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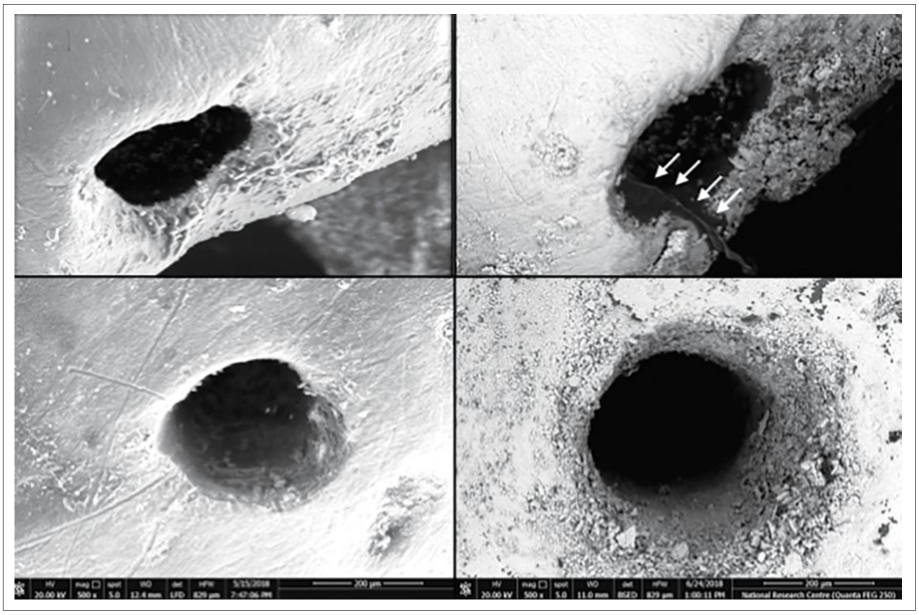

Management of an unusual foreign body in periapical tissues of immature permanent maxillary central incisor

3D Diagnosis and management of external cervical resorption

Original Articles

Using the Periapical Index to evaluate the healing of periapical lesions after root canal treatment

Surface integrity of root ends following apical resection with targeted trephine burs

Analysis of the ability of XP Clean and Easy Clean files to remove Calcium Hydroxide paste from simulated internal tooth resorptions

Case Series

Management of seven external cervical resorptions

Case Reports

Endodontic management of maxillary permanent molar C-shaped morphology

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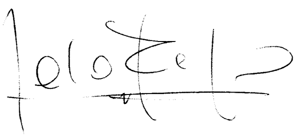

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Editorial

The knowledge cannot have borders: the new challenges of SIE

The recent deconstruction of traditional teaching and research activities has created a moment of great perplexity among professors of all the academic disciplines.
In fact, the guidelines carried out by the ministerial technical committees, basically aimed at reducing the spread of COVID-19, have been mainly based on "social distancing" and "distance learning".
Already in 2011, an article published in Nature highlighted how "the Internet-based degree programs are gaining acceptance, but doubts remain about their suitability for graduate science". However, in the recent years, the growth of online universities, FAD (Distance Learning) courses and webinars, has contributed to making the opinion on such teaching methods (and learning methods, of course) carried out through IT-platforms definitely less hostile. Nonetheless, today the real challenge is to ensure a reliable and effective "hands-on" training, the main training that characterizes and creates professional savoir-faire in clinical practice.
Nowadays, the scientific societies, together with the universities, can no longer neglect this aspect, and must know how to interpret this strong need from an incoercible necessity: the SARS-CoV2 pandemic.
Zygmunt Bauman was the first to define modern society as a "liquid society", and this status is expressed in the concept "that the changing is the only permanent thing, and that the uncertainty is the only certainty".
Therefore, everything flows (panta rei) as Heraclitus said in his famous metaphor describing the immobile but always changing river flow. Similarly, the role of the Universities and the Scientific Societies is to manage the changes "sooner and better" of the subjects who will suffer the consequences of such changes: in this landscape, it is necessary and indispensable to maintain the quality of training excellent, as it has always been in Italy regarding the dental disciplines.
Undoubtedly, Italian dentistry has always been a quality model, a precursor of the best techniques and technologies, pioneer of the most effective experimental procedures: today we are called to combine the old concept of teaching in presence, with the need of distance learning.
SIE has already welcomed this challenge with great enthusiasm: for the first time, the SIE Endodontic Week has been organized (3-7 November 2020) with free webinars, where clinical dentistry topics will be addressed very close-to-patient practice.
The promise is not to stop knowledge, and not to give up on our quality.
Indeed, the sincere hope is to return to teaching and training activities "in person", because a computer can also be an excellent way of transferring information, but it can never replace the enthusiasm of a conference, and it can never replace the empathy of an ex-cathedra lesson. Therefore, knowledge must be considered a "product", just like such products resulting from industrial activities: we can assert that knowledge is undoubtedly the main "asset" of academia and scientific societies and must be protected and valued.
In conclusion, I want to greet all SIE Members with a warm see you soon... hopefully, being able to meet again in person.

Peer review under responsibility of Società Italiana di Endodonzia.

10.32067/GIE.2020.34.02.21

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SIE PRESENTA

ENDODONTIC WEEK

3-7 novembre 2020

CONVENTION VIRTUALE

Martedì 3 novembre
18.00-19.30
J Morita Europe Gmbh
MORITA: Modern treatment & Modern Workshop
Relatore:
Riccardo Tonini

Mercoledì 4 novembre
18.00-19.30
Sweden&Martina
Mtwo a quasi vent'anni dalla nascita: sequenza standard e nuovi strumenti. Minimal. Innovazioni e accorgimenti dallo scouting alla rifinitura apicale
Relatore:
Vito Antonio Malagnino

Giovedì 5 novembre
13.00-14.30
Simit Next
Il protocollo R2C™ "The Root to Crown Solution" e Single Visi
Relatori:
Marco Martignoni, Simone Grandini

Venerdì 6 novembre
14.30-14.45
Cerimonia di apertura lavori a cura del Consiglio Direttivo

Sessione I
Tecniche di otturazione canalare a confronto

14.45-15.45
Maria Teresa Sberna
presenta Giuseppe Cantatore
Tecniche e materiali per l'otturazione canalare: dalla tradizione all'innovazione

15.45-16.45
Luigi Cecchinato
presenta Andrea Polesel
La condensazione verticale: semplice, prevedibile, economica. Perché cambiare?

