through the steps involved in the management of different canal types and shapes including anomalies. This would allow dental students to receive a more personalized education with the opportunity to strengthen areas where they might need further instruction with the possibility to exchange information with other students and supervisors (70).

Several applications of 3D imaging devices in dental education have been described (70, 71). In root canal anatomy education, the 3D imaging tools (CBCT and micro-CT) have been used for teaching third-year undergraduate dental students through integration with virtual reality (72); the students were able to inspect tooth anatomy from every perspective, move

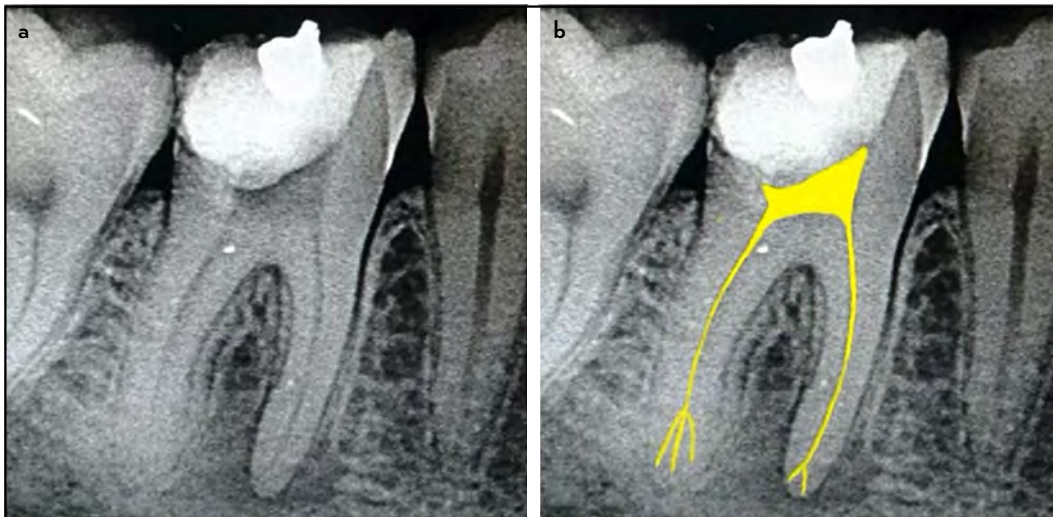


Figure 8. Future applications of AI models may help students to identify challenging anatomical landmarks, and act as a guide for case difficulty assessment. (a) Periapical radiograph of a mandibular first molar with unclear apical anatomy, (b) Manual segmentation of the mesial and distal canal space shows the suspected complex apical anatomy (trifurcation) of distal root canal.

around the tooth, look through it, or even view slices of the tooth. These favourable results were also consistent with another study that used an extended reality (XR) application illustrating the root canal anatomy of teeth (73).

The use of AI models for automated radiographic image analysis of CBCT scans provides detailed information on root and canal anatomy including their complexity, which can aid in their education as well as case difficulty assessment, diagnosis and treatment planning (71). The ability of AI models to create detailed 3D presentations of the roots and canals would help students design a customized canal instrumentation plan based on the unique anatomy of each tooth (74). This approach would minimize the loss of tooth tissue during the various stages of root canal treatment, improve treatment predictability and reduce the risk of complications, especially in complex cases (74).

Advantages and Limitations of AI Applications In Education and the Study of Root and Canal Anatomy

Results of this comprehensive review reveal that AI has potential to enhance the study of root and canal anatomy and its use in education. AI is highly accurate and can help students and clinicians to interpret images and aid treatment planning. The application of fully automated AI models paves the way for tooth analysis in a short time, which would be convenient for students and clinicians. However, there are a number of limitations that can be summarized as follows:

- The development of more advanced, accurate AI models suitable for clinical and educational applications requires large, diverse datasets for training and validation (20, 28, 42). The involvement of high-quality labelled and annotated datasets is expensive and time consuming due to the specialized expertise needed (16). Inter-observer variability when labelling further complicates the training of AI models.
- One of the key factors affecting the accuracy of deep learning models is the sample size (49). There are no clear guidelines for sample size calculation in AI-based root canal

anatomy studies, which could vary according to the area of interest and accuracy of the diagnostic tool.

- AI models do not overcome the inherent limitations of 2D diagnostic imaging tools. Clinical exploration of canals remains an important step for identification and classification of roots and canals.
- The lack of data on rare/uncommon root and canal anatomical variations (such as three-rooted maxillary premolars, double-rooted mandibular premolars and double-rooted canines) may limit the performance of AI models (16).
- Manual and semi-automated segmentation of the roots and canals performed in some of the studies is time consuming since several of the anatomical structures are ill-defined with unclear boundaries of the roots and canals (20, 42, 51), especially on CBCT images with large voxel sizes examining small canals with calcifications (20, 51). This is further challenged with imaging artefacts. The application of other image processing functions should be appraised and included if they yield better results (20).
- The AI systems should be used in dental education with caution, since it may generate incorrect and unsafe treatment recommendations if there is an over-reliance on technology and limited patient data (70).
- AI models often operate as black boxes, making it difficult to comprehend and explain how they arrived at specific conclusions or recommendations, which is one of the important concerns (71).

Direction for Future Studies

AI applications using different deep learning models with large datasets

As mentioned above, the use of small datasets is one of the limitations of AI-based root and canal anatomy studies. One study examined deep learning for a larger dataset of 805 patients collected from 4 institutes across Korea and Japan (25). Future studies should use large datasets on different tooth

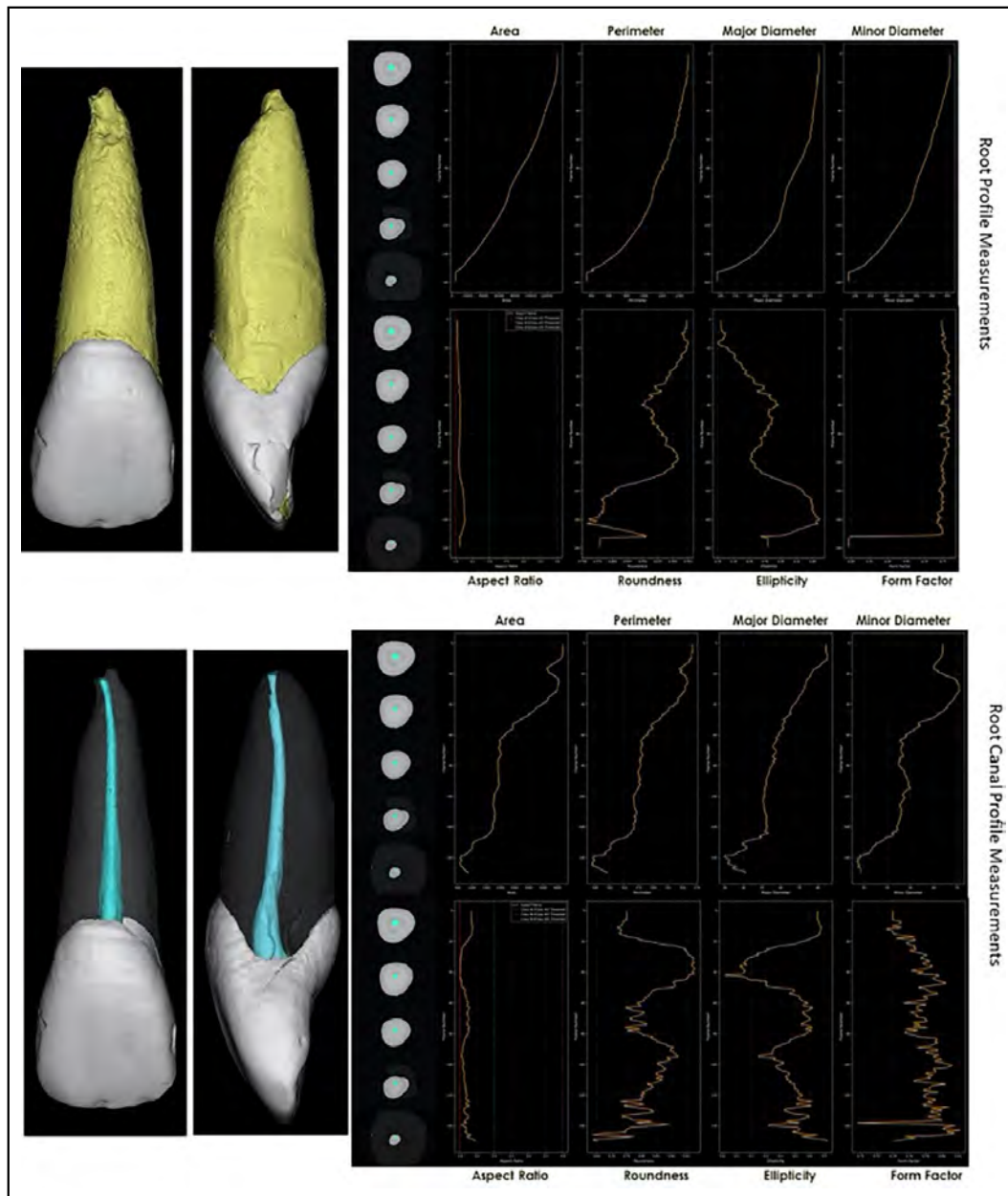


Figure 9. A number of 2D and 3D measurements in graphical format from the axial sections of root (above) and canal (below) in a maxillary central incisor. The geometric parameter calculations were performed through a Python-based image processing pipeline utilizing computer vision techniques. Root and canal profiles were segmented using HSV color thresholding with morphological dilation (5×5 kernel) to enhance structural continuity. Contour analysis was conducted via OpenCV's ellipse fitting algorithm (cv2.fitEllipse) to determine major and minor axes, while perimeter and area measurements were obtained using cv2.contourArea and cv2.arcLength. Three shape descriptors were systematically calculated: (1) aspect ratio, (2) form factor, and (3) ellipticity. The pipeline incorporated quality control measures including minimum area thresholds (10 pixels²) and contour complexity validation. Visualization outputs combined raw images with measurement overlays, maintaining clinical conventions through proper orientation of anatomical axes. All parameters were calculated across axial slices to ensure comprehensive representation of the three-dimensional dental anatomy.

HSV: Hue, Saturation and Value.

types from different institutions of the same country, different ethnic groups in other countries or designed as a multi-centre study. The development of guidelines for sample size calculation is important. The accuracy of AI training and validation using radiographic images of different parameters should be a focus for future investigations.

Further research also is needed to compare the data obtained from deep learning to other accurate analytical models such as histological sections and/or micro-CT (20). The development of fully automated deep learning algorithms may reduce subjectivity and errors, but this also requires sophisticated learning processes using large datasets. Improving the qual-

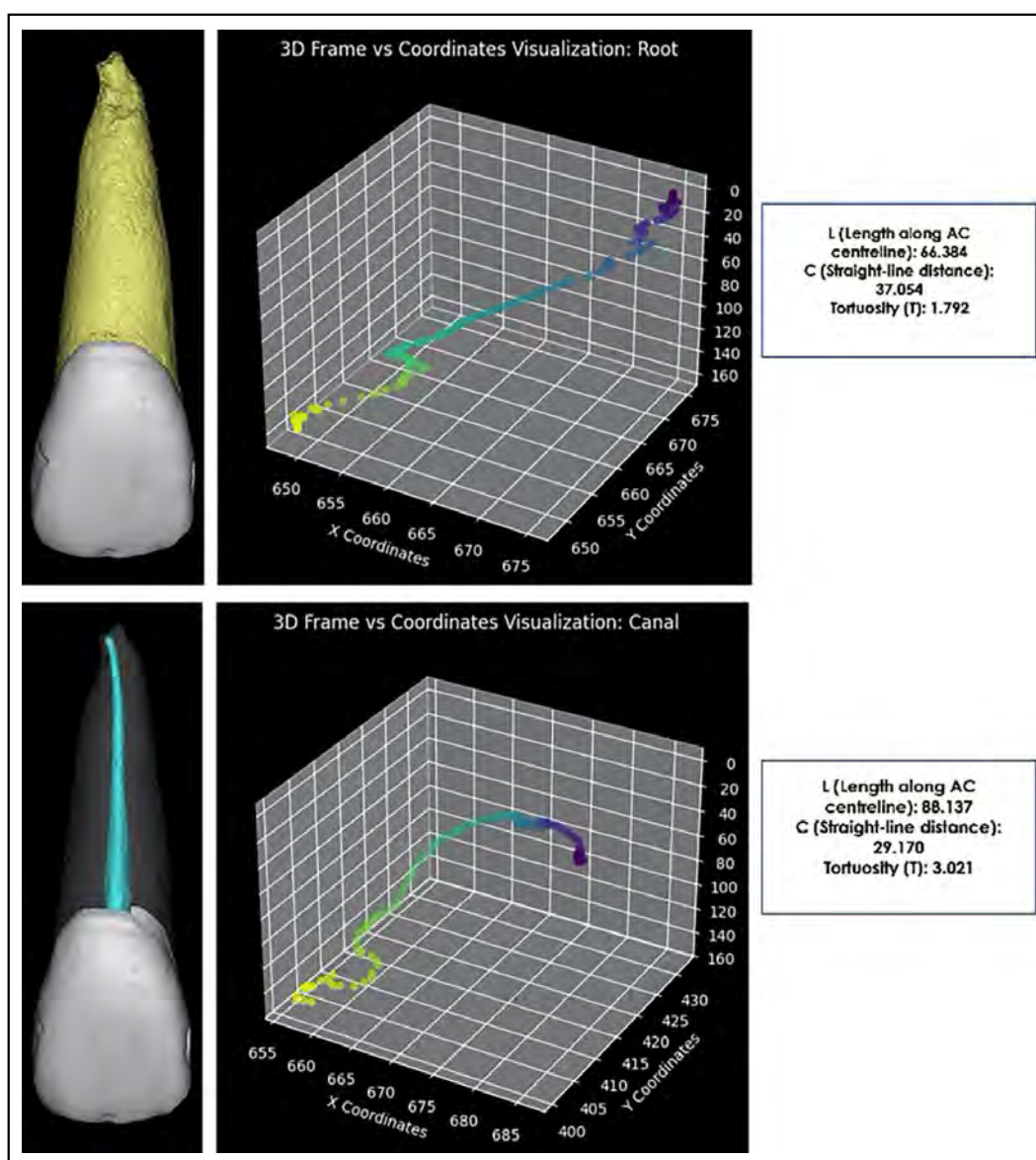


Figure 10. 3D visualization of the root and canal centrelines based on frame and coordinate data. The tortuosity analysis and visualization were implemented in Python (VSCode). The tortuosity index (T) was computed by analyzing the 3D centreline coordinates (x, y in pixel units, z as frame index) extracted from the sample video file. The index T was calculated as the ratio between the actual centreline length (L, in pixels) and the straight-line distance between the start and end points (C, in pixels), yielding a dimensionless measure of curvature (frames 0 and 150 refer to upper and lower ends of the root and canal, respectively).

ity of radiographic images would also facilitate more accurate segmentation and reconstruction (20, 51).

AI applications for quantitative measurements of roots and canals on micro-CT images

In the last two decades, micro-CT has been used extensively to study root and canal anatomy because of its non-invasive nature and high accuracy with opportunities to provide high resolution images and a wide range of 2D and 3D quantitative analyses of roots and canals including area, perimeter, aspect ratio, roundness, form factor etc. (75) (Figs. 9, 10). The future holds promise for the application of AI models for fully automated segmentation of roots and canals and to provide such quantitative analysis in a single AI-powered software designed for detailed measurements that can be

used for research purposes and education. The use of AI models during the classification of root and canal systems can be another direction of future research. Figure 11 shows the application of an AI model (ResNet 50) when classifying root canal anatomy configurations using the Ahmed et al. (59) coding system. The application of AI with this coding system could pave the way for classifying teeth with complex canal anatomy in a fast and accurate manner. Future research is needed to validate this potential application in education, research and clinical practice.

Translation of AI-based root canal anatomy studies to dental education and clinical practice

The data generated from AI studies hold promise for the use of AI in various applications for the identification and classification

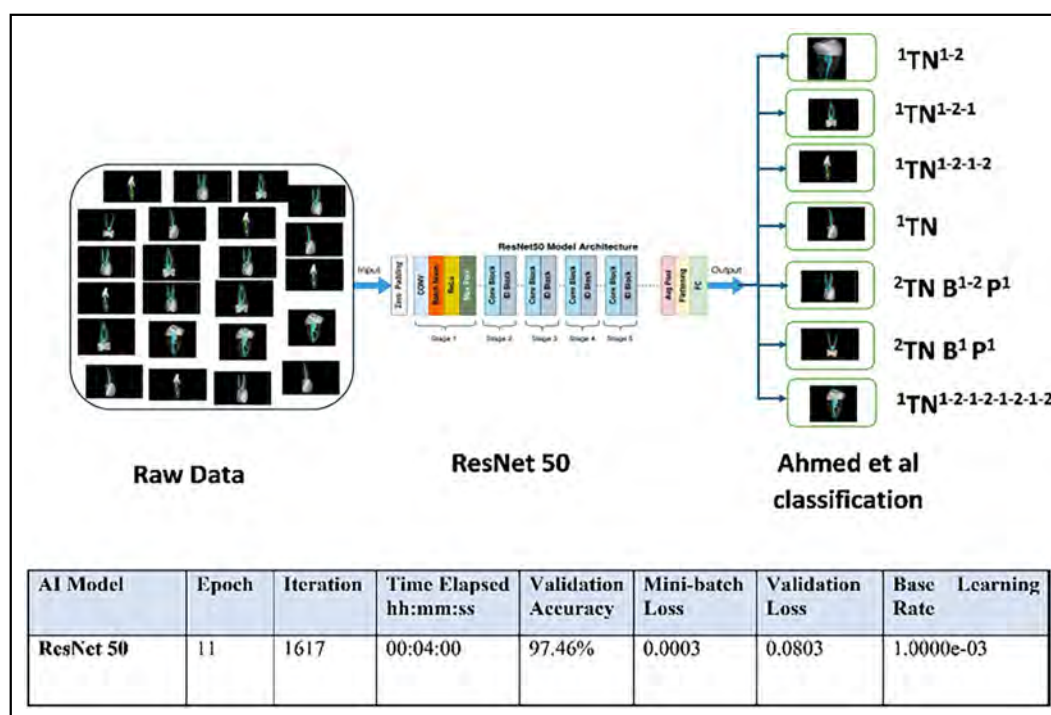


Figure 11. The use of micro-CT scanned teeth images for training ResNet 50 model to classify the samples according to Ahmed et al. coding system (59).

CT: Computed tomography.

of root and canal anatomy and the education of students. Deep learning was reported to have similar accuracy for the detection of C-shaped canals to those of the specialists (36), or even more accurate in identifying C-shaped canals compared with endodontic residents (28). However, whether this deep learning process using several imaging modalities would enhance knowledge on variations of root and canal anatomy, and performance of dental students and general dental practitioners in terms of preclinical training, clinical diagnosis, treatment planning, root canal treatment procedures and clinical outcomes is unclear.

The use of deep learning processes to analyse images obtained from the dental operating microscope (such as identifying locations of root canal orifices through the inter-orifice grooves) and correlating them with data from radiographic findings (including possible outlines for missed roots) is a potential direction for future research (Fig. 12). This is important since root canal treatment is a multi-stage procedure in which new information is gained at every step from initial pre-operative evaluation, access cavity preparation, canal exploration and preparation to root canal filling (Fig. 12).

The integration of AI-assisted interpretation of root canal anatomy with both patient-related factors and tooth-related factors is a direction for future development of a complete case difficulty assessment tool based on AI-optimized rating guidelines (29), which can be embedded in the teaching curriculum for under- and postgraduate dental students as well as general dental practitioners.

In summary, there is a potential for AI applications to enhance clinical translation in root and canal anatomy studies by bridging the gap between research findings and practical applica-

tions through enhanced image quality and interpretation with high consistency and precision. The automated segmentation of root canals provided by AI models (that can continuously learn from new data and refine its accuracy for detection and interpretation) can help students and practitioners to identify the normal and unusual anatomical variations and suggest root canal treatment strategies for teeth with simple as well as complex and curved canals. The automated segmentation of the surrounding anatomical structures (i.e. maxillary sinus and inferior alveolar canal) can alert practitioners when those structures are close to the root apex of related teeth scheduled for root canal treatment. AI-powered platforms can be used to train dental students using virtual root and canal anatomy models developed from training on large datasets of human teeth. However, all of these potential applications require further validations on different clinical scenarios to confirm its usefulness and impact on treatment outcomes.

Ethical considerations for AI applications in the study and education of root and canal anatomy

While AI has become increasingly implemented in endodontic research and education, there are ethical concerns around its usage (16, 76). It is important that users and stakeholders consider these concerns when developing, implementing, or receiving AI applications (76). For studies related to root and canal anatomy, it is imperative for patients to understand that the data obtained from clinical imaging diagnostic tools may be utilised in the implementation of AI models. Given the inherent need of large datasets for AI training and validation of different root and canal anatomical variations, there is a potential risk for data leaks and unauthorised exposure or manipulation of personal details, especially for 2D periapi-

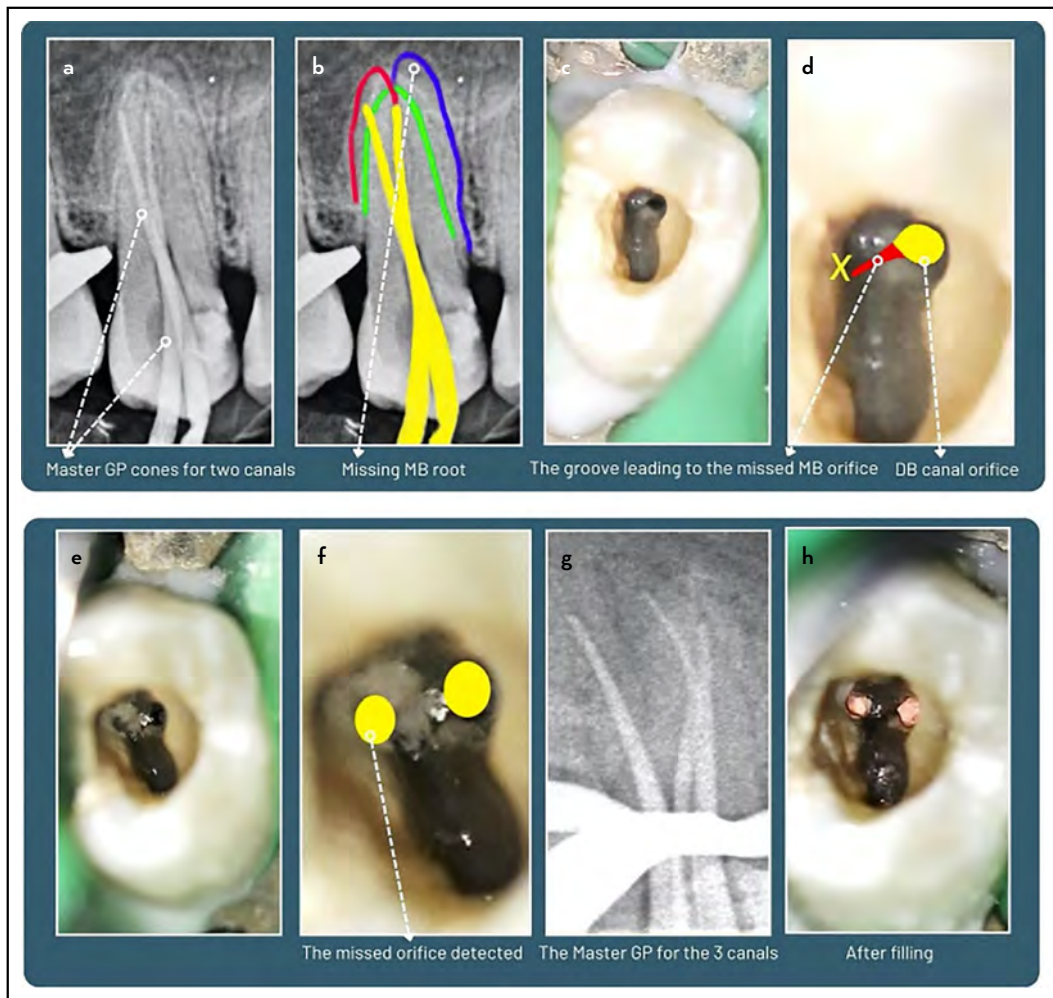


Figure 12. Future directions for AI model applications to detect missed roots and canals. An example of a missed root following root canal treatment of a three-rooted maxillary first premolar (the labelling and segmentation including the outlines of the roots, canal orifices and gutta percha filling material were done manually by an endodontist). (a) A trial gutta-percha radiographic view. (b) After manual labelling. With the aid of deep learning methods of the root and canal outlines in 2D imaging, the AI model may aid in detection of missed roots and canals. The AI models can be also trained with other images obtained from the dental operating microscope on the anatomical landmarks of the pulp chamber floor and canal orifices including the grooves between orifices which can be correlated with radiographic images to help identifying missed canals (c, d). (e-h) Clinical detection of the missed MB root canal orifice and root canal filling.

MB canal: mesio-buccal canal.

cal and panoramic imaging as well as 3D CBCT scans (16). The use of encrypted files and strict access controls is important to preserve patients' identity and details. It is a shared responsibility among users and dental educators to ensure the effective and sustainable implementation of ethics in AI applications. Recently, one study developed a fundamental ethical principles framework and a checklist protocol relevant to dental AI applications (76).

Summary

- AI has the capacity to enhance diagnostic accuracy, streamline case difficulty assessments, and support treatment planning by providing detailed insights into anatomical complexities that may be overlooked in traditional analyses.
- The application of AI in dental education offers promising avenues for personalising student learning and improving

diagnostic competencies through real-time feedback and enhanced image interpretation.

- Despite these advancements, the inherent limitations of AI models must be acknowledged. Challenges include the reliance on high-quality, diverse datasets for training and validation, difficulties in detecting rare or complex anatomical variations, and the "black-box" nature of many AI models that obscures their decision-making processes. Additionally, AI systems are constrained by the limitations of imaging modalities, such as the inability of 2D radiographs to provide critical bucco-lingual information, and the artefacts and resolution constraints of CBCT images.
- Future research should focus on developing robust deep learning models trained on large, multicentre datasets that reflect diverse populations and a wide range of tooth mor-

phology. The integration of AI with advanced imaging techniques, such as super-resolution processing and micro-CT validation, holds promise for improving segmentation accuracy and enabling more precise quantification of anatomical features. Moreover, embedding AI-optimised case difficulty assessment tools into educational curricula could enhance clinical decision-making among students and practitioners, particularly for complex endodontic cases.

This review has limitations. The authors followed a systematic search strategy for the selection of relevant studies. However, neither quality assessment nor meta-analysis was undertaken for the studies involved in this review. The data generated from AI root and canal anatomy studies mainly focused on the segmentation of parts of the tooth structure – enamel, dentine and root canals (Appendix 1-3) with no/limited information on anatomical characteristics such as root canal configurations, isthmuses and accessory canals. Some studies have examined anatomical features such as C-shaped canals, however, the data sources and imaging tools are different. The study of MB2 canals in maxillary molars is limited. The overall data generated from AI studies related to root and canal anatomy can be considered as preliminary findings that require more studies on larger datasets from which a meta-analysis can be undertaken.

CONCLUSIONS

AI applications for the study and teaching of root and canal anatomy on a range of 2D and 3D imaging modalities are promising; however, more studies are needed with larger datasets to provide more accurate, consistent deep learning models. Students and clinicians should understand the inherent limitations of AI data generated from 2D and 3D imaging devices. Future studies are needed to assess what effect deep learning models have on the diagnostic and clinical skills of students and dental practitioners when managing teeth with a range of anatomical complexities.

Disclosures

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APPENDIX 1. A summary of AI studies that used periapical and bitewing radiographs for the study of roots and canals

Authors/ Country	Dataset size (n)	Imaging model	AI model used	Labelling phase	Training phase	Performance levels	Main results and findings
Wu et al., 2024 (28) (China)	271 teeth	271 teeth	Pretrained VGG19, EfficientNet-b5, ResNet18, and ResNet50 implemented either with multiangle projection or python package Augmentor method.	C-shaped fused-rooted mandibular second molars (merging, symmetrical, and asymmetrical types).	70% training (190), 20% testing (54), 10% validation (27).	VGG19 Augmentor= 0.7170, VGG19 multiangle projection= 0.7358, ResNet18 Augmentor= 0.7358, ResNet18 multiangle projection= 0.7925, ResNet50 Augmentor= 0.7358, ResNet50 multiangle projection= 0.7547, EfficientNet-b5 Augmentor= 0.7736, EfficientNet-b5 multiangle projection= 0.7736. Inception v2 and DINO models had the best cross-validation accuracy at 91.05% and 91.04%, respectively. VGG16 and Inception v2 models had the best AUC score, at 94.36% and 92.42%, respectively. VGG16 model had the best overall precision, recall, and accuracy across all models.	• CNNs are more accurate in identifying root canal morphology compared with endodontic residents. • CNNs successfully provided 3D root canal morphology from 2D radiographs. • ResNet18 model with multiangle projection showed highest accuracy among all other AI models (79.25%). • The baseline VGG16 model attained 87.62% accuracy in classifying difficulty. • Self-supervised pretraining did not improve performance. • Regression predicted scores with ± 3.21 score error. • All models outperformed human raters, with poor inter-examiner reliability.
Karkehabadi et al. 2024 (29) (Iran)	1386 radiographs	2D Periapical radiographs	VGG16, ResNet18, ResNet50, and Inception v2.	Root curvature angle, Radix ento/paramolaris, Canal splits in middle or apical third, Mandibular anterior or premolar with 2 roots, S-shaped canal curvature, and C-shaped morphology.	202 cropped tooth images were used for the test using a random stratified sampling method. The model was trained using 10-fold stratified cross-validation to prevent overfitting and assess generalizability.	The mean accuracy levels for root canal detection (single vs combined loss functions): • F1 score = 0.68 vs 0.80 • precision[†] = 0.85 vs 0.81 • sensitivity[†] = 0.64 vs 0.79 • specificity[‡] = 0.99 vs 0.99	• Combined loss functions (Focal Dice, Generalized Dice Focal, and Cross-Entropy Dice) demonstrated significantly superior performance to overcome the imbalance between the predicted and true data compared with the single loss function (Dice, Focal, and Tversky) trained with the three AI models. • The combined Dice focal loss showed the highest performance to detect the label structures among all loss functions.
Büttner et al., 2024 (22) (Germany)	1625 radiographs	Bitewing radiographic views	DeepLabv3+ (ResNet152), LinkNet (Densenet121) models using six different loss functions (Dice, Focal, Focal Dice, Tversky, Generalized Dice Focal, and Cross-Entropy Dice). The AI models were with implemented with MONAI 1.2 and Pytorch 2.0.	Root canal, enamel, dentine, filling, and crown	60% (975) training, 20% (325) validation, and 20 % (325) testing.	Accuracy= 85.45% Precision = 86% Recall= 84% F1 score = 85%	• Hybrid Ensemble Classifier showed better improvements in accuracy, precision, recall, and F1-score compared with other used learning classifiers.
Latke and Narawade, 2023 (30) (India)	900 radiographs	2D Periapical radiographs	Hybrid Ensemble Classifier utilized stacking technique (combining different classifiers: Support Vector Machine (SVM), Decision Tree, Naïve Bayes, Logistic Regression, XGBoost (Extreme Gradient Boosting), and K-Nearest Neighbors (KNN).	Narrow canal, curved canal, long root and calcified root canal.	80% training (720), and 20% testing (180).	Sensitivity = 0.9843 precision = 0.9429 F1 score = 0.9631	• AI model was able to automatically recognize the pulp space in bitewing radiographs with high accuracy levels.

APPENDIX 1. Cont.						
Authors/ Country	Dataset size (n)	Imaging model	AI model used	Labelling phase	Training phase	Performance levels
Lee et al., 2023 (32) (South Korea)	598 radiographs	2D Periapical radiographs	Proposed 17-convolu- tional layer model implemented with self-attention layer and residual blocks, namely as "Periapical Radiograph Explanatory System with Self- Attention Network- PRESSAN-17" compared with residual neural network (RESNET)-18.	Canal visibility, previous root filling, root rest, presence of proximal teeth, full coverage restoration, coronal defect, and periapical radiolucency in single- rooted premolars	75% training (498) and 25% validation (100).	Accuracy= 76% Sensitivity = 83.90% precision =88% F1 score = 85.80% Specificity= 23.10%
			EfficientNet-B0 model used with real images alone and combined with generative adversarial network (GAN)-generated images from the real images. The StyleGAN2- adaptive discriminator augmentation (ADA)) was the GAN model for enhancing the quality of preapical radiographs and increase the trained data.	Classifying C-shaped versus non-C-shaped root canals of mandibu- lar second molars	70% training (1019), 20% validation (291), 10% testing (146).	Accuracy (real alone vs combined with GAN)= 0.805 vs 0.890. Sensitivity (real alone vs combined with GAN)= 0.800 vs 0.910. Specificity (real alone vs combined with GAN)= 0.810 vs 0.870. Precision (real alone vs combined with GAN)= 0.849 vs 0.884.
Yang et al., 2023 (33) (South Korea)	1456 radio- graphs (650 patients)	2D Periapica radiographs and CBCT as control				
Ari et al., 2022 (34) (Turkiye)	1169 radiographs	2D Periapical radiographs	U-Net model imple- mented with the PyTorch library (version 1.4.0).	Dental Caries (352), crown (91), Dental Pulp (975), dental filling (758), periapical. Lesion (266), and root canal filling (627).	80% training (781), 10% testing (97), 10% validation (97).	Sensitivity = 0.97 precision = 0.87 F1 score = 0.92
			CNN (VGG16), SIFT-SVM, and transfer learning (ResNet50, VGG16, and VGG19) with and without ROI region extraction.	Teeth with and without root canal therapy. Teeth without root canal therapy were used as control.	Improved levels, when ROI region extraction is applied on dataset com- pared with no ROI region extraction is applied, as follows: • CNN (VGG16): Accuracy= 90.1% to 96.3%; AUC= 0.96 to 0.99. • SIFT-SVM algorithm: Accuracy= 92.7% to 97.2%; AUC= 0.96 to 0.99. • Transfer learning: Accuracy for VGG16= 93.9% to 96.9%; Accuracy for VGG19= nearly 95% for both; Accuracy for ResNet50= nearly 80% for both; AUC for VGG16 and VGG19 = 0.99 for both; AUC for ResNet50= 0.86 to 0.88.	Learning methods can successfully detect root canal with high accu- racy levels (above 95%), particu- larly when ROI region extraction is applied. • Application of ROI region extraction on the dataset before training the models is significantly improved the overall accuracy. • In transfer learning model, the VGG16 and VGG19 showed higher training effect compared with ResNet50 model, reflecting that ResNet50 requires higher size of dataset.
Xu et al., 2022 (35) (China)	920 radiographs	2D Periapical radiographs				

APPENDIX 1. Cont.						
Authors/ Country	Dataset size (n)	Imaging model	AI model used	Labelling phase	Training phase	Performance levels Main results and findings
Yang et al., 2022 (36) (Korea)	1000 Periapical and panoramic radiographs and 740 CBCT images	2D peri- apical and panoramic radiographs. The CBCT images were used as a gold standard.	EfficientNet (convolu- tional neural network).	Predicting C-shaped canal variation of mandibular second molars.	90% training (900: 504 non-C-shaped and 396 C-shaped canals) and 10% testing (100: 56 non-C-shaped and 44 C-shaped canals).	The highest model performance (which only the root portion) versus specialist performance: • Periapical: AUC = 0.98 vs 0.95 • Accuracy = 0.91 vs 0.95 • Sensitivity = 0.88 vs 0.95 • Specificity = 0.95 vs 0.94 • Precision (Positive prediction) = 0.94 vs 0.94 • Negative prediction = 0.89 vs 0.95 • F1 score= 0.91 vs 0.94 • Panoramic: AUC = 0.95 vs 0.96 • Accuracy = 0.86 vs 0.96 • Sensitivity = 0.85 vs 0.97 • Specificity = 0.88 vs 0.95 • Precision (Positive prediction) = 0.89 vs 0.95 • Negative prediction = 0.83 vs 0.97 • F1 score= 0.87 vs 0.96 • Accuracy= 93% EfficientNet efficiently detected the C-shaped canal when the image showed only the root por- tion of the tooth compared with the images showing the whole tooth. EfficientNet showed similar accuracy levels of C-shaped canal detection to those of the specialists. Using AI model may help in diag- nosis of C-shaped canal configuration.
Saghiri et al., 2012 (37) (USA)	50 teeth	2D Periapical radiographs and stereomi- croscope as gold standard.	Artificial neural network (ANN).	Locating minor apical foramen of single-rooted mandibular incisors and second premolars (root curvature <30); Human extracted teeth were fixed to dry skull.	72% training (36) and 28% testing (14).	• ANN can correctly determine the location of the apical foramen in the radiographs. • ANN can be helpful for working length determination as second opinion.
Saghiri et al., 2012 (38) (USA)	50 teeth	2D Periapical radiographs and stereomi- croscope as gold standard.	Artificial neural network (ANN).	Locating minor apical foramen for working length determination of single-rooted teeth of human cadaver (<30 root curvature).	Used a ready set-up from their previous study "Saghiri et al., 2012a"; 72% training (36) and 28% testing (14).	• ANN can accurately determine the correct working length through locating minor apical foramen. • ANN is more accurate method com- pared than endodontists' decision based on radiographs. • No significant difference between the results of ANN and gold stan- dard stereomicroscope.

†: Calculated from the supplementary file of the study. ADA: adaptive discriminator augmentation, AI: Artificial intelligence, ANN: Artificial neural network, AUC: Area under receiver operating characteristics curve, CNNs: Convolutional neural networks, DDEs: Dental diagnostic expert system, GAN: Generative adversarial network, KNN: K-nearest neighbors, Micro-CT: Micro-computed tomography, NR: Not reported, PRESSAN: Periapical radio-graph explanatory system with self-attention network, RESNET: Residual neural network, SVM: Support vector machine.

APPENDIX 2. A summary of AI studies that used panoramic radiographs for the study of roots and canals

Authors	Dataset size (n)	AI model used	Labelling phase	Training phase	Main results and findings
Jin et al. 2024 (39) (China)	730 patients (1444 MFMs) (367 teeth were three-rooted and 1077 teeth were two-rooted)	ResNet 101, ResNet 50, DenseNet 201, MobileNet-v3, and Inception-v3	Three-rooted MFMs	A ratio of 7:3 was used for training and validation datasets.	• ResNet-101 demonstrated superior diagnostic performance (accuracy, sensitivity, and specificity were 87.5%, 83.6%, and 88.9%, respectively), and the AUC value attained was 0.907, significantly higher than that of all other models (all $P < 0.01$). • DenseNet-201 showed the lowest diagnostic performance among the five models (all $p < 0.01$), with an AUC value of 0.701 and an accuracy of 73.2%. • The performance of the CNNs diminished when using image patches containing only the distal half of MFMs, with AUC values ranging between 0.680 and 0.800. The diagnostic performance of the two clinicians was poorer, with AUC values of 0.680 and 0.632, respectively. • In Group A, all five networks exhibited satisfactory diagnostic performance, with AUC values ranging from 0.875 to 0.916 and accuracies ranging from 81.8 to 86.7%. No statistical differences were detected among the five CNNs. • In Group B, the AUC values reached 0.984–0.996, and the accuracies ranged between 94.5% and 98.1%. • CNNs outperformed dental professionals in classification performance, and the AUC values achieved by dental specialist, novice dentist, and dental graduate student were only 0.806, 0.767 and 0.730, respectively. • The accuracies for Workflows 1, 2, and 3 were 0.770, 0.755, and 0.839, respectively. • The precisions for detecting C-shaped canals for Workflows 1, 2, and 3 were 0.693, 0.676, and 0.802, respectively. • Considering the detection performance of all models, the mAP, accuracy, precision, recall, and F1 scores were recorded up to 0.92, 0.72, 0.91, 0.87 and 0.83, respectively. • The sensitivity, precision, and F1-score values of taurodont tooth segmentation were 0.8650, 0.7898, and 0.8257, respectively. • The accuracy and AUC values of C-shaped root canals on the image data of mandibular second molars were 0.9838 and 0.996, respectively.
Jin et al. 2024 (40) (China)	1340 patients Group A: 730 (1453 MSMs) Group B: 610 (1211 MSMs)	ResNet 101, ResNet 50, DenseNet 121, DenseNet 161, and Inception-v3	C-shaped canals in MSMs	• A ratio of 7:3 was used for training and validation datasets. • In Group A, conventional panoramic images and CBCT images were derived from the same patients. • In Group B, the patients underwent CBCT examinations in the absence of available panoramic images; CBCT images were acquired and utilized to generate simulated panoramic images.	• 80% training, 20% validation.
Yang et al. 2024 (25) (Korea, Japan)	402 patients (750 MSMs teeth) (South Korea), 303 (600 teeth) (3 different centers in Japan)	Workflow 1: EfficientNet Workflow 2 and 3: YOLOv7	C-shaped canals in MSMs	80% training, 10% testing, 10% validation.	
Celik and Celik 2023 (41) (Türkiye)	983 teeth in 636 panoramic radio-graphs	19 models were used	Root Dilaceration	80% (348) training, 10% (43) testing, 10% (43) validation.	
Duman et al. 2023 (24) (Türkiye)	434 images	U-Net model	Taurodontism	80% (348) training, 10% (43) testing, 10% (43) validation.	
Zhang et al. 2022 (42) (China)	725 images (364 non-C-shaped canals, 361 C-shaped canals)	A proposed neural network structure to classify root canal types	C-shaped canals in MSMs	• From the initial data set, 30% of images of each category were tested separately. • A data set composed of 114 images of non-C-shaped root canals and 108 C-shaped root canals was generated. • Then, the remaining images (250 images of non-C-shaped root canals and 253 images of C-shaped root canals) were used for training.	
Jeon et al. 2021 (43) (Korea)	1020 patients (2040 MSMs), 887 C-shaped canals, 1153 non-C-shaped canals.	Xception architecture	C-shaped canals in MSMs	80% (1632) training, 20% (408) testing.	• The accuracy, sensitivity, specificity, and precision were 95.1, 92.7, 97.0, and 95.9%, respectively. • Grad-CAM analysis showed that the model mainly identified root canal shapes converging into the apex to predict the C-shaped canals, while the root furcation was predominantly used for predicting the non-C-shaped canals. • AlexNet demonstrated an accuracy of 87.4%, sensitivity of 77.3%, specificity of 97.1%, a positive predictive value of 96.3%, and a negative predictive value of 81.8%. • GoogleNet demonstrated an accuracy of 85.3%, sensitivity of 74.2%, specificity of 95.9%, a positive predictive value of 94.7%, and a negative predictive value of 80.0%. • The AUCs of the deep learning systems using AlexNet and GoogleNet were 0.87 and 0.85 respectively, while that of the radiologists was 0.74.
Hiraiwa et al. 2019 (44) (Japan)	760 MFMs (400 patients)	AlexNet, GoogleNet, Radiologist	Accessory distal roots in MFMs	80% training, 20% testing.	