16.45-17.45
Denise Pontoriero
presenta Giorgio Vittoria
Indicazioni, limiti, tips & tricks delle tecniche carrier-based

17.45-18.45
Cristian Coraini
presenta Franco Ongaro
Come semplificare l'otturazione canalare con i nuovi cementi bioceramici: dalla letteratura alla clinica

18.45-19.30
Tavola rotonda:
Luigi Cecchinato, Denise Pontoriero, Cristian Coraini
Modera Maria Teresa Sberna

Sabato 7 novembre
9.30-9.45
Apertura lavori
A cura del Past President Francesco Riccitiello, del Presidente Maria Teresa Sberna e del Presidente Eletto Roberto Fornara

Sessione II
Le nuove tecnologie in Endodonzia

9.45-10.45
Maria Teresa Sberna
presenta Emanuele Ambu
Le potenzialità della CBCT: dalla diagnosi al trattamento

10.45-11.45
Roberto Fornara
presenta Stefano Gaffuri
Endodonzia Chirurgica Guidata: approccio semplificato alla resezione apicale

11.45-12.45
Francesco Riccitiello
presenta Massimo Gagliani
Endocrown, una vecchia innovazione nei restauri post endodontici

12.45-13.30
Tavola rotonda:
Roberto Fornara e Francesco Riccitiello
Modera Maria Teresa Sberna

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CASE SERIES

Management of seven external cervical resorptions

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Giulia Boschi[1*]
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ABSTRACT

Aim: *External cervical resorption (ECR) assessment and its management planning are often difficult. This work proposes a standardized workflow for ECR treatment considering the 3D-classification of the lesion by Patel and assesses its clinical applicability to seven preliminary clinical cases.*
Summary: *ECR cases were detected from medical and dental history, clinical data and a conventional radiography; a Cone Beam Computed Tomography (CBCT) with a small field of view (FOV) was performed too. A both 2D and 3D-classification was applied on each resorption. After studying better the clinical aspect, especially the accessibility of the lesion, the restorability of the tooth was assessed and the approach was decided between external repair, with or without endodontic treatment, or internal repair. Reparative tissue was removed and the defect was managed through new generation composite resins, mineral trioxide aggregate (MTA) or other cements. A pulp capping was performed in one case and a canal treatment in the others. Clinical and radiographic checks assessed both quality and adequacy of the treatments immediately and over time.*
Key learning points:
- *A standardized workflow could be useful for ECR treatment.*
- *CBCT is essential for determination of ECR extension and an appropriate treatment planning.*
- *ECR management should be conservative, but should also remove the reparative tissue.*
- *ECR management could be performed within one or two appointments.*
- *A follow-up is required to evaluate the treatment over time. The main outcome is the survival of the element, the secondary outcomes are the absence of resorption progression, no symptoms and healthy periodontal values.*

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KEYWORDS *cone-beam computed tomography, conservative treatment, tooth resorption*

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Introduction

Tooth resorption is a condition associated with a process resulting in a loss of dentin, cementum and/or bone (1). It is physiologic and desirable in primary teeth as it aids exfoliation of the deciduous tooth and thus facilitates eruption of the permanent successor (2), whereas it is pathologic and undesirable in adult teeth as it leads to irreversible damage (3).
A preliminary classification of tooth resorption considers its localization: it could be an internal or external resorption. External resorption may be further subclassified in external inflammatory resorption, external replacement resorption, external cervical resorption (ECR) and external surface resorption (4).
ECR is known also as invasive cervical resorption or cervical root resorption (5). It is a relatively uncommon and highly aggressive form of external tooth resorption that starts from the cervical region, below the epithelial attachment, and progressively destroys the tooth structure (4). It is assumed that there must be damage to the periodontal ligament and cementum in combination with a stimulating factor, that can induce and maintain the activity of clastic cells. The histopathogenesis consists of three main stages: resorptive (initiation), resorptive (propagation) and reparative (remodelling), that can occur in parallel in different areas of the same lesion, while it extends deeper towards the pulp and the middle and apical thirds of the root (3, 6).
Epidemiology shows no difference in sex or age: also young people have been found to be involved (7, 8). Maxillary anterior teeth are usually the most affected (8).
Aetiology is rather unclear. Some potential predisposing factors have been already proposed by Heithersay at the end of the last century: orthodontic treatment, dental trauma, internal bleaching, intraoral surgical and restorative treatments (9). Mavridou investigated also other factors, among which: viruses, systemic diseases, poor oral health, parafunctional habits, orthognathic surgery, music wind instruments, malocclusion, frenulum tension, extraction of a neighboring tooth, eruption disorders and cracks. In the majority of clinical cases, more than one potential predisposing factor was identified, indicating that ECR is multifactorial, not idiopathic. Furthermore, in 99% of the examined cases, almost one potential predisposing factor was recognized (7). Nevertheless, a sure causal relationship has not yet been found for any factors and a minority of cases is still without a plausible explanation.
Clinical features vary from nothing to a small defect at the gingival margin, which is directly detectable through a visual inspection, to a pink coronal discoloration of the crown, as a result of soft tissue color shining through the thin tooth tissue overlying the resorptive cavity, resulting in ultimate cavitation of the enamel. The process is usually painless because the pulp remains protected by a thin layer of predentin and dentin until late, when a pulpal or periodontal infection supervenes (9, 10). As the resorption is usually without symptoms through a very long period or even never become symptomatic, the lesion could proceed without anyone becoming aware of it, until the tooth could not be longer saved. So, due to the nature of this lesion, early detection is essential and difficult at the same time. It is very often discovered thanks to a routine conventional radiography during a visit as an incidental radiographic finding or, alternatively, because the clinic is rather advanced (5).
Until a few years ago, the only way to assess the extent of the lesion was the conventional X-ray. Here the lesion appears as radiolucency; a radiopaque line demarcates the root canal from the adjacent irregular radiolucency when the latter is close to the canal (11). The first classification by Heithersay was two-dimensional: it categorized ECR according to its extension into the root and its proximity to the root canal, as follows.
Class 1: a small invasive resorptive lesion near the cervical area with shallow penetration into dentine.
Class 2: a well-defined invasive resorptive

lesion that has penetrated close to the coronal pulp chamber, but shows little or no extension into the radicular dentine.
Class 3: a deeper invasion of dentine by resorbing tissue, not only involving the coronal dentine, but also extending at least to the coronal third of the root.
Class 4: a large invasive resorptive process that has extended beyond the coronal third of the root canal and may involve almost the entire root (12).
However, the classification above has got limitations, as it does not describe the true nature of ECR (resorptive and reparative) and, above all, it does not read the third dimension; furthermore when there is only a small starting lesion, especially if on the buccal or lingual aspect of the tooth, it may not completely be seen in conventional X-ray (13, 3). Therefore the periodontal radiography can result in inadequate assessment or even misdiagnosis and also poor management of root resorption (14).
Fortunately, the Cone Beam Computed Tomography (CBCT) has become available in Endodontics thanks to a reduced field of view (FOV) with a reasonable exposure dosage and good quality, optimizing exposure parameters on an individual basis: the principle as low as reasonably achievable (ALARA) is therefore followed (15).
Several specialist societies published position statements, among which the European Society of Endodontology (ESE): they recommended the CBCT to assess and/or manage root resorption, which clinically appears to be potentially amenable to treatment (16, 17).
Patel suggested a new three-dimensional classification for ECR based on additional information available from CBCT. It takes into account three parameters thanks to three-dimensional analysis, as follows.

Height (coronal-apical extent) of the lesion: the lesion
1) is at cement-enamel junction level or coronal to the bone crest (supracrestal);
2) extends into coronal third of the root and apical to the bone crest (subcrestal);
3) extends into mid third of the root;
4) extends into apical third of the root.

Circumferential spread, which is graded according to the lesion maximum spread within the root:
A) ≤90°
B) >90° to ≤180°
C) >180° to ≤270°
D) >270°
Proximity to the root canal, which can be best assessed using axial CBCT views:
d) lesion confined to dentine;
p) probable pulpal involvement (18).