MFMs: Mandibular first molars, MSMs: Mandibular second molars, AUC: Area under receiver operating characteristics curve, CNNs: Convolutional neural networks

APPENDIX 3. A summary of AI studies that used CBCT and micro-CT images for the study of roots and canals

Authors	Dataset size (n)	Imaging model	AI model used	Labelling phase	Training phase	Main results and findings
Slim et al. 2024 (45) (Belgium)	66 patients (166 molars)	CBCT	U-Net model	Segmentation of the root canals in mandibular molars	(86 molars) training, (20 molars) validation, (60 molars) testing.	• The AI-driven tool achieved high accuracy, with a DSC of 88% for first molars and 90% for second molars. • The 95% Hausdorff distance (HD) was lower for AI-driven segmentation (0.13) compared to manual segmentation (0.21). • Regarding time efficiency, AI-driven (4.3 s) and R-AI segmentation (139 s) methods were the fastest, compared to manual segmentation (2349 s). • The sensitivity of the MB2 canal segmentation model was 0.92. The precision, F1 score and AUC were 0.83, 0.87 and 0.84, respectively. • The mAP value at 0.5 inter-over union (IoU) was found as 0.88. • The deep-learning algorithm used showed a high success in the detection of the MB2 canal. • In 4-canal maxillary first molar, the identification of MB2 was 72% (18/25) in the CBCT group, 92% (23/25) in the super-resolution CT group, and 100% (25/25) in the micro-CT group. • The deep learning model helps to optimize the root canal morphology of extracted teeth in CBCT, and it may be helpful for the identification of MB2 in the maxillary first molar.
Duman et al. 2024 (26) (Turkiye)	153 patients (922 axial CBCT scans)	CBCT	YOLOv5	Detection of MB2 in maxillary molars	80% (1054) training, 10% (134) testing, 10% (133) validation.	
Ji et al. 2024 (46) (China)	171	CBCT (Extracted teeth)	Basicvsr11	Volume of hard tissue (V1), Volume of pulp chamber and root canal system (V2), Length of visible root canals under orifice (VL-X), and intersection angle between coronal axis of canal and long axis of tooth.	40 maxillary first molars as the training set and 40 maxillary first molars and 91 other teeth as the external test set.	
Tan et al. 2024 (47) (China)	314 patients	CBCT	U-Net model	Segmentation of the enamel, dentine and dental pulp in incisors, canines and molars.	70% training, 10% validation, 20% testing.	• The authors' model can automatically segment tooth enamel, dentine and dental pulp from CBCT images with high Dice and precision values.
Wang et al. 2023 (48) (China)	14 patients (67 teeth)	CBCT	Authors' model DentalNet PulpNet	Segmentation of the root canals in maxillary and mandibular anterior teeth and premolars.	A training set of 5 subjects with 21 teeth, a validation set of 2 subjects with 8 teeth, and a test set of 7 subjects with 27 teeth.	• The DentalNet was able to identify and segment the tooth, which was followed by pulp chamber and root canal space segmentation using PulpNet. • The workflow was applied on two clinical cases (severely curved canal and severely narrow canal), and it took 2 mins to obtain the 3D model of tooth and root canal effectively.
Albitar et al. 2022 (49) (USA)	57 patients (102 teeth)	CBCT	U-Net model	Presence and absence of MB2 (obturated and unobturated canals).	90% training, 10% testing.	• The overall detection performance revealed a sensitivity of 0.71, a specificity of 0.98 with an accuracy of 0.84. • Despite favourable results, the AI algorithm is affected by metallic artifacts and canal calcifications.
Yang et al. 2022b (50) (China)	Not reported	CBCT	U-Net model	Enamel, dentine, cementum, dental pulp cavity, cortical bone, cancellous bone and other tissues.	80% training, 20% validation	• The time of the automatic segmentation process for each CBCT was less than 13 min. The Dice of the evaluation reference image was 98%. • The U-net model combined with the watershed method can effectively segment the teeth.
Lin et al. 2021 (51) (China)	n=30	CBCT and micro-CT	U-Net Neural Network	Segmentation of the root canals in single-rooted single and second maxillary and mandibular premolars.	Training = 25, Test = 5	• The segmentation accuracy of the experimental group measured by the Dice similarity coefficient, precision rate, recall rate, average symmetric surface distance, and Hausdorff distance were 96.20%, 97.31%, 95.11%, 0.09 mm and 1.54 mm in the tooth and 86.75%, 84.45%, 89.94%, 0.08 mm and 1.99 mm in the pulp cavity, respectively. • This study proposed a novel data pipeline based on micro-CT data that can be used to improve the segmentation accuracy of the U-Net segmentation model for tooth and pulp cavity on CBCT images.
Duan et al. 2021 (52) (China)	20 patients	CBCT	U-Net model	Segmentation of the root canals in single and multi-rooted teeth.	Not mentioned.	• The experimental results showed that the proposed method can obtain an average dice 95.7% for ST, 96.2% for MT and 88.6% for pulp of ST, 87.6% for pulp of MT.

APPENDIX 3. Cont.

Authors	Dataset size (n)	Imaging model	AI model used	Labelling phase	Training phase	Main results and findings
Sherwood et al. 2021 (20) (India)	135 patients	CBCT	U-Net, Residual U-Net, Xception U-Net	C-shaped canal anatomy in MSMs.	70% training and validation, 30% testing	• Both Xception U-Net and residual U-Net performed significantly better than U-Net. • The mean sensitivity values were 0.786 Xception U-Net, 0.746 for residual U-Net, and 0.720 for U-Net. • The mean positive predictive values were 77.6% for U-Net, 78.2% for residual U-Net, and 80.0% for Xception U-Net. • The proposed 3D U-Net had higher segmentation performance than other methods (Lower average surface distance (ASD) and maximal surface distance (MSD) than other methods).
Zhang et al. 2021 (53) (China)	78 patients	CBCT	3D Mask RCNN 3D U-net V-net ROI locator+ Dice U-net Proposed ROI locator+Dice U- net+Dice Local	Segmentation of the root canals.	The training = 51 CBCT images with 9120 slices. The test set = 17 CBCT images with 4530 slices	• Applying to the testing dataset, the segmentation accuracy measured by the intersection over union (IOU), dice similarity coefficient (DICE), average precision rate (APR), average recall rate (ARR), and average symmetrical surface distance (ASSD) are 0.914, 0.955, 95.8%, 95.3%, 0.145 mm, respectively. • The F1 Score, AUC, sensitivity, specificity and accuracy were 0.7324, 0.9174, 0.8271, 0.9997 and 0.9996, respectively.
Li et al. 2020 (54) (China)	29 patients	CBCT	UNET Model Training Recurrent neu- ral network	Tooth root segmentation in maxil- lary and mandibular teeth.	Training = 24 CBCT containing 1160 images Testing = 5 sets of CBCT contain- ing 361 Images.	• The proposed method can extract tooth pulp cavity regions from teeth efficiently. The segmentation results of the authors' method are more accurate compared to other methods.
Dumont et al. 2020 (55) (USA)	40 patients	CBCT	UNET Model Training	Segmentation of the root canals in mandibular teeth.	10 models were trained and for each model, 9 folds (36 scans) were used for training and one- fold (4 scans) was used as the validation set.	• The proposed method can extract tooth pulp cavity regions from teeth efficiently. The segmentation results of the authors' method are more accurate compared to other methods.
Wang et al. 2019 (56) (China)	10 patients (50 teeth)	CBCT	Reciprocal cross entropy method (RCE), Active contour- based method (AC), Region grow- ing method (RG) Level set method (LS) Authors' method	Segmentation of the root canals in incisors, canines, premolars and molars.	Not reported.	• The results showed better detection of anatomical features, such as the size, shape, or curvature of the root canal using CNN approaches over reconstruction-based methods.
Hatvani et al. 2018 (57) (Hungary, France)	17 teeth	CBCT	A subpixel network and U-net	Segmentation of the root canals in incisors, canines, premolars and molars.	5680 slices of 13 teeth were selected for the training sets, and four other teeth (an incisor, a pre- molar, and two molars) provided 1824 slices for the test sets.	• The results showed better detection of anatomical features, such as the size, shape, or curvature of the root canal using CNN approaches over reconstruction-based methods.

CBCT: cone beam computed tomography, MB2: Second mesiobuccal canal, AUC: Area under receiver operating characteristics curve, CNNs: Convolutional neural networks, DSC: Dice similarity coefficient

Healing Outcome of Different Aqueous-based Calcium Hydroxide Intracanal Medicament in Patients with Pulpal Necrosis and Symptomatic Apical Periodontitis: A Randomised Controlled Trial

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ABSTRACT

Objective: This study aimed to assess and compare the healing outcome associated with different aqueous-based calcium hydroxide intracanal medicaments in patients with pulpal necrosis and symptomatic apical periodontitis.

Methods: Seventy five patients with pulpal necrosis and symptomatic apical periodontitis in permanent mandibular molar teeth were selected as the part of this study. The participants were randomly allocated to three groups, each comprising 25 patients, based on the type of intracanal medicament used during the treatment procedure. Group 1 consisted of calcium hydroxide (CH) mixed with 0.9% saline (NS), Group 2 contained CH combined with 2% lidocaine, and Group 3 included CH with 2% chlorhexidine (CHX). The Periapical Index Score was utilized to assess the healing of periapical lesions in pre-operative and post-operative periapical radiographs at 3-month intervals for 12 months. The Kruskal-Wallis test was used to determine the significance, with Post Hoc Dunn tests for multiple comparisons.

Results: At the 12-month follow-up, the CH+CHX group demonstrated significantly improved periapical healing, with a mean PAI score of 1.57 ± 0.66 , compared to CH+LA (2.27 ± 0.63) and CH+NS (2.48 ± 0.79), with Kruskal-Wallis $p < 0.05$. The mean time to achieve a healthy periapical status ($PAI \leq 2$) was shortest in the CH+CHX group (8.10 ± 3.28 months), followed by CH+NS (8.23 ± 3.28 months) and CH+LA (8.25 ± 3.31 months), with the multivariate Log-Rank test indicating a statistically significant difference among the groups ($p < 0.05$).

Conclusion: The findings of this study indicate that CH when combined with 2% CHX as an aqueous vehicle demonstrated superior healing of periapical lesions in patients with pulpal necrosis and symptomatic apical periodontitis compared to saline or lidocaine.

Keywords: Calcium hydroxide, chlorhexidine, root canal medicament, periapical lesion

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HIGHLIGHTS

- The combination of calcium hydroxide with 2% chlorhexidine showed noticeably higher healing rate of periapical lesions compared to saline or lidocaine.
- A robust methodology with 75 participants randomised into three groups ensured a reliable comparison of different aqueous-based calcium hydroxide formulations.
- This study demonstrated that the choice of intracanal medication affects the healing outcome in patients with pulpal necrosis and symptomatic apical periodontitis.

INTRODUCTION

Apical periodontitis refers to inflammation of the periodontium at the root apex, which can be acute or chronic, caused by an immune-mediated inflammatory response to the infection lo-

cated within or adjacent to the root canal system (1, 2). It results in loss of bone structure in the apical and periapical regions of the infected tooth (3). As the necrotic root contents lie beyond the body's natural defence mechanism, intervention

is necessary to treat such infections (4). Non-surgical endodontic therapy is considered the most conservative and treatment of choice for pulpal necrosis and symptomatic apical periodontitis (3). The primary aim of root canal therapy is to eradicate inflamed and infected pulpal tissue, fostering an environment that promotes healing, and thereby prevents the advancement of periapical pathology, eventually supporting the long-term survival of teeth (5).

The root canal system undergoes significant ecological changes during an endodontic therapy, which plays an important role in eliminating the microflora. Chemo-mechanical preparation focuses on eliminating necrotic pulpal tissue and infected dentin, with mechanical instrumentation combined with antimicrobial irrigation effectively eradicating most microorganisms within the root canal system. However, some microorganisms may persist, necessitating the use of an inter-appointment intracanal dressing to achieve thorough disinfection of the root canal system prior to obturation (6).

The use of an intracanal medicament is recommended in the treatment of pulpless teeth for several advantages: (a) to eradicate residual bacterial within the root canal; (b) to inhibit bacterial regrowth between appointments; and (c) to serve as a physicochemical barrier, preventing reinfection of the root canal and restricting nutrient availability to any remaining bacteria. Since they remain in the canal longer than irrigants, they can potentially reach bacteria residing in hard-to access areas within root canal system (7).

Calcium Hydroxide (CH), first introduced by Hermann in 1920 is widely utilized in endodontics as an intracanal medicament because of its strong alkaline nature and potent antibacterial activity against oral pathogens (7, 8). Its mechanism of action is attributed to the release of hydroxyl ions, which elevate the pH of the surrounding environment, leading to the inactivation of bacterial lipopolysaccharides found in the outer membrane of Gram-negative bacteria (9). The choice of vehicles combined with CH powder plays a crucial role in the dissociation process as they impact the rate of ionic dissociation, which in turn affects the solubilization and resorption of the paste at different rates by the periapical tissues and within the root canal (10). Typically, three types of vehicles are employed in the preparation of CH paste: viscous, aqueous, and oily (11).

Various aqueous vehicles that have been used to mix CH powder are distilled water, local anesthetic solution (LA), normal saline solution, normal saline (NS), chlorhexidine (CHX) gluconate, and more to enhance the antimicrobial activity of CH. These components encourage a high degree of solubility when the paste interacts with tissues and tissue fluids (11). Some water-soluble compounds that release Ca^{2+} and OH^- ions progressively over a long period of time are known as viscous vehicles. These include glycerine, propylene glycol, and polyethylene glycol. In contrast to aqueous vehicles, they have a lower solubility (11). Oily vehicles, such as camphor, olive oil, silicon oil, and metacresyl acetate have inadequate diffusion and solubility. Their major limitations include decreased alkalinity, slower ion release, limited antimicrobial action, and challenging removal, increasing the chance of canal blockage (11).

The rate of ionic dissociation is inversely proportional to the viscosity of vehicle (9). In clinical scenarios, an aqueous vehicle containing CH paste is indicated when rapid ionic release is required at the onset of treatment, while a viscous vehicle is utilized for controlled and sustained ionic release (12).

While CH is a commonly used intracanal medicament for patients with pulpal necrosis and symptomatic apical periodontitis, most existing studies have largely focused on its efficacy to reduce post-operative pain or bacterial load (13, 14). However, there is currently no universally accepted protocol regarding the optimal clinical protocols and materials used during root canal therapy (14).

A notable gap in the literature pertains to the limited clinical evidence evaluating the impact of different vehicles used for intracanal medicaments on periapical healing. Given that the vehicle can influence the dissociation, diffusion, and antimicrobial effectiveness of CH, understanding this interaction is crucial for optimising therapeutic outcomes in endodontic treatment.

This randomised controlled trial was designed to test the hypothesis that the type of aqueous vehicle - NS, LA, or CHX, when used in combination with CH, significantly affects the rate and extent of periapical healing in patients with pulpal necrosis and symptomatic apical periodontitis.

MATERIALS AND METHODS

Study Design, Trial Registration, and Ethical Approval

The study was officially registered in the Clinical Trials Registry of India (Ref no. CTRI/2024/06/069197) and was approved by the Institutional Ethical Committee OF Manav Rachna Dental College I (MRIIRS/MRDC/FDS/IEC/2023/23) dated 16.05.2023. Both verbal and written informed consent were acquired from the participants. The study followed the CONSORT guidelines (Fig. 1). This study was carried out at the Department of Conservative Dentistry and Endodontics of Manav Rachna Dental College in accordance with ethical guidelines by following the Declaration of Helsinki's principles.

Sample Size Calculation

A sample size of 75 participants (25 per treatment group) was recommended for the present study based on the assumptions and estimates derived from prior studies. With an effect size of $d=0.3683$ and a statistical power of 80% ($1 - \beta = 0.80$), this sample size was determined to achieve a 95% confidence level (15).

Recruitment and Eligibility Criteria

Inclusion criteria

- Healthy individuals between 18–45 years of age with endodontic diagnosis of pulpal necrosis and symptomatic apical periodontitis in mandibular molar teeth were included in the study.
- Thermal testing (Endofrost; Coltene, Whaledent Pvt Ltd, Mumbai, India) and electric pulp testing (Digitest; Parkell Inc. Edgewood, NY, USA) were utilized to evaluate pulp sensitivity. The diagnosis was confirmed by the lack of pulpal bleeding during the access opening.

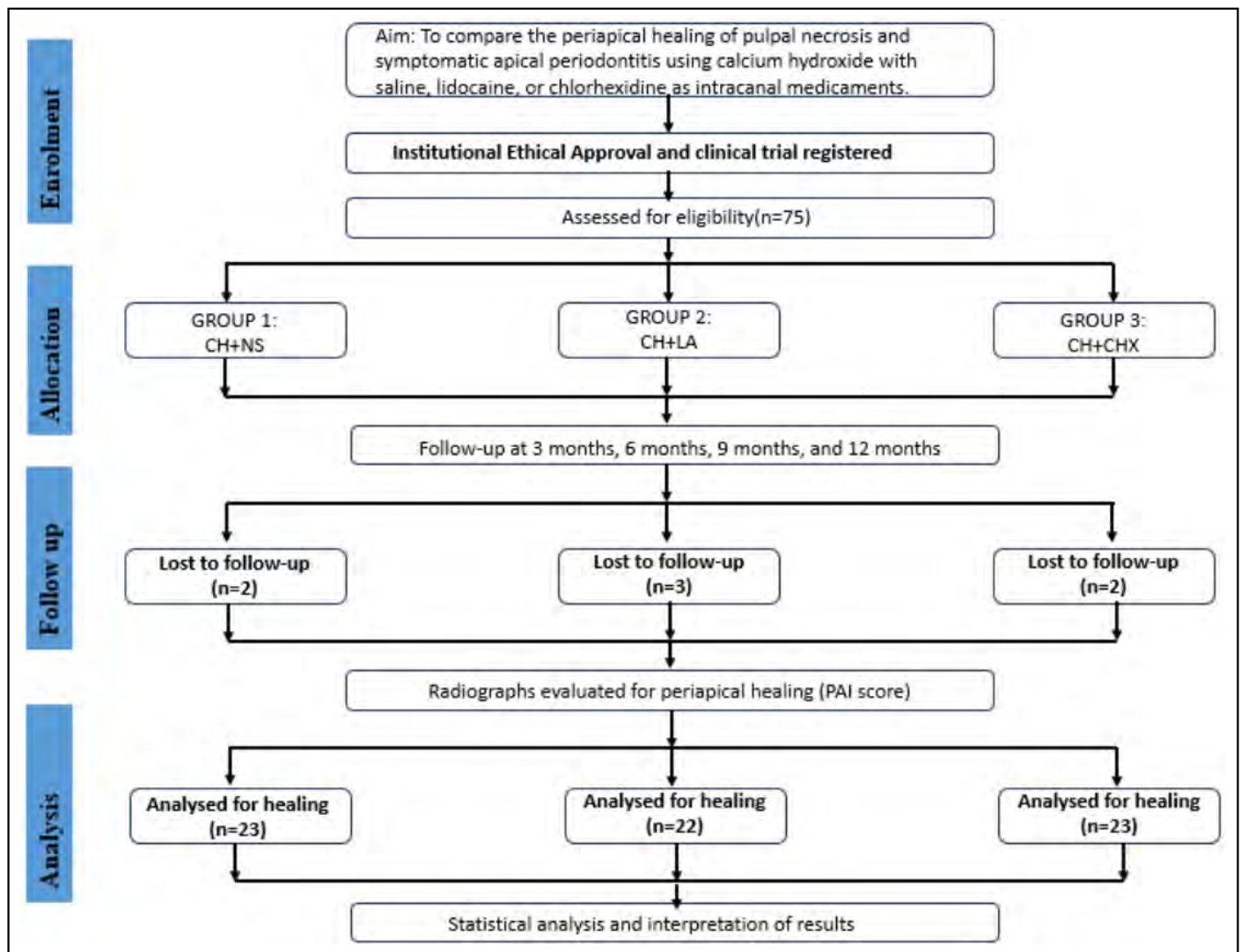


Figure 1. CONSORT flowchart of the study.

CH+NS: Calcium hydroxide and normal saline, CH+LA: Calcium hydroxide and local anesthetic solution, CH+CHX: Calcium hydroxide and chlorhexidine.

- Teeth were tender on percussion with periapical score (PAI) of more than or equal to 2.

Exclusion criteria

- Patients with systemic disorders, periodontal conditions or pregnant patients.
- Patients taking steroids or antibiotics.
- Patients with a history of allergy to any of the components of local anesthesia, CHX, EDTA or sodium hypochlorite.
- Previously endodontically treated or initiated teeth.
- Severely damaged or mutilated teeth.
- Patients not willing to participate in the study and did not sign the consent form.

Randomisation and Blinding

Block randomisation method was carried out to obtain a list of numbers from Research Randomizer Software (<https://www.randomizer.org/>). This online tool randomly allocated 75 participants into 3 groups (n=25), sealed, sequentially numbered, opaque envelopes were prepared before recruitment. During the treatment, the intracanal medicament was freshly prepared

by an assistant and provided to the operator to ensure blinding. Both the operator and the patient were blinded to the specific medicament used. Additionally, outcome assessment was performed by two independent evaluators who were also blinded to the treatment allocation. Therefore, this study followed a triple-blind design, minimising potential bias at the levels of patient care, treatment administration, and outcome evaluation.

Clinical Procedure

Before the clinical procedure, a pre-operative periapical radiograph was recorded to match the inclusion criteria of PAI score more than or equal to 2. A single operator carried out all clinical procedures in compliance with accepted standards. Local anesthesia was administered with 2% lidocaine and 1:80,000 adrenaline (Lignospan Special; Septodont, Maidstone, UK).

The tooth was isolated under a rubber dam, and the access cavity was prepared using a sterile round diamond point. Pulp extirpation was carried out, and apical patency was established. Working length was measured using apex locator (Root ZX Mini; J Morita Corp., Kyoto, Japan) and a #10 K-file (Mani Inc., Tochigi, Japan) and confirmed by radiograph. Biomechanical

TABLE 1. Demographic factors analysis

	Calcium hydroxide and normal saline	Calcium hydroxide and chlorhexidine	Calcium hydroxide and local anesthetic solution	p value
Age distribution (Kruskal-Wallis test)				
Female	30.15±9.54	30.25±6.98	29.08±6.41	0.9275
Male	27.83±6.93	25.00±6.95	28.17±6.19	0.3700
Gender distribution (Chi-Square-test)				
Female	12	13	13	0.9481
Male	13	12	12	
Tooth Number distribution (Chi-Square-test)				
36	8	5	7	0.9573
37	7	7	7	
46	4	6	6	
47	6	7	5	

preparation of canals was performed using the crown-down technique with ProTaper Gold rotary files (Dentsply Maillefer) upto #F2 for mesial canals and #F3 in larger distal canals. For larger distal canals, it was prepared up to size 35 and 0.04 taper. During preparation, a copious irrigation with 3% NaOCl was used, and the canals were flushed for 1 minute with 2 ml of 17 % EDTA using a 30G side-vented needle placed at 1 to 2 mm short of the working length.

Study interventions

The canals were dried using paper points and the freshly prepared intracanal medicament was placed with a size #25 lentulo spiral (Dentsply Maillefer, Oklahoma, USA) that was 25 mm long and at 2 mm short of the working length. According to the randomisation and allocation of groups, the subjects were divided into 3 groups:

Calcium Hydroxide & saline group (CH+NS):

A slurry like paste with P/L ratio of 1:1 was prepared with CH powder (Prevest Denpro; Digiana, Jammu, India) with 0.9% of NaCl (BRAUN Medical Pvt Ltd, Mumbai, India).

Calcium Hydroxide & local anaesthesia group (CH+LA):

A slurry-like paste of CH and 2% Lidocaine (Lignospan Special, Septodont, Maidstone, UK) with a P/L ratio of 0.25mg/0.15ml was used.

Calcium Hydroxide & chlorhexidine group (CH+CHX):

A slurry-like paste of CH and 2% CHX (Prevest Denpro Ltd, Samba, India) in a P/L ratio of 1:1 was used.

The access cavity was then temporarily sealed with Intermediate Restorative Material (Dentsply Ltd, Weybridge, UK). The patients were recalled after 7 days. All the treated teeth were confirmed to be asymptomatic at the time of the second appointment, indicating resolution of clinical symptoms before the next procedure. During the second appointment, the paste was removed with Hedstrom files (Mani Inc, Brussels, Germany) followed by copious irrigation with 5.25% NaOCl (Septodont, Navi Mumbai, India). Canals were dried using paper points and obturated with gutta-percha and sealer (AH plus; Dentsply Sirona, Gurugram, India). The access cavity was restored with flowable and nanohybrid composite resins. An immediate postoperative radiograph was taken using preset exposure

parameters with a Rinn paralleling device (XCP Instruments, Elgin, IL) and processed to maintain angulation consistency and standardization. The patients were instructed to take Ibuprofen 400mg as required. Follow-up clinical and radiographic examinations were performed at every 3-month interval up to 12 months using the same parameters as the initial examination.

Outcome Evaluation

Pre-operative and follow-up radiographs were evaluated by two independent observers (AG and DA) who were unaware of the patient's treatment group, using the PAI scoring system. The final outcome for each tooth was determined based on the root with the highest PAI score. If discrepancies arose, the observers reviewed their assessments together to reach a consensus. Teeth were classified as either healed (PAI ≤2) or improved (with a decreased PAI score).

Statistical Analysis

At a 95% confidence level, statistical analysis was performed using Python 3.11.4 (Python Software Foundation, Wilmington, DE, USA) and Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). This statistical analysis plan involves performing data preprocessing, including cleaning, reduction, and integration, followed by assumption validation for normality using the Shapiro-Wilk test and QQ plot. Descriptive analysis of demographic data was done using mean ± STD. The Kruskal-Wallis test was applied to evaluate the distribution of patients' ages across genders within the three treatment groups and to assess the significance between the three treatment groups (1) CH+NS, (2) CH+LA, (3) CH+CHX across over a 12-month follow-up period with 3-month intervals. This test was employed to assess significance, with Post Hoc Dunn tests for multiple comparisons. For time-period significance, multivariate and pairwise log-rank tests was used. Survival analysis was performed using the Kaplan-Meier curve, with results and interpretations summarized at a 5% significance level.

RESULTS

Out of 25 participants in each group, a total of 7 participants were lost to follow-up: 2 from CH+NS group, 3 from CH+LA group and 2 from CH+CHX group. The results showed no significant difference as the p-values exceeded the significance threshold (0.05), in age among the patients (Table 1), as well as at baseline

TABLE 2. Results of Kruskal Wallis test and Post hoc Dunn test for pairwise comparisons

Time period	CH+CHX	CH+LA	CH+NS	Kruskal Wallis Test p value	CH+CHX vs CH+LA	CH+CHX vs CH+NS	CH+LA vs NS
Pre	2.84±0.62	2.72±0.61	2.84±0.62	0.7290	0.4911	1.0000	0.4911
3 months	2.52±0.59	2.76±0.60	2.84±0.62	0.1492	0.151	0.0627	0.6708
6 months	2.26±0.54	2.70±0.63	2.75±0.74	0.0218*	0.0446*	0.0191*	0.7536
9 months	1.96±0.56	2.41±0.50	2.48±0.79	0.0160*	0.039*	0.0228*	0.8508
12 months	1.57±0.66	2.27±0.63	2.48±0.79	0.0002*	0.0058*	0.0006*	0.5309

*: p<0.05. CH+CHX: Calcium hydroxide and chlorhexidine, CH+LA: Calcium hydroxide and local anesthetic solution, CH+NS: Calcium hydroxide and normal saline

TABLE 3. Results of multivariate and pairwise log-rank test comparing the time taken by treatment groups to achieve a healthy healing score

Treatments	Time (in months) to achieve score ≤2	Multivariate log-rank test p value	CHX vs LA pairwise log-rank test p value	CHX vs SALINE pairwise log-rank test p value	LA vs SALINE pairwise log-rank test p value
CH+CHX	8.10±3.28	0.00032*	0.0050*	0.0050*	0.5100
CH+LA	8.25±3.31				
CH+NS	8.23±3.28				

*: p<0.05. CH+CHX: Calcium hydroxide and chlorhexidine, CH+LA: Calcium hydroxide and local anesthetic solution, CH+NS: Calcium hydroxide and normal saline

and 3-month follow-up. In contrast, the p-values at 6, 9, and 12 months were all less than 0.05, indicating significant differences between the three treatment groups as shown in Table 2.

The Post Hoc Dunn test (Table 2) results indicated no statistically significant difference between the CH+LA group and H+NS group across all time points. However, significant differences were observed between CH+CHX vs. CH+LA group and CH+CHX group vs. CH+NS group, as their p-values were below the significance level (0.05). Additionally, compared to the CH+LA group and the CH+NS group, the CH+CHX group appears to be a more effective intracanal medicament.

A Multivariate Log-Rank test (Table 3) was used to assess the time taken by different treatment groups to achieve a healthy healing score (≤2). With a p-value <0.05, it can be concluded that there are significant differences between treatment groups in the time required to reach a healthy healing score. Specifically, the Pairwise Log-Rank test (Table 3) indicated that the CH+CHX treatment group showed significant differences when compared with the CH+LA and CH+NS group. Descriptive statistics further revealed that the CHX group achieved healthy healing in the quickest time compared to the LA and NS group.

To determine the probability of achieving healthy healing in the shortest time, the Kaplan-Meier curve was utilized. For this analysis, an event was considered to occur if the PAI score had reduced to 2 or less. The Kaplan-Meier curve demonstrated (Fig. 2) that the CH+CHX group had the highest probability of achieving healthy healing within the minimum time period when compared to the other groups.

Figure 3 illustrates the changes in periapical radiolucency observed in the CH+CHX treatment group over the 12-month follow-up period.

DISCUSSION

The healing process depends on restoring the structure and function of areas influenced by intrinsic or extrinsic factors (16). It begins with an inflammatory response, which helps control and manage tissue damage. It progresses toward resolution as the immune system effectively clears the underlying immunogen responsible for triggering the tissue reaction, ultimately restoring homeostasis (17). Inflammation in the periapical region is triggered as a defence mechanism to neutralize the antigen. This inflammatory response also stimulates bone resorption, creating space for the infiltration of immune cells. These immune cells then organize into a structured barrier, effectively isolating and containing the infection (16). Bone resorption and formation are continuous processes governed by the coordinated activity of osteoclasts, osteoblasts, and osteocytes, influenced by systemic and local factors. However, during apical periodontitis, bone homeostasis is disrupted, leading to an accelerated rate of bone resorption (18).

The approach to managing apical periodontitis is influenced by the presence of periapical pathosis and its progression over time (19). The ideal healing process of apical periodontitis should be asymptomatic, marked by the restoration of periradicular tissue integrity, the absence of radiographic abnormalities, and the biological sealing of the foramina through cementum deposition, ensuring complete structural and functional recovery (20). Most periapical lesions heal following meticulous non-surgical endodontic treatments. To evaluate the healing potential, a follow-up period of at least 6 to 12 months after root canal therapy is recommended (21).

A range of therapeutic factors (e.g. biomechanical preparation, quantity and type of irrigant solution, intracanal dressing, root canal filling, apical limit of obturation or expansion of the apical foramen), systemic condition of the patient and physi-

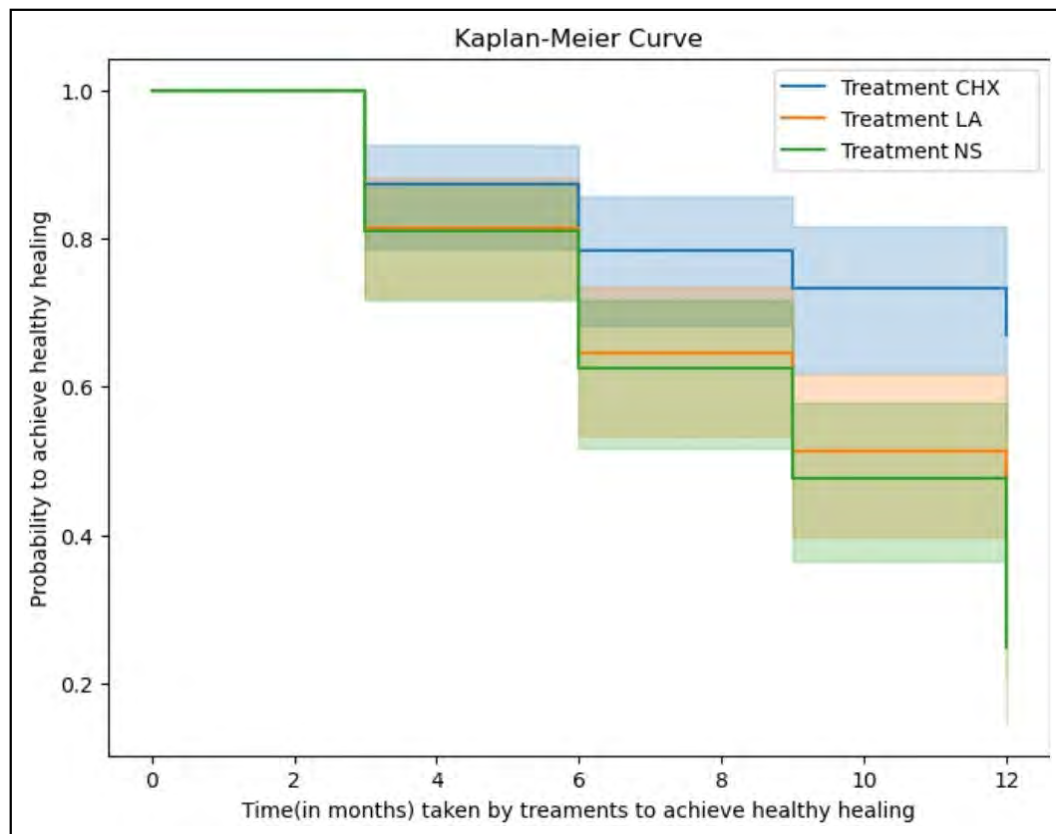


Figure 2. Kaplan-Meier Curve illustrating the probability of different treatments in improving healing scores over various time

CHX: Chlorhexidine, LA: Local anesthetic solution, CH+NS: Calcium hydroxide and normal saline, NS: Saline

ology (e.g. chronic diseases or age) can disturb the periapical healing process and affect the prognosis of treatment.

Various medications have been formulated and utilized as intracanal dressings (22). CH is a widely used intracanal medicament in non-surgical root canal treatment. Numerous studies have highlighted its effectiveness in eliminating bacteria, promoting healing, and facilitating the resolution of periapical lesions when applied after thorough canal preparation and irrigation (23). The high pH of CH, resulting from the release of hydroxide ions, disrupts the structural integrity of bacterial cytoplasmic membranes. Additionally, it indirectly affects anaerobic microorganisms in the root canal through an interaction reaction between calcium ions and aqueous carbon dioxide. Furthermore, CH facilitates the degradation of bacterial lipopolysaccharides, contributing to its antimicrobial efficacy (16). Based on the published research, CH as an intracanal medicament significantly affects the healing of periapical lesions (24).

Despite its numerous indications and advantages, CH also has certain limitations (25). Research indicates that *Enterococcus faecalis* can resist the effects of CH for approximately ten days, while its antimicrobial action remains minimal against facultative anaerobes and *Candida* species but highly effective against obligate anaerobes (26, 27).

CHX is a cationic biguanide with peak antimicrobial efficacy in a pH range of 5.5 to 7.0 (28). It functions by interacting with the negatively charged phosphate groups on microbial cell walls

and the molecule's positive charge. This disruption alters the cell's osmotic balance, increasing cell wall permeability and allowing CHX molecules to penetrate the bacteria (29, 30). At 0.2% concentration, CHX causes leakage of potassium and phosphorus, while at 2%, it becomes bactericidal by causing the cytoplasmic contents to precipitate, leading to cell death (30).

Local anesthetics like lidocaine and prilocaine have been explored for their potential use as intracanal medicaments in root canal therapy owing to their antimicrobial, anti-inflammatory, and analgesic properties (31, 32). However, there is a lack of extensive literature on its antimicrobial properties. A study demonstrated that mixing CH with lidocaine HCl can effectively reduce postoperative pain in teeth with irreversible pulpitis and symptomatic apical periodontitis (33). Hence, the present study employed lidocaine as an aqueous vehicle along with CH as an intracanal medicament for the management of periapical lesions.

This study aimed to evaluate and compare the healing outcomes among three different groups based on aqueous-based combinations of CH as an intracanal medicament: CH+0.9% NS, CH+ 2%LA, and CH+2%CHX. Systemically healthy patients of age 18–45 were enrolled in the study to avoid the confounding influence of age-related factors on the healing outcomes of periapical lesions and to control the systemic influences on the periapical healing. This study was designed to establish a baseline score for healing in systemically healthy patients.

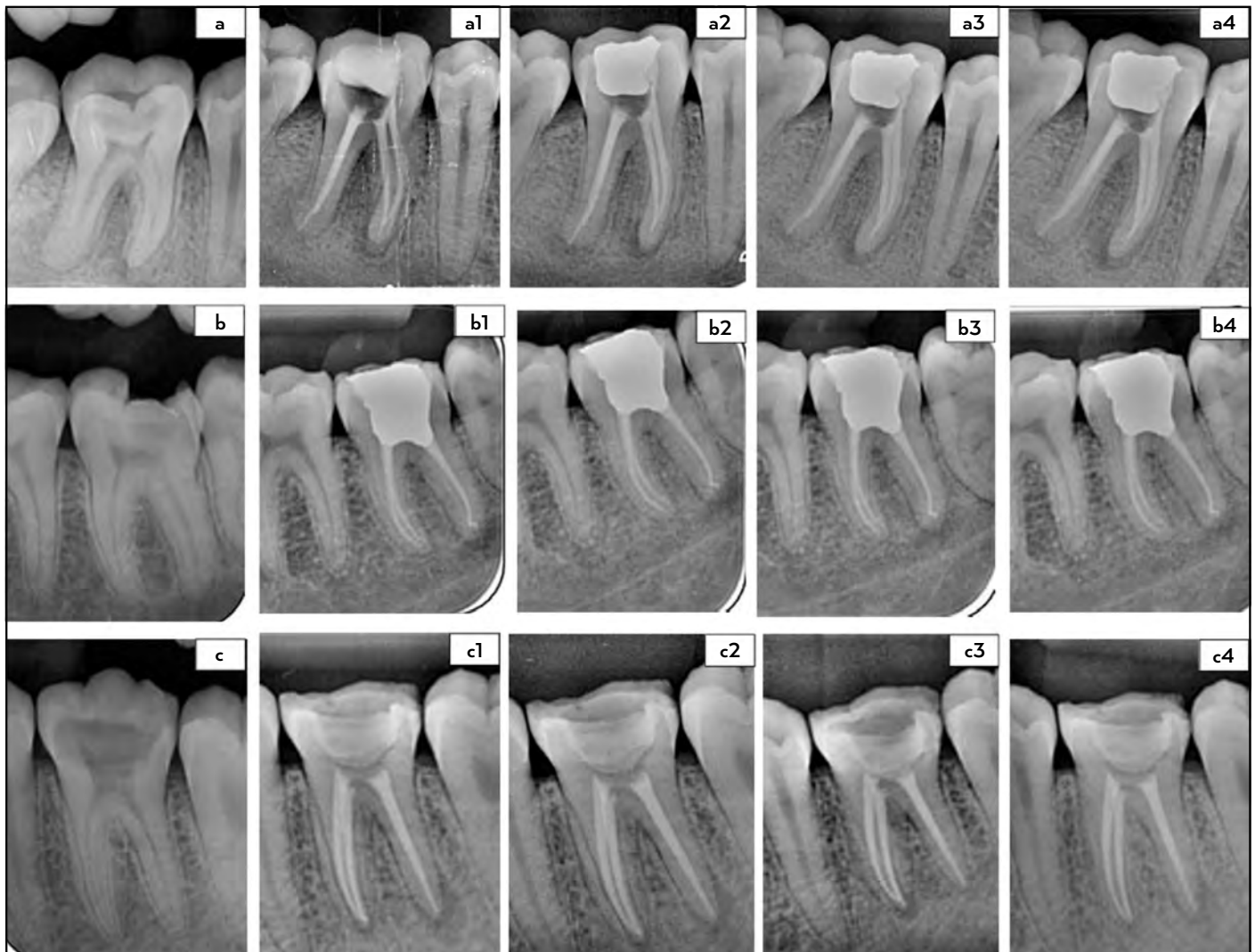


Figure 3. Preoperative and postoperative radiographs for CH+CHX treatment group. (a-c) Preoperative radiograph showing periapical lesion. (a1, b1, c1) 3-month postoperative radiograph. (a2, b2, c2) 6-month postoperative radiograph. (a3, b3, c3) 9-month postoperative radiograph. (a4, b4, c4) 12-month postoperative radiograph

CH+CHX: Calcium hydroxide and chlorhexidine

The presence of systemic disease may influence baseline score variations associated with different aqueous vehicles and introduce confounding effects due to systemic medications that could potentially alter the healing of periapical lesions.

The anatomical structure of mandibular molars is frequently complicated, including multiple bifurcations and trifurcations in the apical third, as well as fins, isthmuses, and accessory canals. These features may hinder the periapical healing resulting in lower success rates compared to single-rooted teeth. Therefore, in the current study, specifically mandibular molars with pulpal necrosis and symptomatic apical periodontitis were included to assess the treatment outcomes in some of the most challenging clinical situations (34).

In the present study, the intracanal medicament was placed for one week, as the literature suggests that applying CH for this duration is highly effective in bacterial elimination, achieving complete (100%) elimination of bacteria from the root canal system (35).

The healing outcome in the current study was assessed radiographically using PAI score, a standardized system for evaluating periapical health and determining the success of endodontic treatment. The PAI scale ranges from 1 to 5, based on comparisons with reference radiographs, reflecting the severity of periapical pathology (36).

Given that the significance level was set at $p \leq 0.05$, the statistical analysis revealed no significant differences among the groups at baseline and the 3-month follow-up ($p > 0.05$), indicating comparable initial treatment responses. However, as the follow-up period progressed, significant differences emerged at 6, 9, and 12 months ($p < 0.05$), suggesting a divergence in treatment outcomes over time.

Various studies have demonstrated that CH intracanal dressing has significantly reduced intracanal microbes from root canals with periapical lesions (37). The success rate of root canal treatment after the use of CH as an intracanal medicament ranged from 73.8% to 80.8% (38).

Literature has reported that CHX is more effective than CH in eliminating *Enterococcus faecalis* from dentinal tubules when used as an intracanal medicament (29). Studies have shown that all tested CHX formulations, including a 50:50 combination of CHX and CH, successfully eradicated *Enterococcus faecalis* from dentinal tubules (39). Kaplan-Meier survival analysis reinforces these results, showing that CHX promotes faster and more effective healing than LA or saline, likely due to its strong antimicrobial action.

The emergence of significant differences at later stages indicates that the choice of vehicle may influence the long-term effectiveness of treatment. These findings highlight the potential role of adjunctive agents in modulating the therapeutic properties of CH, possibly affecting factors such as antibacterial efficacy, tissue healing, and inflammatory response.

Nevertheless, there is sparse evidence in the literature regarding the evaluation of periapical radiolucency using different aqueous combinations of CH. Before interpreting the results, it is imperative to recognize the limitations of the study, which includes the relatively small sample size, limited follow-up duration, and the use of periapical radiographs as the primary method for evaluating treatment success.

CONCLUSION

Based on the findings of this study, it can be concluded that the use of different aqueous-based CH intracanal medicaments influences the healing outcome in patients with pulpal necrosis and symptomatic apical periodontitis. Among the tested medicaments, the CH+CHX combination demonstrated a significantly better periapical healing response over time compared to CH+LA or CH+NS. The findings suggest that the choice of vehicle for CH may impact its efficacy in eliminating infection and promoting periapical healing.

Disclosures

Ethics Committee Approval: The study was approved by the Manav Rachna Dental College Ethics Committee (no: MRIIRS/MRDC/FDS/IEC/2023/23, date: 16/05/2023).

Informed Consent: Informed consent was obtained from all participants.

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Survival Outcomes of Crowns with and without Repaired Endodontic Access Cavities: A Retrospective Propensity Score Matching Study

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ABSTRACT

Objective: This retrospective study aimed to compare the survival outcomes between crowns with repaired endodontic access cavities and intact crowns and to identify factors that influence restoration longevity.

Methods: Clinical records of patients who underwent root canal treatment through existing crowns (crowns with repaired access cavities, CRA) or received crowns after root canal treatment (intact crowns, IC) between 2012 and 2023 were analysed. A 1:1 propensity score matching was applied based on age, sex, tooth type, and crown type. The outcomes of the matched cases were classified as survival or non-survival. Kaplan–Meier analysis and log-rank tests were used to compare outcomes between the two groups over time. For CRA, multivariable Cox proportional hazards regression analysis was conducted to identify potential predisposing factors.

Results: Among 608 eligible endodontically treated teeth, 120 CRA and 488 IC met the inclusion criteria. After matching, 120 samples per group were analysed. The survival rate was significantly lower for CRA (85.8%) than for IC (91.7%) ($p=0.004$). Occlusal parafunctional habits or interferences were the only significant factors affecting CRA survival.

Conclusion: CRA demonstrated lower survival rates than IC, with occlusal parafunctional habits or interferences as key factors influencing their longevity.

Keywords: Dental crowns, dental prosthesis repairs, endodontically treated teeth, outcomes, survival rates

HIGHLIGHTS

- The survival rate of crowns with repaired endodontic access cavities was lower than that of intact crowns in propensity score-matched cases.
- The presence of occlusal parafunctional habits and interferences significantly impacted the longevity of repaired crowns.
- Understanding survival differences and key influencing factors can aid clinical decision-making and enhance long-term outcomes for post-endodontic restorations.

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INTRODUCTION

Endodontic treatment is occasionally required as a consequence of restorative procedures, particularly tooth preparation, which can pose risks to the dental pulp. Approximately 10% of teeth require endodontic therapy following full-coverage restorations (1). The combination of mechanical irritation during tooth preparation and dentin removal facilitates bacterial invasion, increasing the risk of infection and inflammation,

which may require endodontic treatment (2). The survival rate of vital pulp in crowned teeth is approximately 80–90% over 10–25 years (3, 4). A systematic review identified the loss of pulp vitality as a common biological complication associated with metal-ceramic and all-ceramic single crowns (5). In some cases, root-filled teeth may require endodontic retreatment, often necessitating access through the existing crown due to persistent interradicular infections (6).

Managing pulpal or root canal complications in crowned teeth requires either replacing the restoration after treatment or repairing the access cavity through the existing crown (6). Treating through the existing crown can delay the need for a new restoration, improving patient satisfaction and cost-effectiveness (6). However, it can cause unnecessary loss of tooth structure, difficulty in locating canals, missed detection of cracks or fractures, and potential damage to the restoration to some extent (7). An *in vitro* study has indicated that endodontic access may compromise crown integrity and retention, increasing the risk of fractures or dislodgement (8). Although composite repairs yield clinically satisfactory outcomes (9–12), the absence of standardised guidelines and limited evidence regarding the survival of crowns in endodontically treated teeth (ETT) with repaired access cavities pose challenges for clinical decision-making.

A retrospective study reported survival rates of full-coverage restorations with composite-repaired endodontic access cavities as 82.7%, 71.5%, 67.3%, and 48.8% at 2, 5, 7, and 10 years, respectively (9). Other studies on ETT with repaired crowns reported survival rates of 51–99%, reflecting variations in study criteria and observation periods (10–12). Although studies have evaluated the survival of intact crowns in ETT (crowns without repaired access cavities), their findings remain inconsistent (13–15). No clinical study has directly compared the survival of repaired and intact restorations under uniform evaluation criteria, limiting understanding of how damage to existing restorations affects crown longevity in ETT.