The combination of each of the three parameters (e.g. 1Ad) provides to the clinician a more accurate determination of the whole extension of ECR and can lead to a more appropriate treatment planning.
The same author proposed different strategies for the management of ECR: external repair with or without endodontic treatment, internal repair, intentional reimplantation and periodic review until extraction. He suggested materials and he tried to connect each strategy with both the Heithersay and the three-dimensional classification, as far as possible (19).
Some case reports have already been published about ECR and its treatment (20-27), but without following a specific workflow.
We arranged a preliminary workflow since 2015, then we upgraded it according the ECR classification by Patel (18) and the last ECR review (19). Here we suggest the last version of the workflow for a standardized approach in the anterior as well as in the posterior mouth, as schematized in the diagram of Figure 1. We have also already applied this workflow to new seven cases of ECR, as described below.
We found the cases of ECR thanks to medical and dental anamnesis, clinical data - including probing - and a conventional radiography or directly thanks to conventional radiography. We asked the patient about any symptoms, if not directly referred. Then we performed a CBCT with a small FOV, giving a relatively low effective dose and obtaining specific and clear information that made us know the real three-dimensional extension of the lesion. Only in one case we directly chose a larger CBCT FOV, as the patient needed also a cysts removal in the right half-jaw, and

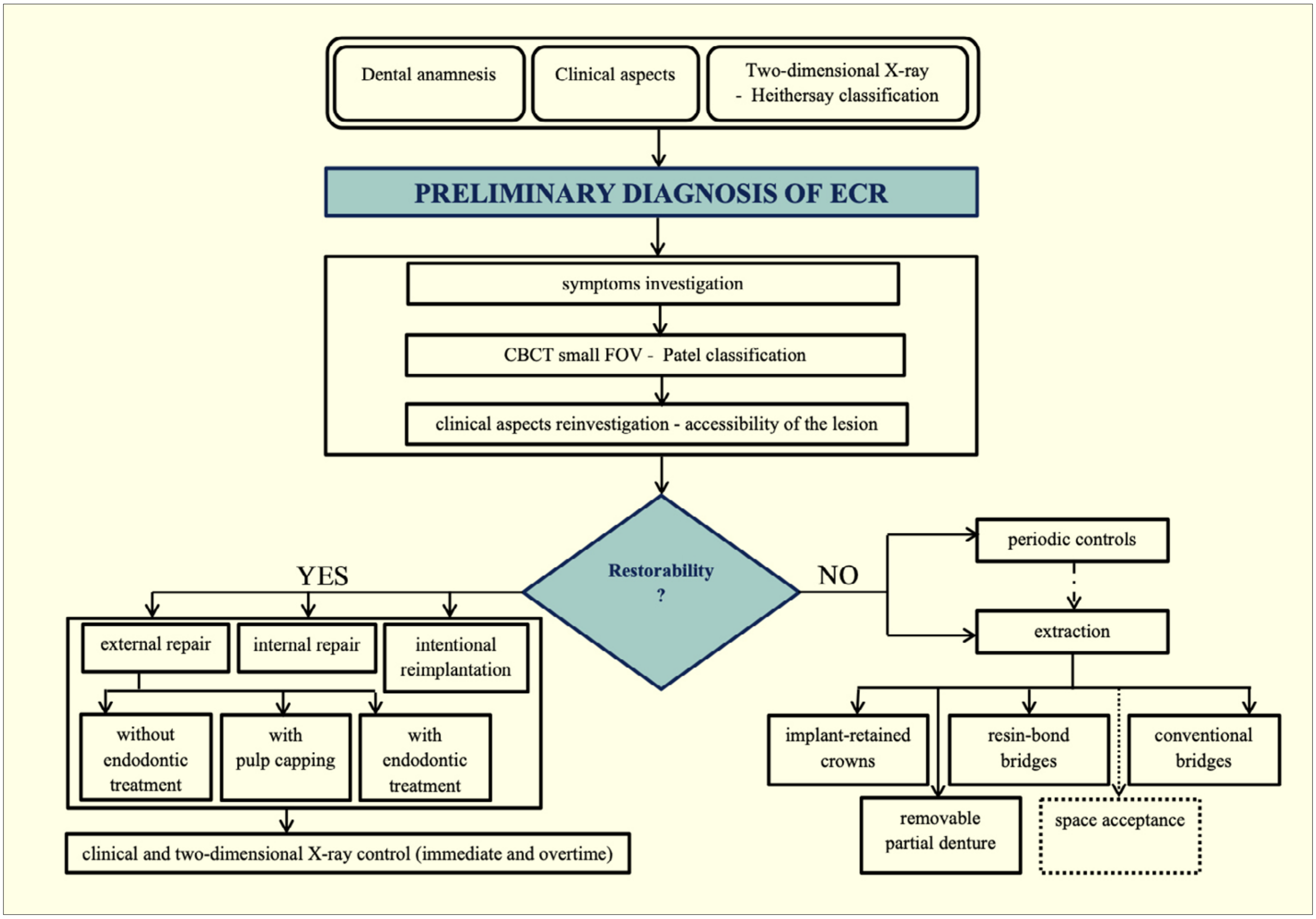

Figure 1.
Workflow diagram.

in another case an ECR was discovered in a CBCT for an implant plan. Consequently we applied a double classification, both by Heithersay and Patel.
We studied again and better the clinical aspect, especially the accessibility of the lesion, and subsequently we assessed the restorability of the tooth, that was fortunately always possible. Then we decided the approach between external repair, with or without endodontic treatment, or internal repair, following instructions and advices in the review by Patel (19). Especially, we tried to remove resorptive tissue when possible and managed the resorptive defect through new generation composite resins, MTA or other cements. We applied a calcium hydroxide cement for pulp capping in one case and used warm gutta-percha, endodontic cement and eventually mineral trioxide aggregate (MTA) for the canal treatment in the others.
Clinical and radiographic checks assessed both quality and adequacy of the treatments immediately and over time.

Report

#Case1
It was the first case in chronological order (year 2015).
A 17-years-old male presented requiring an orthodontic treatment. At the first visit, the oral health seemed good. Any soft oral tissues lesions, any caries or tooth abnormalities were directly detectable; some previous conservative and endodontic therapies were present. Periodontal pocket values were between 1 mm up to 3 mm, without bleeding or suppuration on probing. Instead, a malocclusion was assessed: therefore we prescribed an orthopantomography in order to plan the orthodontic treatment. Here we noticed a strange

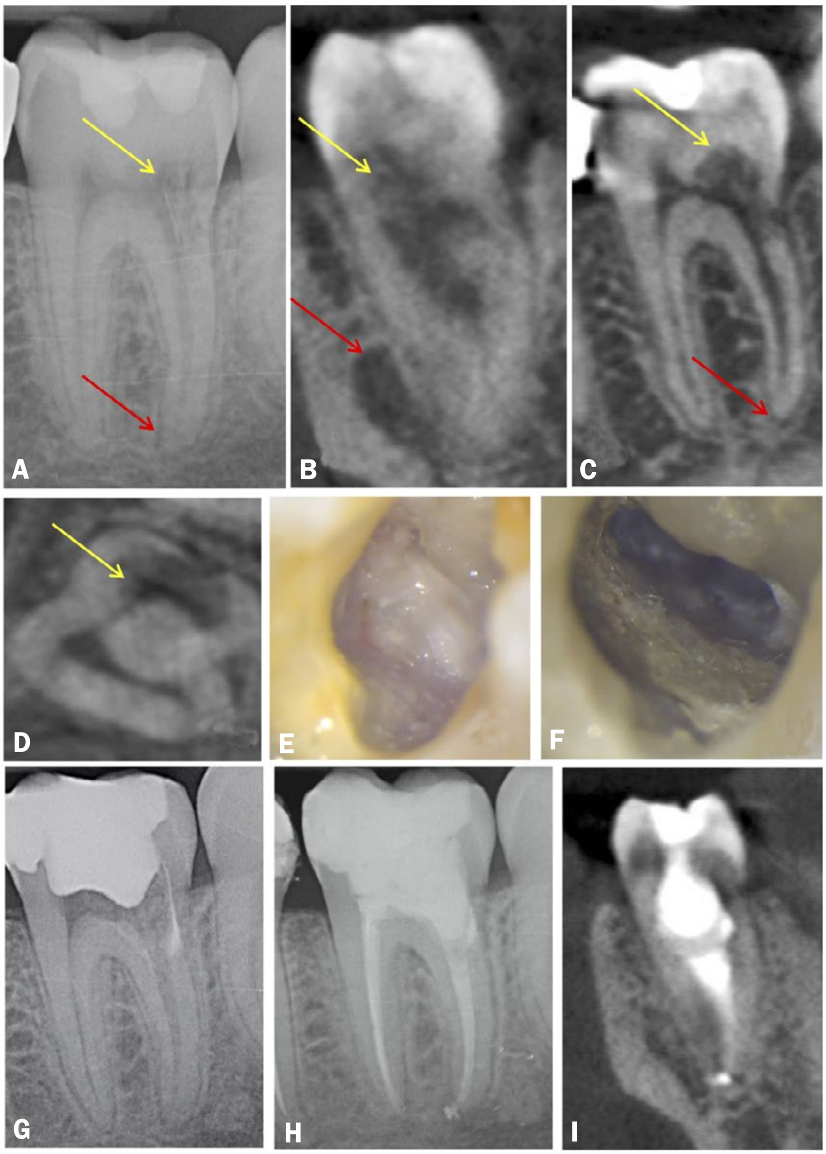

Figure 2 (video 1).
ECR case 1. **A)** Preoperative radiograph of a 3.7 showed ECR (yellow arrow) and preliminary signs of apical periodontitis (red arrow). **B)** Frontal, **C)** sagittal and **D)** axial CBCT slices confirmed the extensive nature of ECR which extends apically and completely around the distal canal (yellow arrows). **E)** Access cavity preparation revealed the partially reparative nature of ECR. **F, G)** MTA was located all around the access of the distal canal. **(H)** Immediate post-fill radiograph. **I)** Frontal.

radiolucency at the distal cervical area of his second lower left molar 3.7.
We suspected an ECR. The tooth was a normal responder to both the vitally test (we used the cold test) and the percussion test. The general medical anamnesis was negative; the patient could not recall a history of dental trauma, another previous orthodontic treatment or any of the other possible predisposing factors and he did not report any symptoms. The clinic aspect of the tooth confirmed to be silent: any pink spots, any gingival irregularities, any strange small cavitations in the crown third part and any deeper periodontal probing values could be detected.
However, we decided to investigate better taking a periodontal radiography with a paralleling technique (Figure 2A): it revealed a more widespread radiolucent area in the distal side of the crown starting from cervical area and extending to the coronal third of the root. Besides, a small suffering area could be seen around the last third of the distal root, whereas it was not detectable in the orthopantomography. According to this further information the resorption was really present and was classified as a class 3 of ECR, following Heithersay classification.
We took a CBCT (Figure 2B-D, M) to understand its real extension. A 3D classification was not available in 2015, however now we could further classify the lesion as a 3Dp stage following Patel classification. The CBCT showed also a more important radiolucency into the wave bone around the distal root, therefore the positive answer to vitality test could be due to the mesial part of the teeth. These findings could be explained by CBCT high sensitivity: CBCT imaging was demonstrated to have twice the odds of detecting a periapical lesion than traditional periapical radiography (28).
We investigated again the clinical aspects, we decided for the restorability of the element 3.7, but, because the direct accessibility was limited, we concluded that an external repair of the root (the first choice in our workflow for restorable teeth) may have been really difficult. Therefore we chose an internal repair with MTA thanks to its biocompatibility and sealing ability (29), followed by a conventional canal treatment with warm gutta-percha.
Therefore, at the first appointment, we applied a stamp technique: we impress the occlusal anatomy and then we performed a classical cavity access (30, 31) with diamond burs before and ultrasonic (US) tips

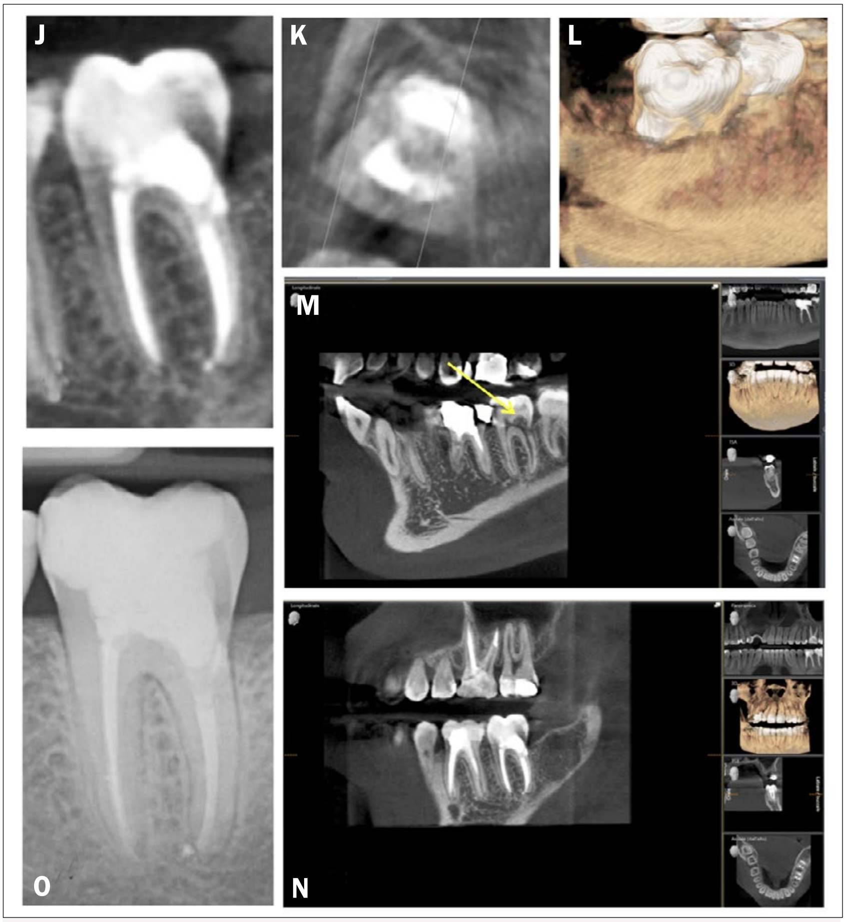

Figure 2.
ECR case 1. **J)** sagittal, **K)** axial CBCT slices and **L)** 3D rendering of two years recall. Comparison between **(M)** pre-operative and **(N)** two years recall CBCT. **O)** Four years recall periapical X-ray.

later (Figure 2E). Thanks to the microscope, we directly saw the reparative tissue and we removed it with an endodontic dedicated US tip, also to have access to the coronal third of the canal into the distal root. We located MTA both to the mesial and to the distal aspect of the canal access and we compacted with a micro-brush (Figure 2F). A periodontal X-ray was taken to make us sure that the MTA was correctly located (Figure 2G, video 1) and we waited 7 days to allow the complete hardening of the material under a temporary restoration. After a week the patient underwent the second appointment: we checked the MTA seal, we completed the root canal treatment and performed a direct restoration. An immediate post-operative periapical radiography (Figure 2H) was taken. After 2 years another CBCT was prescribed in order to plan an implant guided surgery of 1.3 and the imaging was extended to the lower jaw, according with the patient: it confirmed a good seal of the previous resorptive lesion and the radiolucent periapical area was no longer detectable (Figure 2I-N). Meanwhile, clinical controls and periapical X-rays have been taken until 2019 (4 years recall): the tooth has always been clinically asymptomatic, the periapical radiographs confirmed the healing bone process and the absence of new areas of resorption (Figure 2O).