Propensity score matching is a statistical method that minimises selection bias by balancing confounding variables between treatment groups, particularly in non-randomised controlled studies. Estimating the probability of treatment assignment based on observed covariates facilitates the comparison of groups with balanced characteristics (16). To date, no clinical studies on post-endodontic restoration survival have applied propensity score matching.

Apart from survival rates, tooth-related variables such as the amount of remaining tooth structure and cavity type; factors related to occlusal forces, including tooth type, tooth location, opposing dentition, and presence of parafunctional habits; and crown type may influence the longevity of restorations. However, these factors have not been thoroughly analysed in current studies (9–12).

This study aimed to evaluate the survival outcomes of crowns with repaired access cavities (CRA) compared with those of intact crowns (IC) using propensity score matching and to investigate potential factors influencing restoration longevity.

MATERIALS AND METHODS

Study Design

This retrospective observational study investigated the survival rates of dental crowns in ETT, comparing CRA and IC. CRA referred to restorations damaged by endodontic access cavities and subsequently repaired with direct restorations, whereas IC included crowns in ETT without access cavities. Data were collected from patients who underwent non-surgical endodontic treatment performed by postgraduate students or endodon-

tists at the Endodontic Clinic, Mahidol University, between January 2012 and December 2023. For CRA, patients received root canal treatment performed through existing crowns, which were later repaired with direct restorations, and attended recall appointments within the study period. In IC cases, patients received dental crowns following the completion of initial root canal treatment or retreatment at the Main Clinic, Advanced General Dentistry Clinic, or Prosthodontic Clinic. Treatment was performed by undergraduate students, postgraduate students, or specialists, and patients attended recall appointments within the same timeframe. This study adhered to the guidelines and checklist of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement (17) and the Preferred Reporting Items for Observational Studies in Endodontics (PROBE) 2023 guidelines (18). The study protocol was approved by the Ethics Committees of the Faculty of Dentistry and the Faculty of Pharmacy, Mahidol University, Institutional Review Board (Number: MU-DT/PY-IRB 2024/DT017). The study was conducted in accordance with the Declaration of Helsinki.

Sample Size Calculation

The required sample size was determined using data from previous research (9). A significance level of 0.05 and a statistical power of 0.8 were used for the calculation. The effect size proportion for CRA was estimated at 0.827. An allocation ratio of 1:1 between CRA and IC was established, with 27 cases in each group following the propensity score matching process.

Case Selection

Patient records were reviewed to determine their eligibility.

Inclusion Criteria

1. Dental records with sufficiently detailed and complete clinical and radiographic examinations.
2. A minimum follow-up period of at least 1 year after the final restoration was required. For CRA, this period began after the repair of access cavities with direct restorative materials. For IC, the follow-up period started after crown placement.

Exclusion Criteria

1. For CRA, where the existing restoration exhibited marginal leakage, secondary caries, or was not intact before the root canal treatment or during the endodontic procedure.
2. Teeth diagnosed with root fractures, cracks, or severe periodontal conditions classified as stage III or IV periodontitis based on the 2017 classification of periodontal and peri-implant diseases and conditions (19).
3. Teeth with procedural errors that compromised the structural integrity of the coronal or radicular tooth structure, such as crown or root perforation.
4. Teeth with incomplete root formation or root resorption.

Endodontic and Restorative Procedures

Endodontic and restorative procedures were performed under a dental operating microscope (Zeiss Surgical and Dental Microscopes, Carl Zeiss Meditec AG, Jena, Germany), following standard institute protocols as detailed in previous studies (13–15). The selection of direct restorative materials for CRA access cavity repair, including direct resin composite, glass

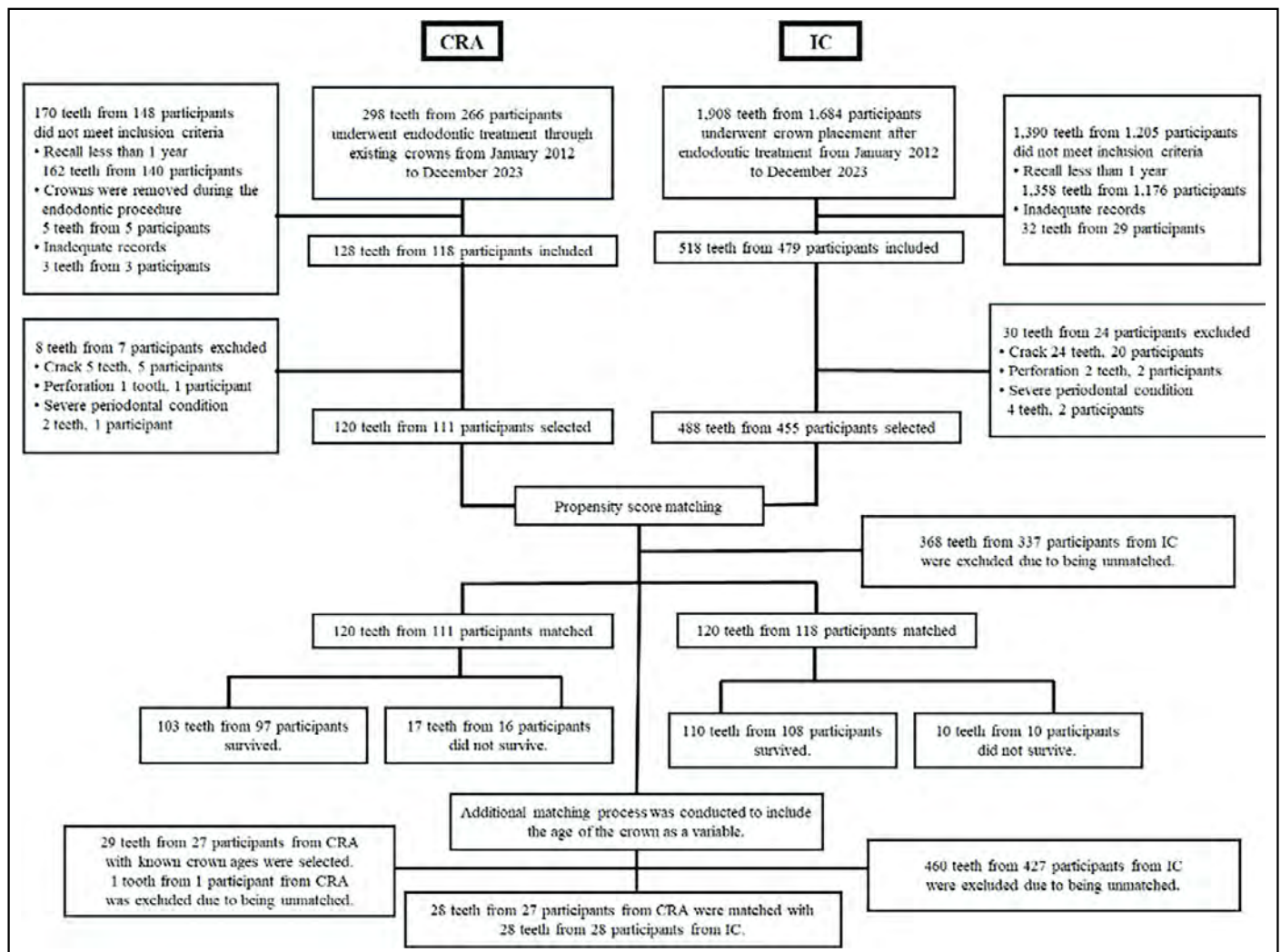


Figure 1. Flowchart illustrating sample inclusion and exclusion criteria.

CRA: Crowns with repaired access cavities, IC: Intact crowns.

ionomer cement combined with resin composite, core build-up, or post and core, was determined based on the operator's clinical judgment and case-specific considerations.

Data Collection

The following data were collected:

Demographic information including sex (male/female), age (years), crown placement date for IC and CRA (if available), and duration (months) following the repair of access cavities after endodontic treatment for CRA.

Clinical and radiographic information including tooth type (anterior/premolar/molar), tooth location (maxillary/mandibular), type of non-surgical root canal treatment (initial root canal treatment/root canal retreatment), crown type (full metal/porcelain-fused-to-metal [PFM]/ceramic), opposing dentition (natural tooth/fixed prosthesis/removable prosthesis), function as an abutment for prosthesis (none/dental bridge/removable prosthesis), presence of adjacent teeth (none/one side/two sides), occlusal parafunctional habits or occlusal interferences (yes/no), operator (postgraduate student/qualified endodontist), and direct restorative material for repairing access cavities in CRA (amalgam/resin composite/core/post and core).

Outcome Assessment

Outcomes were classified as survival or non-survival as follows: Survival was defined as the retention of the crown with an intact substructure, no signs of loosening or fracture, and no fractures in the natural teeth or root structure. Teeth with porcelain chipping or marginal leakage due to dental caries were classified as surviving cases.

Non-survival included cases where the crown became loose, dislodged, or structurally compromised due to fracture. This category also encompassed instances where a fracture resulted in the destruction of the natural teeth or root structure. For non-surviving cases, additional information was gathered, including the fracture pattern (restoration dislodgement/restoration fracture/tooth fracture/restoration-tooth fracture), restorability (restorable/non-restorable), and the treatment provided (e.g., crown replacement/extraction).

Statistical Analysis

Data analysis was conducted using SPSS v.22 for Windows (SPSS Inc., Chicago, IL, USA) and STATA 17 (StataCorp LLC, College Station, TX, USA). Statistical significance was set at $p < 0.05$. Numerical data are presented as means and standard deviations (SDs).

TABLE 1. Characteristics and distribution of ETT before and after propensity score matching

Factors	Total population				Propensity score-matched pairs			
	CRA (n=120)	IC (n=488)	p value	ASD	CRA (n=120)	IC (n=120)	p value	ASD
Age (years) (mean±SD, median [IQR])	61.4±13.0 63 [54–71]	56.2±13.0 59 [49–65]	<0.001[†]	0.403	61.4±13.0 63 [54–71]	61.2±12.2 63.5 [56.5–69]	0.894 [†]	0.017
Sex, n (%)			0.500 [‡]	0.068			0.896 [‡]	0.017
Male	52 (43.3)	195 (38.6)			52 (43.3)	51 (42.5)		
Female	68 (56.7)	293 (61.4)			68 (56.7)	69 (57.5)		
Tooth type, n (%)			0.032[‡]	0.255			0.957 [‡]	0.024
Anterior	15 (12.5)	87 (17.8)			15 (12.5)	15 (12.5)		
Premolar	33 (27.5)	173 (35.5)			33 (27.5)	35 (29.2)		
Molar	72 (60.0)	228 (46.7)			72 (60.0)	70 (58.3)		
Crown type, n (%)			<0.001[‡]	0.447			0.959 [‡]	0.036
Full metal	35 (29.2)	84 (17.2)			35 (29.2)	33 (27.5)		
PFM	82 (68.3)	344 (70.5)			82 (68.3)	84 (70.0)		
All-ceramic	3 (2.5)	60 (12.3)			3 (2.5)	3 (2.5)		
	CRA (n=29)	IC (n=488)	p value	ASD	CRA (n=28)	IC (n=28)	p value	ASD
Crown age (months) (mean±SD, median [IQR])	86.7±43.5 80 [53–115.5]	41.9±31.5 30.5 [19–55]	<0.001[†]	1.179	84.8±43.0 78.5 [53–106]	82.6±49.9 70 [55–134.5]	0.860 [†]	0.048
Age (years) (mean±SD, median [IQR])	63.2±8.0 62 [57–69.5]	56.2±13.0 59 [49–65]	0.004[†]	0.651	63.1±8.1 62 [57–69.5]	62.3±10.2 63 [55–69.5]	0.719 [†]	0.097
Sex, n (%)			0.879 [‡]	0.029			0.783 [‡]	0.072
Male	12 (41.4)	195 (38.6)			11 (39.3)	10 (35.7)		
Female	17 (58.6)	293 (61.4)			17 (60.7)	18 (64.3)		
Tooth type, n (%)			0.522 [‡]	0.222			0.370 [‡]	0.050
Anterior	3 (10.3)	87 (17.8)			3 (10.7)	4 (14.3)		
Premolar	10 (34.5)	173 (35.5)			10 (35.7)	9 (32.1)		
Molar	16 (55.2)	228 (46.7)			15 (53.6)	15 (53.6)		
Crown type, n (%)			0.629 [‡]	0.158			0.943 [‡]	0.065
Full metal	7 (24.2)	84 (17.2)			6 (21.4)	5 (17.9)		
PFM	19 (65.5)	344 (70.5)			19 (67.9)	20 (71.4)		
All-ceramic	3 (10.3)	60 (12.3)			3 (10.7)	3 (10.7)		

Bold values indicate statistical significance ($p < 0.05$). [†]: Independent samples t-test; [‡]: Two-sided Pearson's chi-square test or Fisher's exact test. ETT: Endodontically treated teeth, CRA: Crowns with repaired access cavities, IC: Intact crowns, ASD: Absolute standardised difference, SD: Standard deviation, IQR: Interquartile range, PFM: Porcelain-fused-to-metal

or as medians and interquartile ranges (IQRs). Categorical data are summarised using frequencies and percentages.

Survival rates of restorations in matched CRA and IC were assessed and compared using Kaplan–Meier survival analysis. Log-rank tests were conducted to evaluate the univariable effect of potential predisposing factors in CRA. Variables with a $p < 0.25$ were further analysed using the multivariable Cox proportional hazards model.

Propensity Score Matching

A 1:1 propensity score matching was performed for both CRA and IC using four variables: age, sex, tooth type, and crown type. The balance between groups was assessed by calculating absolute standardised differences (ASD) before and after matching. An $ASD < 0.1$ indicated an acceptable balance.

For CRA with available crown placement dates, an additional matching process was conducted separately from the primary matching process. This analysis incorporated crown age, de-

fined as the duration since crown placement, as an additional variable to enhance matching precision.

RESULTS

The initial screening included 298 CRA and 1,908 IC. After applying the inclusion and exclusion criteria, 608 ETT were eligible for analysis, consisting of 120 CRA and 488 IC. A 1:1 propensity score matching was conducted based on four variables, resulting in 120 IC being matched with CRA. Among CRA, crown placement dates were available for only 29 teeth (age in months: mean=86.7±43.5; median=80.0; IQR=53.0–115.5), as most crowns had been placed at external clinics. Consequently, an additional matching process incorporating crown age as a variable resulted in 28 samples per group (Fig. 1).

Following matching, no significant differences were observed between the two groups (Table 1). Across all variables, ASD decreased from >0.1 before matching to <0.1 after matching, indicating a successful balance between the groups (Fig. 2).

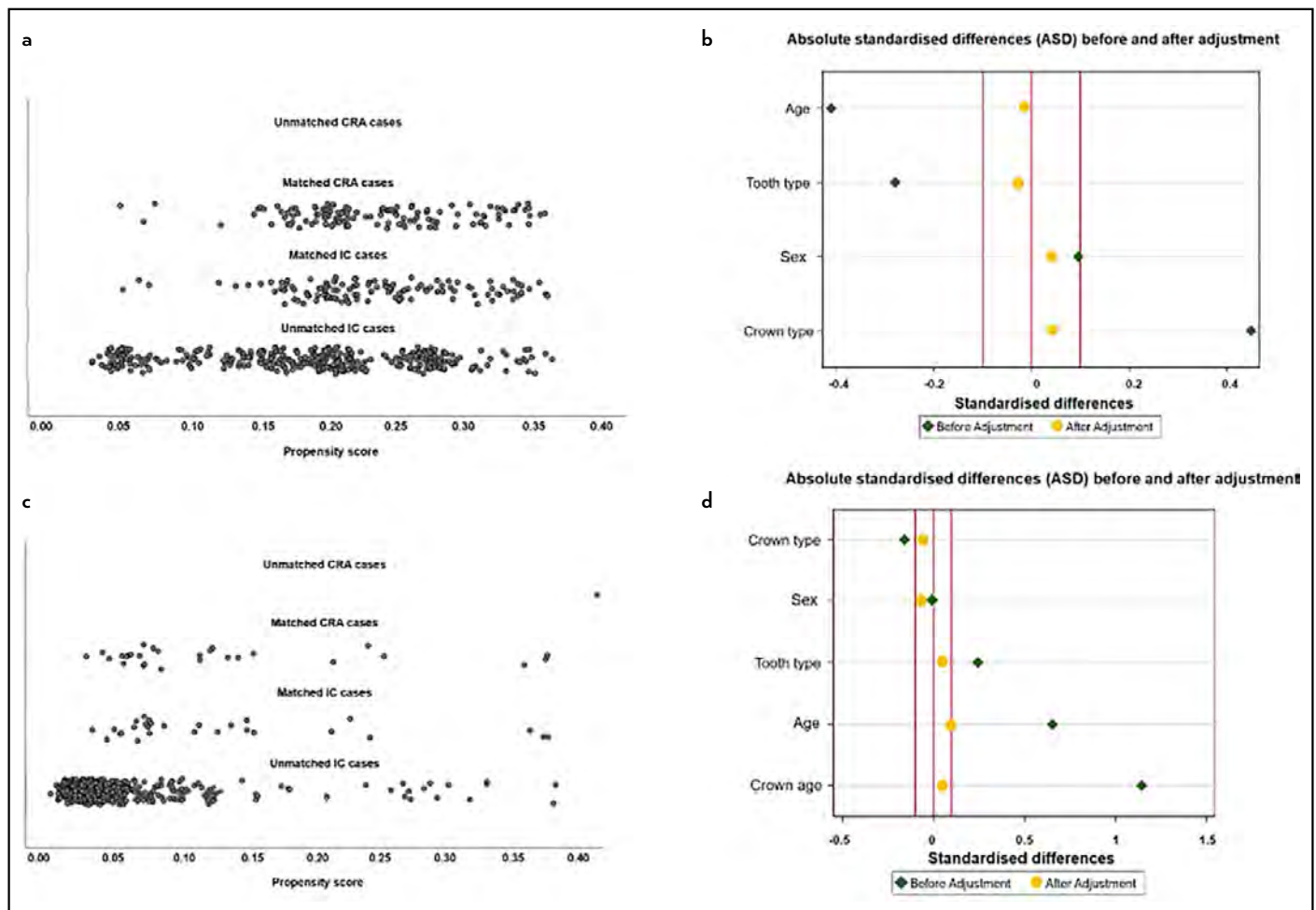


Figure 2. Results of propensity score matching for CRA and IC. (a) Distributions of propensity scores for matched and unmatched cases. (b) Standardised differences for covariates between the two groups before and after matching. (c) Distributions of propensity scores for matched and unmatched cases, with additional analysis accounting for controlled crown age. (d) Standardised differences for covariates between the two groups before and after matching, with additional analysis accounting for controlled crown age.

CRA: Crowns with repaired access cavities, IC: Intact crowns.

Survival Rates of Crowns with and without Repaired Access Cavities

Based on matching age, sex, tooth type, and crown type, with a recall period ranging from 12 to 167 months, the overall survival rates of CRA (mean recall period of 38.13 months) and IC (mean recall period of 45.15 months) ($n=120$) were 85.8% (103/120 teeth) and 91.7% (110/120 teeth). The mean survival times for CRA and IC were 84.62 and 129.87 months. Log-rank tests revealed significant differences in overall survival between CRA and IC ($p=0.004$). Within the first two years, CRA showed comparable survival rates to IC ($p=0.175$). The cumulative survival rate of CRA was 97.19% at 24 months, gradually decreasing to 78.40% and 53.06% at 60 and 96 months. However, IC survival rates were 100%, 92.98%, and 77.61% at 24, 60, and 96 months (Fig. 3a).

Among non-surviving CRA, non-restorable fractures affecting only the tooth structure accounted for the majority of failures (13/17 teeth). Restoration-tooth fractures led to extractions in 2/17 teeth. Crown dislodgement occurred in 2/17 teeth, both of which were subsequently managed with crown recementation and crown replacement (Table 2).

After crown age matching, log-rank tests indicated no significant differences in overall survival rates ($p=0.749$). The mean survival time was 151.81 months for CRA and 154.75 months for IC, with an 85.70% overall survival rate in both groups (Fig. 3b).

Potential Predisposing Factors Affecting the Survival of Crowns with Repaired Access Cavities

Univariable analysis identified occlusal parafunctional habits or interferences as the only significant factor influencing the survival rate ($p<0.001$) (Table 3, Fig. 4c). No statistically significant differences in longevity were observed across different crown types ($p=0.223$) or among the direct restorative materials used for repairs ($p=0.336$), although slight variations in survival curves appeared in the Kaplan–Meier survival graphs (Fig. 4a, b). The Cox proportional hazards regression analysis identified occlusal parafunctional habits or interferences as a significant factor affecting the survival rate. The hazard ratio (HR) revealed that ETT with occlusal parafunctional habits or interferences were 7.186 times less likely to survive compared to those without (95% confidence interval, 2.265–22.798; $p=0.001$) (Table 4). Crown type was excluded from the model due to no recorded fractures in all-ceramic crowns.

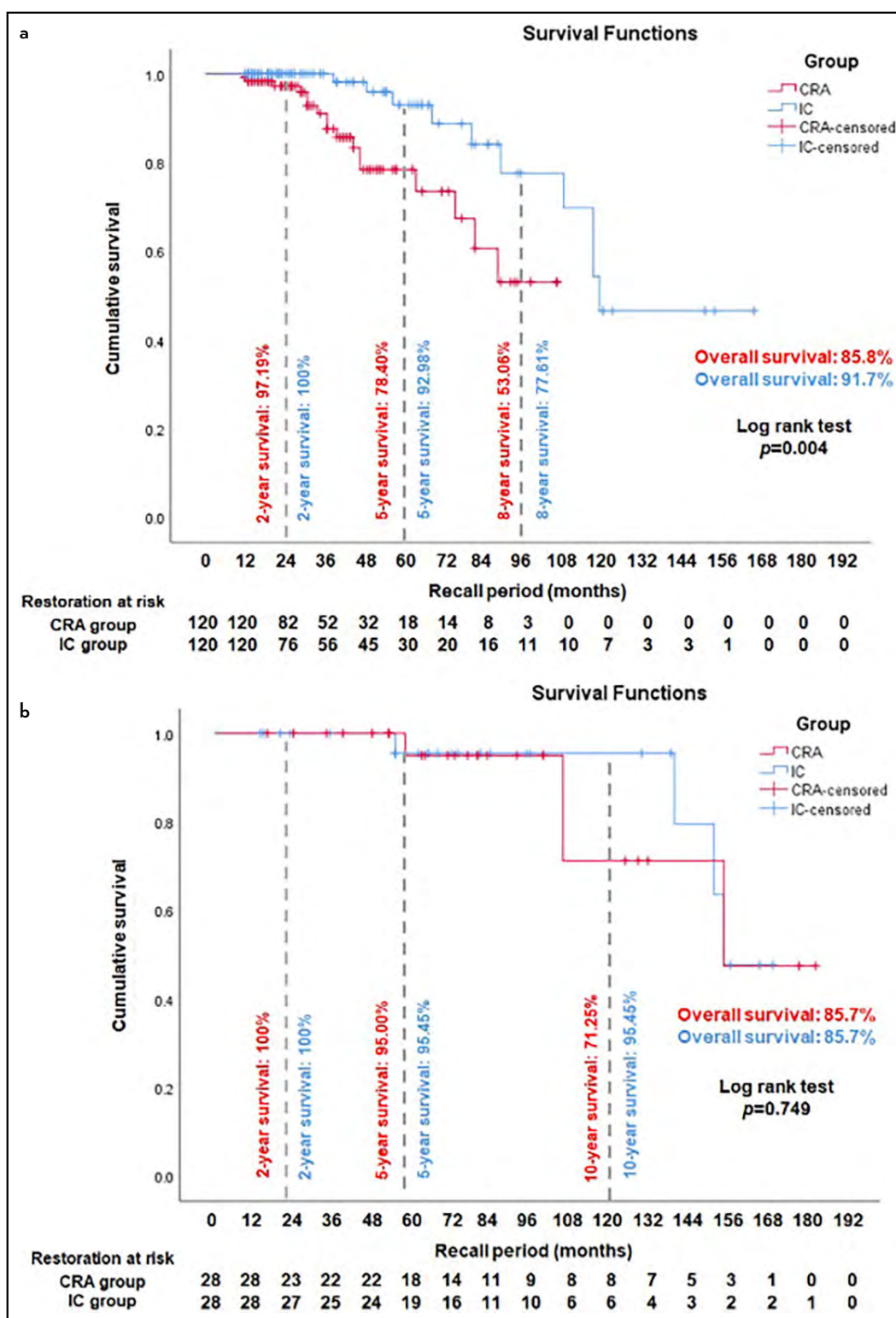


Figure 3. Kaplan–Meier survival curves depicting the cumulative survival rates of CRA and IC. Red annotations indicate the survival rates of CRA, whereas blue annotations represent the survival rates of IC. (a) Kaplan–Meier survival curve for matched CRA and IC ($n=120$). (b) Kaplan–Meier survival curve for matched CRA and IC ($n=28$), with additional analysis accounting for controlled crown age.

CRA: Crowns with repaired access cavities, IC: Intact crowns.

DISCUSSION

In this study, CRA exhibited a lower overall survival rate than IC. Within the first two years, CRA showed a high survival rate of 97.19%, comparable to the 100% observed in IC. The sur-

vival rate of CRA declined more than that of IC after 2 years, with 5- and 8-year survival rates of 78.40% and 53.06%, which are comparable to the 5- and 10-year survival rates of repaired restorations reported at 71.5% and 48.8% in a previous study

TABLE 2. Demographic data of non-surviving CRA (n=17)

Case	Sex	Tooth type	Crown type	Fracture pattern	Restorability after fracture	Type of treatment provided
1	M	Molar	Full metal	Crown dislodgement	R	Crown recementation
2	F	Molar	PFM	Crown dislodgement	R	Crown replacement
3	M	Molar	PFM	Tooth fracture	NR	Extraction
4	F	Molar	Full metal	Tooth fracture	NR	Extraction
5	F	Premolar	PFM	Tooth fracture	NR	Extraction
6	F	Premolar	PFM	Tooth fracture	NR	Extraction
7	F	Premolar	PFM	Tooth fracture	NR	Extraction
8	F	Premolar	PFM	Tooth fracture	NR	Extraction
9	F	Molar	PFM	Tooth fracture	NR	Extraction
10	F	Molar	PFM	Tooth fracture	NR	Extraction
11	F	Molar	PFM	Tooth fracture	NR	Extraction
12	F	Molar	PFM	Tooth fracture	NR	Extraction
13	F	Molar	PFM	Tooth fracture	NR	Extraction
14	F	Molar	PFM	Tooth fracture	NR	Extraction
15	F	Molar	PFM	Tooth fracture	NR	Extraction
16	M	Premolar	PFM	Restoration-tooth fracture	NR	Extraction
17	M	Molar	PFM	Restoration-tooth fracture	NR	Extraction

CRA: Crowns with repaired access cavities, M: Male, F: Female, PFM: Porcelain-fused-to-metal, R: Restorable, NR: Non-restorable

(9). Although definitions vary, survival is generally based on the clinical functionality and long-term prognosis of a crown (9, 11). We adopted this definition, considering porcelain chipping as a survival outcome, as it typically does not compromise the restoration's integrity and can be managed with minor repairs. Therefore, differences in failure definitions may influence variations in reported survival outcomes (9–12).

The reduced longevity of CRA compared to that of IC may be attributed to the risks associated with performing endodontic treatment through existing crowns, which can compromise the integrity and retention of the restoration and the underlying abutment, potentially causing fractures or dislodgement (8). However, this comparison was limited by the inability to control for crown age. In many CRA cases, the date of crown placement was unknown, and some crowns in the CRA group may have been placed earlier than those in the IC group, affecting the accurate assessment of restoration longevity. An additional propensity score matching analysis was performed to control for crown age. This supplementary analysis revealed no significant difference in survival between CRA and IC, different from the primary findings. The time from crown cementation was not consistently available for most CRA, resulting in a small sample size (28 teeth) in the crown age-matched analysis, thereby limiting the generalisability of these findings.

For non-surviving ETT, approximately 88% (15/17 teeth) exhibited unrestorable fractures involving the crown and/or tooth structure. This finding is consistent with failure patterns observed in non-surviving IC, where fractures represented the primary cause of failure, aligning with the findings of previous studies (20, 21). Notably, crown restorations affected by access cavities rarely failed due to loss of retention (loosening or dislodgement). Instead, fractures were the predominant failure mechanism, emphasising the importance of preserving the remaining tooth structure and minimising

damage to the crown during access cavity preparation to maintain restorability and overall tooth survival.

This study identified occlusal parafunctional habits or interferences as significant factors influencing restoration outcomes (HR=7.186). Excessive forces on ETT may increase the risk of fractures in both the tooth and restoration compared to normal forces (22). Patients without occlusal parafunctional habits or interferences seem to experience better crown survival than those with such conditions. However, the limited number of restorations exhibiting parafunctional habits constrains the overall reliability of this finding. Additionally, no significant differences in survival outcomes were observed for other occlusal force-related factors, including opposing dentition type, prosthetic abutment function, or the number of adjacent teeth. These findings contrast with the results of previous studies that reported reduced survival rates for telescopic crowns or fixed dental prostheses compared to those for single crowns (9, 11). Although there is limited data on the impact of these factors on the survival of repaired crowns, this study highlights the importance of effectively managing occlusal forces to enhance the long-term restoration success.

Univariable analyses revealed no significant effect of tooth type, tooth location, or crown type on survival, aligning with previous studies (9, 10, 23, 24). The variations in survival rates among different crown types may be attributed to the differing definitions of failure used across studies (20, 25). Although the sample primarily consisted of molars with PFM crowns, potentially limiting generalisability, the findings likely reflect outcomes observed in most clinical cases. Approximately 90% of CRA cases were repaired using resin composite or core material without posts, with no significant impact on survival rates. These materials exhibit adhesive properties and a modulus of elasticity comparable to that of dentin, contributing to enhanced fracture resistance of the

TABLE 3. Univariable analysis of potential predisposing factors for CRA (n=120)

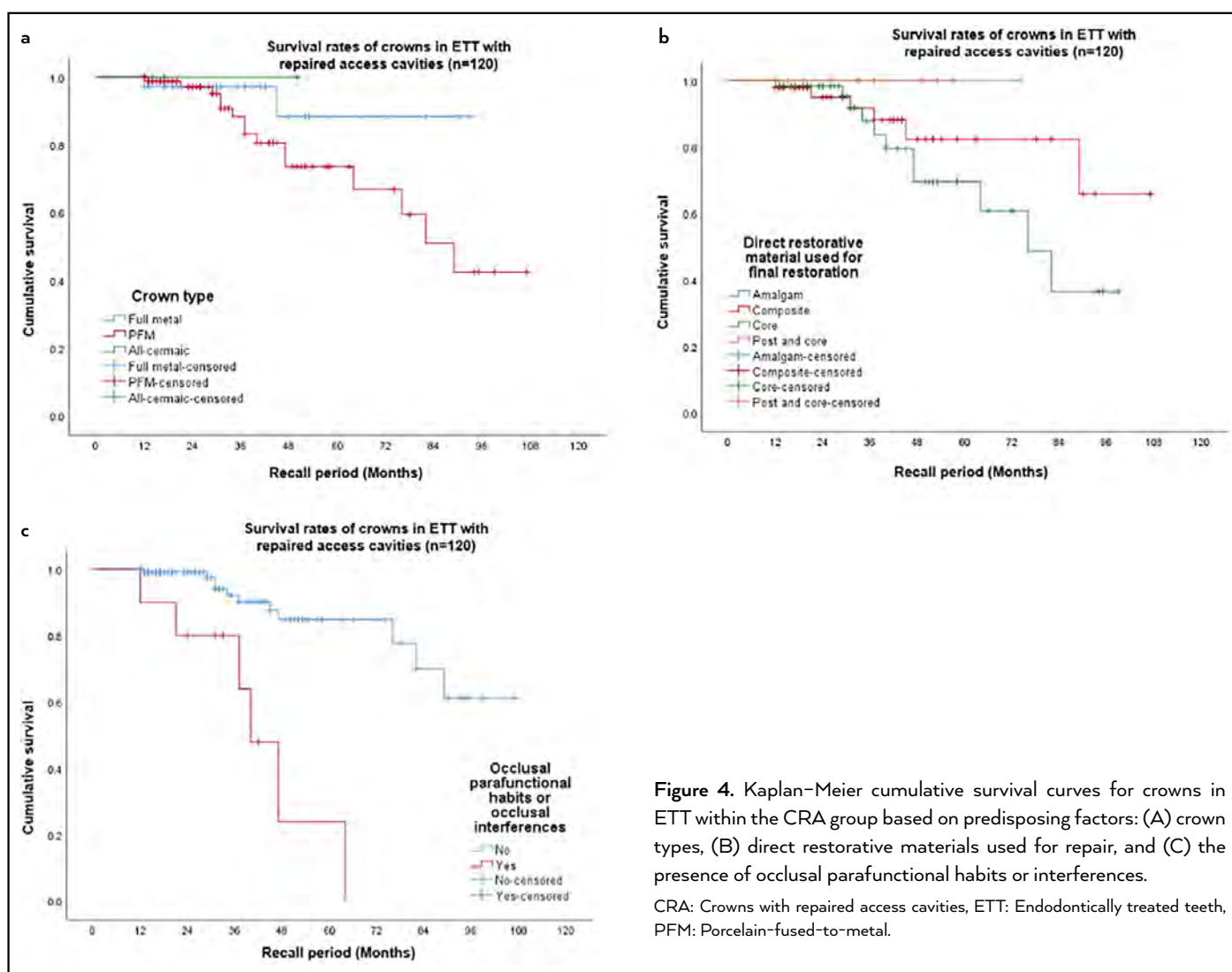
Factors	Total (n=120)		Non-survival (n=17)		Survival (n=103)		p value
	n	%	n	%	n	%	
Sex							0.134
Male	52	43.3	4	7.7	48	92.3	
Female	68	56.7	13	19.1	55	80.9	
Tooth type							0.542
Anterior	15	12.5	0	0.0	15	100.0	
Premolar	33	27.5	5	15.2	28	84.8	
Molar	72	60.0	12	16.7	60	83.3	
Tooth location							0.829
Maxillary	51	42.5	6	11.8	45	88.2	
Mandibular	69	57.5	11	15.9	58	84.1	
Type of root canal treatment							0.850
Initial root canal treatment	98	81.7	14	14.3	84	85.7	
Root canal retreatment	22	18.3	3	13.6	19	86.4	
Crown type							0.223
Full metal	35	29.2	2	5.7	33	94.3	
PFM	82	68.3	15	18.3	67	81.7	
All-ceramic	3	2.5	0	0.0	3	100.0	
Opposing dentition							0.564
Natural tooth	62	51.7	6	9.7	56	90.3	
Fixed prosthesis	48	40.0	10	20.8	38	79.2	
Removable prosthesis	10	8.3	1	10.0	9	90.0	
Function as an abutment for prosthesis							0.547
No	79	65.8	9	11.4	70	88.6	
Bridge	30	25.0	7	23.3	23	76.7	
Removable prosthesis	11	9.2	1	9.1	10	90.9	
Presence of adjacent teeth							0.778
0 sides	9	7.5	3	33.3	6	66.7	
1 side	48	40.0	6	12.5	42	87.5	
2 sides	63	52.5	8	12.7	55	87.3	
Occlusal parafunctional habits or occlusal interferences							<0.001
No	110	91.7	11	10.0	99	90.0	
Yes	10	8.3	6	60.0	4	40.0	
Direct restorative used for repair							0.336
Amalgam	2	1.7	0	0.0	2	100.0	
Resin composite	48	40.0	6	12.5	42	87.5	
Core	61	50.8	11	18.0	50	82.0	
Post and core	9	7.5	0	0.0	9	100.0	
Operator							0.988
Postgraduate student	106	88.3	14	13.2	92	86.8	
Qualified endodontist	14	11.7	3	21.4	11	78.6	

Bold values indicate statistical significance ($p < 0.05$). All p-values were derived from the log-rank test. CRA: Crowns with repaired access cavities, PFM: Porcelain-fused-to-metal

TABLE 4. Multivariable Cox regression analysis of variables significant in univariable analyses

Factors	Hazard ratio (95% CI)	p value
Sex		0.586
Male	1	
Female	1.408 (0.411–4.822)	
Occlusal parafunctional habits or occlusal interferences		
No	1	
Yes	7.186 (2.265–22.798)	0.001

Bold values indicate statistical significance ($p < 0.05$). All p-values were derived from the multivariable Cox regression model. CI: Confidence interval



remaining tooth structure (26). Notably, survival curves beyond 5 years (Fig. 4b) indicated a greater decline for core material repairs compared to resin composite repairs. This trend may be influenced by a selection bias, as clinicians may prefer core materials for deeper or larger cavities, potentially affecting long-term outcomes. However, this study lacked data on surface conditioning methods, such as silica coating or primers, which have been associated with improved survival and bond strength in previous research (27, 28).

No non-surviving case of CRA repaired with a post and core was identified in this study. Typically, the use of a post is associated with intraradicular retention of the core material and reinforcement of the root structure, which may help prevent tooth fractures (29). However, unnecessary post placement can significantly weaken the tooth structure (30). Here, the rationale for post placement remains unclear, as only limited information was available regarding the amount of remaining tooth structure, including cavity type, dentin wall thickness, and the number of remaining walls. This limitation may introduce potential bias in the survival comparisons, as post placement was more frequently observed in IC than in CRA (Tables 3, 5).

To evaluate the longevity of repaired versus intact crowns, propensity score matching was used to balance potential confounding factors and enhance comparability between groups (16). In general, crown longevity is influenced by multiple factors, including patient-related, tooth-related, occlusal, and restoration-related factors. However, due to the variability of clinical cases, increasing the number of matching variables in the model reduces the number of matched pairs, as more cases are excluded due to mismatches, leading to a smaller sample size for comparison.

Since information on cavity type and amount of remaining tooth structure was available for only a limited number of cases, we included as many other relevant variables as possible in the matching process to account for potential confounding factors, while ensuring adequate representation of each group for meaningful comparison. The selected variables included age, sex, tooth type, and crown type, all of which have been previously reported to influence survival outcomes (9–11, 23–25, 31). Examples of the matching variables used in various models, along with the corresponding number of cases in each comparison group, are presented in Table 6. Future studies with larger sample sizes should incorporate a

TABLE 5. Demographic characteristics of IC by relevant factors before (n=488) and after propensity score matching (n=120)

Factors	Before propensity score matching						After propensity score matching					
	Total (n=488)		Non-survival (n=23)		Survival (n=465)		Total (n=120)		Non-survival (n=10)		Survival (n=110)	
	n	%	n	%	n	%	n	%	n	%	n	%
Sex												
Male	195	38.6	12	6.2	183	93.8	51	42.5	4	7.8	47	92.2
Female	293	61.4	11	3.8	282	96.2	69	57.5	6	8.7	63	91.3
Tooth type												
Anterior	87	17.8	1	1.1	86	98.9	15	12.5	0	0.0	15	100.0
Premolar	173	35.5	7	4.0	166	96.0	35	29.2	2	5.7	33	94.3
Molar	228	46.7	15	6.6	213	93.4	70	58.3	8	11.4	62	88.6
Tooth location												
Maxillary	249	51.0	10	4.0	239	96.0	61	50.8	5	8.2	56	91.8
Mandibular	239	49.0	13	5.4	226	94.6	59	49.2	5	8.5	54	91.5
Type of root canal treatment												
Initial root canal treatment	453	92.8	21	4.6	432	95.4	119	99.2	10	8.4	109	91.6
Root canal retreatment	35	7.2	2	5.7	33	94.3	1	0.8	0	0.0	1	100.0
Crown type												
Full metal	84	17.2	9	10.7	75	89.3	33	27.5	2	6.1	31	93.9
PFM	344	70.5	13	3.8	331	96.2	84	70.0	8	9.5	76	90.5
All-ceramic	60	12.3	1	1.7	59	98.3	3	2.5	0	0.0	3	100.0
Opposing dentition												
Natural tooth	317	65.0	11	3.5	306	96.5	85	70.8	6	7.1	79	92.9
Fixed prosthesis	132	27.0	7	5.3	125	94.7	26	21.7	3	11.5	23	88.5
Removable prosthesis	39	8.0	5	12.8	34	87.2	9	7.5	1	11.1	8	88.9
Function as an abutment for prosthesis												
No	361	74.0	5	1.4	356	98.6	93	77.5	1	1.1	92	98.9
Bridge	29	6.0	7	24.1	22	75.9	10	8.3	5	50.0	5	50.0
Removable prosthesis	98	20.0	11	11.2	87	88.8	17	14.2	4	23.5	13	76.5
Presence of adjacent teeth												
0 side	21	4.3	7	33.3	14	66.7	4	3.3	2	50.0	2	50.0
1 side	145	29.7	11	7.6	134	92.4	27	22.5	6	22.2	21	77.8
2 sides	322	66.0	5	1.6	317	98.4	89	74.2	2	2.2	87	97.8
Occlusal parafunctional habits or occlusal interferences												
No	447	91.6	10	2.2	437	97.8	115	95.8	6	5.2	109	94.8
Yes	41	8.4	13	31.7	28	68.3	5	4.2	4	80.0	1	20.0
Restorative foundation												
Core	26	5.3	1	3.8	25	96.2	5	4.2	0	0.0	5	100.0
Post and core	462	94.7	22	4.8	440	95.2	115	95.8	10	8.7	105	91.3
Operator												
Undergraduate student	103	21.1	3	2.9	100	97.1	36	30.0	1	2.8	35	97.2
Postgraduate student	346	70.9	17	4.9	329	95.1	79	65.8	8	10.1	71	89.9
Qualified endodontist	39	8.0	3	7.7	36	92.3	5	4.2	1	20.0	4	80.0

IC: Intact crowns, PFM: porcelain-fused-to-metal

broader range of influencing factors to improve the reliability and generalizability of the results.

The retrospective data lacked detailed information on tooth condition prior to crown placement, including the amount of remaining tooth structure, status of the abutment, and size or depth of access cavities. For instance, significant structural loss, particularly in Class II cavities involving marginal ridge loss, increases the risk of fracture compared to a more preserved structure of teeth (32). Due to the retrospective nature of the data and limitations in clinical documentation, the assessment of pre-existing conditions in CRA may have been inaccurate or

incomplete. As a result, access openings may have been performed through crowns on teeth with varying types and degrees of structural loss, influencing the survival outcomes. This lack of reliable baseline information may introduce bias and limit the validity of comparisons between groups. In addition to tooth condition, patients' medical histories, comprising further factors influencing the durability of the restoration, were also not available in most cases (33). These variations may introduce bias and potentially affect the observed survival outcomes.

Operator variability, including clinical judgment, decision-making, and treatment protocols (such as crown cementa-

TABLE 6. Examples of alternative propensity score matching models based on different matching variables and the number of matched CRA and IC

Model	Factors	Primary propensity score matching (without crown age)	Additional propensity score matching (with crown age)
1	Tooth type		
	Crown type	120 cases matched	29 cases matched
2	Age		
	Tooth type		
	Crown type	120 cases matched	28 cases matched
3*	Age		
	Sex		
	Tooth type		
	Crown type	120 cases matched	28 cases matched
4	Age		
	Sex		
	Tooth type		
	Crown type		
	Occlusal parafunctional habits or occlusal interferences	73 cases matched	12 cases matched
5	Age		
	Sex		
	Tooth type		
	Crown type		
	Tooth location		
	Occlusal parafunctional habits or occlusal interferences	70 cases matched	11 cases matched
6	Age		
	Sex		
	Tooth type		
	Crown type		
	Tooth location		
	Occlusal parafunctional habits or occlusal interferences		
	Opposing dentition	43 cases matched	7 cases matched

*: Model used in this study. CRA: Crowns with repaired access cavities, IC: Intact crowns

tion techniques and adhesive systems), may have also influenced survival outcomes. The decision to retain or remove the crown was based on individual experience. Although such variability is difficult to control in retrospective studies, all procedures in our study followed standardised clinical protocols and were performed under dental operating microscopes in an academic setting. The operators, who were students, were supervised by instructors, which helped minimise inconsistencies. As corresponding treatment records were unavailable, some restorations may have been replaced or repaired in other settings during the follow-up period without our knowledge. This lack of information may have affected the accuracy of the survival analysis.

The limited sample size may have reduced the ability to detect failures in specific subcategories such as anterior teeth, all-ceramic crowns, amalgam restorations, or post-and-core restorations. Future studies with larger sample sizes should investigate these variables to enhance the identification of significant treatment factors influencing clinical outcomes.

Despite these limitations, our findings provide a valuable foundation for guiding clinical decision-making. By highlighting key factors that influence the longevity of restora-

tions, they support more informed choices and treatment planning. From a cost-effectiveness perspective, considering both financial costs and clinical outcomes, we conducted a brief analysis using the incremental cost-effectiveness ratio (ICER) (34). Based on data from our institute, the ICER suggests that an additional expenditure of approximately USD 60 is associated with a 1% increase in survival probability for CRA. Therefore, CRA may offer greater economic efficiency in certain clinical scenarios. However, a comprehensive cost-effectiveness analysis is warranted to better assess the acceptability of the lower survival rate, given the reduced treatment costs, and support evidence-based decision-making in restorative treatment planning.

CONCLUSION

Post-endodontic CRA exhibited lower overall survival compared to IC. However, this finding is limited by the absence of crown age data and limitations inherent to the retrospective study design. Performing endodontic access through existing crowns may compromise the underlying abutment structure and crown integrity, potentially shortening restoration longevity. Occlusal parafunctional habits and interferences emerged as potential predisposing factors influencing restoration outcomes.

Disclosures

Ethics Committee Approval: The study was approved by the Ethics Committees of the Faculty of Dentistry and the Faculty of Pharmacy, Mahidol University, Institutional Review Board (no: MU-DT/PY-IRB 2024/DT017, date: 21/03/2024).

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SEM Analysis and Pulp Tissue Dissolution Following Retrograde Preparation and Irrigation in Surgical Endodontics: A Novel Approach

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ABSTRACT

Objective: This study aimed to assess the efficacy of a novel retrograde irrigation protocol involving ultrasonic activation, compared with conventional techniques, using two complementary experimental models.

Methods: Experiment 1: Sixty extracted human mandibular premolars were endodontically prepared, obturated, and randomly assigned to three groups (n=20): Group 1 (saline irrigation), Group 2 (ultrasonically activated 17% EDTA gel and 5.25% NaOCl gel), and Group 3 (no irrigation). Following 1 mm apical resection and 3 mm retrograde cavity preparation, root-end cleanliness was assessed using scanning electron microscopy (SEM). Three calibrated, blinded examiners evaluated smear layer and debris scores. Experiment 2: A 3d-printed artificial canal system with lateral extensions containing bovine pulp tissue was used to simulate organic material. Groups received: Group 1 (saline), Group 2 (ultrasonically activated 5.25% NaOCl gel), Group 3 (no irrigation). Pulp dissolution was measured using a high-precision analytical microbalance. Data were analysed using the Kruskal-Wallis test and Dunn-Bonferroni post hoc (Experiment 1), and one-way ANOVA with Tukey HSD (Experiment 2). A significance level of $p < 0.05$ was adopted.

Results: In Experiment 1, Group 2 showed significantly lower debris and smear layer scores than Groups 1 and 3 ($p < 0.001$). In Experiment 2, only Group 2 achieved complete pulp tissue dissolution ($p < 0.001$).

Conclusion: Ultrasonically activated EDTA and NaOCl gel significantly enhance cleaning efficacy and organic tissue dissolution in retrograde endodontic surgery, allowing for a more conservative apical resection approach.

Keywords: Dentin debris, EDTA, endodontic surgery, sodium hypochlorite, ultrasonics

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HIGHLIGHTS

- A novel retrograde irrigation protocol using ultrasonic activation of EDTA and NaOCl gel significantly improved smear layer and debris removal compared to traditional saline irrigation or no irrigation.
- The experimental approach achieved complete pulp tissue dissolution in an artificial canal model, outperforming conventional methods.
- This enhanced cleaning efficacy allows for a more conservative apical resection, potentially preserving more of the root structure.
- Ultrasonic activation of irrigants improves debridement even in anatomically complex areas such as lateral canals.
- The protocol could lead to better outcomes in surgical endodontics, especially for teeth with compromised crown-to-root ratios.

INTRODUCTION

Endodontics is a branch of dentistry dedicated to the study and treatment of dental pulp and periapical tissues, addressing associated pathologies and their management (1). The main objective of root canal therapy is to achieve instant success and ensure positive long-term clinical outcomes. While revitalisation procedures may be considered for patients with necrotic permanent teeth with incomplete development and with or without apical periodontitis, root canal treatment is still strongly recommended (2, 3). Achieving this requires thoroughly cleaning the complex endodontic space following mechanical preparation of the canals before complete obturation (4). Despite the high success rates of conventional orthograde endodontic therapy, certain clinical scenarios remain challenging due to anatomical complexities, such as isthmuses, lateral canals, and apical ramifications, which can harbor persistent microbial biofilms. Additionally, factors like canal calcification, procedural errors, or obstructed anatomy may prevent adequate cleaning and sealing of the root canal system. These limitations can result in persistent periapical pathology, necessitating a surgical approach when orthograde retreatment is not feasible or has failed. Surgical endodontics has therefore evolved as a critical adjunctive option, offering direct access to the apical region and allowing for debridement, retrograde preparation, and sealing of the root end (5, 6). Prior to surgery, clinicians must assess whether the surgical approach offers significant advantages over prior orthograde treatment and evaluate the likelihood of success in the specific case (7).

Advancements in surgical techniques have transformed endodontic surgery into a predictable treatment modality, with a reported success rate of 91.6% after one year of follow-up (8). Surgical intervention is especially valuable for managing teeth with periapical lesions that have received adequate orthograde treatment or teeth with complex restorations (9).

During the decision-making phase, cone-beam computed tomography (CBCT) is an invaluable tool for endodontists. It enhances surgical planning by enabling precise evaluation of missed canals, resorptions, perforations, and complex canal anatomies, as well as the proximity of vital anatomical structures (10). The introduction of the surgical operating microscope in the 1990s (11), combined with ultrasonic retro-tips, revolutionised surgical endodontics, heralding a new era of precision and predictability (11).

In endodontic surgery, the operating microscope offers unmatched precision, allowing for minimal damage during root apex exposure (12). The use of ultrasonic instruments has significantly advanced this field by minimising the amount of bone that needs to be removed. These tools enable smaller resection angles, which help preserve cortical bone and the length of the roots. Additionally, they allow for the creation of cavities aligned parallel to the long axis of the roots and extend perpendicularly into the canals for several millimetres (5). Moreover, adequate magnification and illumination aid in identifying anatomical variations in the resected root, including isthmuses, confluent canals, microfractures, and lateral canals, all of which can contribute to treatment non-success (13).

The current study introduces and evaluates a novel irrigation protocol, Retro Ultrasonic Activation (RUA), aimed at optimising retrograde debridement in surgical endodontics. The effectiveness of this approach was assessed through two complementary experimental models: one using human teeth to evaluate smear layer and debris removal, and another employing a synthetic canal system to investigate organic tissue dissolution. In the natural tooth model, the protocol combines ultrasonic activation with 17% EDTA and 5.25% NaOCl to enhance cleaning of the apical dentinal walls. In the synthetic model, only 5.25% NaOCl gel was used, given the absence of smear layer formation in plastic blocks. The overarching goal of this protocol is to improve apical disinfection and cleaning efficacy while preserving periapical structures by minimizing the need for extensive root-end resection.

This study's null hypothesis proposes that the experimental protocol, conventional saline irrigation, and the control group do not produce statistically significant differences in either smear layer removal or pulp tissue dissolution.

MATERIALS AND METHODS

First Experiment

Sample selection

Sixty mandibular premolars ($n=60$) were selected. The sample size was determined using G*Power version 3.1 for Windows (Heinrich Heine University, Düsseldorf, Germany), with an effect size of 0.4, an α level of 0.05, and a desired power of 80%.

Sample Size Justification

The effect size of 0.4 used in the G*Power sample size calculation was selected based on Cohen's conventional criteria for a moderate effect size (14), which is considered appropriate for *in vitro* experimental designs with ordinal or continuous outcome measures. This choice was further supported by previous studies in the endodontic literature that compared different irrigation or activation protocols and reported moderate to large effect sizes (1, 15). In the absence of directly comparable preliminary data for the specific retrograde irrigation approach assessed in this study, the selection of an effect size of 0.4 provided a methodologically sound and conservative estimate for detecting clinically meaningful differences.

The study was conducted in accordance with the Declaration of Helsinki, the extractions were performed as part of necessary orthodontic treatment plans that were unrelated to the current study. Every patient provided written informed consent, ensuring their understanding and agreement to participate.

The appropriate institutional ethics committee granted ethical approval for this study prior to its initiation, ensuring that our research adheres to the highest ethical standards. Ethical approval was granted by the University of Naples, Federico II (Approval Code: N. 00008975).

Teeth selected for the study exhibited a single, straight root canal and normal root morphology. They were extracted for orthodontic purposes without any underlying pathology. Specimens were excluded if they showed signs of structural damage,

such as fractures or cracks, evidence of resorption or periapical disease, prior endodontic treatment, evidence of previous dental injury, incomplete root development, or canal calcification.

The root surfaces were cleaned to remove any remaining periodontal tissue and then thoroughly rinsed. Following this, the samples were stored in a 10% formaldehyde solution at room temperature for one week before further procedures.

Root Canal Preparation

An experienced operator performed all procedures with over 20 years of clinical expertise in endodontics. Premolars were sectioned using a diamond disk (Horico, Berlin, Germany) to remove the coronal portion and standardise sample lengths at 18 mm, ensuring uniform root length.

Working length (WL) was determined using a #10 K-file (Coltene/Whaledent Inc., Cuyahoga Falls, OH, USA). The file was inserted until visible at the apex and then adjusted to 0.5 mm short of this length.

Root canals were instrumented using nickel-titanium (NiTi) rotating files (Hyflex EDM; Coltene/Whaledent Inc.). Files #10/0.05, #20/0.05, and #25/0.08 were utilised to full WL. During shaping, canals were irrigated with 3% sodium hypochlorite (NaOCl) (Canal Pro, Coltene/Whaledent Inc.) with a 30G open-ended vented needle. An entirety of 5 ml of NaOCl was delivered per canal, reactivated every 60 seconds. Irrigation was followed by a rinse with physiological saline solution and 3 ml of 17% EDTA (Coltene/Whaledent Inc.) for smear layer removal, left for 60 seconds. Finally, canals were flushed with 3 ml of normal saline.

The canals were irrigated utilising a side-vented 30 G needle (Canal Tip, Coltene/Whaledent Inc.). Sterile paper points were used to dry the canals before the obturation phase with the single-cone technique using Bioseal sealer (Coltene/Whaledent Inc.) and #25/0.08 gutta-percha cones (Coltene/Whaledent Inc.). The coronal access cavity was sealed using a provisional restorative material (Cavit, 3M ESPE, St. Paul, MN, USA). The specimens were then preserved at 37°C in 100% humidity for one week to allow complete sealer setting and ensure optimal curing.

Before the experiment began, all specimens were randomly assigned to groups using a sequence generated by Random Allocation Software 2.0. This ensured allocation concealment from investigators and maintained the experiment's fairness.

Root Canal Cleanliness Assessment

Surgical Procedures

Specimens were divided into three groups (n=20 each). Apicoectomy was performed by resecting 1 mm of the apices with a carbide bur with multiple blades. Retro-preparations were performed to a 3 mm depth using the P14D retro tip (Satelec, Mérignac, France) under 8× magnification (Kaps SOM 32, Karl Kaps GmbH, Germany).

Group 1 (Traditional): Retro-cavities were irrigated with 2 ml of physiological saline using a 30G needle and dried using sterile paper points.

Group 2 (Experimental): Retro-cavities were irrigated with 17% EDTA gel (Canal Pro, Coltene, Whaledent Inc.) and subjected to ultrasonic activation for 30 seconds using a specially modified ultrasonic tip (25/02 Ultra Smart AI, Coxo, China).

The ultrasonic activation was performed using a cordless ultrasonic device (Ultra Smart AI, Coxo, China), which enables the tip to oscillate at a frequency of 40,000 Hz (40 kHz), ensuring consistent and high-frequency energy delivery during irrigant activation.

The tip was pre-bent to a 100° angle at its apical 3 mm using an Endo-bender (Kerr, CA, USA) and further adjusted with a goniometer. Following the EDTA activation, the cavities were irrigated with 1 mL of saline, dried using sterile paper points, then rinsed with 1 mL of 5.25% sodium hypochlorite gel (Chloraxid 5.25% Gel, Cerkamed, Stalowa Wola, Poland). The NaOCl gel was reactivated following the same ultrasonic procedure. A final rinse with a total of 2 ml of saline solution was performed using a 30G needle. The cavities were then thoroughly dried with sterile paper points.

Group 3 (Control): Retro-cavities without irrigation.

SEM Analysis

The apical 3 mm of all the roots were sectioned and prepared for scanning electron microscopy (SEM) analysis. The samples were divided lengthwise into two halves using a diamond bur while maintaining water cooling, followed by immersion in liquid nitrogen and fracturing with a stainless-steel chisel.

Specimens were then air-dried overnight at room temperature, gold-coated, and examined with SEM (EVO MA 10, Carl Zeiss SMT AG, Germany) at 1000× magnification. Thirty photomicrographs were taken per sample (10 from each coronal, middle, and apical area).

Assessment of Debris and Smear Layer Score

In this study, three blinded evaluators assessed the presence or the dissolution of debris and smear layer using criteria adapted from Hülsmann et al (15). To ensure consistency and minimise subjectivity in the SEM image analysis, inter- and intra-examiner calibration was performed. This process involved a preliminary training session where evaluators analysed a subset of images and discussed discrepancies to establish a consensus. Following this, inter- and intra-examiner agreement was quantified using the Cohen's Kappa coefficient (κ), which measures the degree of concordance beyond chance. The interpretation of Kappa (κ) values followed standard criteria: values above 0.75 reflected excellent agreement, those between 0.40 and 0.75 indicated fair to good agreement, and values below 0.40 were interpreted as poor agreement. This calibration process ensured consistent and reliable evaluations (12).

Debris Scoring:

Score 1: Canal walls appear clean with almost no debris.

Score 2: Presence of a few small clusters of debris.

Score 3: Moderate debris detected, covering under half of the canal surface.

Score 4: Debris extends across more than 50% of the canal walls.

Score 5: Canal walls are almost entirely, or completely, coated with debris.

Smear Layer Scoring:

Score 1: No smear layer visible; dentinal tubules are entirely open.

Score 2: A thin smear layer is present; some tubules remain open.

Score 3: Smear layer is uniform; only a few tubules are visible.

Score 4: All canal surfaces are evenly covered; tubules are not visible.

Score 5: A dense smear layer completely masks the canal walls.

Statistical Analysis and Results

The statistical evaluation was performed using SPSS Statistics 21.0 (Armonk, New York: IBM Corp.) and the Kruskal-Wallis test was selected, a non-parametric approach for comparing independent groups with ordinal data. This test was chosen due to the nature of the collected data, specifically the categorical and non-normally distributed scores for debris and smear layer presence. Post hoc pairwise comparisons were conducted utilising the Dunn-Bonferroni test to identify specific differences among groups, ensuring control of the Type I error rate.

The Kruskal-Wallis test demonstrated statistically significant differences in debris and smear layer scores across the three groups ($p<0.001$). Post hoc analysis further confirmed the superior cleaning efficacy of the experimental retro-irrigation protocol compared to both the traditional method and the control group. This combination of statistical methods provided a robust framework for validating the study's conclusions.

The debris scores in Group 1 (Traditional) were consistently high across all root thirds, recorded as 5 (± 0 SD) for the coronal, middle, and apical thirds. Similarly, Group 3 (Control) also demonstrated debris scores of 5 (± 0 SD) across all thirds, reflecting no improvement in cleaning with the traditional saline irrigation.

In contrast, Group 2 (Experimental) showed significantly lower debris scores, recorded as 1.2 (± 0.41 SD) for the coronal third, 1.1 (± 0.31 SD) for the middle third, and 1.2 (± 0.41 SD) for the apical third. These results indicate the enhanced efficacy of ultrasonic activation with 17% EDTA gel and 5.25% NaOCl.

For the smear layer scores, Groups 1 and 3 both recorded consistent scores of 5 (± 0 SD) across all root thirds. However, Group 2 demonstrated lower scores, with values of 1.2 (± 0.41 SD) for the coronal third, 1.15 (± 0.37 SD) for the middle third, and 1.15 (± 0.37 SD) for the apical third, confirming the superior cleaning achieved with the experimental protocol.

Statistical analysis using the Kruskal-Wallis test revealed significant differences between groups' debris and smear layer scores ($p<0.001$). Post hoc comparisons confirmed that Group 2 performed significantly better than Groups 1 and 3 in debris and smear layer removal. No significant differences were observed within the root thirds of the same group (Table 1, Figs. 1-3).

TABLE 1. The debris and smear layer scores for each group

Group	Root third	Debris score ($\pm$ SD)	Smear layer score ($\pm$ SD)
1	Coronal	5 (± 0)	5 (± 0)
	Middle	5 (± 0)	5 (± 0)
	Apical	5 (± 0)	5 (± 0)
2	Coronal	1.2 (± 0)	1.2 (± 0.41)
	Middle	1.1 (± 0)	1.15 (± 0.37)
	Apical	1.2 (± 0)	1.15 (± 0.37)
3	Coronal	5 (± 0)	5 (± 0)
	Middle	5 (± 0)	5 (± 0)
	Apical	5 (± 0)	5 (± 0)

SD: Standard deviation

It is noteworthy that the standard deviation was zero in Groups 1 and 3 because all specimens uniformly received the maximum score (5) for both debris and smear layer across all root thirds. This complete consistency reflects the absence of active irrigation or chemical agents in these groups, resulting in uniformly poor canal cleanliness.

Key Findings:

- Group 2 (Experimental) demonstrated significantly lower debris and smear layer scores in all thirds of the root canal compared to Groups 1 and 3 ($p<0.001$).
- Groups 1 and 3 exhibited identical scores of 5 across all regions, indicating no improvement in cleaning efficacy when using saline alone.
- The Kruskal-Wallis test confirmed significant differences between the groups ($p<0.001$), with post hoc analyses highlighting the superior cleaning efficacy of the experimental protocol.

Second Experiment

Sample size determination

Sample size was estimated using G*Power version 3.1 for Windows (Heinrich Heine University, Düsseldorf, Germany). The parameters included a significance level (α) of 0.05, a statistical power of 80%, and an effect size of 0.4, as recommended by Cohen. The analysis indicated that a minimum of 30 samples (10 per group) would be necessary to detect statistically significant differences among the experimental groups.

Artificial Canal System Design and Fabrication

The artificial root canal model was designed using AutoCAD software (© 2024 Autodesk Inc.) and fabricated with a high-resolution 3D printer (Imprinter Form 3B, Formlabs, Somerville, MA, USA) operating at a 0.05 mm resolution. To enhance visibility during experimental procedures, a bio-compatible, transparent resin (Biomed Clear Resin, Formlabs) was employed for manufacturing.

The standardised canal configuration included an apical diameter of 0.30 mm, a 6% taper, and a total length of 16 mm. Thirteen millimetres of the canal (from the coronal towards the apical end) were filled with resin to simulate endodontic obturation.

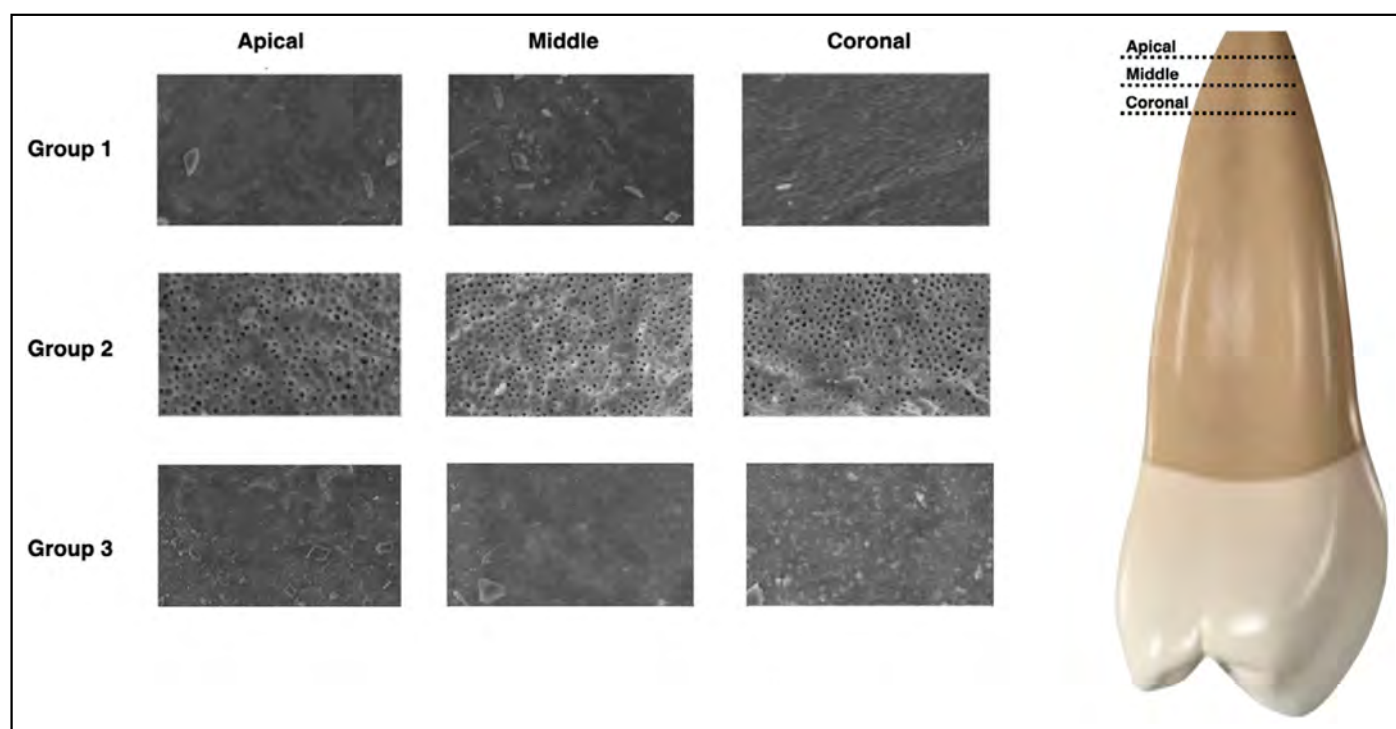


Figure 1. Representative scanning electron microscope (SEM) Images at 1000× Magnification: Group 1, traditional: Coronal, middle, and apical thirds of traditional retro-preparation cleaned using conventional techniques. Group 2, experimental: Coronal, middle, and apical thirds of traditional retro-preparation cleaned with retro-ultrasonic activation. Group 3, control: Coronal, middle, and apical thirds of retro-preparation without irrigation.

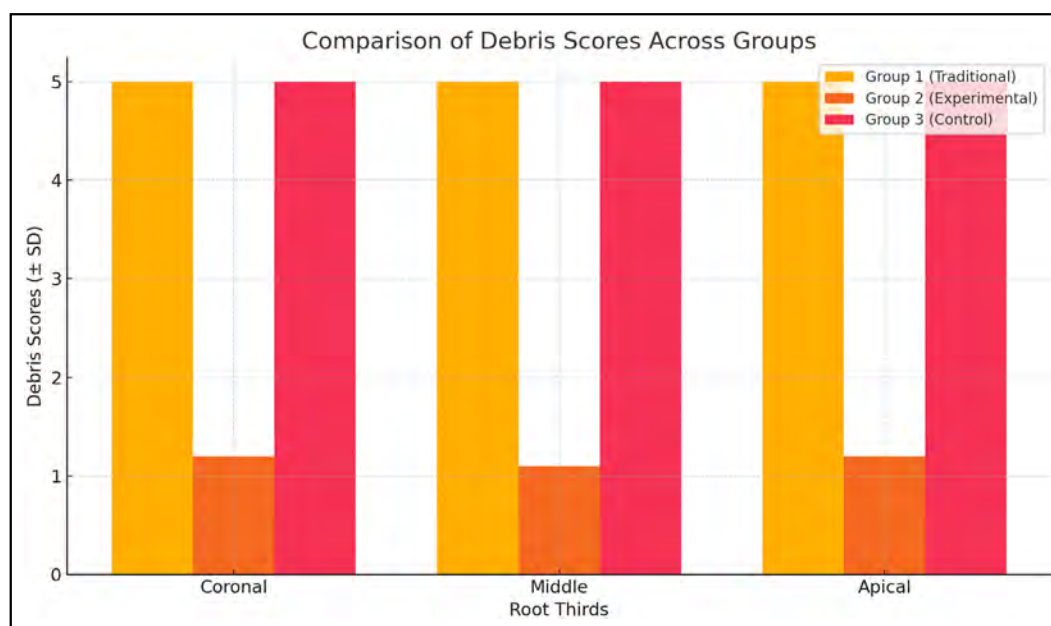


Figure 2. Graph of the distribution of Debris in the coronal, middle, and apical thirds between Group 1, Group 2, and Group 3.

SD: Standard deviation.

Additionally, a lateral canal-like space was also integrated, measuring 1 mm in length and located 3 mm from the apex. Its dimensions included a minor base of 0.48 mm, a major base of 0.54 mm, and a depth of 0.5 mm.

The canal system was printed in two interlocking parts to facilitate specimen placement and observation. This step enabled precise positioning of pulp tissue within the lateral extension.

This modular design also allowed for direct visualisation of tissue dissolution during the experiment, optimising assessment of the irrigation protocols' performance.

Preparation of Pulp Specimens

Fresh bovine pulp tissue was harvested post-mortem from the mandibular incisors of animals designated for human consumption; therefore, no ethical approval for animal experi-

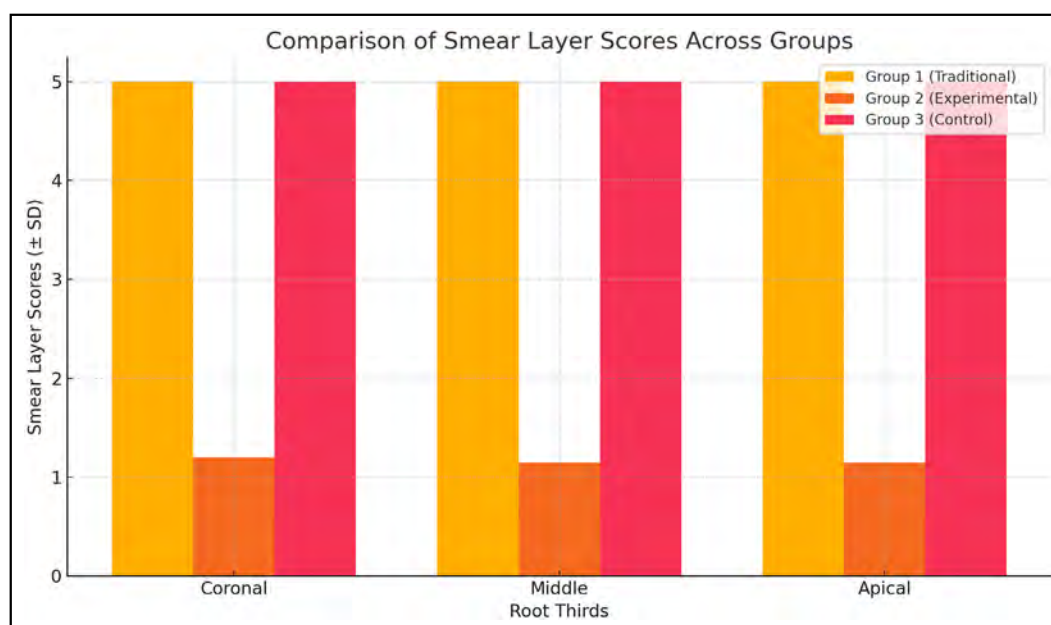


Figure 3. Graph of the distribution of Smear Layer in the coronal, middle, and apical thirds between Group 1, Group 2, and Group 3.

mentation was required. Teeth were extracted within 36 hours of slaughter and immediately immersed in 0.1% thymol solution to preserve tissue viability.

To avoid thermal damage, crowns were cut at the cemento-enamel junction using a high-speed diamond bur (Komet, Lemgo, Germany) under continuous water cooling. The pulp was carefully removed, rinsed with distilled water, and stored individually in 1.5 ml Eppendorf tubes holding 1 ml of distilled water at -20°C .

Prior to experimentation, specimens were thawed at room temperature for 30 minutes, followed by incubation in a 37°C water bath for 15 minutes to simulate intraoral temperature. Each sample was then standardised to dimensions of $1 \times 0.5 \times 0.5$ mm under $8\times$ magnification (SOM 32, Karl Kaps GmbH, Germany), using millimetre graph paper and a surgical scalpel blade (Braun, Tuttlingen, Germany) for precise trimming and measurement.

Experimental Groups and Setup

Samples were weighed using an analytical microbalance with 0.00001 g precision (Explorer Semi-Micro, OHAUS CORPORATION, Parsippany, NJ, USA) at three stages: pre-insertion, post-insertion, and following each irrigation cycle. Complete pulp tissue dissolution was defined as 100% mass loss, as determined by the disappearance of measurable tissue weight on the microbalance. (Fig. 4). The following groups were tested:

Evaluation of Root Canal Cleanliness

Surgical simulation procedures

Specimens were randomly assigned to three groups ($n=10$ per group). Before each test, the pulp fragment was placed into the lateral canal extension, and the two parts of the canal model were assembled.

Retrograde cavity preparations were carried out to a depth of 3 mm using a P14D ultrasonic retro tip (Satelec, Mérignac, France) under $8\times$ magnification (Kaps SOM 32, Karl Kaps GmbH, Germany).

- **Group 1 (Conventional Irrigation):** Retro-prepared cavities were irrigated with 2 ml of physiological saline delivered via a 30G needle, followed by drying with sterile paper points.
- **Group 2 (Experimental Protocol):** Cavities were filled with 1 ml of 5.25% sodium hypochlorite gel (Chloraxid 5.25% Gel, Cerkamed, Stalowa Wola, Poland). The gel was activated ultrasonically for 30 seconds using a customised ultrasonic tip (25/02 Ultra Smart AI, Coxo, China), pre-bent to 100° at its apical 3 mm using an Endo-bender (Kerr, CA, USA) and adjusted using a goniometer. The gel was allowed to remain within the cavity for an additional 60 seconds to maximise its chemical action. Final irrigation was performed using 2 ml of physiological saline solution, using a 30G needle, and then paper points were used to dry the cavities.
- **Group 3 (Negative Control):** No irrigation procedure was applied after cavity preparation.

Each experiment involved 10 sequential irrigation cycles.

Statistical Analysis

All the tests were performed using SPSS Statistics 21.0 (Armonk, New York: IBM Corp.). The Shapiro-Wilk test verified the normality of the data distribution. One-way ANOVA was conducted to compare the groups' extent and time of pulp tissue dissolution. Post hoc comparisons were made using Tukey's HSD test. A p-value of less than 0.05 was considered statistically significant.

Although the Shapiro-Wilk test indicated mild deviations from normality in Groups 1 and 3, one-way ANOVA was em-

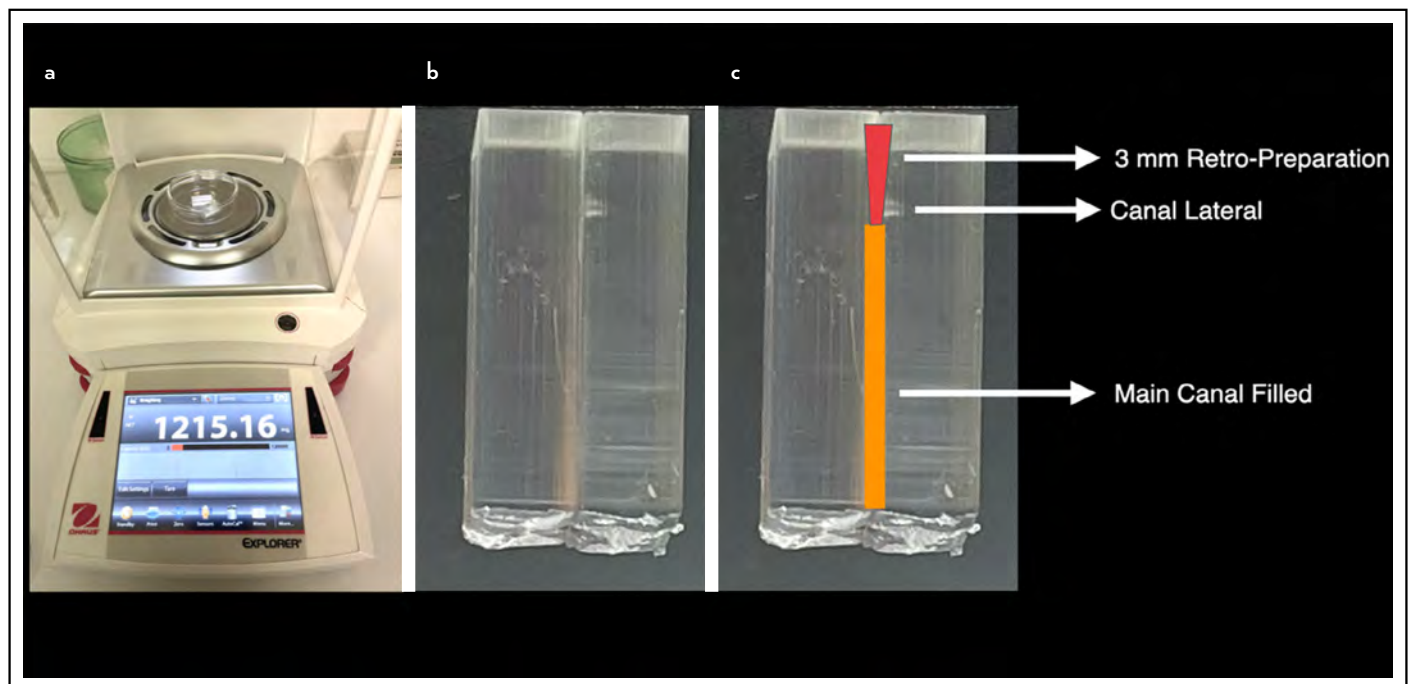


Figure 4. (a) High-precision microbalance used for quantitative measurement of pulp tissue dissolution. (b, c) 3D-printed resin artificial canal system featuring a lateral canal located in the apical third, designed to simulate complex anatomical conditions for evaluating irrigation protocols.

ployed due to its demonstrated robustness under such conditions, particularly when group sizes are equal and sample sizes are moderate (16, 17). Prior studies have shown that ANOVA maintains reliable Type I error rates even when the assumption of normality is mildly violated, especially in experimental designs with balanced groups. Nevertheless, this limitation is acknowledged, and results should be interpreted with this consideration in mind.

RESULTS

The Shapiro-Wilk test confirmed normal distribution for Group 2 (Experimental), while Groups 1 (Conventional) and 3 (Control) exhibited mild deviations from normality ($p < 0.05$). Nevertheless, parametric testing was deemed appropriate given the robustness of ANOVA with equal sample sizes ($n=10$).

A statistically significant difference was revealed through One-way ANOVA in pulp tissue dissolution among the three groups ($p < 0.001$). Tukey's HSD post hoc analysis showed that Group 2 (Experimental) achieved significantly greater pulp dissolution compared to both Group 1 (Conventional) (mean difference = 0.122 mg, $p < 0.001$) and Group 3 (Control) (mean difference = 0.122 mg, $p < 0.001$). No significant difference was observed between Groups 1 and 3 ($p = 1.000$).

These findings demonstrate that only the experimental protocol, involving 5.25% sodium hypochlorite gel activated ultrasonically, was effective in dissolving pulp tissue within the lateral canal space (Figs. 5, 6, Table 2).

DISCUSSION

The null hypothesis was rejected, as the proposed novel protocol demonstrated superior outcomes compared to the traditional technique and the control group.

Surgical endodontics represents an essential therapeutic approach that effectively addresses complex dental issues by removing a portion of the apical third of the root. This targeted approach can significantly alleviate pain and promote better oral health (18). This segment is anatomically complex, making adequate cleaning challenging, especially in cases where a complete seal cannot be achieved via non-surgical means (19). The primary objectives of surgical endodontics include confining microorganisms within the root canals by creating an apical seal, removing the intricate apical portion of the root, and eradicating existing periapical lesions (20). However, surgical retreatment is indicated only in specific cases, such as when canals are obstructed or when there is a significant risk of harming the tooth crown or restoration (21).

Historically, the success rate of endodontic surgery was approximately 60% (22), a limitation often linked to challenges in accessing, cleaning, and sealing the apical region of the root canal and reliance on outdated obturation techniques. The adoption of surgical microscopes in the 1990s, along with the development of ultrasonic retro-tips, significantly advanced the precision and outcomes of surgical endodontic procedures (23).

Using operative microscopes in endodontic surgery has significantly increased success rates by enhancing precision at every procedure stage. Ultrasonic retro-tips have further refined root-end preparation, offering a more conservative and meticulous approach. These micro-ultrasonic advancements have enabled clinicians to achieve more predictable surgical outcomes with higher success rates (24).

Despite these improvements, the retro-cavity cleaning phase remains an area of potential enhancement to improve the overall success rate of surgical endodontic treatment (25).

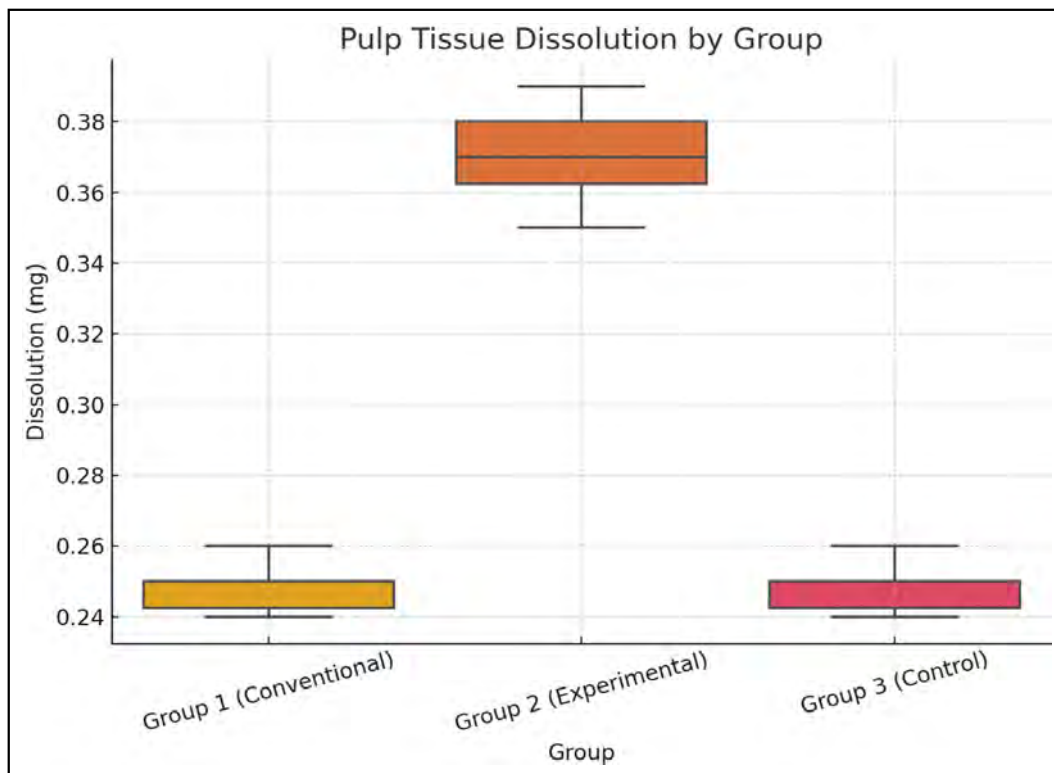


Figure 5. Boxplot of pulp tissue dissolution (mg) across the three experimental groups. Group 2 (Experimental), which received 5.25% NaOCl gel with ultrasonic activation, showed significantly greater tissue dissolution compared to Group 1 (Conventional saline irrigation) and Group 3 (Control, no irrigation). No significant difference was observed between Groups 1 and 3.

NaOCl: Sodium hypochlorite.

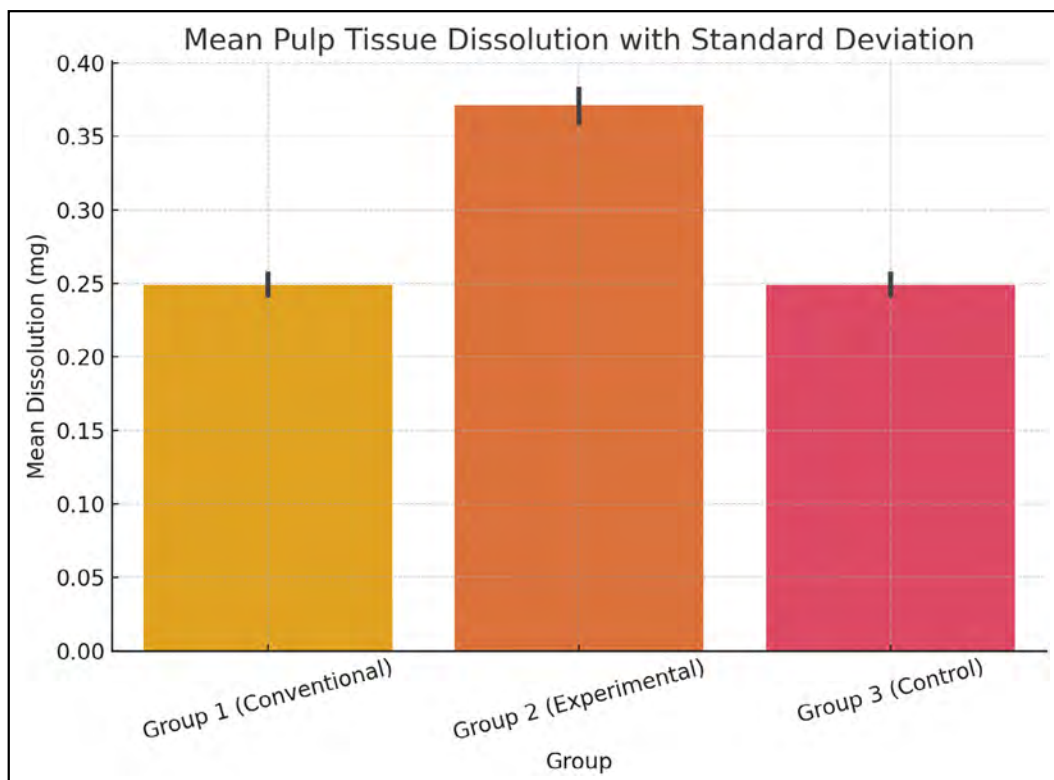


Figure 6. Mean pulp tissue dissolution (mg) by group with standard deviation error bars. The bar chart highlights the superior efficacy of the experimental irrigation protocol (Group 2) in achieving tissue dissolution. Conventional irrigation (Group 1) and no irrigation (Group 3) yielded similarly low dissolution outcomes.

TABLE 2. Tukey HSD post hoc results

Comparison	MD (mg)	95% CI		p value
		Lower	Upper	
Group 1 vs Group 2	0.122	0.1118	0.1322	<0.001*
Group 1 vs Group 3	0.000	-0.0102	0.0102	1.000
Group 2 vs Group 3	-0.122	-0.1322	-0.1118	<0.001*

*: Statistically significance. HSD: Honestly significant difference, MD: Main difference, CI: Confidence interval, Group 1: Conventional, Group 2: Experimental, Group 3: Control

Ultrasonic activation during irrigation in orthograde treatment enhances the delivery and agitation of irrigants through physical phenomena such as flow and cavitation (1, 26, 27). *In vitro* studies have demonstrated that ultrasonic activation improves canal cleaning, increases irrigant penetration into the canal system, facilitates tissue debridement, and enhances the removal of biofilm and smear layer (26, 28). Additionally, the adjunctive application of EDTA further removed smear layers due to its chelating and decalcifying properties (1).

Building on previous findings, this study introduces a new retro-cavity irrigation protocol utilising activated irrigants. Integrating modern ultrasonic tools with advanced activation techniques aims to improve the clinical success of surgical endodontic procedures, which are currently reported to be around 91% (8).

One ongoing debate in apicoectomy relates to the optimal extent of root removal. There is currently no clear agreement on the exact amount that should be excised while respecting biological guidelines. According to Gilheany et al. (29), removing at least 2 mm from the apex may help reduce bacterial leakage into the canals. In contrast, Kim et al. (11) recommend removing at least 3 mm to eliminate 98% of apical ramifications and 93% of lateral canals, as observed in anatomical studies. Most current literature supports a 3-mm resection, maintaining about 7–9 mm of root to ensure proper resistance and structural integrity in teeth of standard length (30).

Resections under 3 mm may leave behind untreated lateral and apical canal branches, which can increase the chance of reinfection and treatment failure (7). This study evaluates two different approaches, emphasising a more conservative technique that removes less apical tissue than conventional methods.

The described approach involves conducting a conventional retro-preparation using retro-tips in combination with saline irrigation. This step is followed by ultrasonic activation of 25.02 EDTA and sodium hypochlorite gel to enhance debridement. The gel's viscosity helps limit the risk of extrusion into the surrounding alveolar bone and allows more accurate control during application.

Previous research evaluating EDTA activation alone has shown promising results (25, 28). This study builds on that by incorporating NaOCl gel alongside EDTA. The addition of NaOCl gel further reduces bacterial load after EDTA removes the smear layer.

The *in vitro* results demonstrate a statistically significant reduction in debris between teeth irrigated using the traditional cleaning protocol (Group 1), the control (Group 3), and the spicemens with the proposed irrigation protocol (Group 2). Group 2 exhibited significantly less debris on the walls of the canal. The differences between the protocols can be attributed to variations in the apicoectomy procedure and the cleaning of the retrocavity. Specifically, the new protocol involves resecting only 1 mm from the apical third, in contrast to the standard 3 mm.

This conservative approach is justified by the enhanced cleaning achieved through ultrasonic activation of EDTA and NaOCl gel, allowing better irrigant diffusion into lateral anatomies. Consequently, extensive apical tissue removal becomes unnecessary. Moreover, the proposed approach preserves the apical portion of the tooth, especially in cases with an unfavourable crown-to-root ratio.