#Case 2

The second case began in 2017.

A 17-years-old female was seen in urgency because of a poorly located strong spontaneous pain. Her lower left canine 3.3 showed a pink discoloration – therefore we supposed a resorption (11, 10, 3) – and the vitality test led to the following diagnosis: pulpitis (32). Any caries could be detected. A periodontal radiography was taken using a paralleling technique (Figure 3A): it revealed a radiolucent lesion with irregular and poorly defined borders in the cervical aspect of tooth extending to the coronal third of the root. Besides, a radiolucency could already be seen into the alveolar bone around the distal aspect of the first third of the root. The diagnosis of ECR was confirmed. According to this preliminary information the resorption was classified as class 3 following Heithersay classification. The patient confirmed that her general medical anamnesis was negative; her main predisposing factor was a previous fixed orthodontic treatment (9, 7, 33), followed by a post-orthodontic splinting and periodical recalls. She highlighted that she did not experienced any symptoms before, but this finding is not strange as ECR becomes symptomatic (if it happens) at its last stages (11). We opened immediately the access cavity in urgency, we put a temporary restoration and we planned a CBCT to further confirm the diagnosis of ECR and assess the true position and extension of the radiolucency. We took a CBCT (Figure 2B-E, video 2) some days later. A 3D classification was not yet available, however now we could classify

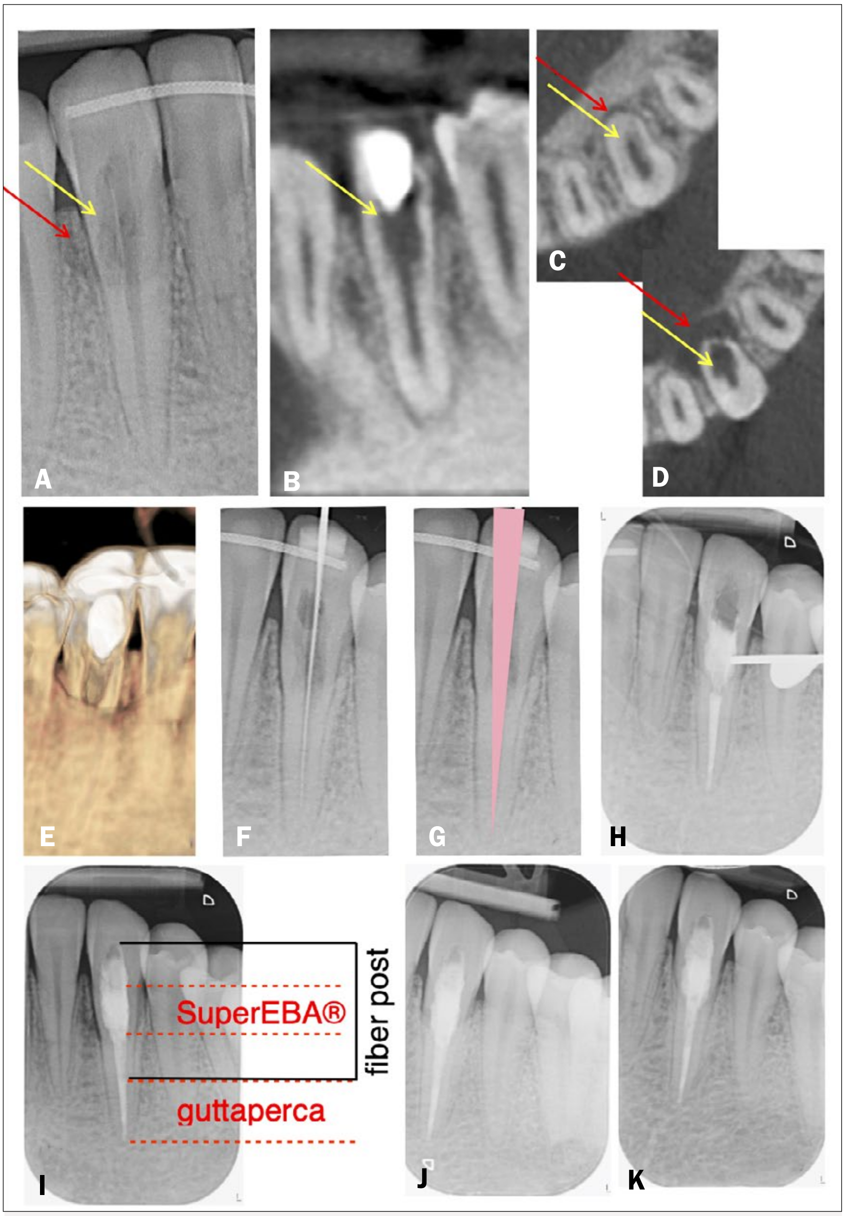

Figure 3 (video 2).
ECR case 2. **A)** Preoperative radiograph of a 3.3 showed ECR (yellow arrow) and signs of lateral periodontitis (red arrows). **B)** Frontal and **C, D)** axial CBCT slices confirmed both the extensive nature of ECR (yellow arrows) which extends apically the bone crest and a lingual bone lesion (red arrows). **E)** 3D rendering of the tooth. **F)** Radiographic working length. **G)** A gutta-percha cone was temporarily put into the canal. **H)** Defect restoration using SuperEBA® Cement and gutta-percha apical seal. **I)** Schematic view of the fully restored tooth: a fiber post was put into the middle and coronal third of the root. **J)** One year recall. **K)** Two years recall.

the lesion as a 2Bp stage following Patel classification. The CBCT showed also an important lingual radiolucency into the wave bone, that broke off the neighboring cortical bone (28).
We reinvestigated the clinical aspects, we decided for the restorability of the element 3.3 thanks to external repair and endodontic treatment. Therefore we removed the temporary restoration and we recorded the working length through a periapical X-ray (Figure 3F) as electronic apex detector did not work correctly because of the periodontal tissues interference. We shaped the canal and put a temporary gutta-percha point in it (Figure 3G). We opened a surgical flap and we removed both granulomatous tissue that was profusely bleeding and the resorbing tissue thanks to US inserts and operative microscope. Before filling the resorptive defect with SuperEBA® Cement, we controlled the integrity of gutta-percha point inserted into the canal to prevent cement from sliding downward. We put SuperEBA® Cement in the defect and closed the flap. Another appointment followed: the apical third of the tooth was filled with endodontic cement and warm gutta-percha (Figure 3H), an hollow fiber post was located because of its favorable mechanical properties (34) and a direct composite restoration was performed. An immediate post-operative periapical radiography (Figure 3I) was carried out. Clinical and periapical X-ray has been taken until 2019 - 1 (Figure 3J) and 2 (Figure 3K) years recall: the tooth has been clinically asymptomatic; the periapical radiographs show a healing bone process around the coronal third of the root and no new areas of resorption.