The additional experiment using a synthetic canal model further reinforces the efficacy of the proposed technique. In this model, only 5.25% NaOCl gel was used, without EDTA, given the absence of a smear layer in the plastic blocks. The results confirmed that ultrasonic activation of NaOCl alone was sufficient to achieve complete pulp tissue dissolution in lateral extensions, unlike the traditional and control groups, which showed no dissolution. These findings support the protocol's ability to effectively remove organic material from complex anatomical areas, even with minimal resection.

In summary, improving the quality of retro-cavity cleaning can reduce the extent of apical root resection, enabling effective treatment of teeth with unfavourable crown-to-root ratios, poor periodontal support, or prior surgical interventions without excessive apical reduction.

Safety Considerations of Retrograde NaOCl Gel Irrigation

The use of NaOCl in endodontic procedures has historically raised concerns regarding its potential extrusion and associated tissue damage. However, in the present protocol, NaOCl was used in a high-viscosity gel formulation, which allows for controlled placement and significantly reduces the risk of apical extrusion. Throughout the experimental phase, no extrusion of irrigant was observed during ultrasonic activation. This safety is further enhanced when procedures are performed under high magnification—ideally with an operative microscope—which provides superior visibility and control. Notably, retrograde irrigation in an open surgical field offers greater safety margins compared to orthograde irrigation, where several anatomical and procedural variables increase the risk of inadvertent extrusion. In addition, the proposed technique aims to limit the extent of apical resection, offering a conservative alternative particularly suited to teeth with a compromised crown-to-root ratio. While clinical application requires adequate training, as with all advanced endodontic techniques, the protocol is considered both safe and reproducible when proper safeguards are employed.

Limitations and Future Directions

One limitation of the present study is the reliance on manual assessment of SEM images for smear layer and debris scoring. Although evaluations were conducted by three blinded and calibrated examiners following a standardized protocol, and inter- and intra-examiner reliability was confirmed through Cohen's Kappa coefficient, the absence of software-based quantitative image analysis introduces a degree of subjectivity. Future investigations should consider incorporating digital analysis tools or artificial intelligence-based systems to minimize observer bias and improve reproducibility in SEM evaluations.

The current study did not evaluate the antimicrobial efficacy of the proposed technique. Future studies should include antimicrobial testing, micro-CT analysis, and additional validation methods to confirm these findings. Moreover, the retro-cavities were prepared under controlled laboratory conditions, which may not fully replicate the technical challenges encountered in clinical apicoectomy procedures on mandibular premolars. This should be considered a study limitation, as surgical constraints, such as restricted access and surrounding anatomical structures, were not fully simulated. Future investigations should aim to reproduce more realistic surgical conditions to enhance clinical applicability.

CONCLUSION

The conservative technique produced statistically significant differences among the groups, suggesting that this approach in endodontic surgery not only maintains a favourable crown-to-root ratio but also improves treatment outcomes by enhancing retro-cavity debridement through ultrasonic irrigant activation.

Utilising limited root-end resection alongside ultrasonically activated irrigants in retro-cavities presents a valuable strategy for retaining teeth with reduced crown-to-root ratios, cases that might otherwise be considered non-restorable and extracted.

Disclosures

Ethics Committee Approval: The study was approved by the University of Naples, Federico II Ethics Committee (no: N. 00008975, date: 28/03/2023).

Informed Consent: Informed consent was obtained from all participants.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Use of AI for Writing Assistance: The authors declare that no artificial intelligence (AI)-assisted technologies, including large language models (LLMs), chatbots, or image generation tools, were used in the preparation of this manuscript.

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Effect of the Chelating Agent Alendronic Acid versus EDTA on the Physicochemical Properties of Dentine

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ABSTRACT

Objective: The present study aimed to evaluate the changes in the physicochemical properties of dentine after irrigation with a solution of 0.22% alendronic acid (AA) as a chelating agent compared to 17% ethylenediaminetetraacetic acid (EDTA).

Methods: A total of 48 extracted premolars and molars that were intact, free of caries or cracks, without root canal treatment and restorations were collected. The roots were randomised into three groups (n=16): Group A: Distilled Water (dH₂O); Group B: 17% EDTA, and Group C: 0.22% AA. Longitudinal sections of the dentine with a root of 1x1x10 mm were made with a diamond disc and a low-speed handpiece for bending tests (n=9). For morphological analysis, images were taken with a scanning electron microscope, crystallographic analysis with X-ray diffraction, and chemical analysis with Fourier Transform Infrared Spectroscopy (FTIR) and Vickers Hardness. For this purpose, cross-sections were made through the root using the Isomet to obtain 3 mm thick dentine discs (n=14). The samples were stored in dH₂O for up to 24 h before use and dried at room temperature before exposure to chelating solutions for 1 h in a Stuart STR6D mixer at 50 rpm. For data comparison, the Kruskal-Wallis statistical test was used ($\alpha=0.05$).

Results: The chelating solutions of EDTA and AA cause alterations in the physicochemical structure of dentine, attacking mainly the inorganic part (Hydroxyapatite), which was observed in the decrease in intensity of the peaks in the X-ray diffraction pattern of hydroxyapatite. This generated a greater exposure of the collagen fibres that were observed in SEM and the increase in the bands characteristic to Collagen Type I in the infrared spectrum at 1645, 1550, and 1240 cm⁻¹ belonging to amide I (C=O), amide II (N-H) and amide III (C-N), significantly affecting its dentine hardness ($p=0.001$).

Conclusion: AA can be used as a chelating agent in the area of dentistry. It does not generate a significant demineralising effect that modifies the physicochemical properties of dentine, as observed with EDTA.

Keywords: Alendronic acid, chelating solution, EDTA, flexural strength, vickers hardness

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HIGHLIGHTS

- Alendronic acid solution 0.2% is a potent chelating agent that does not modify the hardness of dentine.
- The solution generates minimal changes in chemical properties and does not cause the decalcification of dentine.
- An alendronic acid solution of 0.2% produces minimal erosion on the dentine surface compared to 17% EDTA.
- Alendronic acid solution 0.2% has the potential to be proposed as an alternative to 17% EDTA.

INTRODUCTION

The success of root canal treatment is associated with instrumentation and chemical-mechanical irrigation for disinfection and shaping of the root canal before three-dimensional filling. During root canal instrumentation, the formation of a residual layer known as the smear layer is inevitable. This layer consists of organic and inorganic debris, including dentinal particles, remnants of pulp tissue, and potential microbial contaminants (1). Therefore, irrigation with biocompatible, cost-effective solutions during instrumentation is essential for the elimination of necrotic tissue, reduction of bacterial load, decontamination of areas where files cannot be accessed, elimination of the smear layer, and lubrication of the root canal (2, 3). However, there is currently no irrigating solution that meets all these characteristics. Therefore, it is necessary to irrigate with a sequence of irrigating agents, including agents with chelating properties, to remove the smear layer, because it prevents the adhesion capacity of the cement sealer.

Ethylenediaminetetraacetic acid (EDTA) and citric acid are the most commonly used strong chelating solutions in the endodontic area (3–5). However, their action is not limited to the smear layer and causes demineralisation, leading to changes in the physicochemical properties of dentine, which is a hard-mineralised tissue composed of Type I collagen with hydroxyapatite and carbonates that provide rigidity and resistance. These changes in the properties of dentine can have a negative effect on restoration, leading to fracture and compromising the adhesion of the restorative material (6, 7). To achieve this aim, alternative chelating solutions have been evaluated, such as chemical compounds from the bisphosphonate family approved by the Food Drug Administration (FDA) such as etidronate (1-hydroxyethylidene-1,1-disphosphonate) and alendronic acid ((4-amino-1-hydroxybutane-1,1-diyl) bis (phosphonic acid)-AA) which contain phosphate groups that give them the ability to act as a chelating agent and bind to transition metal ions through coordination bonds (7, 8). In the case of etidronate, it was proposed by Zehnder et al. (9), within a continuous irrigation protocol in combination with sodium hypochlorite (NaOCl), giving rise to various investigations. In the case of AA, it has only been reported as a promising new chelating agent due to its effectiveness in eliminating intracanal medication (10). However, the lack of literature highlights the need for studies focused specifically on evaluating its effects on the changes that it could generate in the physicochemical properties of dentine after exposure. Therefore, the purpose of this study was to evaluate the effect of EDTA and AA chelating solutions through a comparison of their impact on flexural strength, Vickers hardness, and changes in chemical composition and crystallinity. The null hypothesis of this study was that no differences were observed in the physicochemical properties of dentine in samples irrigated with EDTA and AA.

MATERIALS AND METHODS

This research was conducted in full accordance with the World Medical Association Declaration of Helsinki and has been approved by the Research Ethics Committee of the Faculty of Stomatology, UASLP, with code: CEI-FE-033-023 (approved on 04/05/2023).

Preparation of 0.22% AA Solution

The 0.22% AA solution was prepared by dissolving 4 Dronadil® tablets (Laboratorio Alpharma, Ciudad de México, México) with 0.070 g of the active ingredient (AA) in 130 mL of dH₂O and adjusting the pH to 7 (0.195 g/tablet).

Preparation of 17% EDTA Solution

The 17% EDTA solution was prepared by dissolving 17g EDTA (Hycel; Jal, México) in 100 mL of dH₂O and adjusting the pH to 7 (9.25 mL 5 M sodium hydroxide) (11).

Sample Preparation

48 recently extracted molars and premolars that were intact, free of caries or cracks, and without root canal preparation and restorations were collected and stored in dH₂O until further use. Teeth with curved roots, anatomical or morphological deformities, resorptions, cervical abrasions, calcifications, cracks or fractures and immature apices were excluded. The teeth were randomly divided into three groups (Group A: dH₂O; Group B: 17% EDTA and Group C: 0.22% AA). For X-ray diffraction, FTIR, and Vickers hardness tests, 3mm thick vertical coronal dentine discs (one buccal and one lingual/palatal) were obtained from each tooth using the Isomet (Buehler, Illinois, USA). For mechanical analysis, 1×1×10 mm dentine blocks were made longitudinally with a diamond disc and a low-speed handpiece. Each dentine block and disc were measured with a Digital Vernier (Mitutoyo UK Ltd, Andover, Hampshire, UK) with a precision of 0.01 mm. The samples were washed with dH₂O in a sonicator bath for 4 min (5 washes, until a translucent liquid was obtained, each with a volume of 40 mL) to eliminate the smear layer generated by the cuts. They were stored in dH₂O, and 24 h before use, they were dried at room temperature before being exposed to chelating solutions for 1 h in a mixer (Stuart, STR6D, Delaware, USA) at 50 rpm Figure 1 shows the flowchart of the experimental procedures of the study). For data comparison, the Kruskal-Wallis statistical test was used ($\alpha=0.05$).

Morphological Characterization

For Scanning Electron Microscope (SEM), three roots were randomly selected and divided into two halves along the coronal-apical axis. Grooves were made along the longitudinal axis of each root using a diamond blade and a low-speed handpiece. The roots were then divided longitudinally into two halves using a chisel and mallet. Dentine morphology was evaluated using a scanning electron microscope (SEM; JSM-6510, JEOL, Tokyo, Japan) operating at 10kV, and images of the top surfaces of each root were captured at 3500x and 7000x magnification (two areas from each root were randomly selected and analysed in the coronal third). For this, the samples were dehydrated in serial amounts of 20 to 100% alcohol for 10 min. The samples were cut in half and placed vertically on pins using carbon tape with double adhesive and coated in gold for 40 s.

Chemical Analysis

The chemical changes generated by the irrigating agents were evaluated using the Fourier Transform Infrared Spectroscopy (FTIR) in the attenuated total reflectance mode (ATR) in the intermediate infrared region from 4000 to 400 cm⁻¹ (Thermo

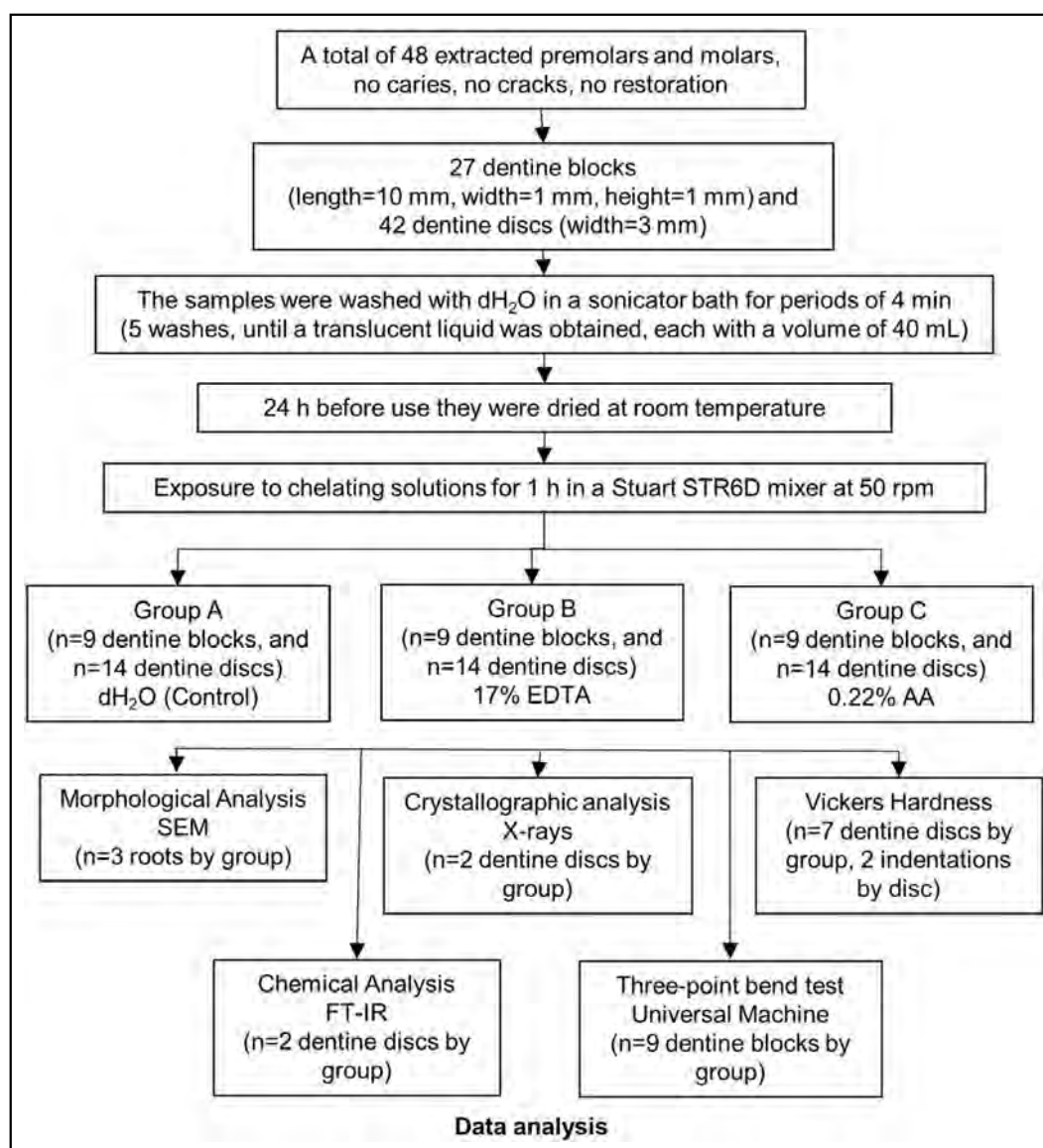


Figure 1. The flowchart of the experimental procedures of the study.

EDTA: Ethylenediaminetetraacetic acid, AA: Alendronic acid.

Fisher Scientific, Nicolet IS50, Waltham, USA), at a resolution of 4 cm⁻¹ and 32 scans. For FTIR-ATR, two discs were randomly selected from each experimental group.

X-ray Diffraction Test

Changes in the crystalline structure on the dentine surface after irrigation were evaluated by X-ray diffraction. Post-irrigation dentine discs were analysed on a PANalytical X'Pert PRO X-ray diffractometer (PANalytical; Empyrean, Houston, TX, USA) with a Cu-Kα monochromatic radiation source at a wavelength of 1.54 Å in a range of 10 to 70° in 2θ, at intervals of 0.02° and an acquisition time of 12 s/step. They were also analysed using the X'Pert HighScore Plus PANalytical software. For the X-ray diffraction test, two discs were randomly selected from each experimental group.

Mechanical Analysis

The sample size per group for the mechanical tests was calculated using G*Power v.3.1.9.7 software for Windows 10

(Heinrich Heine, University of Düsseldorf, Düsseldorf, Germany) using the Wilcoxon-Mann-Whitney test. An alpha error of 0.05, the beta power of 0.95, and an N2/N1 ratio of 1 were considered. The test calculated a total of 9 samples/group. The mechanical changes generated by the action of the irrigants were evaluated in a universal testing machine (Shimadzu, AGS-X500, Carlsbad, USA) using a three-point bending test. 1x1x10 mm dentine bars were evaluated at a speed of 1mm/min and with a load of 0.1N.

Vickers Hardness

Vickers test was performed to measure the surface hardness of dentine after irrigation. Indentations were made 0.5 mm from the root canal space using the Shimadzu Vickers durometer (Digital Microhardness Tester, HMV-G, Mammelzen, Germany) with a load of HV 0.1 (980.7 mN) for 10 s. To increase the power of the Vickers hardness test, the decision was made to increase the sample size to 14 measurements per group (n=7 dentine discs by group, 2 indentations per disc).

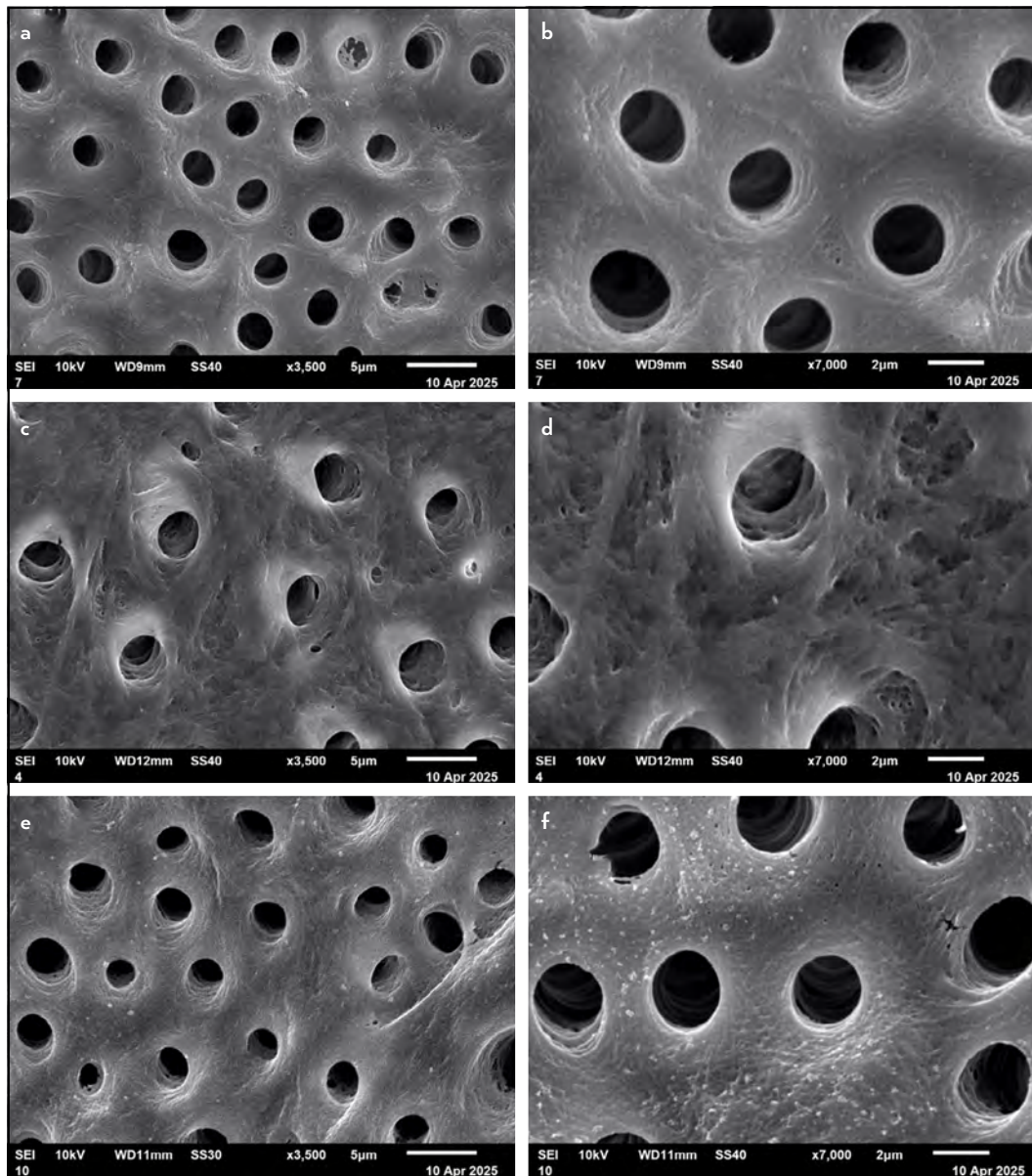


Figure 2. Scanning electron microscopy (SEM) images of the surface with secondary electrons. (a, b) dH₂O, (c, d) EDTA and (e, f) AA at 3500x and 7000x magnification.

EDTA: Ethylenediaminetetraacetic acid.

Statistical Analysis

The statistical analysis was performed using the Minitab software version 18 (Minitab, LLC is a privately owned company headquartered in State College, State College, PA, USA), where the Shapiro-Wilk normality test was performed to evaluate the normality of the data, where the non-normality of the data was determined. After this, the Kruskal-Wallis statistical test was performed ($\alpha=0.05$). The flow chart of the experimental procedures is presented in Figure 1.

RESULTS

Morphological Characterization

Micrographs obtained by SEM, show the morphological changes in the surface of the dentine after irrigation (Fig. 2). In Group B (Fig. 2c, d), the exposed collagen fibres are observed in the intertubular dentine. While in Group A (Fig. 2a, b), the

morphology is observed with a smooth surface, free of exposed collagen fibres, like that of Group C (Fig. 2e, f).

Chemical Analysis

After baseline correction and normalization, the FTIR spectra were analysed. Figure 3 shows the infrared spectrum of dentine after irrigation with irrigating solutions. In Group A (Fig. 3a), bands corresponding to the asymmetric stretching of the phosphate group (PO_4^{3-}) are observed around 1033 cm^{-1} , which constitutes hydroxyapatite. Likewise, another characteristic band of the phosphate group at 962 cm^{-1} is associated with symmetric stretching. A medium-intensity band is observed in the region $565\text{--}566\text{ cm}^{-1}$ and at $603\text{--}604\text{ cm}^{-1}$, associated with an asymmetric bending of the O-P-O bond (12). Peaks in the region of $960\text{ to }1100\text{ cm}^{-1}$, and the bands of $603\text{ and }565\text{ cm}^{-1}$ correspond to the decrease in the phosphate of hydroxyapatite in Group B (Fig.

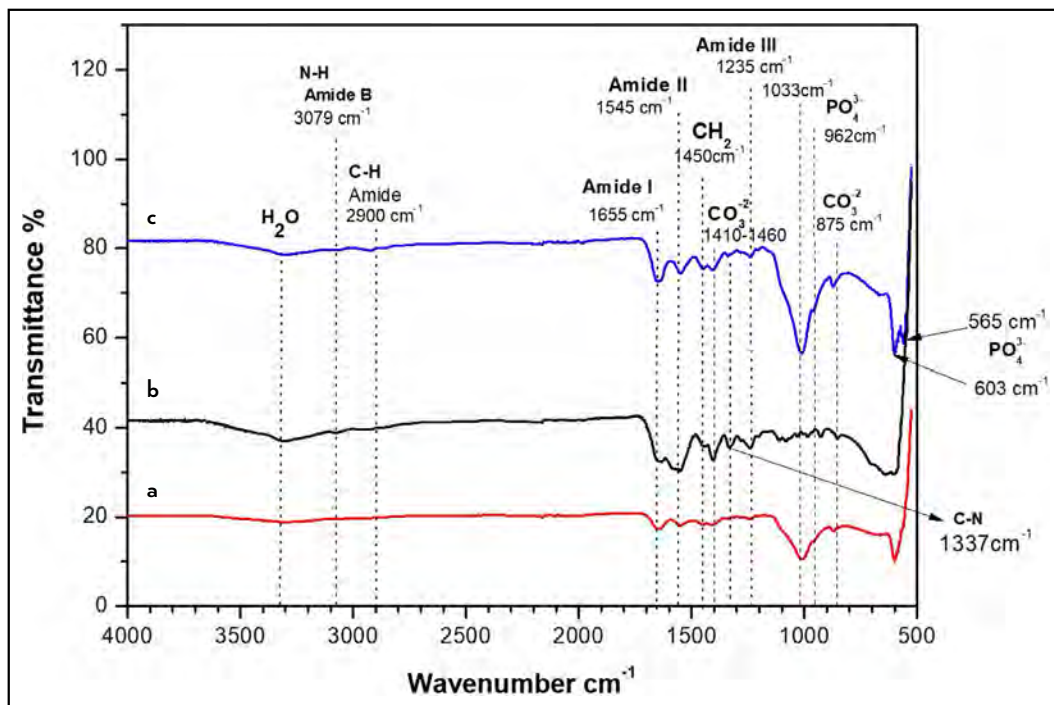


Figure 3. Fourier transform infrared spectrum of dentine after irrigation with chelating solutions. (a) dH₂O, (b) EDTA and (c) AA.

EDTA: Ethylenediaminetetraacetic acid, AA: Alendronic acid.

3b). Regarding Collagen Type I, another constituent of dentine, characteristic collagen bands can be observed around 1655, 1550, and 1235 cm⁻¹, which belong to amide I (C=O), amide II (N-H) and amide III (C-N) (12). Also, a shoulder is observed at 3079 cm⁻¹ associated with free N-H stretching for amide II. Around 2900 cm⁻¹, another band appears associated with asymmetric C-H stretching of the amide group (13, 14). The CH₂ group appears at 1450 cm⁻¹ with a deformation vibration (12, 13). Regarding the characteristic bands of amide I, the vibration of the amide carbonyl groups along the polypeptide backbone can be found (15) between 1650–1680 cm⁻¹, which correspond to the vibration of C=O bonds, a C-N stretching and a N-H deformation usually reported at 1653 cm⁻¹ (13).

Amide II, with a stretching movement of the C-N bond and a N-H swing, presents a characteristic band at 1545 cm⁻¹ to the amide groups of the collagen triple helix. Amide III is found between 1240–1242 cm⁻¹ and corresponds to the N-H deformation associated with tertiary amines. Therefore, the ratio between Amide III/Amide I can be used to infer the relationship between carbon and nitrogen present in collagen. In addition, a medium intensity band associated with C-N stretching is also observed at 1337 cm⁻¹. Regarding the infrared spectrum, the ratio of Amide III (1235 cm⁻¹), CH₂ (1450 cm⁻¹) and CO₃²⁻ group (1410 cm⁻¹) increased in intensity in Group B, due to the loss of inorganic structure, as well as a stretching and amplitude of the Amide II peak (1545 cm⁻¹), which corresponds to the changes in N-H and the stretching of the average intensity of C-N. To the spectrum of Group C, only an increase in the Amide I signal is observed.

X-ray Diffraction Test

The crystallographic properties of dentine were evaluated by post-irrigation X-ray diffraction. The diffraction pattern of each of the treatments can be seen in Figure 4. It can be observed that Group A presents peaks of greater intensity at 25.89, 32.05, 39.67, 46.78, 49.49, and 53.54 in 2θ. According to the X'Pert HighScore Plus PANalytical software database, the pattern corresponds to the crystallographic card 00-001-1008 of the JCPDS-International Centre for Diffraction Data database, and according to the Crystallography Open Database with card 1521038, both correspond to a hydroxyapatite in which the main peaks correspond to the crystallographic planes (002), (210), (211), (112), (022), (310), (222) and (213) respectively (16). Type I collagen, one of the other main components of dentine, corresponds to a low crystallinity polymer. Regarding Groups A and B, a decrease in the intensity of the peaks of the crystalline planes can be observed, indicating a reduction in the degree of crystallinity, possibly due to the loss of Ca²⁺ ions.

Mechanical Analysis and Vickers Hardness

Table 1 shows the results obtained from the bending test, in which it is observed that the irrigating solutions did not produce significant effects on the bending of the dentine (35.32, 35.41 and 33.39N, respectively). According to the Kruskal-Wallis statistical test using the Minitab software version 18 (Minitab, LLC is a privately owned company headquartered in State College, State College, PA, USA), no statistically significant differences were found (p=0.409). However, regarding the hardness of the dentine, a decrease of 43.7% was observed when it was irrigated with EDTA (25.53 HVN), compared to the control sample (54.92 HVN). According to the Kruskal-Wallis statistical test, statistically significant differences were found (p=0.001).

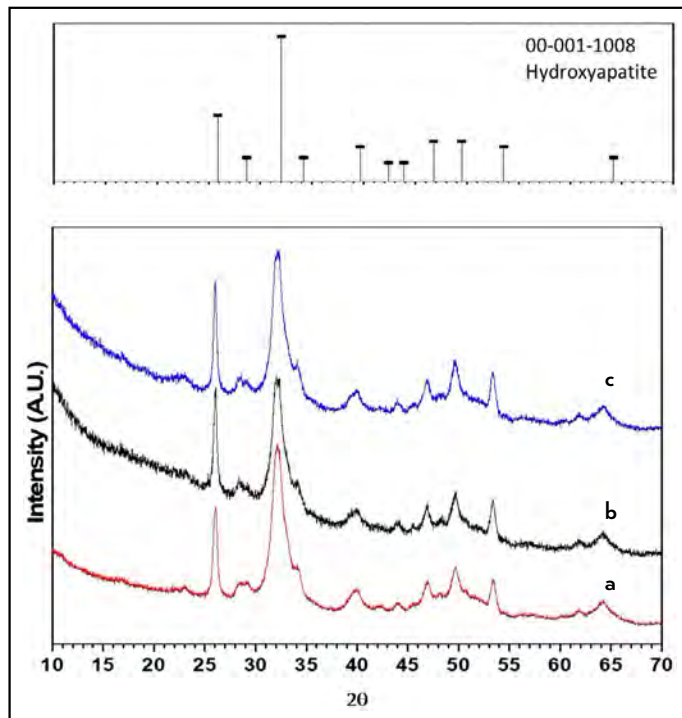


Figure 4. Diffraction pattern of dentine after irrigation with chelating solutions. (a) dH₂O, (b) EDTA and (c) AA.

EDTA: Ethylenediaminetetraacetic acid, AA: Alendronic acid.

DISCUSSION

Dentine is the principal dental structure that absorbs mechanical loads (17). Its chemical composition is up hydroxyapatite crystals, which represent 70% of dentine and correspond to the mineral part, as well as 20% organic matrix in the form of type I collagen and 10% water (18, 19). Dentine is presented in the form of a dense collagen network covered by hydroxyapatite (20). Collagen is a fibrous and insoluble protein composed of three polypeptide chains formed by the repetition of amino acids such as glycine, proline, and hydroxyproline, and the union of the 3 chains occurs through hydrogen bonds forming the triple helix (21). Dentine hydroxyapatite is composed of smaller calcium phosphate crystals, richer in carbonates and poorer in calcium, for which the chemical formula is Ca₁₀(PO₄)₆(OH)₂ (22).

Histologically, dentine contains dentinal tubules, which are cylindrical structures that extend from the pulp to the amelo-

dentinal or cementodentinal junction, and house the odontoblastic processes. These tubules are covered by peritubular dentine, which in turn are joined together by intertubular dentine composed of a matrix of collagen and apatite fibres. For Intertubular dentine, much of the dentine and its structure is made of collagen fibres at right angles to the tubule, in which hydroxyapatite crystals are deposited (23). These channels vary in number and can increase as they are closer to the pulp, which gives it anisotropic behaviour, that is, its properties are different depending on the direction. Therefore, this composition and structure of dentine provide it with three physical properties, namely hardness, resistance, and elasticity, which are essential to understanding masticatory function.

In endodontic treatment, the elimination of microorganisms from the root canal system is essential. In addition to mechanical instrumentation, chemical disinfection using irrigating solutions plays a crucial role. (2, 3). In the instrumentation processes, a layer is created that covers the walls of the canal, composed mainly of remains of dentine and pulp tissue, and microorganisms, called the smear layer (24). Therefore, using irrigating solutions is essential, and among the most used in endodontic treatment is sodium hypochlorite (NaClO), due to its bactericidal effect and ability to dissolve organic matter and necrotic tissue (24). Therefore, when used as an irrigating solution in the preparation of the canal, the dentine walls are left with fewer organic remains but are covered with inorganic particles (25). To remove the inorganic components of the dentine barrel, it is important to combine it with a chelating solution such as EDTA. This was reported by the study of Ligeng Wu et al. (26), where combinations of NaOCl with chelating agents were performed, and it effectively removes the dentine smear layer. However, the physicochemical properties of dentine can be affected. This causes decalcification of the dentine of the canal, which can increase when using strong chelating solutions, which can compromise the sealing of the root canal (25, 27).

The hardness of dentine was evaluated by the Vickers hardness test, which is a universal test that uses a diamond indenter to affect the sample at a defined force, allowing the hardness of the material to be measured, since the larger the indentation, the softer the material. Regarding the results of this research, the hardness decreased in Group B, which was irrigated with EDTA, from 58.45 to 25.53 HVN, which represents a statistically

TABLE 1. Median, first quartile (Q1) and third quartile (Q3) of the flexural strength and microhardness (VHN) of dentine after irrigation with chelating agents

Specimen	n	Flexural strength		n	HVN	
		Median	Q1-Q3		Median	Q1-Q3
Group A	9	35.32	31.84–52.08	14	54.925	52.83–61.53
Group B	9	35.41	32.61–38.83	14	25.535*	23.15–28.83
Group C	9	33.39	28.04–37.60	14	58.450	39.68–63.93
p		0.409			0.001	

The Kruskal-Wallis test; *: Indicates a significant difference between the control group and the chelating agent (vertical) group ($p < 0.05$). VHN: Vicker's hardness number, HVN: Vickers hardness number

significant change in said property of dentine. However, these significant changes were not appreciated in Group C. Furthermore, the action of EDTA is not self-limiting, meaning that its chelating effect continues until all the available solution forms complexes between the salt and the calcium of the dentine, generating a chemical change in the dentine. This was corroborated by FTIR, by decreasing the bands associated with hydroxyapatite, as well as the bands that appear at 1100 cm^{-1} and 1005 cm^{-1} of low intensity, which are identified by the deformations of the C-O-H, C-O, and C-O-C groups of carbohydrate residues and out-of-plane torsions of carboxylic acids (15). According to the studies reported by Barón in 2020, they are associated with the amide III/ CH_2 that increased significantly, and this is because dentine is richer in collagen after exposure to EDTA (13). These facts are corroborated in the micrographs obtained with the SEM, where there is a decrease in hydroxyapatite (inorganic matter), which covers the collagen fibres, which are mostly exposed when the hydroxyapatite is lost due to the effect of these solutions.

This is in accordance with the study carried out by Liñan et al. (28), where they observed the degree of erosion of EDTA in the different thirds. The presence of dentinal canaliculi can be seen due to the destruction of peritubular and intertubular dentine.

Likewise, in 2002, Calt and Serper reported that EDTA causes demineralisation effects on peritubular and intertubular dentine over time (29). Other studies show that the use of EDTA results in the elimination of the dentine barrel, but results in a demineralisation of the superficial dentine of 4 to $6\text{ }\mu\text{m}$ in the coronal, middle, and apical third of the canal (30). Furthermore, these changes were confirmed in the tests of the analysis of the crystallinity of the dentine, since they are related to the changes observed in the diffraction patterns. In X-ray diffraction, a decrease in the crystallinity of the dentine is observed where there is a decrease in the signal of the crystalline planes of hydroxyapatite, which is the part that mainly provides hardness to dentine, since it is a biocrystal composed of calcium, phosphorus and hydrogen atoms.

However, although dentine is less mineralised than enamel, collagen is what gives it the capacity for compression and traction, so in the bending test, statistical changes were not appreciated (31). These physicochemical changes of dentine after exposure to chelating solutions are due to minerals present in dentine, mainly phosphate and calcium, which are soluble in water. The chelating interacts with the calcium ions present in the structure of hydroxyapatite, generating complexes and not interacting with the organic part of dentine (13). These changes generated by EDTA have been reported by Beltz et al. (32), where they observed that NaOCl dissolves 90% of the organic components of dentine, and EDTA at 17% dissolves 70% or more of the inorganic matrix. EDTA is a strong chelating agent because it has six potential sites in its chemical structure to bind to metal ions, in addition to its high concentration. In contrast, AA is a compound of the bisphosphonate family, for which the mechanism of action is an antiresorptive effect, that is, it prevents the function of osteo-

clasts, decreasing bone resorption. It is characterized by having two phosphoric groups and an amine group in its chemical structure, in addition to its hydroxyl group (OH)-, which gives it the property of a strong chelating agent, attracting metal ions. However, the AA solution used in this work, as well as being an FDA-approved compound, does not affect the physical properties of dentine. Since its toxicity is low, its clearance from plasma and soft tissues is fast, making it easy for the kidney to eliminate it quickly. In addition, its absorption decreases when taken with food, especially if it contains calcium, iron, coffee, or tea. Between 20–80% is deposited in the bone, the rest is excreted in the urine (31).

Bisphosphonates are synthetic compounds with structural characteristics similar to pyrophosphates, so they bind to the hydroxyapatite of the bone, where the oxygen element (P-O-P) is replaced by the carbon in the bisphosphonate (P-C-P), which makes it resistant to hydrolysis. Therefore, the solubility of hydroxyapatite is reduced by this double phosphoric acid, which reduces osteoclast activity, decreases bone resorption, and stimulates osteoblasts to produce inhibitors of osteoclast formation.

Similarly, some studies have been carried out using chemical compounds of the bisphosphonate family as chelating agents in dentistry, such as those carried out by Lottanti et al. (25), where they evaluated the effect of EDTA, etidronic acid, and peracetic acid. It showed that EDTA presents greater demineralisation at 3 min, while Etidronic acid used as a final irrigant with NaOCl did not show demineralisation of the canal dentine. De-Deus et al. (33) compared the chelating effect of Etidronate at 9 and 18% and EDTA at 17%, reporting that after 60 s all the smear is eliminated. However, an increase is shown in the size of dentinal tubules over time, characteristic of demineralisation, with a faster activity than bisphosphonate.

This study has some limitations. One of them is the anisotropic characteristics of dentine, which can influence mechanical properties such as hardness and tensile strength. The contact time and volume of the irrigation solution used during root canal treatment may be less than those used in the trial. However, each group was subjected to the same conditions, as well as the randomisation of the teeth, allowing for comparable results among the chelating solutions evaluated. Another important aspect is the need for further *in vitro* and *in vivo* studies to evaluate the efficacy of the AA chelating agent in removing the smear layer from root canal walls and its antibacterial efficacy. This also includes an assessment of the implications this irrigating agent may have on restoration processes (34, 35).

CONCLUSION

EDTA and AA chelating solutions cause alterations in the physicochemical structure of dentine, attacking mainly the inorganic part (Hydroxyapatite). These alterations cause greater exposure of collagen fibres, with the EDTA solution being where the greatest changes are generated. However, these alterations do not compromise the bending of dentine, but rather its hardness when irrigated with EDTA. It is also

concluded that AA can be used as a chelating agent in the dentistry area since it does not have a significant demineralising effect that modifies the physicochemical properties of dentine, as observed with EDTA.

Disclosures

Ethics Committee Approval: The study was approved by the Research Ethics Committee of the Faculty of Stomatology, UASLP (no: CEI-FE-033-023, date: 04/05/2023).

Informed Consent: Informed consent was obtained from all participants.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Human Stem Cells of Apical Papilla Viability Following the Removal of Triple Antibiotic Paste in a 3D Root Canal Culture Model

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ABSTRACT

Objective: This study investigated the residual effects of various concentrations of triple antibiotic paste (TAP) on the viability of human stem cells of the apical papilla (hSCAPs) in a 3D root canal culture model.

Methods: Sixty-four single-rooted segments were prepared and allocated into five groups (n=12): four concentrations of TAP (1 mg/ml, 2.5 mg/ml, 5 mg/ml, 10 mg/ml) and a control group with vehicle alone. TAP was prepared by mixing USP-graded antibiotic powder with vehicle (macrogol and propylene glycol). The canals were filled with the prepared medication for 28 days. After removing TAP, fibrin gels containing hSCAPs were loaded into the canal and incubated for 7 days. Cell morphology was observed using confocal laser scanning microscopy (CLSM), whilst cell viability was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Results: The vehicle alone and the 1 mg/ml TAP groups showed viable and proliferative cell morphology. However, higher concentrations of TAP displayed non-proliferative cells as observed by CLSM. For the cell viability test, 1 mg/ml TAP did not demonstrate a different percentage of cell viability from the control group. However, 2.5 mg/ml, 5 mg/ml and 10 mg/ml TAP exhibited significantly lower percentages of cell viability compared with the control (p<0.001).

Conclusion: Cells can survive at low TAP concentrations of ≤1 mg/ml. However, harmful effects become evident at TAP concentrations of ≥2.5 mg/ml.

Keywords: 3D culture, cell viability, confocal laser scanning microscopy, human stem cells of apical papilla, regenerative endodontics, triple antibiotic paste

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HIGHLIGHTS

- This was the first study to investigate TAP cytotoxicity at concentrations of 1-10 mg/ml in a 3D root canal model.
- TAP concentrations ≥ 2.5 mg/ml generated a cytotoxic effect on hSCAPs.
- The control (vehicle alone) and low concentration TAP (1 mg/ml) were not toxic to hSCAPs.
- The CLSM analysis confirmed that the control and low concentration TAP (1 mg/ml) cells were viable and able to form a spindle shape in the fibrin scaffold.

INTRODUCTION

Triple antibiotic paste (TAP) is the medicament of choice for Regenerative Endodontic Procedures (REPs) because of its effective antimicrobial activity (1). Previous studies demonstrated that high concentration TAP (≥10 mg/ml) completely eradicated bacterial biofilm in root

dentine (2–4). However, the use of TAP showed a detrimental effect on stem cells and might impair the regeneration phase of REPs that are crucial for the treatment outcome (5–8). Therefore, the optimum concentration of TAP used in REPs has been discussed to balance the bactericidal efficacy and the adverse effects on stem

cells. The cytotoxic effect of various concentrations of TAP on stem cells of the apical papilla (SCAPs) has been investigated. A study using a direct contact method suggested that a non-toxic concentration of TAP should be 0.1 mg/ml, whereas 1 mg/ml and 10 mg/ml TAP showed a dramatic reduction in SCAP survival (5, 8). Recently, the residual effect of TAP in a 3D culture model demonstrated that 1 mg/ml TAP did not affect SCAPs, whereas 1,000 mg/ml resulted in no viable cells. However, this study did not investigate the cytotoxic effect of TAP between 1–1,000 mg/ml (6).

To our knowledge, 10 mg/ml TAP is effective to eradicate bacterial biofilm, however, it might be harmful to stem cells. In contrast, 1 mg/ml TAP, which showed less cytotoxic effect on stem cells, was insufficient to eradicate the bacterial biofilm. Moreover, most previous studies employed a direct contact method and a 2D culture model, which may not be directly applicable to clinical situations. Therefore, the purpose of this study was to evaluate the residual effect of freshly-mixed 1 mg/ml, 2.5 mg/ml, 5 mg/ml and 10 mg/ml TAP on human stem cells of the apical papilla (hSCAPs) survival in a 3D root canal culture model.

MATERIALS AND METHODS

Tooth Preparation

This study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Faculty of Dentistry and Faculty of Pharmacy, Mahidol University, Institutional Review Board (MU-DT/PY-IRB 2022/029.2705). Sixty-four single-rooted mandibular premolars were collected. Teeth with root fracture, root caries, root resorption, immature root formation, multiple root canals, canal calcification and previous root canal treatment were excluded. The teeth were stored in 0.1% (w/v) Thymol solution until used. To obtain 3-mm root segments, the teeth were cut at the cemento-enamel junction (CEJ) and 3 mm apically from the CEJ with a low-speed precision cutter (Isomet 1000, Buhler, New York, USA). The root canals were standardized to a 1.7 mm diameter by sequentially enlarging the canal with a Peeso reamer No. 1–6 (Dentsply Maillefer, USA) under irrigation with sterile phosphate-buffered saline (PBS; Sigma Aldrich, St. Louis, MO, USA). Smear layer removal was performed as previously described (9). The specimens were sterilized by autoclaving at 121°C 15 psi for 20 min. Four specimens were randomly collected and incubated in cell culture medium at 37°C for 24 hours to confirm the sterile condition by the absence of turbidity.

TAP Preparation

TAP was prepared at 1 mg/ml, 2.5 mg/ml, 5 mg/ml and 10 mg/ml using the antibiotic powder (USP-grade; Sigma Pharmaceutical, North Liberty, Iowa, USA) together with macrogol and propylene glycol (M:P ratio = 1:1) as a vehicle. The composition of a 1 mg/ml TAP preparation is shown in Table 1. The prepared TAP was used immediately after preparation.

Medication Procedure

The specimens were randomly allocated into 5 groups (n=12), i.e., 1 mg/ml, 2.5 mg/ml, 5 mg/ml, 10 mg/ml TAP and vehicle alone. The medication preparation was performed in a biosafety cabinet with a laminar flow (NU-126-300E, NUAIRE, Minnesota, USA). Each specimen was placed in a 48-well plate, 600 µl TAP or vehicle was added into the root canal using micropipettes and incubated at 37°C with 5% CO₂ and 95% humidity for 28 days. After incubation, the medicament was removed by irrigating the canals with 20 ml of 17% EDTA for 5 min and 20 ml of sterile normal saline for 5 min using a sterile syringe and 25-gauge needle before cell seeding.

Cell Seeding in Fibrin Gel

Primary hSCAPs were isolated from extracted human mandibular third molars and cultured as previously described (10). Cells at passages 3–6 were used in this study. The cells were stained with green fluorescent cytopainter dye (ab176735: Abcam, Cambridge, UK) for 30 min before loading into a fibrin gel. Eight mg/ml fibrinogen, 2.5 mM Ca++ and 2 NIH Units/ml thrombin were diluted (Tisseel kit Baxter, Zurich, Switzerland) in Tris-Buffered saline, pH 7.4. To prepare hSCAPs in 1% fibrin gel (11), 6×10³ hSCAPs were added to a thrombin solution prior to mixing with fibrinogen solution at a 1:1 ratio. Immediately after mixing, 15 µl of the mixture was loaded into the root canal and incubated for 30 min to allow complete gelation of the fibrin scaffold. Subsequently, 800 µl culture medium was added and the specimens were incubated at 37°C for 7 days.

Fluorescent Imaging

To investigate the cell morphology of viable hSCAPs, four specimens from each group were randomly selected and observed using a confocal laser scanning microscope (CLSM; Stellaris 8, Leica Microsystem, Wetzlar, Germany) at 10X and 20X magnification and resolution of 1024×1024 pixels. The images were analyzed with Leica Application Suite X (LAS X) software.

TABLE 1. The composition of TAP in each concentration for 1 ml preparation

	Antibiotic powder (mg)			Vehicle (ml)	
	Metronidazole	Ciprofloxacin	Minocycline	Macrogol	Propylene glycol
1 mg/ml TAP	0.33	0.33	0.33	0.5	0.5
2.5 mg/ml TAP	0.83	0.83	0.83	0.5	0.5
5 mg/ml TAP	1.66	1.66	1.66	0.5	0.5
10 mg/ml TAP	3.33	3.33	3.33	0.5	0.5

TAP: Triple antibiotic paste

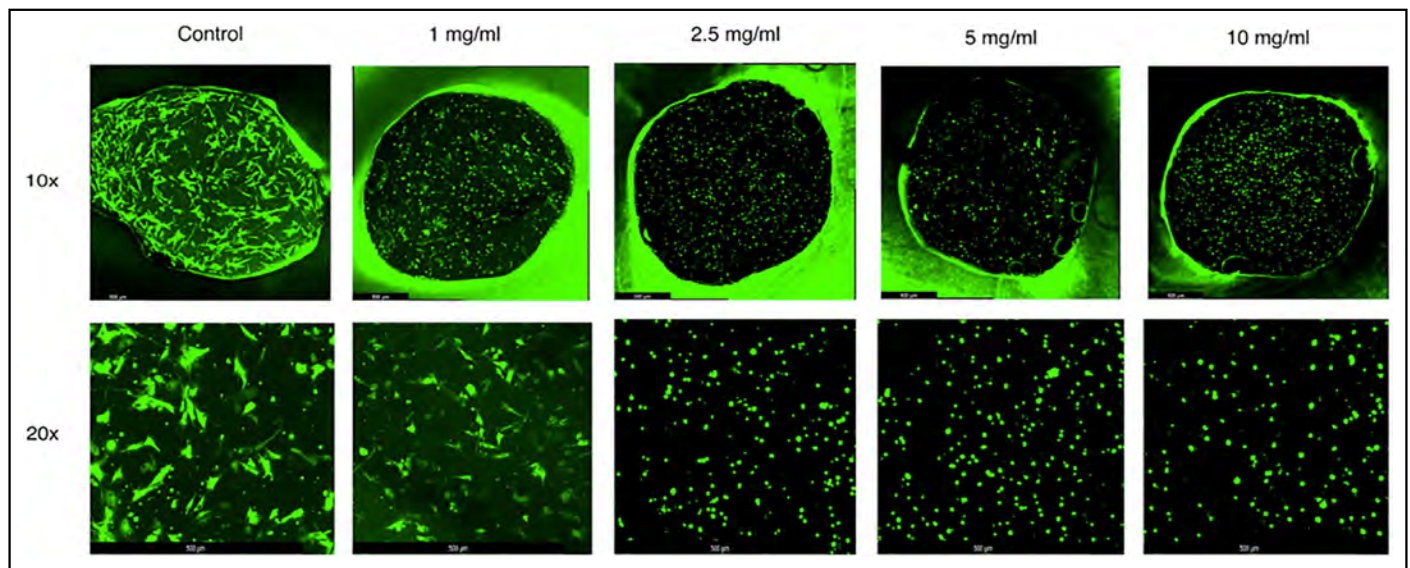


Figure 1. Representative CLSM images of viable hSCAPs (green colour) in each experimental group ($n=4/\text{group}$) under 10x and 20x magnifications. CLSM: Confocal laser scanning microscopy, hSCAPs: Human stem cells of apical papilla.

MTT Assay

After removing the old culture medium, the specimens were washed twice with PBS. Eight hundred microliters of 0.5 mg/ml MTT (Sigma, Berlin, Germany) in DMEM (Gibco, Life Technologies, Grand Island, NY, USA) were added to each well and incubated at 37°C for 4 hours. After discarding the supernatant, the precipitation was dissolved by adding 400 μl dimethylsulfoxide (DMSO) and mixed for 1 hour to ensure complete solubilization. The solution was transferred to a 96-well plate to measure the absorbance at OD570 nm by a spectrophotometer. The percentage of cell viability was calculated according to the following equation.

$$\text{Percentage of cell viability} = \frac{\text{OD570-OD690 value of each experimental group} \times 100}{\text{OD570-OD690 value of control}}$$

Data Analysis

Statistical analysis was performed using SPSS version 23.0.0.0 (SPSS Inc., IL, USA). One-way ANOVA and Games-Howell test were used to analyse the cytotoxic effect of residual TAP. The statistical significance level was set at $p < 0.05$. The cell morphology of each experimental group was also described.

RESULTS

Effect of Residual TAP on hSCAP Morphology As Determined by CLSM

The effects of 1 mg/ml, 2.5 mg/ml, 5 mg/ml and 10 mg/ml TAP concentrations on cell morphology and viability were evaluated (Fig. 1). In the control group, the cells appeared densely populated with spindle-shaped cells and that formed cell-to-cell interactions. Likewise, 1 mg/ml TAP demonstrated a similar cell density and morphology to the control group. In contrast, a reduction in cell density and structural integrity was observed with increasing TAP concentrations. At higher concentrations, the cells were small and round and isolated from each other, indicating decreased viability or increased cell death.

Effect of Residual TAP on hSCAP Viability As Determined by MTT Assay

The mean percentage of cell viability, with the control group (vehicle alone) set at 100%, is presented in Figure 2. TAP at a concentration of 1 mg/ml resulted in $82.24 \pm 22.28\%$ cell viability, which was not significantly different from the control group. In contrast, higher TAP concentrations exhibited a percent cell viability of 15.90 ± 12.22 , 18.96 ± 6.30 , and 14.25 ± 6.58 for 2.5, 5, and 10 mg/ml, respectively. A notable decrease in cell viability occurred as the TAP concentration increased from 1–2.5 mg/ml. Statistical analysis demonstrated that the percentage of viable cells in the 2.5 mg/ml, 5 mg/ml, and 10 mg/ml groups was significantly lower compared with the 1 mg/ml group and the vehicle alone ($p < 0.001$).

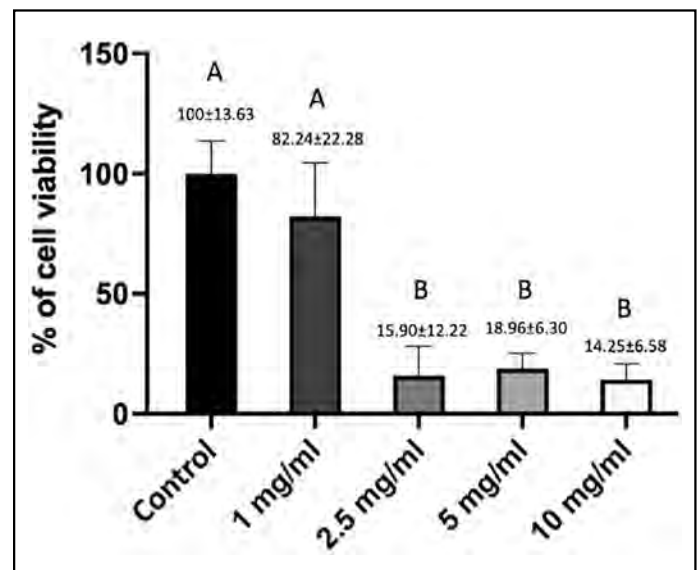


Figure 2. The percentages of cell viability in each experimental group ($n=12/\text{group}$) are represented as means and standard deviations. One-way ANOVA and Games-Howell test were used for statistical analysis. The different uppercase letters indicate a statistically significant difference at $p < 0.05$.

DISCUSSION

High concentrations of TAP are effective in eradicating bacterial biofilm, however, its cytotoxicity may adversely impact the viability of stem cells that migrate from the apical region into the root canal space during pulp tissue regeneration (12). An appropriate concentration of TAP has been discussed in order to balance the bactericidal efficacy and the adverse effects on stem cells. To evaluate the cytotoxicity of TAP on SCAPs, various concentrations of TAP were investigated in this study. The *in vitro* results demonstrated that the antibiotics exhibited cytotoxic effects on stem cells at high concentrations, resulting in significantly reduced cell viability and proliferation. The concentration at 1 mg/ml or lower was non-toxic, similar to the control group loading only vehicle into the canal. It is essential to create a microbe-free environment using the lowest effective concentration of medicament to support pulp-dentine regeneration.

The current study supports the idea that high concentrations of TAP, whilst bactericidal, can harm stem cell viability. Our MTT results demonstrated that TAP at 1 mg/ml maintained acceptable cell viability, consistent with a previous study using a 3D culture model, which found no significant impact on SCAP survival at this concentration (6). However, 1 mg/ml TAP was insufficient to eradicate the intracanal bacteria, which requires at least 10 mg/ml, a concentration detrimental to SCAP viability (2–4). Similarly, direct contact studies have shown that TAP concentrations ≥ 10 mg/ml significantly reduce stem cell survival, whilst lower concentrations (0.01–0.5 mg/ml) exhibit minimal cytotoxic effects (3, 5, 8).

The CLSM results revealed that cells in the 1 mg/ml TAP and control groups maintained a viable, spindle-shaped morphology, with a denser cell population in the control group. This confirms that mesenchymal stem cells can reside in fibrin gel, where they transform into spindle-shaped cells under non-toxic conditions (13). In contrast, cells in fibrin gel with TAP ≥ 2.5 mg/ml remained round, indicating reduced metabolic activity and a dormant state (14). Combined with the low cell viability from the MTT assay, this suggests potential apoptosis.

This study aimed to replicate the clinical environment for stem cell-based regeneration by addressing the limitations of previous approaches for evaluating the cytotoxicity of TAP and optimizing the use of fibrin gel as a biocompatible scaffold. Most studies on TAP cytotoxicity use direct contact methods (3, 5, 7, 8), which differ from clinical scenarios where TAP must be washed out to reduce toxicity before stem cells can be added. Moreover, the irrigation protocol was also performed according to the AAE guideline with some modification by additionally irrigating with 20 ml of normal saline (15). In the clinical setting, stem cells reside within a blood clot scaffold, forming a 3-dimensional (16). To replicate this, fibrin gel was chosen for its biocompatibility, nontoxic degradation and ability to form a porous, cross-linked network that supports cell adhesion, nutrient exchange, dental stem cell growth and facilitates pulp-like tissue formation (17).

The formulation of TAP, including the choice of antibiotics and vehicle, plays a critical role in determining its cytotoxic effects on SCAP viability, with differences in the preparation method potentially influencing outcomes. Although USP-grade an-

tibiotic powder was used to ensure accurate concentrations, previous studies have shown higher cytotoxicity in TAP made from USP-grade antibiotics compared with tablet or capsule forms (18). In the present study, MP was used as the vehicle for TAP due to its penetration properties and mild antimicrobial activity of propylene glycol (19, 20). Additionally, TAP with MP demonstrated lower acidity and reduced cytotoxicity compared with those prepared with distilled water (18). Our results also revealed that MP alone had no cytotoxic effect on stem cells, as seen from the control group.

In our study, as well as in a previous study (6), TAP concentration was limited to 1 mg/ml, which is below the effective range for REP, and reducing it further could compromise disinfection. Clinical studies found that full-strength TAP is associated with higher root development rates (15, 21), emphasizing the importance of disinfection in REPs. Although regeneration can still occur with high TAP concentrations, the survival rate may be affected by either infection or the toxicity of the antibiotic medicament. The current study supported the idea that high-concentration TAP produced high bactericidal efficacy and might be harmful to the viability of stem cells. However, reducing the antibiotic concentration may compromise disinfection efficacy. Therefore, the removal of TAP from the root canal should be a key consideration. This approach allows TAP to be used at an effective bactericidal concentration, followed by its removal prior to stem cell migration. Future research should focus on exploring more efficient TAP removal methods, incorporating adjunctive irrigation techniques, as conventional needle irrigation may be insufficient due to the deep penetration of TAP into dentinal tubules (22, 23).

CONCLUSION

TAP at 1 mg/ml had no significant effect on hSCAP viability, whereas 2.5 mg/ml, 5 mg/ml and 10 mg/ml TAP showed cytotoxicity to hSCAPs, as determined using a 3D root canal culture model.

Disclosures

Ethics Committee Approval: The study was approved by the Faculty of Dentistry and Faculty of Pharmacy, Mahidol University, Institutional Review Board Ethics Committee (no: MU-DT/PY-IRB 2022/029.2705, date: 27/05/2022).

Informed Consent: Informed consent was obtained from all participants.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Comparative Buckling Strength and Metallurgical Analysis of Five Classic NiTi Endodontic Rotary Files

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ABSTRACT

Objective: Nickel-titanium (NiTi) rotary instruments have revolutionized endodontic practice through continuous advancements in metallurgy and design. Despite these improvements, mechanical failure remains a clinical concern. This study aimed to evaluate and compare the design features, metallurgical properties, and buckling resistance of five widely used NiTi rotary endodontic systems.

Methods: A total of 250 new NiTi rotary instruments from five systems (ProTaper Next, Mtwo, ProFile, EndoSequence, and GT Series X) were analyzed. Design features were assessed using dental microscopy and scanning electron microscopy. Metallurgical properties were evaluated through energy-dispersive X-ray spectroscopy and differential scanning calorimetry. Buckling resistance was measured using a universal testing machine equipped with a 1 kN load cell, applying a compressive load at 1 mm/min until 1 mm of lateral displacement was achieved. Statistical analysis was performed using the Shapiro–Wilk test to assess normality, followed by the non-parametric Kruskal–Wallis test to compare groups. A significance level of $p < 0.05$ was adopted.

Results: ProFile instruments exhibited the highest number of spirals (19) and spiral density (1.19 spirals/mm), while GT Series X featured the shortest cutting blade length (≤ 12 mm). All systems demonstrated near-equiatomic nickel-titanium ratios. ProTaper Next and GT Series X showed higher R-phase and austenitic transformation temperatures. Buckling resistance was significantly greater in the ProFile (0.04 and 0.06 taper) and EndoSequence 35/.06 and 40/.06 instruments ($p < 0.05$). In contrast, EndoSequence 0.04 files, Mtwo, and ProTaper Next exhibited lower resistance.

Conclusion: Design features, taper, and metallurgical composition significantly influence the buckling resistance of NiTi rotary endodontic instruments.

Keywords: Buckling, differential scanning calorimetry, endodontics, energy-dispersive X-ray spectroscopy, scanning electron microscopy

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HIGHLIGHTS

- Advanced metallurgical treatments do not guarantee superior performance, ProTaper Next and GT Series X files with elevated R-phase and austenitic transformation did not show improved buckling resistance.
- The metallurgical treatments may influence the capabilities of an endodontic NiTi files, however the spiral density, cross sectional design and cutting blade length significantly affect the buckling strength.
- NiTi files with higher spiral density and greater taper showed superior buckling resistance but have reduced flexibility and potentially lower cutting efficiency.
- The study highlights the importance of selecting instruments to match specific clinical scenario, striking a balance between strength and flexibility.

INTRODUCTION

Mechanical instrumentation in endodontics has undergone significant advancements, particularly with the introduction and evolution of nickel-titanium (NiTi) files (1). In recent years, notable improvements in the quantity, quality, and design of these instruments have been achieved. Advances in metallurgy and design have led to the development of a wide variety of systems whose physical and metallurgical characteristics are fundamental to their clinical performance (2). These properties enable precise canal shaping, which is essential for effective irrigation and disinfection, particularly in anatomically complex root canals containing isthmuses, fins, and accessory canals (3, 4). The integration of mechanical instrumentation with appropriate disinfection protocols is critical for thorough microbial elimination, ultimately improving the success and quality of endodontic treatments (5, 6). Thus, the effectiveness of root canal therapy is closely tied to the physical and metallurgical attributes of the instruments employed (7).

Despite these advancements, all endodontic instruments remain prone to mechanical failure, particularly due to torsional stress and cyclic fatigue, which presents a significant challenge for clinicians (8, 9). Prior to the introduction of NiTi alloys, such failures were more frequently associated with stainless steel instruments, whose limited flexibility and fatigue resistance restricted their performance. The development of NiTi metallurgy has enabled the production of instruments with diverse designs, tapers, and heat treatments, which have significantly improved clinical outcomes by reducing the risk of iatrogenic complications such as canal deviation and perforation, while simplifying the shaping process (10). Nevertheless, NiTi instruments are not immune to deformation and fracture, necessitating ongoing innovation in their design and manufacturing to enhance both mechanical resilience and clinical reliability (11). To achieve these goals, manufacturers have employed techniques such as heat treatment, surface modification, and adjustments in instrument geometry and kinematics (12).

Among the many available systems, five widely used rotary NiTi instruments - ProTaper Next, Mtwo, ProFile, EndoSequence, and GT Series X - demonstrate notable differences in their metallurgical properties, cross-sectional design, and clinical performance. According to manufacturers and existing literature, ProTaper Next (Dentsply Maillefer, Ballaigues, Switzerland) incorporates proprietary M-Wire technology and an off-centered rectangular cross-section that facilitates debris removal while enhancing flexibility and cutting efficiency (13, 14). Mtwo (VDW, Munich, Germany) features an S-shaped cross-section with two active cutting edges and a small pitch, promoting preparation efficiency, stability, and debris elimination (15, 16). ProFile (Dentsply Maillefer, Ballaigues, Switzerland), the oldest system included in this study, is known for its excellent centering ability, particularly in canals with sharp curvatures. EndoSequence (Brasseler, Savannah, GA, USA) possesses a triangular cross-section with alternating contact points, electropolished surfaces for durability, variable pitch and helical angles, and a non-cutting tip to minimize canal aberrations (17). Finally, the GT Series X system (Dentsply

Maillefer, Ballaigues, Switzerland) features variable radial lands and an open-blade design that reduces threading and enhances cutting precision.

Despite these technological advancements, variability in instrument design, alloy treatment, and manufacturing processes can adversely affect mechanical properties such as flexibility, cyclic fatigue resistance, and cutting efficiency, ultimately impacting clinical outcomes (11). These inconsistencies may arise from pre-existing manufacturing defects or inappropriate clinical use. While most manufacturers adhere to rigorous quality control standards and perform *in vitro* testing before product release, batch-to-batch variability during mass production may still result in instruments with significant performance discrepancies.

One critical, yet often, overlooked mechanical property of endodontic instruments is buckling strength, which refers to the instrument's ability to withstand axial compressive forces without lateral deformation. During initial canal negotiation, glide path preparation, or retreatment procedures, particularly in constricted or calcified canals, files are frequently subjected to compressive stresses that may lead to buckling. An instrument with low buckling resistance may deflect prematurely, compromising directional control, reducing cutting efficiency, and increasing the risk of canal transportation or instrument fracture. Therefore, evaluating buckling resistance is essential to better understand the mechanical behavior of rotary NiTi files under clinically relevant loading conditions and to guide appropriate instrument selection based on procedural demands.

The NiTi systems examined in this study are considered "classic" instruments in contemporary endodontic practice, maintaining widespread clinical use years after their initial release. They have become reference standards against which newer systems are developed. Therefore, this study aims to evaluate and compare these five rotary NiTi systems in terms of design characteristics, metallurgical properties, and buckling strength, providing valuable insights for clinicians and researchers in selecting appropriate instruments for clinical applications.

MATERIALS AND METHODS

This study was conducted following the PRILE laboratory study guidelines (Fig. 1), a total of 250 brand-new 25-mm NiTi rotary endodontic instruments, differing in size, taper, and alloy heat treatment, were collected from five distinct multifile systems (ProTaper Next, Mtwo, ProFile, EndoSequence, GT Series X) for buckling testing, the inclusion of varying sizes and tapers was aimed to reflect the design differences among the brands included in the study as most endodontic files systems do not offer a directly comparable file with identical dimensions, and clinicians often choose instruments based on system-recommended protocols rather than uniform size specifications. Each endodontic file was inspected under a dental operating microscope (Opmi Pico, Carl Zeiss Surgical, Jena, Germany) at 13.6× magnification for any major defects that would necessitate exclusion from the

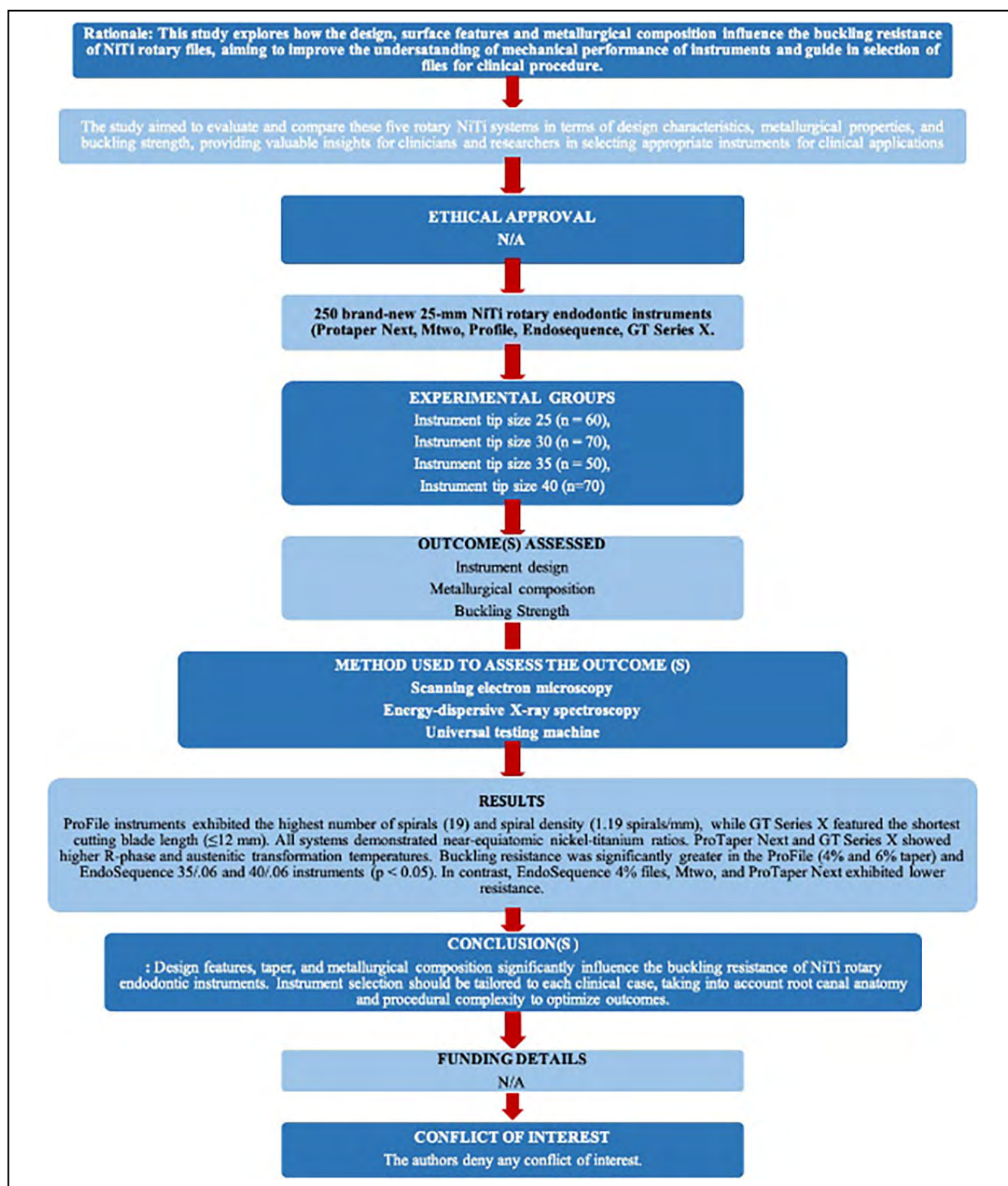


Figure 1. PRILE flow chart.

study; however, all instruments met the inclusion criteria.

Instrument's Design Assessment

Four files of each size were randomly chosen and inspected under a dental operating microscope (13.6× magnification) (Opmi

Pico, Carl Zeiss Surgical, Jena, Germany), with images recorded using a digital camera (Canon EOS 500D; Canon, Tokyo, Japan) to assess the cutting blade length, the total number of spirals, spirals per millimeter, and cutting spiral orientation. Next, the files were secured in a file holder and examined with a scanning elec-

tron microscope (150× magnification) (SEM) (S-2400, Hitachi, Tokyo, Japan) to evaluate surface finish and machining marks.

Metallurgical Assessment

The metallurgical features assessment was conducted on five reference endodontic files from each system (ProTaper Next X3 30/.07v, MTwo 30/.06, ProFile 30/.06, EndoSequence 30/.06 and GT Series X 30/.06). Only one reference file was selected per system, as each within a specific system undergoes identical metallurgical treatment.

A semi-quantitative elemental analysis was performed on three instruments from each system, utilizing energy-dispersive X-ray spectroscopy (EDS) with a standard scan electron microscope unit (DSM-962, Carl Zeiss Microscopy GmbH, Jena, Germany) equipped with an Inca X-act EDS detector (Oxford Instruments NanoAnalysis, Abingdon, United Kingdom). The device was operated at 20 kV and 3.1 amperes after a 10-minute vacuum preparation. Data collection took place over a 500 µm × 500 µm area for one minute, with a working distance set at 25 mm. Using the ZAF correction, the relative proportions of metallic elements were determined with specialized software (Microanalysis Suite v.4.14; Oxford Instruments NanoAnalysis, Abingdon, United Kingdom).

Differential scanning calorimetry (DSC) testing (DSC 204 F1 Phoenix; Netzsch-Gerätebau GmbH, Selb, Germany) was conducted to assess phase transformation temperatures, in accordance with ASTM F2004-17 (18) standards. A fragment of each instrument, 4 to 5 mm long and weighing 5 to 10 mg, was taken from the active blade and immersed in an etching solution (composed of 45% nitric acid, 25% hydrofluoric acid, and 30% distilled water) for 2 minutes. After neutralization in distilled water, each sample was placed in an aluminum pan within the DSC device, with an empty pan used as the control. The thermal cycle, spanning 1 hour and 40 minutes, was conducted under a nitrogen gas atmosphere. The temperature range for the cycle was set from -150°C to 150°C, increasing/decreasing at a rate of 10°C per minute. Netzsch Proteus Thermal Analysis software (Netzsch-Gerätebau GmbH) was used to process and generate DSC data and phase transformation temperatures graphs.

Buckling Assessment

The sample size was calculated based on the largest difference observed between two instruments in the initial five buckling tests. This calculation included the seven endodontic files with a tip size of 30 (ProTaper Next X3 30/.07v, MTwo 30/.06, ProFile 30/.04, ProFile 30/.06, EndoSequence 30/.04, EndoSequence 30/.06, and GT Series X 30/.06). With an alpha level of 0.05, a power of 80%, an effect size of 4.56, and a standard deviation of 2.63 (EndoSequence 30/.04 vs. ProFile 30/.06), a sample size of 7 instruments was calculated. As a result, 10 instruments per group were included in the final sample.

The buckling tests were conducted using a universal testing machine with a 1 kN load cell (Instron Corporation 4502; serial no. H3307, Bucks, England). Each instrument was positioned vertically, secured by the handle to the machine head, with the tip pointing downward into a small slot on a stainless-steel test base for stabilization (19). During testing, a compressive

load was applied at a rate of 1 mm per minute along the instrument's axis, from the handle toward the tip, until it achieved a lateral displacement of 1 mm. The peak buckling load was recorded in Newtons (N).

Statistical Analysis

The outcomes of the buckling test indicated a non-Gaussian distribution, as determined by the Shapiro-Wilk test. Results were presented as medians and interquartile ranges, and comparisons between groups, with the same apical size, were made using the nonparametric Kruskal-Wallis test. The significance level was set at 0.05 (SPSS v.22 for Windows; IBM SPSS Statistics, Chicago, IL, USA).

RESULTS

Instrument's Design Assessment

Analysis of the instrument designs showed that, with the exception of the GT Series X instruments, which had a cutting blade length of 12 mm or less, all other files had blades of 16 mm or more. The ProFile instruments had the highest number of spirals (19) and spirals per millimeter (1.19), while MTwo and EndoSequence files had the fewest (Table 1). All instruments had cutting blades oriented in a clockwise direction. In terms of microscopic surface finish and machining marks, the ProFile files displayed the most irregular surface, while the EndoSequence instruments had the smoothest surface (Fig. 2).

Metallurgical Assessment

EDS analysis indicated that all tested instruments were composed of a NiTi alloy, with near to equiatomic nickel and titanium ratios (ProTaper Next: 1.014; MTwo: 1.032; ProFile: 1.026; EndoSequence: 1.022; and GT Series X: 1.017), with no traces of other metallic elements (Fig. 3).

In the DSC tests, ProTaper Next and GT Series X instruments had the highest R-phase start (45.3°C and 50.2°C, respectively) and R-phase finish temperatures (16.3°C and 11.8°C, respectively), while the other instruments displayed R-phase starts at 27.9°C (EndoSequence) or lower. For the heating curves, ProTaper Next and GT Series X files also exhibited the highest austenitic start and finish temperatures (Fig. 3).

Buckling Assessment

The buckling test showed that ProFile 0.04 and 0.06 constant taper instruments, as well as the EndoSequence 35/.06 and 40/.06 files, had significantly higher results ($p < 0.05$) (Figs. 4, 5). The lowest results were observed in the EndoSequence 0.04 files, as well as in the MTwo and ProTaper Next instruments ($p < 0.05$) (Figs. 4, 5).

DISCUSSION

This study provides valuable insights into the relationship between design features, metallurgical properties, and buckling strength in five widely used NiTi rotary endodontic systems. The results revealed significant differences in mechanical performance, which appear to be closely related to variations in instrument design, surface characteristics, and metallurgical behavior.

The mechanical properties of endodontic instruments are known to be influenced by several key physical characteris-

TABLE 1. Instrument geometric features and buckling results (presented as median and interquartile range) of the assessed instruments

Endodontic instruments	Apical taper/variation*	Lot reference*	Cutting blade length (mm)	Number of spirals	Spirals per millimetre	Cutting blades direction	Buckling strength (Newton)
Instrument tip size 25 group							
ProFile	0.04	6519600	16	19	1.19	Clockwise	4.00 (3.87–4.03)
EndoSequence	0.04	0198	16	8	0.50	Clockwise	1.85 (1.43–2.03)
ProFile	0.06	1720487	16	19	1.19	Clockwise	3.80 (2.90–6.63)
EndoSequence	0.06	9376	16	7	0.44	Clockwise	2.80 (2.63–2.90)
ProTaper Next X2	0.06v	1515010	17	8	0.47	Clockwise	2.35 (2.10–2.40)
MTwo	0.07	363436	17	6	0.35	Clockwise	2.40 (2.08–2.83)
Instrument tip size 30 group							
ProFile	0.04	2725220	16	19	1.19	Clockwise	5.50 (4.58–6.00)
EndoSequence	0.04	0198	16	8	0.50	Clockwise	2.65 (2.48–2.90)
ProFile	0.06	060308511	16	19	1.19	Clockwise	6.50 (4.98–7.68)
EndoSequence	0.06	9376	16	7	0.44	Clockwise	3.60 (3.30–3.95)
GT Series X	0.06	SXRA525	12	8	0.67	Clockwise	4.10 (3.55–4.75)
ProTaper Next	0.07v	1515010	17	7	0.41	Clockwise	3.75 (3.50–3.90)
MTwo	0.06	362601	17	7	0.41	Clockwise	3.70 (3.38–3.80)
Instrument tip size 35 group							
ProFile	0.04	7235110	16	19	1.19	Clockwise	7.05 (5.70–8.00)
EndoSequence	0.04	0198	16	7	0.44	Clockwise	3.80 (3.40–4.10)
ProFile	0.06	1712662	16	19	1.19	Clockwise	7.75 (6.70–8.45)
EndoSequence	0.06	9376	16	6	0.38	Clockwise	7.65 (6.95–8.00)
MTwo	0.06	0903310641	17	8	0.47	Clockwise	4.40 (4.05–4.90)
Instrument tip size 40 group							
ProFile	0.04	7051790	16	19	1.19	Clockwise	9.55 (8.27–10.08)
EndoSequence	0.04	0198	17	6	0.35	Clockwise	5.70 (5.38–6.03)
ProFile	0.06	1686802	16	19	1.19	Clockwise	9.35 (8.60–10.70)
EndoSequence	0.06	9376	17	5	0.29	Clockwise	8.80 (7.85–9.73)
GT Series X	0.06	SXRA525	10	7	0.70	Clockwise	6.45 (5.80–7.10)
ProTaper Next X4	0.06v	1529960	18	7	0.39	Clockwise	6.15 (5.00–6.43)
MTwo	0.06	0904310642	17	8	0.47	Clockwise	5.05 (4.78–5.68)

*: Information from the manufacturer

tics, including tip diameter, taper, and manufacturing methods (20–22). Additionally, the length, number, and depth of spirals, as well as the orientation of the cutting blades, have a substantial impact on clinical performance (23, 24). An inverse relationship is often observed between spiral density and flexibility: while a greater number of spirals can theoretically enhance instrument resistance to deformation, lower resistance typically promotes smoother and more efficient canal shaping (25). This pattern was reflected in our findings. The ProFile instruments (with both 0.04 and 0.06 taper), along with the EndoSequence 35/.06 and 40/.06 files, exhibited superior buckling resistance—likely due to their higher spiral density and design geometry. In particular, ProFile's greater number of spirals per millimeter and total spiral count seemed to confer increased structural rigidity. However, SEM analysis revealed a relatively irregular surface finish, which, under high stress, has been identified as a critical factor contributing to fatigue failure (11).

These findings support prior research suggesting that spiral configuration and density are key determinants of instrument stiffness and deformation resistance (23, 24). However, they also raise a critical consideration: while increased structural integrity may improve resistance to buckling, it may simulta-

neously reduce cutting efficiency and hinder debris removal. This underscores the need for balance between mechanical robustness and clinical functionality.

The metallurgical assessment revealed a possible link between phase transformation temperatures and mechanical behavior. ProTaper Next and GT Series X instruments exhibited higher R-phase and austenitic transformation temperatures compared to the other systems. Nevertheless, this did not correspond to higher buckling resistance, suggesting that metallurgical factors alone may not be the primary determinants of axial stability. Instead, features such as cross-sectional design and surface finish may play more significant roles. These findings challenge the assumption that advanced heat treatments automatically translate to superior mechanical performance. Moreover, the near-equiatomic nickel-titanium ratios observed across all systems indicate that compositional differences were minimal and unlikely to drive mechanical variability.

The lower buckling resistance observed in EndoSequence 0.04 files, Mtwo, and ProTaper Next instruments presents both clinical benefits and drawbacks. Despite EndoSequence's smooth surface finish, its 0.04 taper variants demonstrated lower resistance compared to their 0.06 counterparts, suggesting that

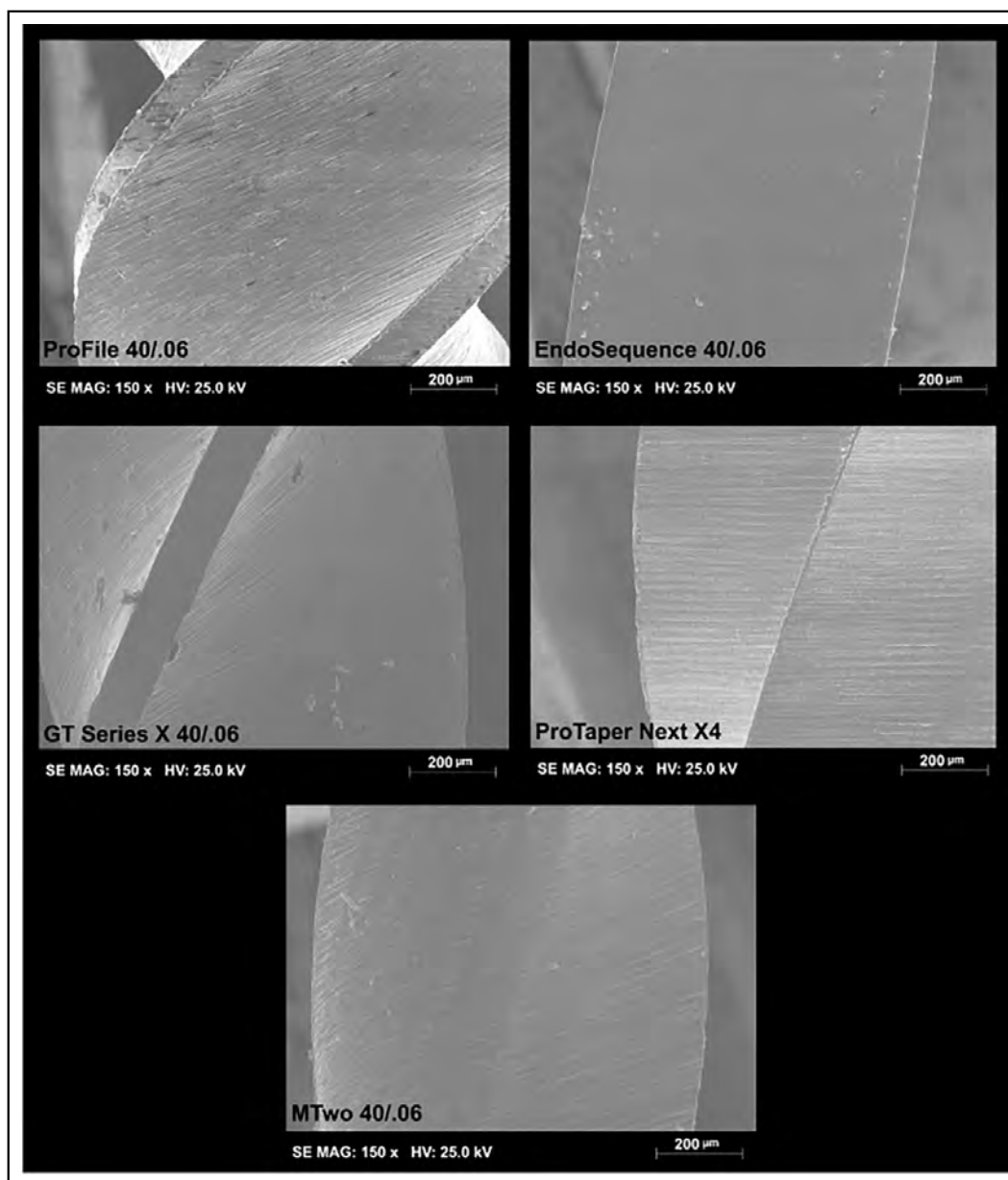


Figure 2. Representative images of the surface finish for instruments from each multifile system. ProFile instruments generally showed the most irregular surfaces, while EndoSequence instruments appeared to have the smoothest.

taper has a more substantial impact on mechanical behavior than surface treatment alone. Similarly, the reduced buckling resistance of ProTaper Next, despite its M-Wire technology, may be attributed to its off-centered rectangular cross-section. While this design enhances flexibility and debris removal, it appears to compromise axial stiffness. These observations raise further questions about the trade-offs inherent in instrument design. Some clinicians may favor increased flexibility in curved canals to avoid transportation or perforation, while others may prioritize structural stiffness to ensure control in calcified or straight canals (26, 27). This ongoing debate emphasizes the importance of selecting instruments based on case-specific anatomical and procedural demands.

An interesting observation arose from the analysis of cutting blade length. According to Euler's buckling theory, longer instruments are more susceptible to lateral deformation under

axial load (28). Based on this principle, GT Series X files, having the shortest cutting blade length (≤ 12 mm), might be expected to show greater resistance to buckling. However, our findings did not support this hypothesis. These instruments did not outperform those with longer blade lengths (≥ 16 mm), suggesting that the correlation between blade length and buckling resistance is more complex than previously thought. It may be that the formula applies more directly to the total instrument length rather than the active blade segment. Other design parameters, such as cross-sectional geometry and core diameter, likely also influence axial stability (26, 29, 30).

From a clinical standpoint, our findings have important implications for instrument selection and usage. Instruments like ProFile and EndoSequence with a 0.06 taper, which demonstrated superior buckling strength, may be more suitable in cases that require enhanced structural rigidity, such as retreat-

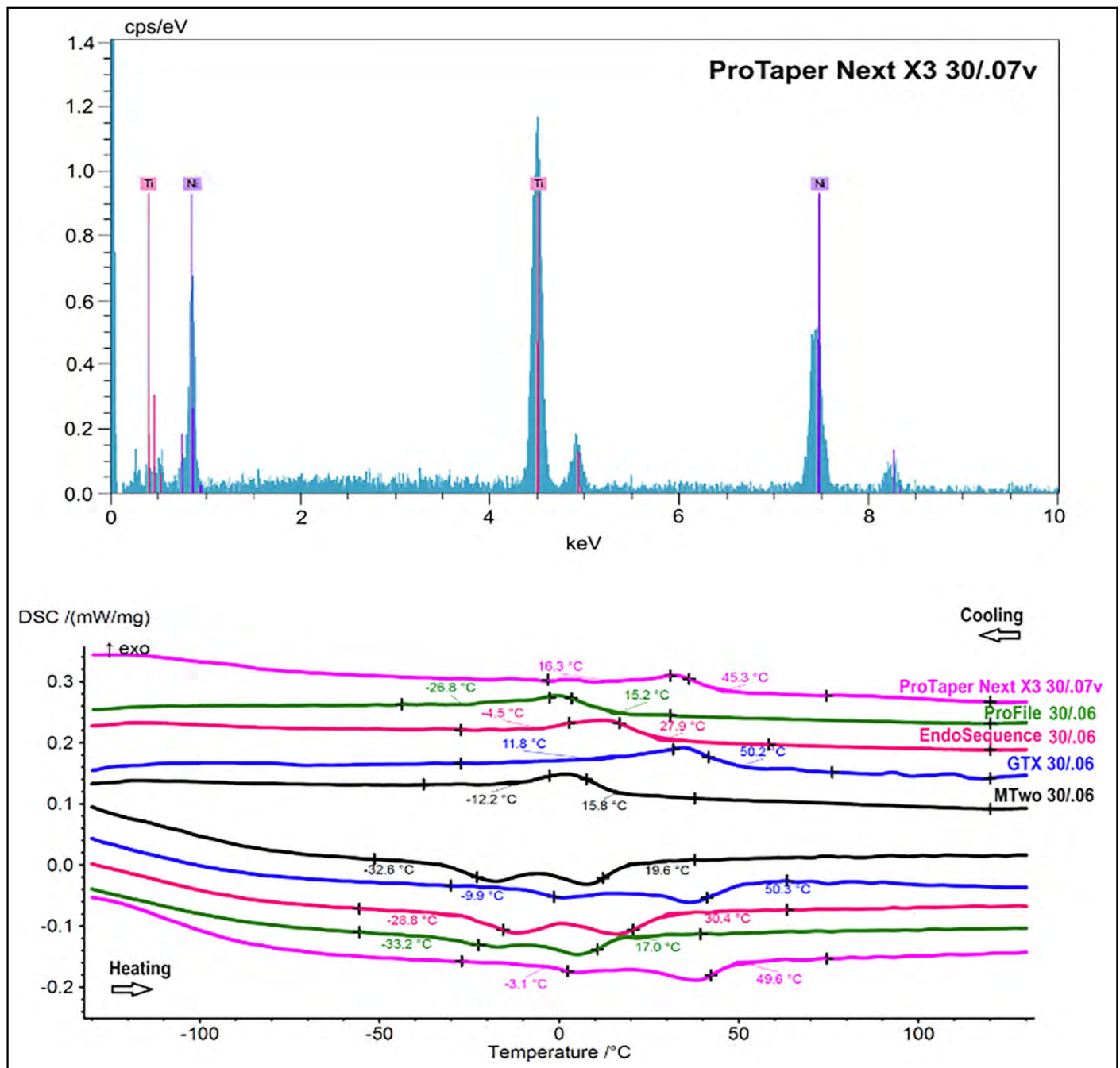


Figure 3. The metallurgical assessment confirmed the presence of NiTi alloys in all tested instruments, each with distinct phase transformation temperatures. At the top, a representative EDS spectrum highlights the nickel and titanium elements; spectra from all instruments were consistent with this example. Below, the DSC chart shows that GT Series X and ProTaper Next instruments had the highest phase transformation temperatures (the chart reads right to left on the cooling curve and left to right on the heating curve).