#Case 3
The third case was discovered in November 2018.
A 12-years-old female showed a discoloration on her second upper right incisor tooth 1.2 during her fixed orthodontic treatment. We immediately thought that a resorption was happening, as in that case the resorbed structure is replaced by highly vascular tissue which could be seen through a thinner enamel, especially in anterior teeth (11, 10, 3). The element was not symptomatic; however endodontic tests were performed: it responded to cold like her neighboring teeth and it was non-tender to pressure and percussion.
The general medical anamnesis was nega-

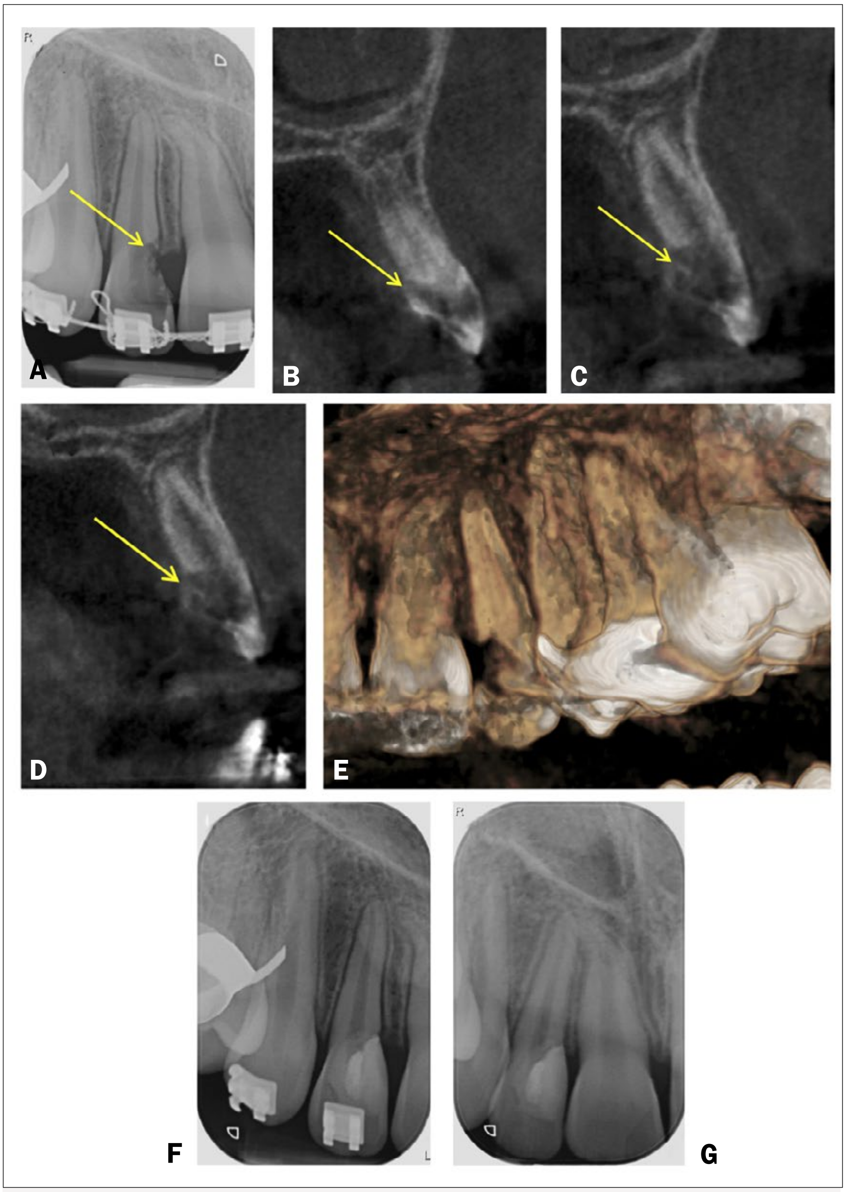

Figure 4 (video 3).
ECR case 3. **A)** Preoperative radiograph of a 1.2 showed ECR (yellow arrow). **B, C, D)** Sagittal CBCT slices let know the real extension of ECR (yellow arrows) that was mainly palatal and supracrestal. **E)** 3D rendering of the tooth. **F)** Immediate post-operative X-ray. **G)** One year recall.

tive; about her dental history, any other predisposing factors could be detected, except for the orthodontic treatment (9, 7, 33). A periodontal radiography was taken using a paralleling technique (Figure 4A): it revealed an irregular radiolucency in the mesial side of the crown just above the bone crest, not extending apically into the radicular dentine, but quite deeper through the coronal dentine, maybe touching the pulp chamber. According to this preliminary information, the lesion was classified as class 2 ECR, following Heithersay classification.

We took a CBCT (Figure 2B-E) to understand the real extension of the resorption: it was mainly palatal and supracrestal and fortunately did not reach the endodontic system. A 3D classification was recently available and we classified the lesion as a 1Bd stage following Patel classification.

We investigated again the clinical aspects, especially the palatal accessibility and we decided for the restorability of the element 1.2. We proposed a direct external repair, trying to maintain tooth vitality.

Therefore we opened a surgical flap and we removed both granulomatous tissue that was profusely bleeding and the resorbing tissue thanks to US inserts and operative microscope. The pulp of the chamber was directly observable only under the microscope. So we decided to try a quick direct pulp capping through calcium hydroxide (Dycal, Dentsply Sirona) (35). Then we performed a direct composite restoration followed by many finishing and polishing steps to remove respectively main irregularities and minute scratches (36): we used diamond burs, flexible and semi-flexible abrasive instruments (cups, points and wheels), instruments coated with abrasives (abrasive disks and strips) and abrasive polishing paste compounds (37). The flap was closed.

An immediate post-operative periapical radiography (Figure 4F) was taken. Clinical checks with annual periapical X-ray were planned: the tooth has been clinically asymptomatic, has kept its vitality and 1 year recall X-ray (Figure 4G) and probing (video 3) have been satisfactory.

#Case 4

The fourth case was discovered in November 2018 too.

A 52-years-old smoker female was referred by her oncologist. She suffered from lung cancer with multiple metastases, mainly localized to the spine, as well as hypertension and panic attacks. She needed to begin an intravenous bisphosphonate thera-

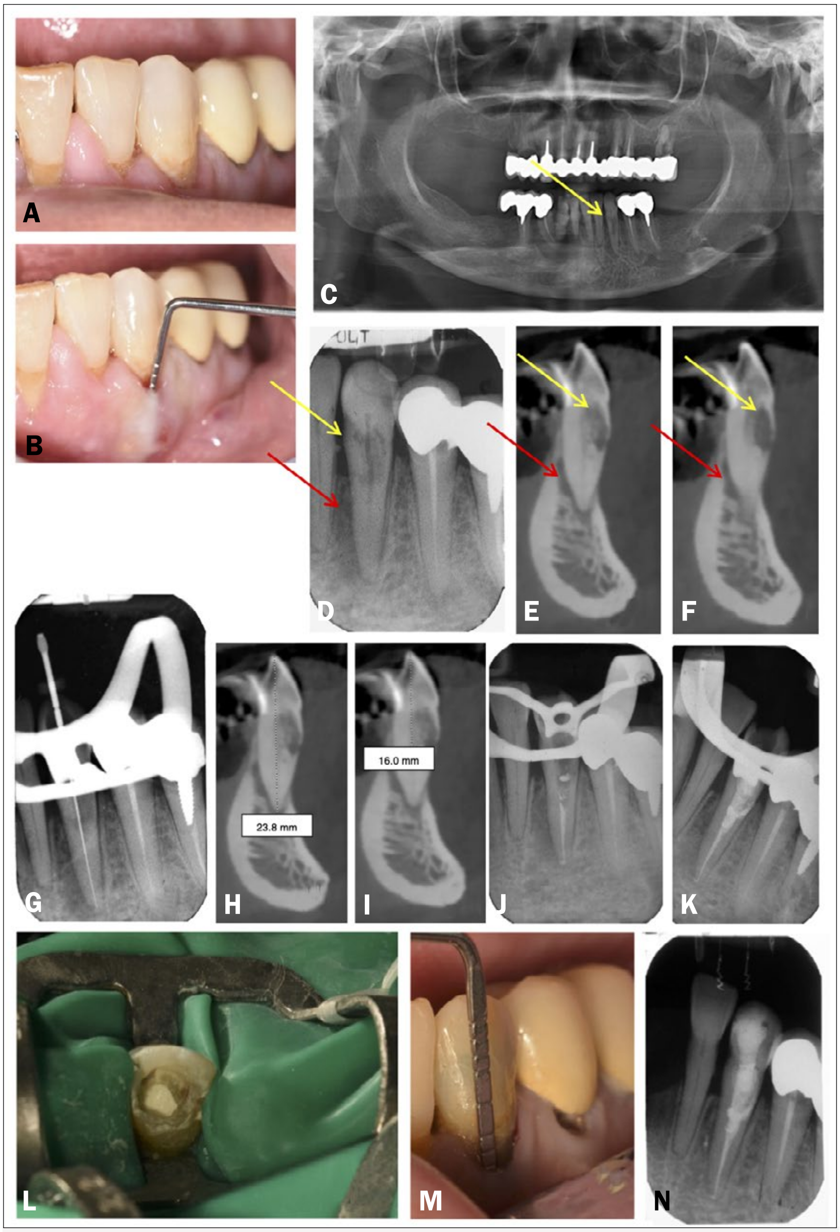

Figure 5 (video 4).
ECR case 4. **A)** Preoperative aspect and **B)** probing of 3.3. **C)** Root of 3.3 showed a strange radiolucency in the orthopantomography (yellow arrow). **D)** Preoperative radiograph of a 1.2 highlighted an ECR (yellow arrow) and a bone lateral radiolucency along the mesial aspect of the root (red arrow). **E, F)** CBCT sagittal slices showed the exact extension of both the resorptive lesion in the coronal and, partially, middle third of the root (yellow arrows) and the periradicular lesion (red arrows). **G, H)** Radiographic working length was taken. **I)** Radiographic resorption level was recorded too. **J)** The apical third was filled with warm gutta-percha. **K)** The middle and coronal third were filled with MTA. **L)** Occlusal view before composite coronal restoration. **M)** One year probing and **N)** Two-dimensional X-ray.

py and the oncologist prescribed a previous dental control to prevent osteonecrosis of the jaws (ONJ). Our dental visit detected a very poor oral hygiene, a single metal-ceramic multiple-unit fixed dental prosthesis on the upper jaw and two conventional metal-ceramic bridges in the lower jaw. Some caries were evident apically to the metal-ceramic crowns and the loss of tooth-supporting structures around elements 4.2, 4.1 and 3.1 resulted in tooth mobility. There was a generalized loss of clinical attachment, mainly through recessions, with no periodontal pocket depths greater than 3 mm, except for the buccal aspect of element 3.3 (Figure 5A), where we recorded the value of 7.5 mm (Figure 5B) with an immediate suppuration on probing. The same element did not respond both to the vitality and percussion test. The patient did not report any symptoms here or elsewhere in the mouth; however, this data was relative as she was taking a high-dose morphine therapy because of her systemic condition.

The patient gave us her latest orthopantomography (Figure 5C) that showed some extremely decayed teeth behind the metal-ceramic crowns, a mandibular cyst behind the elements 4.3, 4.2 and 4.1 and 3.1 and a radiolucent area around the root of the element 3.3, as a sign of periodontitis. Besides tooth 3.3 had got a strange radiolucency also inside the root, just below the cervical area.

A periodontal radiography of 3.3 was immediately carried out using a paralleling technique (Figure 5D): it confirmed a mottled resorptive lesion into the coronal third of the root, and showed better the bone radiolucency, especially along the distal aspect of the tooth. According to this preliminary information, the lesion was classified as a class 3 of ECR, following Heithersay classification.

We supposed the occlusal trauma because of the metal-ceramic opposite prosthesis and the poor oral hygiene as ECR predisposing factors.

A CBCT scan (Figure 5E, F, video 4) was prescribed to assess the true nature, extension, and position of both the mandibular cist and the radiolucency inside 3.3. The slices confirmed the provisional diagnosis of ECR that had spread at the beginning of the middle third of the root and had reached the pulp; besides the bone radiolucency appeared to be in the lingual side above all. We used the new Patel classification: it was a 3Cp lesion. We investigated again the clinical aspects, and we initially proposed a direct external repair of the root: we would have begun with an endodontic access without removing the previous distal third class restoration, put a temporary gutta-percha point into the root canal, opened a flap, directly removed the resorptive tissue and repaired with MTA or SuperEBA® Cement, closed the flap and concluded the canal therapy. We also explained that this therapy might have been performed together with 4.3, 4.2, 4.1 and 3.1 extraction and mandibular cyst removal. But the patient refused any unnecessary oral surgery, so we decided for an internal reparation. We opened the endodontic access and we performed a preliminary shaping at the radiographic working length (Figure 5G, H), as the electronic apex detector did not work correctly because of the periodontal tissues interference, as well as in case 2. We performed canal and resorption filling through a sandwich technique, the same proposed in order to deal with root perforation (38): we closed the apical third (the last 8 mm: working length of 24 mm minus resorption length of 16 mm) (Figure 5H, I) with warm gutta-percha (Figure 5J), then we used MTA up to the cervical area. The MapSystem (Simit Dental) was very useful. As more than 5 mm of MTA were necessary, we put the material partially in a first appointment and partially in a second one (Figure 5K, L), so that the material was able to become completely hard. We ultimate the therapy with a common composite coronal restoration. Subsequently we removed elements 4.3, 4.2, 4.1 and 3.1 because of their advanced mobility, the mandibular cyst and the other teeth with deep caries in the upper jaw. After two months the patient started the intravenous bisphosphonate and we planned almost monthly dental visits. The tooth has always been asymptomatic until today and the buccal periodontal probing depth (PPD) has become shorter: it was 5 mm two months later, 4.5 mm three months later, 1 mm seven month later and 0.5 mm one year later (Figure 5M). At the same time, the two-dimensional radiographic aspect became better and no bone lesions could be seen at 1 year recall (Figure 5N).