DCS: Differential scanning calorimetry, GTX: GT Series X, EDS: Energy-dispersive X-ray spectroscopy.

ment procedures or the removal of gutta-percha. However, it is important to recognize that greater buckling resistance may limit flexibility, which can be a disadvantage in curved or narrow canals. Therefore, maintaining a versatile set of instruments is crucial to accommodate a variety of anatomical and procedural scenarios.

Moreover, our results emphasize that when evaluating endodontic instruments, clinicians and researchers must consider multiple factors. While metallurgical treatments and

surface modifications certainly contribute to performance, basic design features such as taper, spiral configuration, and cross-sectional shape may exert more significant influence on buckling behavior.

A limitation of this study is its exclusive focus on buckling resistance as the primary mechanical parameter. While valuable, this single metric provides an incomplete view of an instrument's clinical performance. Other factors, including core diameter, cutting efficiency, torsional strength, and

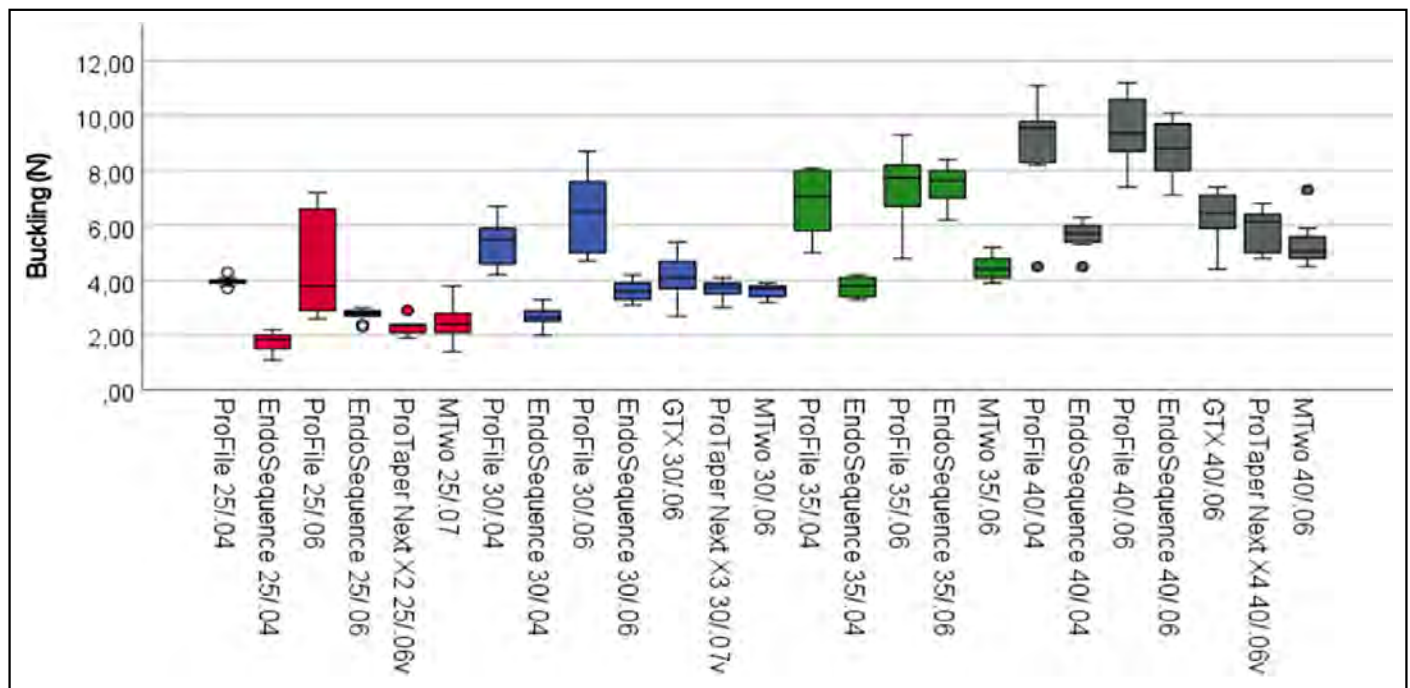


Figure 4. Box plot charts displaying the buckling test results reveal a trend of higher values for ProFile 0.04 and 0.06 instruments and lower values for EndoSequence 0.04 files.

GTX: GT Series X.

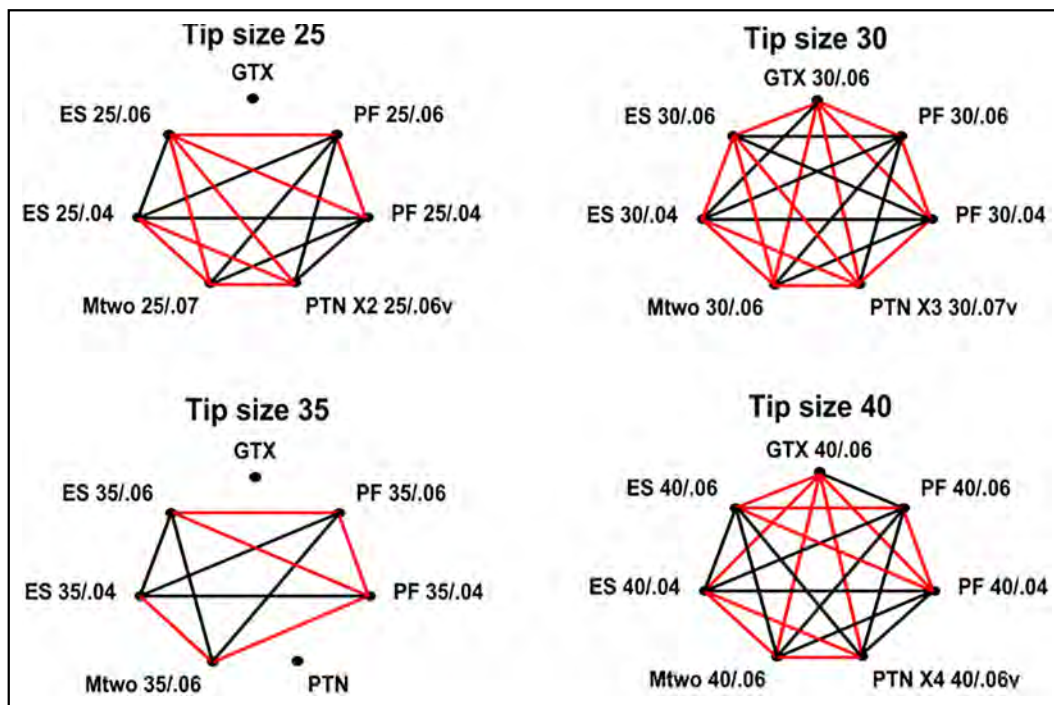


Figure 5. Pairwise comparisons between groups with the same apical size (GTX GT Series X, PF ProFile, PTN ProTaper Next, ES EndoSequence).

GTX: GT Series X, PF: ProFile, PTN: ProTaper next.

fatigue resistance, are also critical to endodontic success. Nonetheless, our findings offer useful data that may inform future instrument development and support improvements in design for enhanced clinical reliability. Additionally, as this investigation was conducted under controlled laboratory conditions, it may not fully replicate the challenges

presented in clinical environments. Future research should consider exploring the influence core diameter of these endodontic files and also incorporate studies in simulated or real canal anatomies to better validate these *in vitro* findings and expand our understanding of how these instruments perform under diverse clinical conditions.

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Impact of Different Nickel-titanium Instruments on Apical Micro-cracks Formation and Residual Amount of Root Canal Filling Materials Following Retreatment Procedure

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ABSTRACT

Objective: This study aimed to evaluate and compare the dentinal defects caused by three different retreatment systems [Endostar RE Endo, XP-endo Retreatment, and MANI Gutta-Percha Removal (GPR)] and to assess the percentage of residual root canal filling material following the retreatment procedure.

Methods: Sixty extracted mandibular second premolars with straight oval canals were used. The roots were standardized to a length of 14 mm. All samples were instrumented with NiTi instruments up to size 30.04, then filled using single cone technique with AH plus sealer and gutta-percha. Four groups were created from the samples. Group 1 served as the negative control (n=15), while the remaining three groups (n=15 each) were categorized based on the retreatment system used. Stereomicroscope at magnification 45 x was used to evaluate the incidence of crack formation and propagation at apex of the roots and the residual volume percentage of root filling materials in groups of Endostar RE, MANI GPR, Xp retreatment systems assessed using CBCT. Fisher's exact test was used to analyze the incidence and propagation of cracks, while one-way ANOVA and Tukey's post hoc test were applied to assess differences in residual filling material volume among the groups P values at 0.05.

Results: There is significance difference ($p=0.028$) between the Endostar and Xp retreatment system. The highest of dentinal defect (10\15) with Endostar retreatment system followed by (4\15) with GPR system and (2\15) with XP endo retreatment system. Highest residual filling material mean at Endostar RE retreatment system (51.97) and lowest mean (39.07) at Xp retreatment system. A statistically significant difference was observed between the groups ($p<0.001$), particularly between the Endostar and XP retreatment systems.

Conclusion: No system was capable of completely eliminating obturated materials. The XP-endo Retreatment system showed the lowest incidence of crack formation and propagation and proved to be the most effective in removing gutta-percha and sealer.

Keywords: Crack formation, endodontic retreatment, root-filling material remnant

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HIGHLIGHTS

- No system exhibited a zero incidence of crack formation.
- No system was capable of completely removing the gutta-percha and sealer.
- The XP-endo Retreatment system exhibited the lowest incidence of crack formation and propagation, demonstrating superior efficacy in the removal of gutta-percha and sealer.

INTRODUCTION

Failure after root canal treatment may occur due to the persistence of infection from persistent microorganisms or the reactivation of pathogens within the root canal system via coronal or apical pathways (1). A successful re-

treatment process involves removing of existing root canal filling material, followed by cleaning, shaping, and obturation stages (2).

Due to their advantages over stainless steel files, NiTi rotary files are being used more and

more in retreatment procedures and root canal therapy. Despite numerous advantages, NiTi rotary systems may lead to dentinal defects, such as crack formation, both during canal shaping and retreatment procedures. There is a concern that compromised dentin resulting from widening procedures may ultimately lead to adverse outcomes such as vertical radicular fractures (3).

Dentinal cracks may form as a result of stress concentration caused by the contact between endodontic instruments and dentin, which, with repetitive occlusal stresses, can propagate and result in complete fracture. Vertical root fractures, a severe complication in endodontic treatment, often result from the gradual propagation of initially undetectable dentinal cracks. Consequently, a primary objective in endodontic research is to minimize the risk of dentinal fracture formation during instrumentation with rotary instruments (4).

Recent studies have focused on the effect of glide path preparation on dentin preservation. The micro-computed tomography images were used to assess the microcracks induced after the application of the different glide path techniques before root canal instrumentation, as reported in study (5). Their results showed that some glide path solutions could have a strong impact on causing dentinal microcracks. Therefore, it is crucial to emphasize careful technique selection when using rotary instruments during retreatment to minimize structural damage.

For many years, a major area of research has been the behavior of rotating instruments in defect creation. The formation and propagation of cracks during procedures using manual, rotary, and reciprocating instruments have been reported in numerous studies (6). Dentinal cracks are structural defects characterized by lines extending from the internal root canal wall to the external root surface, typically occurring when the tensile stress within the canal wall exceeds the strength of the dentin (7, 8).

Successful removal of gutta percha and sealers from the root canal system during retreatment methods can be approached in several ways, some of which involve the use of supplementary chemical solvents and others without. Tools such as lasers, ultrasonic cleaners, heated instruments, nickel-titanium rotary files, gate glidden burs, and stainless steel hand files are utilized in these procedures (8–10).

In spite of the efficacy of all these systems, complete removal of root canal filling materials remains a clinical challenge regardless of the retreatment technique used (11, 12). On the other hand, the type of filling has a direct effect on its elimination (13).

Few studies have compared the Endostar RE Endo System (ERE; Poldent Co Ltd, Poland), XP-endo Retreatment system (FKG Dentaire, Switzerland), and the MANI Gutta-Percha Removal system (MANI, Japan) in terms of their effectiveness in removing filling material and their tendency to induce crack formation and propagation.

This study aimed to evaluate and compare the effect of using three different Niti rotary retreatment systems (The Endostar RE Endo, XP-endo, Mani gutta-percha removal system) on the incidence of microcrack formation and propagation and on the amount of residual root canal filling material following retreatment.

The null hypothesis of this study that the type of NiTi retreatment instrument used has no effect on the occurrence of microcrack initiation or propagation, nor on the percentage of residual root canal filling material.

MATERIALS AND METHODS

Tooth Selection

Sixty extracted human mandibular premolars with comparable lengths, straight roots, and no caries were selected. All teeth were extracted for orthodontic reasons. Ethical approval for this study was obtained from the College of Dentistry Research Ethics Committee at Mustansiriyah University (Approval No. MUOPR29, dated May 1, 2023; REC Reference: REC130). The study was conducted in accordance with the declaration of Helsinki. Radiographs were obtained from bucco-lingual and mesio-distal aspects to confirm the existence of only one canal. The root surfaces of each tooth were examined under a stereomicroscope at 20X magnification for signs of crack lines, open apices, or anatomical anomalies, which should be eliminated if any of these features were present. Teeth with an initial canal size corresponding to a #15 K-file were included in the study. The specimens were stored in purified water for three months throughout the study.

Sample Preparation

For standardization purposes, the selected 60 teeth were measured from the apex toward the cemento-enamel junction (CEJ), and each root was sectioned at 14 mm from the apex perpendicular to its long axis using a diamond disc under water cooling (14).

With waterproof 320-grit silicon carbide abrasive paper, 1 mm of each tooth's apical section was ground perpendicular to the tooth axis. Waterproof silicon carbide abrasive paper with grits of 1000 and 1200 was used to polish the apical surface in order to minimize small scratches and produce a sharp, greatly enlarged image.

Acrylic blocks were constructed with dimensions of 10×20 mm. The coronal 1 mm of each root remained uncovered, while the apical 2 mm were exposed to allow for intraoperative image acquisition, as described in a previous study (15).

To avoid dehydration, the exposed apical section of the root was submerged in water during instrumentation (16). Humidity was maintained during all procedures by immersing the roots in water.

A baseline image of the apical surface of each specimen was examined using a stereomicroscope (45X), and photographs were documented. In order to ensure standardization, all root canal preparations were performed using the EdgeFile X7 system (EdgeEndo®, USA). The instrumentation protocol

began with a #17/04 file in one or more passes, alternating with smaller hand files as needed, until the working length was reached. This was followed by passive use of #25/04 and #30/04 files to the full working length.

A new file was used for each tooth in instrumentation and re-treatment step to ensure cutting efficiency and prevent any influence of instrument fatigue.

The canals were irrigated with 2 mL 2.5% sodium hypochlorite (NaOCl) between each file size. Irrigation was performed using a 27-gauge side-vented needle and syringe. After completion of the preparation, the canals were irrigated with 5 mL 17% EDTA for 1 minute and subsequently washed with 5 mL distilled water. After preparation, photographs of the apical regions of roots were obtained, and crack initiation was verified through stereo microscope, and the specimens showing evidence of visible cracks were uniformly distributed among the experimental groups.

Root Canal Obturation

Root canals were obturated using the single cone technique with AH Plus sealer and a size 30/.04 gutta-percha master cone (Dentsply, Germany). Samples were stored at 37°C with 100% humidity for two weeks to ensure complete sealer setting (Sure Dent, Korea).

Samples Grouping

1. Crack formation and propagation

Sixty samples were randomly divided into four experimental groups (n=15) G power analysis were performed.

- Group 1: Control group
- Group 2: Endostar RE Endo Retreatment
- Group 3: MANI GPR Retreatment
- Group 4: XP-endo Retreatment

2. Percent volume remanent of filling material

The last three group (without control group).

Evaluation of the Amount of Obturation Filling Material Using Cone Beam Computed Tomography Prior to Endodontic Retreatment

The provisional filling was removed from each specimen. Acrylic resin was then removed from all obturated teeth, which were subsequently embedded in a U-shaped dental wax rim for CBCT evaluation. CBCT imaging was performed using Vatech equipment in high-resolution dental mode (90 kV, 10.0 mA). The total scan time was 15 seconds. Furthermore, Each scan had a voxel size of 0.120 mm. The images were reviewed using EZ3D-I software. The volume of the "root filling material" was calculated using the volume measurement tool in the EZ3D-I software by defining the minimum and maximum intensity thresholds (17–19).

Experimental Groups and Retreatment Procedures

Group 1: Control (n=15 teeth)

Group 2: Endostar RE Endo (n=15 teeth)

Instruments used: File 2 (30/.08) and File 4 (30/.04), as per the manufacturer's instructions.

Group 3: MANI GPR (n=15 teeth)

From crown to middle portion of the canal, 2S #50 file was selected. From middle to apical, 4N #30 file was selected. Set a rubber stopper length of 1–2mm up to the apex. The files were moved in pull-stroke motion to remove the debris.

Group 4: XP-endo Retreatment (n=15 teeth)

The procedure followed the manufacturer's guidelines at 37°C to enable phase transformation. DR1 (30/.10) was used in the coronal third, followed by XP-endo Shaper (30/.04) to reach working length, and XP-endo Finisher R for final cleaning.

During the removal of root filling materials, the root canals were irrigated with 2 ml of a 2.5% sodium hypochlorite irrigation solution following the use of each file. After that, the XP-endo Finisher R (30/00) was employed; this file was utilized (for 1 minute) with slow and longitudinal movements to full length of the canal then the canal was irrigated with 1ml 2.5 % NaOCl (10).

Assessment of Crack Formation and Propagation

Photographs were captured of each tooth across all experimental groups following the retreatment procedure. The photos were obtained at 45X magnification utilizing a digital camera Nikon (Tokyo, Japan) connected to a stereo-microscope (MEIJI Techno).

The cracks were classified as follows:

- 1-crack: a single visible line extending from the root canal wall toward the outer surface without reaching it.
- 2-crack: two distinct crack lines observed.
- 1-crack Propagation: an increase in the length of a pre-existing crack observed in sequential imaging.
- 2-crack Propagation: two distinct crack propagation.

A scoring system was applied to categorize the severity and extent of crack formation and propagation based on stereomicroscopic images at each treatment stage.

Each specimen in the experimental groups had four photos (baseline, post-preparation, post-filling, post-retreatment). Each image was compared with the preceding one, and any detectable crack line on the apical surface that was absent in the prior image would be classified as a crack. Any increase in crack length from the preceding step is defined as crack propagation as showed in Figures 1-3.

Amount of Gutta-percha Residual After Retreatment Procedure Using Cone Beam Computed Tomography

Following the removal of obturation material from the teeth, CBCT images were acquired as previously stated. The volume of the residual obturating material was evaluated as showed in Figures 4-6. The percentage of residual volume of obturating material was measured.

Statistical Analysis

Statistical analysis was performed using SPSS version 22. Descriptive statistics included mean, standard deviation (SD), standard error (SE), and range. Data normality was verified us-

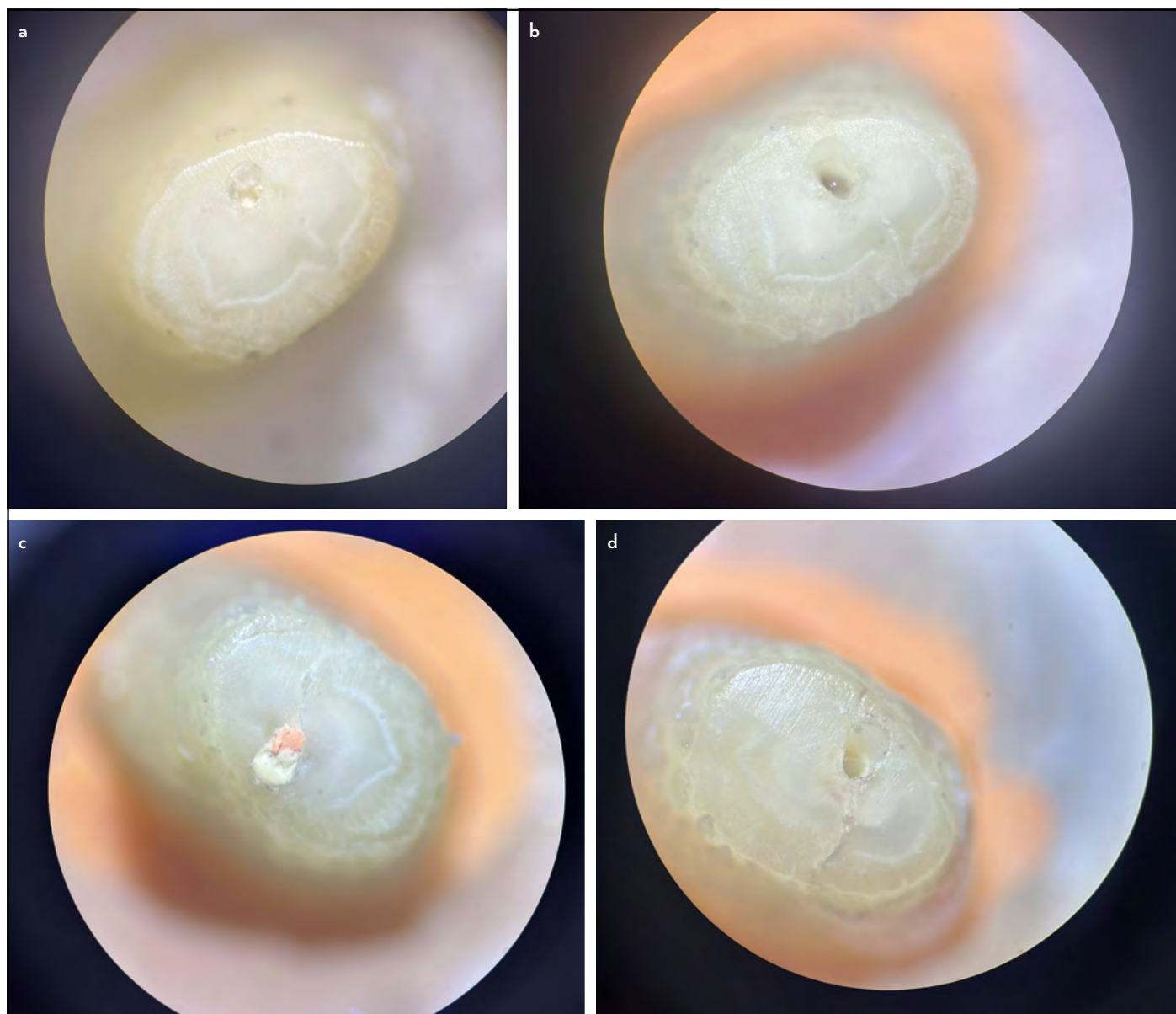


Figure 1. Image taking at 45X in stereoscope, (a) basal line image, (b) after instrumentation, (c) after obturation, (d) after retreatment with endostar retreatment system.

ing the Shapiro–Wilk test, and homogeneity of variances was assessed using Levene’s test. For comparing the percentage of residual filling materials among groups, a one-way analysis of variance (ANOVA) was conducted, followed by Tukey’s HSD post hoc test to identify pairwise differences. The incidence and propagation of cracks were analyzed using Fisher’s exact test. A significance level of $p < 0.05$ was used for all tests.

RESULTS

1. Crack Formation and Propagation After Retreatment

The incidence and propagation of dentinal cracks following retreatment were evaluated under a stereomicroscope at 45X magnification. Table 1 and Figure 7 provide detailed distributions for each group.

The XP-endo Retreatment group exhibited the lowest incidence of crack formation and propagation. Only 1 specimen

(6.67%) showed a new crack, and 1 specimen (6.67%) showed crack propagation. In contrast, the Endostar RE Endo group had the highest occurrence of cracks, with 6 out of 15 samples (40%) exhibiting new cracks and 4 out of 15 samples (26.67%) demonstrating propagation. The MANI GPR group recorded intermediate values, with 3 samples (20%) showing new cracks and 1 sample (6.67%) showing propagation.

The statistical analysis using Fisher’s exact test showed a significant difference between the XP and Endostar groups ($p = 0.028$), indicating a lower incidence of cracks with the XP-endo system. No significant differences were observed between the Endostar and GPR systems ($p = 0.166$) or between XP and GPR ($p = 0.793$).

2. Percentage of Residual Root Filling Material

Descriptive statistics for the percentage of residual filling material are summarized in Table 2. The XP-endo Retreatment

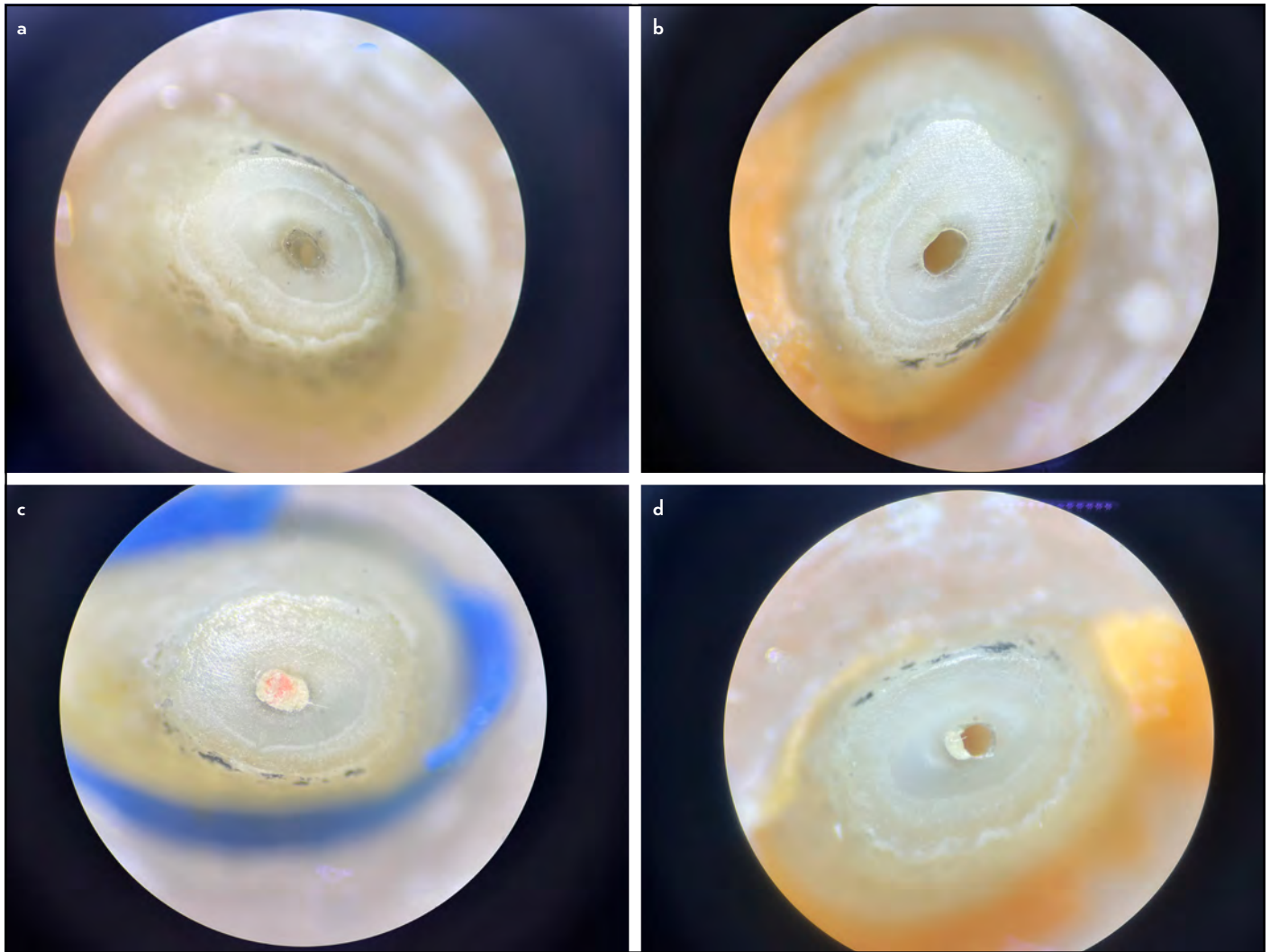


Figure 2. Image taking at 45X in stereoscope, (a) basal line image, (b) after instrumentation, (c) after obturation, (d) after retreatment with MANI GPR retreatment system.

MANI GPR: MANI gutta percha removal system.

group had the lowest mean residual volume ($39.08\% \pm 2.93$), followed by the MANI GPR group ($44.51\% \pm 7.11$), and the Endostar group with the highest residual volume ($51.97\% \pm 9.26$).

Shapiro wilk test used and percentage of residual filling material among groups was normally distributed ranged between 0.252 and 0.702.

Regarding homogeneity of variance, the variance for all groups was homogenous based on Levene's test, ($F=3.084$), ($p=0.056$).

A one-way ANOVA revealed a statistically significant difference among the three groups ($F=13.02$, $p<0.01$), as shown in Table 3. Post hoc Tukey HSD testing (Table 4) identified a significant difference between the Endostar and XP groups ($p<0.001$). However, differences between Endostar and GPR ($p=0.058$) and between GPR and XP ($p=0.094$) were not statistically significant.

These findings indicate that the XP-endo Retreatment system was more effective in removing root canal filling materials and caused significantly fewer dentinal cracks compared to the Endostar system.

DISCUSSION

Process retreatment may result in the development of crack lines and microcracks as well as harm to the tooth's remaining structure. Endodontic therapy may fail as a result of these crack lines and microcracks spreading in response to stress, such as that caused by occlusal pressures and restorative or endodontic procedures, and developing root fractures (3, 12).

The effective removal of root filling materials is an essential aspect of the retreatment process, as it allows access to necrotic tissue and bacteria-containing spaces that could be responsible for endodontic failure (20, 21). Many previous studies showed no technique was able to eliminate it completely (22–26).

Oval-shaped canals represent a problem for removing of all intracanal filling materials due to limited contact between the endodontic file and the root canal wall (27).

In this study, a consistent apical size of #30 was maintained for all instruments during retreatment to eliminate the influence of increasing the size of the retreatment file compared to

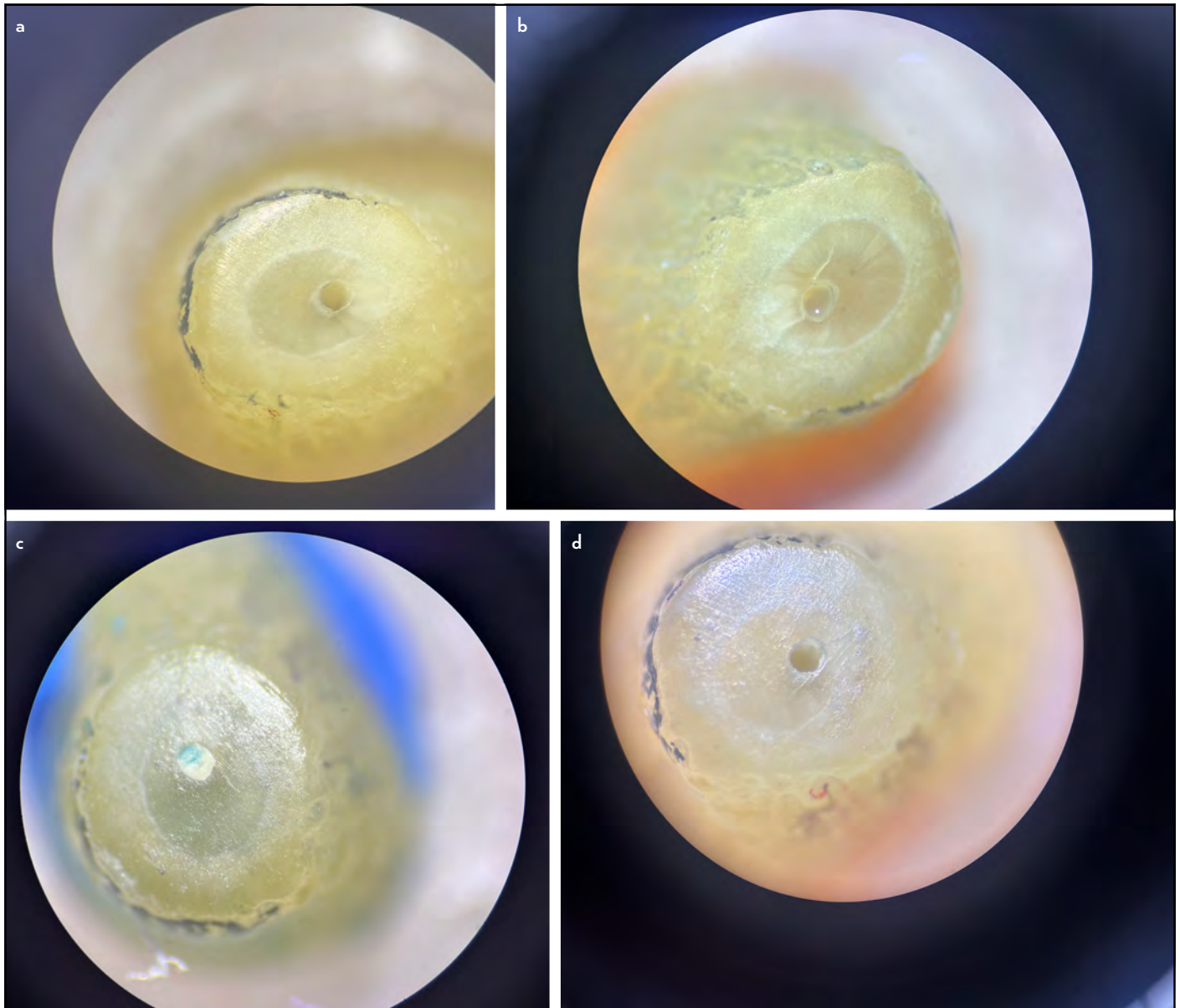


Figure 3. Image taking at 45X in stereoscope, (a) basal line image, (b) after instrumentation, (c) after obturation, (d) after retreatment with xp re-treatment system.

the last file used for instrumentation, and to allow for uniform comparison between the various retreatment file systems.

After each step we take picture for the image in stereo-microscope to compare with baseline image to know there is new crack or its crack from the previous step and propagation happened.

In this study, AH Plus sealer was used because it is widely regarded as the gold standard among endodontic sealers due to its excellent physicochemical properties (12, 13).

The use of instrumentation techniques without solvent in retreatment is more effective and easier than using of solvent. The material's structure may become viscous and highly adhesive, leading to the formation of softened gutta-percha films on the root canal surface (8, 28).

Cone beam computed tomography was employed to assess the volume of residual filling materials. The accurate determi-

nation of the precise location and volume of residual filling material is crucial during retreatment procedures (29).

A significant difference was observed between the XP and Endostar groups; therefore, the null hypothesis was rejected.

This study findings indicate that retreatment with the XP retreatment system resulted in the lowest dentinal defects in the apical region when compared to the same taper of the Endostar retreatment file. This outcome can be attributed to the XP-endo file's high flexibility, zero tapers, and NiTi MaxWire technology. It will be capable of navigating every corner and wall of the root canal with reduced stress on the canal wall, effectively removing debris left inside without deviating from the natural path of the root canal (10, 30, 31).

The GPR retreatment group generates and propagates fewer microcracks and exhibits lower residual remanent compared

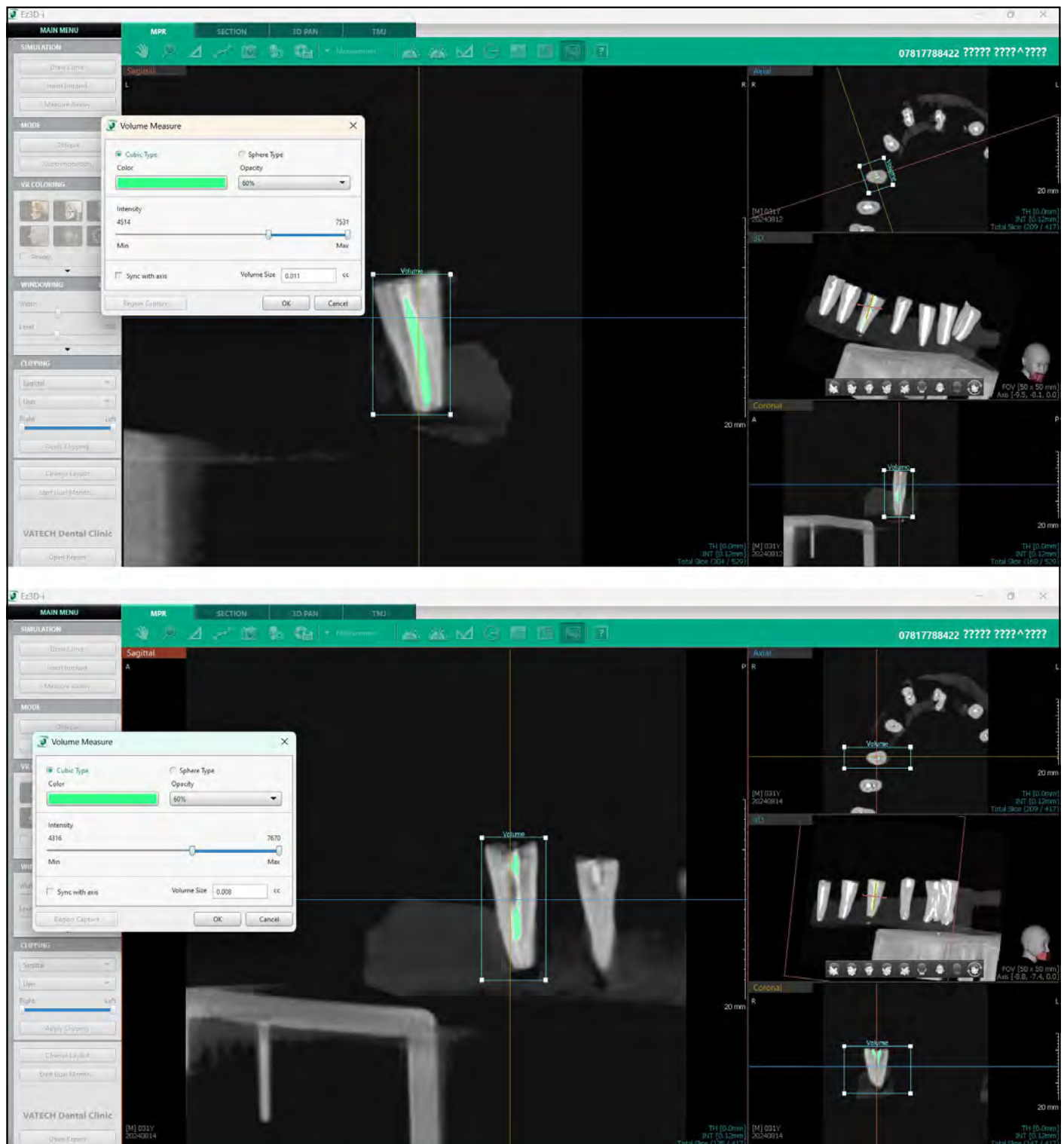


Figure 4. CBCT before and after retreatment with endostar RE endo retreatment system for assessment of residual volume remanent. CBCT: Cone-beam computed tomography.

to the RE Endo retreatment group. The observed phenomenon may be attributed to the S type blade of the RE Endo files, characterized by two cutting edges, further enhances cutting efficiency. This increase in cutting edges correlates with heightened stress on the canal wall and a increase incidence of crack formation, and this design causes the gutta-percha to be cut in a circular cross-section. In contrast, the GPR system, with its low cutting efficiency and high speed,

generates heat that plasticizes the gutta-percha, allowing it to accumulate in the flutes and be removed in bulk (32, 33).

The K type files exhibited four cutting edges with tapers of 8%, representing the highest taper (34) when compared to GPR files, which maintain a constant taper. This increases the contact area and elevates stress on the root canal wall, resulting in a higher incidence of crack formation and propagation (32).

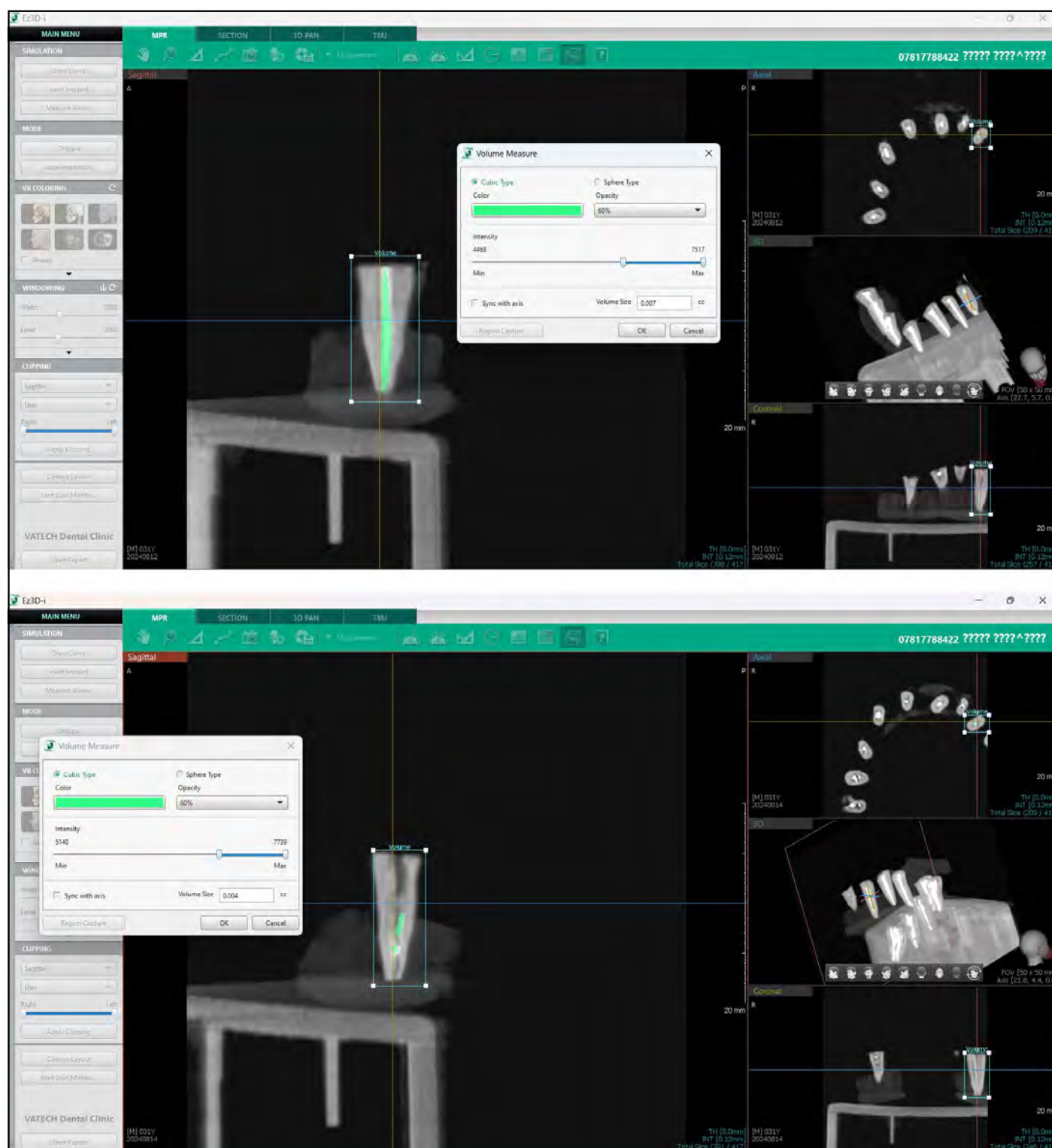


Figure 5. CBCT before and after retreatment with MANI GPR retreatment system for assessment of residual volume remanent.

CBCT: Cone-beam computed tomography, MANI GPR: MANI gutta percha removal system.

The XP-endo Retreatment system, which incorporates the DR1 file with its active tip design, facilitates efficient and rapid penetration of gutta-percha (GP) (35). The XP-endo shaper features a slim profile, a narrow taper, and a booster tip. This design, combined with GP plasticization at high rotational speeds, may facilitate the removal of gutta-percha (36). The mechanical action of the XP-endo Finisher R can enhance the

dislodgement of residual materials, potentially leading to a more rapid removal of root filling materials (31).

Moreover, the XP-endo Finisher R — the final file in this system — features an innovative MaxWire alloy and a non-tapered design, which allow it to expand at body temperature and adapt more effectively to the canal walls, and as a result, during instrument motion the resistance exerted by canal anatomy

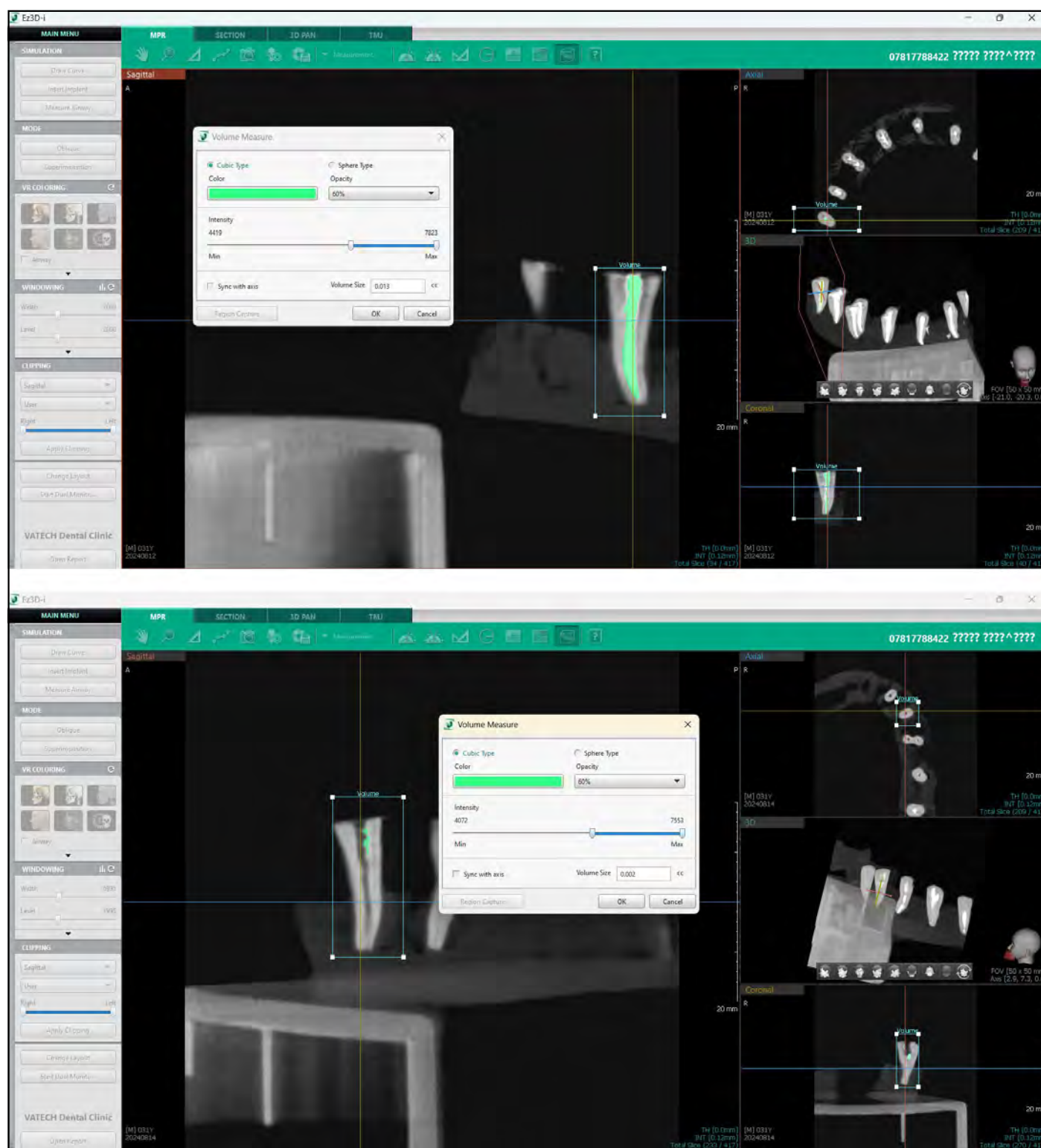


Figure 6. CBCT before and after retreatment with XP endo retreatment system for assessment of residual volume remnant.
CBCT: Cone-beam computed tomography.

compresses the elliptical portion of the instrument. Consequently, the tip of file is pressed against the canal walls. Instrument expansion within the canal may enable the semiactive tip of the file to engage and dislodge obturating root materials from the canal walls, which can subsequently be removed during canal irrigation (31). This result is consistent with previous studies reported by (10, 31, 37, 38).

XP-endo Shaper was associated with significantly more cases with complete filling removal due to its design that surround the gutta percha and withdrawal as one piece.

Clinical Implications

The choice of retreatment file can have a critical impact on both the effectiveness of the procedure and the long-term

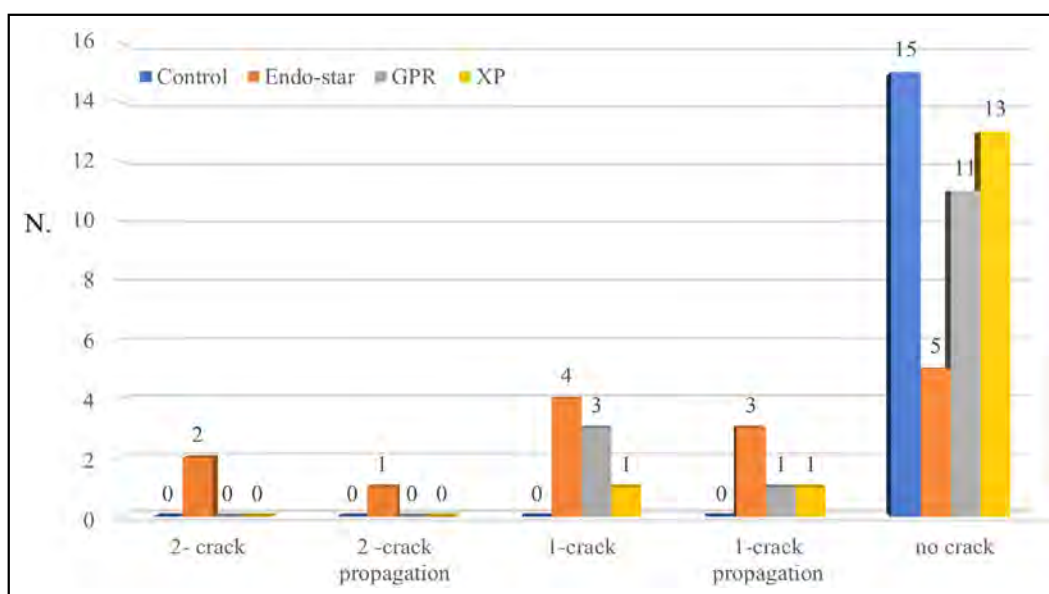


Figure 7. Bar chart for distribution of crack formation and propagation.

GPR: Gutta-percha removal.

TABLE 1. Distribution of crack formation and propagation

	Control		Endo-star		GPR		XP		Total		Fisher exact p value
	n	%	n	%	n	%	n	%	n	%	
2- crack	0	0.00	2	13.33	0	0.00	0	0.00	2	3.33	0.006 Sig.
2 -crack propagation	0	0.00	1	6.67	0	0.00	0	0.00	1	1.67	
1-crack	0	0.00	4	26.67	3	20.00	1	6.67	8	13.33	
1-crack propagation	0	0.00	3	20.00	1	6.67	1	6.67	5	8.33	
No crack	15	100.00	5	33.33	11	73.33	13	86.67	44	73.33	
Endo-star X GPR											0.166 NS
Endo-star X XP											0.028 Sig.
XP X GPR											0.793 NS

$p \leq 0.05$ Significant (S) $p > 0.05$. n: Number, GPR: Gutta-percha removal, Sig.: Significant, NS: Non-significant

TABLE 2. Descriptive statistic of percentage of residual filling material

Groups	n	Mean	±SD	±SE	Min	Max
Endo-star	15	51.973	9.259	2.391	39.500	69.200
GPR	15	44.507	7.106	1.835	35.300	55.500
XP	15	39.079	2.932	0.757	34.700	45.300

n: Number, SD: Standard deviation, SE: Standard error, Min: Minimum, Max: Maximum, GPR: gutta-percha removal

TABLE 3. ANOVA test of percentage of residual filling material among groups

	Sum of squares	df	Mean square	F	p
Between groups	1257.311	2	628.656	13.023	<0.01
Within groups	2027.492	42	48.274		
Total	3284.804	44			

ANOVA: Analysis of variance, df: Degrees of freedom, F: F-statistic

prognosis of the treated tooth. The XP-endo system's minimal crack propagation and superior cleaning efficacy suggest it may be preferable, especially in teeth with thin dentinal walls or those at risk of vertical root fractures.

This study has several limitations. First, it was conducted *ex vivo*, which does not fully replicate intraoral conditions such as thermal variations, periodontal ligament simulation, or masticatory forces. Second, all specimens were standardized to a uniform root length and canal size, which may not reflect natural

TABLE 4. Tukey HSD of percentage of residual filling material among groups

(I) Groups	(J) Groups	Mean difference	p
Endo-star	GPR	7.467	0.058 NS
	XP	12.894	<0.001 Sig.
GPR	XP	5.427	0.094 NS

HSD: Honest significant difference, GPR: Gutta-percha removal, NS: Non-significant, Sig.: Significant

anatomical variability encountered in clinical practice. Third, procedures were performed by a single operator, eliminating operator variability but potentially introducing performance bias. Fourthly, the crack observation method used (stereomicroscope) is limited in its ability to detect subsurface micro-cracks.

This technique is only effective for identifying surface cracks. More advanced, non-destructive 3D imaging techniques like micro-computed tomography (micro-CT) are superior for revealing these hidden defects. Future studies should include a larger sample size, a multi-operator design, and dynamic simulation of clinical conditions to further validate and expand on these findings.

CONCLUSION

No system exhibited a zero incidence of crack formation, nor was there a system capable of completely removing obturated materials. The XP-endo Retreatment system exhibited the lowest incidence of crack formation and propagation, demonstrating superior efficacy in the removal of gutta-percha and sealer, followed by the MANI GPR system and the Endostar retreatment system.

Disclosures

Ethics Committee Approval: The study was approved by the Mustansiriyah University Ethics Committee (no: MUOPR29, date: 01/05/2023).

Informed Consent: Informed consent was obtained from all participants.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Effects of Procedural Errors on Root Canal Treatment Outcomes: A Retrospective Cohort Study of Cases Treated by Sixth-year Dental Students

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ABSTRACT

Objective: This study assessed the effects of various types of procedural errors arising from root canal instrumentation on initial root canal treatment outcomes.

Methods: This retrospective cohort study analysed data from sixth-year dental students performing initial root canal treatment on mature permanent molars between 2015 and 2019. Treatment records and radiographic images were reviewed to identify procedural errors during root canal instrumentation and other potential confounding factors affecting treatment outcomes. Subsequently, uni- and multivariate logistic regression analyses were performed to identify predictors of treatment outcomes.

Results: A total of 142 teeth (343 roots) met the inclusion criteria, with an average follow-up period of 35.84 ± 16.72 months. According to stringent assessment criteria, overall healing rates were 72.5% for the teeth (103 of 142) and 80.5% for the roots (276 of 343). Multivariate logistic regression analysis revealed that among the procedural errors considered, only errors related to under-instrumentation (root canal deviation and lateral perforation) were significant predictors of treatment outcomes ($p=0.002$).

Conclusion: Root canal treatment outcomes are significantly influenced by procedural errors, particularly those affecting the root canal preparation length. Procedural errors related to under-instrumentation can compromise root canal disinfection and increase the risk of post-treatment disease by up to eightfold.

Keywords: Apical periodontitis, procedural errors, root canal preparation, root canal treatment, treatment outcome

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HIGHLIGHTS

- Procedural errors impact root canal outcomes, with under-instrumentation posing the highest risk.
- In this retrospective study, healing rates were 72.5% for teeth and 80.5% for roots, with a mean follow-up duration of 35.84 months.
- Errors related to under-instrumentation increase post-treatment disease risk by up to eightfold.

INTRODUCTION

Root canal instrumentation is a crucial step in root canal treatment, as it involves the removal of infected tissue, bacterial biofilm, and their toxic by-products. It also creates space for antimicrobial irrigants, which enhance the cleaning of untreated root canal walls and ad-

dress anatomical irregularities. Additionally, instrumentation shapes the canal to accommodate the available obturation material and technique, thereby establishing conditions that support the preservation of healthy apical tissues or promote the healing of apical periodontitis (1, 2). It is widely recognised that

optimal root canal preparation should result in a continuous, tapered shape that closely follows the original root canal anatomy, free from procedural errors (3).

However, due to various factors, including the complexity of root canal anatomy, accessibility, instrument flexibility, and operator experience, procedural errors may arise during root canal preparation (4). These errors are typically categorised as root perforation, instrument separation, and uncontrolled canal shaping (5). It is important to note that these errors can vary not only between categories but also within the same category. For instance, variability can occur in the location and size of perforations, in the ability to regain apical patency following instrument fracture, or in the effects of uncontrolled canal shaping on the length of root canal instrumentation. Consequently, these errors can have distinct and varying impacts on the overall success of root canal treatment.

Current clinical studies demonstrated that root perforation significantly reduces the healing rate of initial root canal treatment (5, 6). This is because root perforation causes injury and inflammation to the adjacent periodontium, particularly when it occurs near the alveolar crestal bone, as usually observed in cases with furcal and strip perforations. These conditions can lead to epithelial downgrowth and attachment loss, potentially resulting in tooth loss if not properly managed (7–10). In cases of instrument fracture, clinical studies demonstrated that the inability to regain apical patency in teeth with preoperative radiolucency significantly decreases the healing rate of initial root canal treatment (5, 6, 11). This is attributed to the fact that fractured instrument inside the root canal could make further disinfection of the apical portion of the root canal beyond the fracture site rather challenging (6, 12).

Procedural errors within the category of uncontrolled canal shaping remain insufficiently studied, with only indirect histopathological and *in vitro* studies suggesting potential trends that may impact the success of root canal treatment. For instance, root canal deviation may result in the persistence of bacteria and debris in the untreated apical portion of the root canal (13), while apical perforation may facilitate the migration of bacteria beyond the root apex, potentially compromising the prognosis (14). However, to date, no well-controlled clinical studies have been conducted to examine procedural errors within this category.

Therefore, this study aimed to assess the effect of various procedural errors during root canal instrumentation, performed by sixth-year dental students using stainless-steel hand files, on the outcome of initial root canal treatment. The null hypothesis was that each type of error had no effect on treatment outcomes.

MATERIALS AND METHODS

Study Population

The Institutional Review Board of the Faculty of Dentistry/Faculty of Pharmacy, Mahidol University, approved the study protocol (MU-DT/PY-IRB 2020/017.1603). The study was also conducted in accordance with the Declaration of Helsinki. The

study population comprised patients who underwent initial root canal treatment in mature permanent molars by sixth-year dental students at the Faculty of Dentistry between 2015 and 2019, using stainless steel hand files for root canal instrumentation. This retrospective study adhered to the “Strengthening the Reporting of Observational Studies in Epidemiology” (STROBE) statement and checklist and the “Preferred Reporting Items for Observational Studies in Endodontics” (PROBE) 2023 guidelines.

Case Selection

This study included patients with a follow-up period of at least 1 year and a comprehensive endodontic chart, including pretreatment information, intraoperative records, and follow-up visit documentation. The chart also needed to contain a complete set of radiographs, including preoperative, working length, master apical file (MAF), main gutta-percha cone, postoperative, and follow-up radiographs. Exclusion criteria included patients with poor-quality radiographs, teeth extracted for reasons unrelated to endodontic disease, or those lacking periapical status information at extraction.

Treatment Protocol

All procedures were conducted under rubber dam isolation conditions. The tooth and rubber dam (M Dent, Bangkok, Thailand) were disinfected sequentially with 5% iodine tincture, followed by 70% ethyl alcohol. Any deteriorated dental restorations or carious lesions were removed, and the teeth were subsequently restored using a resin composite (Z250; 3M ESPE, Seefeld, Germany). After access cavity preparation, the root canal orifices were located and explored using pre-curved size 8 or 10 stainless-steel hand files (Dentsply Maillefer, Ballaigues, Switzerland) with a watch-winding motion.

Root canal preparation protocol was systematic, beginning with coronal flaring, followed by apical preparation and complete shaping. Coronal portions of the root canals were enlarged using size 2 or 3 Gate-Glidden burs (Dentsply Maillefer, Ballaigues, Switzerland) or size 40 – 60 K- files using the crown-down technique. Working length was determined using the initial apical file (IAF), electronic apex locators (EAL; Root ZX, J. Morita USA Inc., Irvine, California, United States), and periapical radiographs. Apical preparation continued sequentially using hand files at the working length until the root canal size reached the desired MAF (which is at least 3 sizes larger than IAF, or at least size 30), followed by a sequential 1 mm step-back preparation for complete shaping. Subsequently, the length and direction of the root canal instrumentation were verified using MAF, EAL, and periapical radiographs.

All root canals were frequently irrigated with 2.5% sodium hypochlorite solution (M Dent, Bangkok, Thailand) using a gauge No. 25 needle and a syringe. Calcium hydroxide (M Dent, Bangkok, Thailand) was used as an intracanal medication between treatment sessions, and the access cavity was sealed with temporary filling materials, including Caviton® (GC Corporation, Tokyo, Japan) and IRM® (Dentsply Maillefer, Ballaigues, Switzerland).

Root canal filling was initiated after the patient was asymptomatic. Before root canal filling, the canals were irrigated with 3 mL of 17% ethylenediaminetetraacetic acid (M Dent, Bangkok, Thailand) for 1 minute, followed by 2.5% sodium hypochlorite to remove the smear layer. Gutta-percha (Dentsply Maillefer, Ballaigues, Switzerland) and zinc oxide eugenol sealer (M Dent, Bangkok, Thailand) were used to obturate the root canals using the lateral compaction technique. The root canal orifices were sealed with glass ionomer cement (Fuji VII; GC Corporation, Tokyo, Japan), and the access cavity was restored using resin composite filling (Z250; 3M ESPE, Seefeld, Germany) as an intermediate restoration.

Data Collection

Data were acquired from treatment records and radiographs in each patient's endodontic chart, including demographic characteristics (age, sex, and systemic disease) and preoperative clinical assessments (presence of cracks, clinical signs and symptoms, periodontal pocket formation, sinus tract opening, and preoperative radiolucency), intraoperative details (including number of visits, procedural errors, and quality of root canal fillings), and postoperative follow-up data (clinical findings similar to the preoperative assessments).

Radiographic Assessment

Conventional radiographs were digitised using a scanner (HP Scan G4010, HP Inc., Palo Alto, California, United States) and evaluated by a single examiner (S.T.) using ImageJ software (National Institutes of Health, Bethesda, Maryland, United States). Radiographic evaluation was performed by a single reviewer who was calibrated with a certified endodontist before the assessment process began. The examiner received training from a certified endodontist using a set of 30 radiographs. Subsequently, the examiner reviewed the same set of radiographs twice, with a 2-week interval between reviews. Inter- and intra-examiner agreement in radiographic evaluations was assessed using Cohen's kappa coefficients. Notably, prior to the study, there was nearly perfect agreement between examiners and the certified endodontist, as well as within the same examiner ($k=0.81-0.99$, reference for nearly perfect agreement).

Detection of Procedural Errors

A comprehensive radiographic assessment was performed after identifying procedural errors in the treatment records, especially ledges that may not have been visible on radiographs. This assessment focused on detecting errors that occurred during root canal instrumentation, starting with a comparison of the lengths and directions of the IAF and MAF. Working length radiographs provided the length and direction of the IAF, representing the full extent of the root canal requiring instrumentation; MAF radiographs indicated the portion of the canal that had been instrumented.

The instrumented length was classified as adequate if the MAF matched the IAF in both length and direction. Over-instrumentation was recorded when the MAF extended in the same direction as the IAF but was longer (with the tip of the MAF closer to or extending beyond the radiographic apex). Under-

instrumentation was noted when the MAF deviated in the direction from the IAF. The length of the uninstrumented root canal was also recorded, measured in millimetres, from the point where the MAF deviated from the original canal path.

Subsequently, the radiographs were examined to detect root perforations, indicated by the presence of MAF or root canal filling extending beyond the root, and any broken instrument fragments within the root canal were noted.

Data from these assessments were used to classify procedural errors. The classification criteria were modified from those of Balto et al. (15) and were categorised into four groups. The first group included errors unrelated to the root canal instrumentation length but resulted in altered root canal shape, such as ledge formation and zipping. A ledge was noted if a visible step or irregularity appeared on the root canal wall (Fig. 1a), while zipping was noted when the apical part of the filled root canal appeared elliptical and shifted toward the outer root wall (Fig. 1b, c).

The second group included errors related to under-instrumentation, such as root canal deviation and lateral perforation. A deviation was recorded if the direction of MAF differed from that of the IAF (Fig. 1d, e). Lateral perforation was noted when deviation was observed alongside the extrusion of the misdirected MAF or root canal filling beyond the root (Fig. 1f, g). The third group involved errors associated with over-instrumentation, specifically apical perforation, which was recorded if the MAF was longer than the IAF or if there was extrusion of the root canal filling through the apical foramen (Fig. 1h, i).

The final group consisted of errors with distinct characteristics, including strip perforations and separated instruments. Strip perforation was indicated if extrusion of the root canal filling was observed at the inner root wall (Fig. 1j), and a separated instrument was indicated if a broken instrument was found inside the root canal (Fig. 1k).

Treatment Outcome Assessment

Preoperative and follow-up periapical status were assessed separately based on the presence/absence of periapical radiolucency. The maximum extent of the apical lesion was measured in millimetres.

Treatment outcomes were assessed by analysing preoperative and follow-up data and classified according to Friedman and Mor criteria (16). Treatment outcomes were classified as "healed" when both clinical and radiographic presentations were normal (Fig. 2a-i), "healing" when a reduction in apical radiolucency size was observed alongside normal clinical findings, and "disease" if apical radiolucency emerged or persisted without change, regardless of normal clinical findings, or if clinical signs or symptoms were present despite normal radiographic findings (Fig. 2j-o). This evaluation considered outcomes at the individual root and overall tooth units, with the overall outcome for each tooth determined by the root with the least favourable outcome.

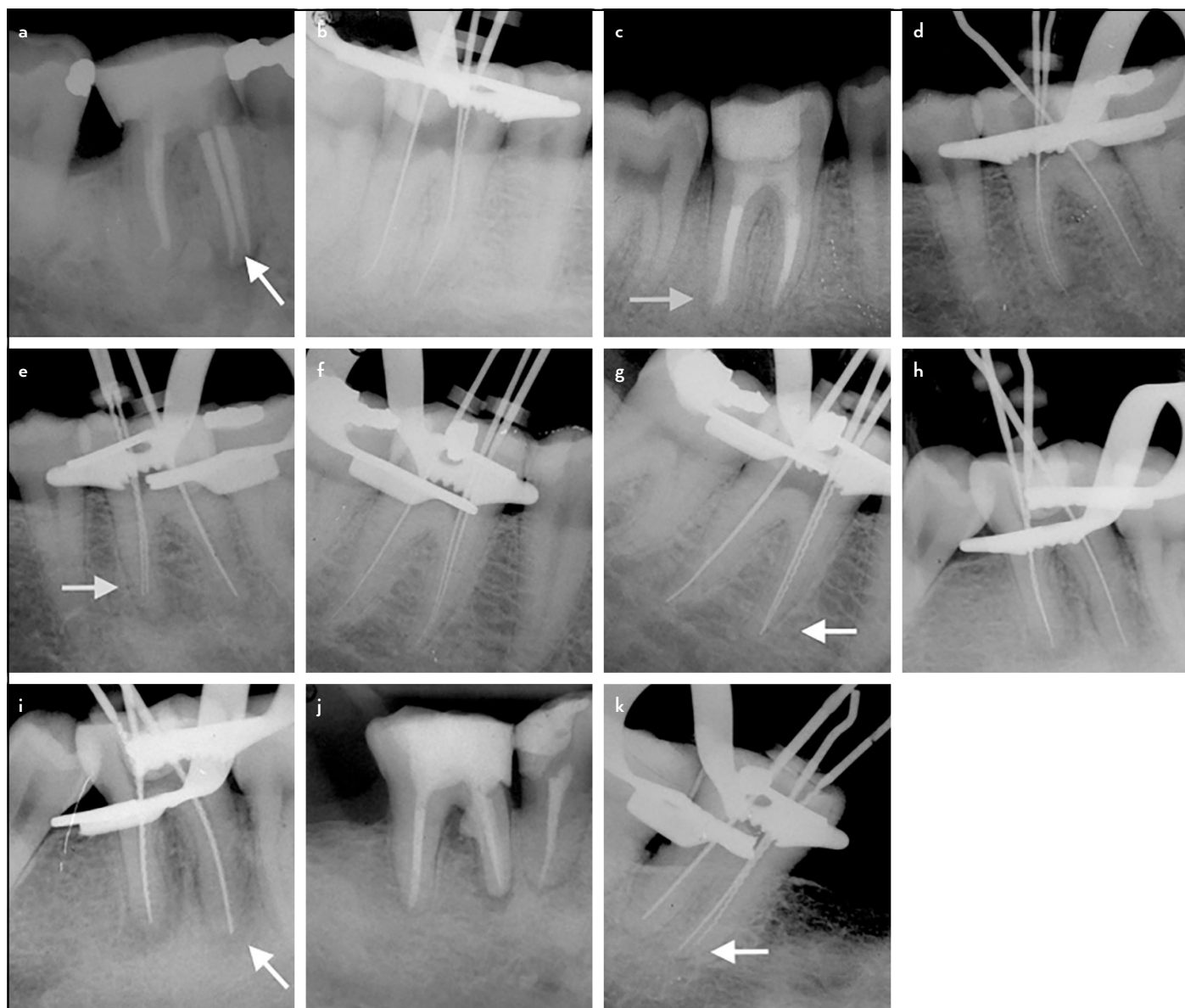


Figure 1. Detection of procedural errors. (a) A ledge was observed in the mesial root of the right mandibular first molar (tooth 46), indicated by a visible step on the canal wall (arrow). (b) Working length determination revealing the original root canal morphology of the right mandibular first molar (tooth 46). (c) Zipping, characterised by an elliptical shape of the apical portion of the distal root (arrow), resulting from apical transportation towards the outer wall of the curved canal, as shown in (b). (d) Original canal morphology of the left mandibular first molar (tooth 36), demonstrated by the IAF during working length determination. (e) Canal deviation was evident in the mesial root (arrow), with the MAF diverging from the IAF, as shown in (d). (f) Working length determination of the right mandibular first molar (tooth 46), showing the initial canal path. (g) Lateral perforation was identified at the mesial root (arrow), as the MAF deviated from the IAF (as seen in f) and extended beyond the root surface. (h) Working length of the left mandibular first molar (tooth 36), demonstrated by the IAF. (i) Apical perforation was evident in the distal root (arrow), marked by the MAF extending beyond the IAF, as shown in (h). (j) Strip perforation in the mesial root of the right mandibular first molar (tooth 46), indicated by extrusion of filling material through the inner root wall. (k) A separated instrument fragment was visible in the mesial root of the right mandibular first molar (tooth 46) (arrow).

IAF: Initial apical file, MAF: Master apical file.

Statistical Analysis

Treatment outcomes based on stringent criteria (normal clinical and radiographic findings) were analysed descriptively for both individual roots and teeth. Predictors of outcomes were identified through uni- and multivariate logistic regression analyses performed at a 5% significance level using Stata software (Stata/SE 17.0, StataCorp LLC, College Station, Texas, USA). Initial analyses covered the entire dataset, followed by sub-analyses of teeth with and without preoperative radiolu-

ency, focusing on the root as the evaluation unit. Logistic regression, with a cluster sandwich estimator for robust standard errors, was used to assess the clustering effect of multiple roots within the same tooth.

RESULTS

Between January 2015 and December 2019, 302 molars underwent root canal treatment from sixth-year dental students. A total of 156 teeth met the inclusion criteria;

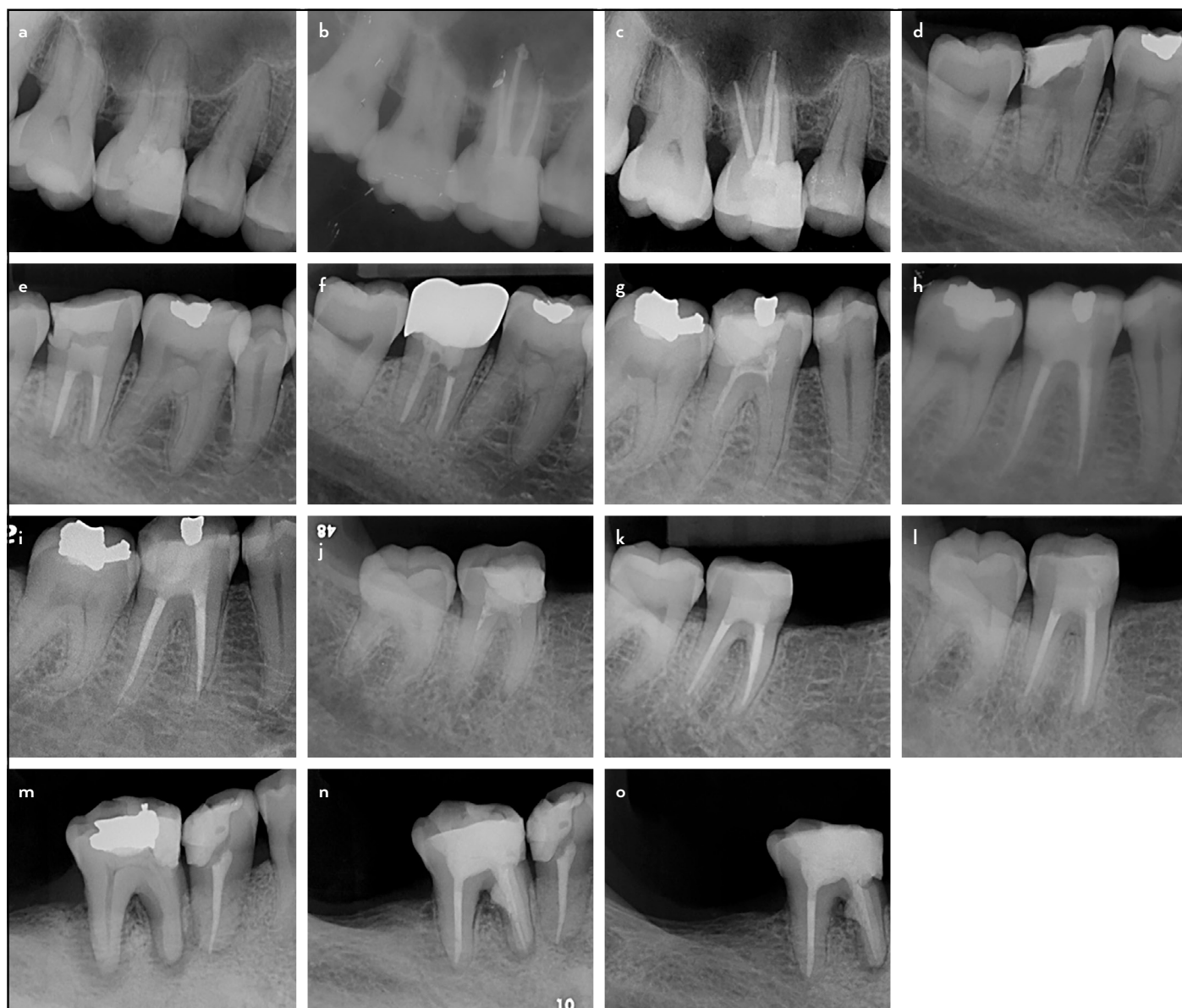


Figure 2. Treatment outcome assessment. (a-c) The preoperative radiograph (a) of the right maxillary first molar (tooth 16) showed normal apical tissues around the palatal root. The postoperative radiograph (b) revealed an apical perforation. At the 2-year follow-up (c), the periapical structures remained normal, and the outcome was classified as “healed.” (d-f) The preoperative radiograph (d) of the right mandibular second molar (tooth 47) showed an apical lesion at the distal root. A postoperative radiograph (e) revealed an apical perforation. At the 6-year follow-up (f), the apical tissues appeared normal, and the outcome was classified as “healed.” (g-i) The preoperative radiograph (g) of the right mandibular first molar (tooth 46) showed normal apical tissues at the mesial root. A lateral perforation was identified in the postoperative image (h). At the 3-year follow-up (i), the periapical structures remained normal, and the outcome was classified as “healed.” (j-l) The preoperative radiograph (j) of the right mandibular second molar (tooth 47) showed an apical lesion at the mesial root. A lateral perforation was evident in the postoperative image (k). At the 4-year follow-up (l), lesion progression resulted in the classification of the outcome as “disease.” (m-o) The preoperative radiograph (m) of the right mandibular first molar (tooth 46) revealed a periradicular lesion involving the furcation and apical region of the mesial root. A strip perforation was evident in the postoperative image (n). At the 3-year follow-up (o), persistence of the radiolucency led to the classification of the outcome as “disease”.

however, 10 teeth were subsequently excluded due to the unavailability of periapical status information at the time of extraction, and an additional four teeth were excluded because of poor-quality radiographs. Thus, 343 roots from 142 teeth were analysed for treatment outcomes. The mean follow-up duration was 35.84 months, ranging from 12 to 72 months. Based on stringent assessment criteria, the overall healing rates were 72.5% for teeth (103 of 142) and 80.5% for roots (276 of 343).

Univariate analysis identified several significant factors, including patient age, clinical signs and symptoms, preoperative radiolucency, and restoration quality. Among procedural errors, only root canal deviation was significantly associated with treatment outcomes (Table 1). Instances of zipping and instrument separation were rare, occurring in only 1.46% (5/343) and 0.29% (1/343) of roots, respectively, and were not observed in the disease group, limiting their impact assessment. In the multivariate analysis, only procedural errors re-

TABLE 1. Association between potential factors and the healing rate using univariate logistic regression analysis (n=343)

	All	Healed (%)	Disease (%)	Crude OR for disease (95% CI)*	p
Procedural errors					
Ledge					
Absent	320	80.6	19.4	Ref	0.777
Present	23	78.3	21.7	1.156 (0.424, 3.150)	
Deviation					
Absent	318	83.0	17.0	Ref	<0.001
Present	25	48.0	52.0	5.296 (2.117, 13.252)	
Lateral perforation					
Absent	332	81.3	18.7	Ref	0.053
Present	11	54.6	45.4	3.629 (0.982, 13.412)	
Apical perforation					
Absent	276	79.3	20.7	Ref	0.291
Present	67	85.1	14.9	0.674 (0.324, 1.402)	
Strip perforation					
Absent	331	81.3	18.7	Ref	0.105
Present	12	58.3	41.7	3.099 (0.788, 12.188)	
Demographic data					
Age (41.11±14.96 years)				0.964 (0.940, 0.989)	0.005
Gender					
Female	223	83.0	17.0	Ref	0.256
Male	120	75.8	24.2	1.551 (0.728, 3.308)	
Systemic disease					
Absent	263	82.1	17.9	Ref	0.291
Present	80	75.0	25.0	1.532 (0.694, 3.383)	
Preoperative data					
Crack					
Absent	321	80.4	19.6	Ref	0.910
Present	22	81.8	18.2	0.910 (0.178, 4.641)	
Clinical signs and symptoms**					
Absent	110	70.9	29.1	Ref	0.032
Present	233	85.0	15.0	0.431 (0.200, 0.930)	
Periodontal probing depth					
≤ 5 mm	330	80.3	19.7	Ref	0.691
> 5 mm	13	84.6	15.4	1.229 (0.446, 3.385)	
Sinus tract opening					
Absent	334	81.1	18.9	Ref	0.074
Present	9	55.6	44.4	3.441 (0.889, 13.323)	
Preoperative radiolucency					
Absent	153	92.8	7.2	Ref	<0.001
Present	190	70.5	29.5	5.395 (2.242, 12.984)	
Intraoperative data					
Treatment sessions				1.041 (0.905, 1.197)	0.576
Length of root canal filling***					
Adequate	323	80.5	19.5	Ref	0.959
Inadequate	20	80.0	20.0	1.032 (0.311, 3.418)	
Void of root canal filling					
Absent	332	81.0	19.0	Ref	0.179
Present	11	63.6	36.4	2.44 (0.664, 8.965)	
Follow-up data					
Dental post placement					
Absent	329	79.9	20.1	Ref	0.257
Present	14	92.9	7.1	0.306 (0.040, 2.364)	
Quality of coronal restoration					
Adequate	318	84.0	16.0	Ref	<0.001
Inadequate	25	36.0	64.0	9.307 (2.689, 32.217)	

Bold font indicates statistical significance. *: Confidence interval for odds ratios was estimated using robust standard error to allow clustering within the tooth. **: Clinical signs and symptoms comprised tenderness on percussion, tenderness on palpation, or increased tooth mobility. ***: The criterion for evaluating the adequate length of the root canal filling was a distance within 2 mm from the radiographic apex. OR: Odds ratio, CI: Confidence interval

TABLE 2. Significant predictors of treatment outcomes identified using multivariate logistic regression analysis

	Adjusted OR for disease (95% CI)*	p
Procedural errors		
Errors unrelated to instrument length (0=absent, 1=present)	0.997 (0.300, 3.316)	0.996
Errors related to under-instrumentation (0=absent, 1=present)	5.329 (1.895, 14.981)	0.002
Error related to over-instrumentation (0=absent, 1=present)	0.767 (0.269, 2.185)	0.620
Error with distinct characteristics (0=absent, 1=present)**	2.122 (0.363, 12.408)	0.404
Demographic data		
Age (year)	0.952 (0.923, 0.981)	0.001
Preoperative data		
Clinical signs and symptoms (0=absent, 1=present)	0.327 (0.136, 0.787)	0.013
Preoperative radiolucency (0=absent, 1=present)	7.120 (2.457, 20.633)	<0.001
Follow-up data		
Quality of restoration (0=adequate, 1=inadequate)	16.542 (2.956, 92.584)	0.001

Bold font indicates statistical significance. *: Confidence interval for odds ratios was estimated using robust standard error to allow clustering within the tooth. **: The error with distinct characteristics in this study was strip perforation. OR, odds ratio, CI: Confidence interval

TABLE 3. Significant predictors of treatment outcomes from sub-analysis of teeth with preoperative radiolucency

	Adjusted OR for disease (95% CI)*	p
Procedural errors		
Errors unrelated to instrument length (0=absent, 1=present)	0.479 (0.098, 2.334)	0.362
Errors related to under-instrumentation (0=absent, 1=present)	8.045 (2.094, 30.909)	0.002
Error related to over-instrumentation (0=absent, 1=present)	0.925 (0.276, 3.096)	0.899
Error with distinct characteristics (0=absent, 1=present)**	3.261 (0.366, 29.057)	0.290
Demographic data		
Age (year)	0.949 (0.914, 0.984)	0.005
Systemic disease (0=absent, 1=present)	4.515 (1.244, 16.379)	0.022
Preoperative data		
Clinical signs and symptoms (0=absent, 1=present)	0.259 (0.101, 0.664)	0.005
Follow-up data		
Quality of restoration (0=adequate, 1=inadequate)	17.839 (2.698, 117.933)	0.003

Bold font indicates statistical significance. *: Confidence interval for odds ratios was estimated using robust standard error to allow clustering within the tooth. **: The error with distinct characteristics in this study was strip perforation. OR: Odds ratio, CI: Confidence interval

lated to under-instrumentation were significant predictors of all procedural errors, with patient age, clinical signs and symptoms, preoperative radiolucency, and restoration quality also identified as predictors (Table 2).

In the sub-analysis of teeth with preoperative radiolucency, errors related to under-instrumentation (odds ratio for disease=8.045, $p=0.002$), patient age, clinical signs and symptoms, and restoration quality persisted as significant predictors. Systemic disease was also an important predictor (Table 3). Furthermore, when the length of the uninstrumented root canal was included in the sub-analysis, it was also identified as a significant predictor of treatment outcome, with an odds ratio of 4.268 for disease for each 1 mm uninstrumented length ($p=0.015$).

Conversely, in teeth without preoperative radiolucency, only restoration quality remained a significant predictor (odds ratio for disease=25.871, $p=0.006$). Neither errors related to under-instrumentation nor the length of the uninstrumented root canal retained their predictive values.

DISCUSSION

Various types of procedural errors occurring during root canal instrumentation exhibit distinct characteristics, potentially

leading to diverse impacts on treatment outcomes. This study expands the understanding of these effects, emphasising the importance of preventing errors that significantly deteriorate treatment outcomes. Additionally, clinicians can deliver a more precise prognosis to patients when errors occur.

Given that multiple factors influence root canal treatment outcomes, this study required the collection of other potential prognostic factors for analysis. Our findings indicated that preoperative radiolucency remained a significant predictor, consistent with previous studies (5, 6, 17–19), and its presence reduced the healing rate by up to 20%. Consequently, it was imperative to analyse the data separately for the groups with and without preoperative radiolucency. Coronal restoration quality also emerged as another significant predictor, as restorations with visibly exposed root canal fillings reduced the healing rate by more than 50%. However, the low occurrence rate of inadequate restorations (only 7.3%) rendered similar sub-analyses infeasible.

The null hypothesis that each type of procedural error would have no effect on treatment outcome was rejected. Univariate analysis indicated that root canal deviation was the only con-

sidered procedural error significantly associated with treatment outcomes. Lateral perforation resulted in a lower healing rate, although without a significant relationship ($p=0.053$), likely due to its infrequent occurrence (11/343 roots). As both root canal deviation and lateral perforation result in incomplete root canal preparation, they were categorised together as procedural errors related to under-instrumentation for subsequent analysis. In the multivariate analysis, this category emerged as a significant predictor of treatment outcomes ($p=0.002$). Bacterial biofilms in unprepared root canal sections likely represent the primary causative factor (13), as supported by the sub-analysis, indicating that these errors specifically affected the treatment outcomes in teeth with preoperative radiolucency (Fig. 2g–l). This finding aligns with that of Ng et al. (6), who observed a 12% decrease in the healing rate for each 1 mm reduction in the apical extent of the MAF.

Previous studies considered short root canal filling to be under-instrumentation; however, their findings were inconsistent despite being from the same researchers (18, 20). This inconsistency may result in short root canal filling being an unreliable indicator of under-instrumentation. For example, debris blockage or gutta-percha cone displacement during obturation can result in a short filling (18, 21). Additionally, evaluating optimal root canal filling length based on a distance within 2 mm from the radiographic apex may be inappropriate, as it does not accurately reflect the correct apical constriction position in each tooth (22, 23). Therefore, evaluating instrument direction changes during the treatment, along with the use of an electronic apex locator, could have enhanced the accuracy of the assessment in this study.

Procedural errors related to over-instrumentation or apical perforations were most frequently encountered in this study. This increased detection rate may have resulted from improved methods, particularly comprehensive radiographic examinations, which allowed for the identification of apical perforations that might have gone undetected in previous studies (15, 21). However, the results of this study indicated that apical perforation had no significant impact on treatment outcomes, likely because this error does not obstruct root canal instrumentation, allowing for effective bacterial biofilm removal (Fig. 2a–f).

Strip perforation, which results from overpreparation in the furcal region of the root canal near the crestal bone, increases the risk of bacterial contamination and attachment loss, ultimately leading to a poor prognosis (7, 8). Although a reduced healing rate was observed in cases of strip perforation, univariate analysis revealed no significant association with treatment outcomes in the present study. This could be attributed to the low incidence rate (12 of 343 roots) and varied management approaches, ranging from immediate repair to delayed repair (up to 370 days). Furthermore, the study lacked information on critical factors, such as perforation size, location, and repair materials (7, 8), limiting the ability to draw definitive conclusions regarding the impact of strip perforation on treatment outcomes.

This retrospective cohort study faced challenges in controlling variables, such as treatment modalities and medications used,

alongside potential issues with incomplete data due to inadequately recorded treatments or missing radiographs. However, the selection of patients treated by undergraduate dental students from the dental faculty ensured stringent adherence to treatment protocols and comprehensive data collection, including detailed treatment records and radiographic documentation throughout the treatment duration. The selection of molars treated with stainless steel hand files also facilitated the inclusion of cases with procedural errors in the analysis.

The mean follow-up time of 36 months in the present study ensured an accurate reflection of prognosis, aligning with the recommendation of Ng et al. (24) that a 3-year follow-up period is suitable for stringent outcome evaluations. Nevertheless, limitations persisted due to the inherent two-dimensional nature of periapical radiographs, which restricted visualisation of the buccal or lingual aspects of the root canal and potential overlap with anatomical structures (4). The accuracy of radiographic angulation may have further influenced the assessment of periapical tissues (25, 26).

Further studies may be necessary to examine the impact of procedural errors with low incidence rates, potentially using different study designs or populations to enhance the detection and analysis of these infrequent events. Investigating predictors, such as patient age, systemic disease, and clinical signs and symptoms, would be valuable to establish definitive conclusions in this area where current knowledge remains limited (5, 6, 17, 19).

CONCLUSION

The impact of specific procedural errors on the outcome of root canal treatment is largely determined by their effect on root canal preparation length. This study identified procedural errors related to under-instrumentation as significant predictors. These errors, such as root canal deviation and lateral perforation, impede instrument insertion into the apical portion of the root canal, compromising root canal disinfection and increasing the risk of post-treatment disease by up to eightfold.

Disclosures

Ethics Committee Approval: The study was approved by the Faculty of Dentistry/Faculty of Pharmacy, Mahidol University Ethics Committee (no: MU-DT/PY-IRB 2020/017.1603, date: 16/03/2020).

Informed Consent: Informed consent was obtained from all participants.

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