#Case 5

The fifth case was detected in January 2019.

The patient, a 40-years-old male, with good oral health, presented in urgency with the chief complaint of intermittent spontaneous pain related to his lower left side. Any tooth showed directly detectable caries or abnormalities. We performed the cold test on every element and we find that element 3.6 suffered from pulpitis.

A periodontal radiography was carried out using a paralleling technique (Figure 6A): it revealed a radiolucent lesion with irregular and poorly defined borders in the cervical mesial aspect of tooth crown extending to the coronal third of the root. An ECR was diagnosed. According to this preliminary information, the resorption was classified as class 3 following Heithersay classification.

The patient general medical history was negative; any ECR predisposing factor could be detected. He highlighted that he did not experienced any symptoms before, until this late stage (11).

We immediately took a CBCT (Figure 6B-D), which confirmed that the resorptive lesion was mainly in the cervical mesial aspect of the teeth and that it had developed deeper and deeper into the dentin until it had reached the pulp chamber. We classified the lesion as a 3Cp stage according with Patel classification.

We reinvestigated the clinical aspects, we decided for the restorability of the element 3.6 and we proposed an endodontic treatment with internal repair.

In the same appointment, we saved a copy of the occlusal anatomy in order to perform a stamp technique (30, 31) for the following coronal restoration, so that it could be quick and precise; then we opened a conservative access cavity and we removed both the pulp tissue and the reparative tissue. We performed a classical canal therapy with warm gutta-per-

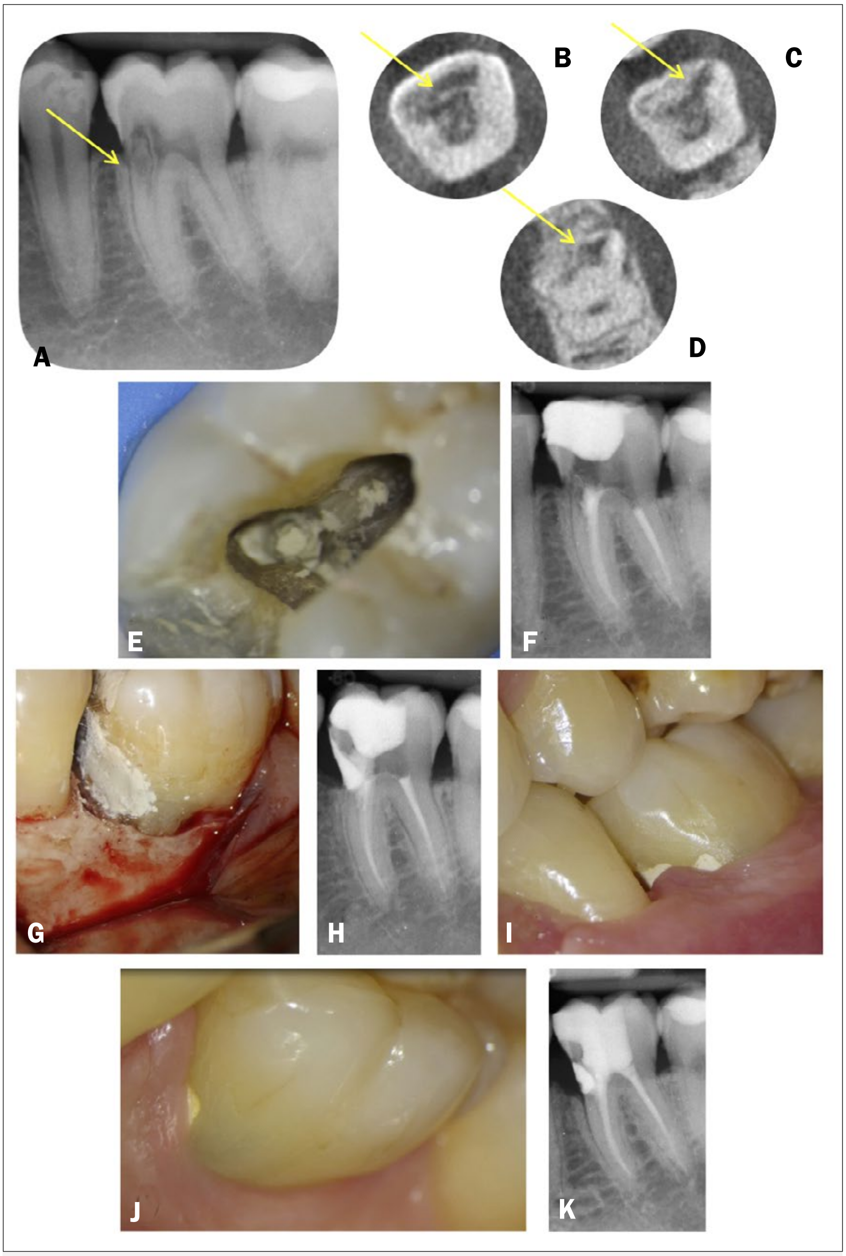

Figure 6 (video 5).
ECR case 5. **A)** Preoperative radiograph of a 3.6 showed ECR (yellow arrow) on the mesial aspect of the tooth. **B, C, D)** Axial CBCT slices let know the real extension of ECR (yellow arrows) that reached the endodontic system. **E, F)** Internal filling of the lesion with MTA. **G, H)** External filling of the lesion with SuperEBA® Cement. **I)** One week clinical control. **J)** Ten moths clinical and **K)** radiographic control.

cha, except for the lesion, which we tried to fill with MTA (Figure 6E), but we did not achieve the whole defect reparation. Therefore we put a moistened micro-brush head in order to allow MTA hardening and a temporary coronal restoration, we took an X-ray in order to understand how much material was located and exactly where (Figure 6F), and we planned also an external approach. In the following appointment we opened a flap, we located SuperEBA® Cement in the defect (Figure 6G) and closed the flap again. An X-ray confirmed that now the defect was completely filled (Figure 6H). We put the rubber dam and completed the case with direct composite restoration, using the stamp technique. 1 week later soft tissues were very good (Figure 6I). A 10 months recall showed a good clinical (Figure 6J) and radiographic healing (Figure 6K): especially, we noticed that interdental papilla was grown; only a small defect was produced on the mesial aspect of the SuperEBA® Cement filling because of an error during the professional oral hygiene. The patient has been asymptomatic until today.
All the steps until the last control are better reported in video 5.

#Case 6
The sixth ECR case was found in July 2019.
The patient, a 21-years-old female, presented in urgency complaining about a spontaneous pain in her left lower side. She had also noticed a small localized swelling between teeth in her lower jaw. At the clinical control, the patient was caries free and had never had a restoration before. All the teeth were pulp tested using refrigerant spray and electrical pulp test, and all responded within normal limits. Teeth were not tender to percussion and gave a normal sound on percussion, except for element 3.6. On its mesial buccal aspect there was the soft tissue swelling the girl was complaining about. Periodontal probing was consistently 3 mm or less and without bleeding or suppuration on probing, but when the periodontal probe reached the mesial buccal site of 3.6 the value became 7 mm with suppuration. We diagnosed a periodontal abscess in a healthy mouth.
A periodontal radiography was carried out using a paralleling technique (Figure 7A): an irregular radiolucent area was discernible on the mesial aspect of 3.6 crown, especially in the cervical area. An ECR was diagnosed. According to this preliminary informa-

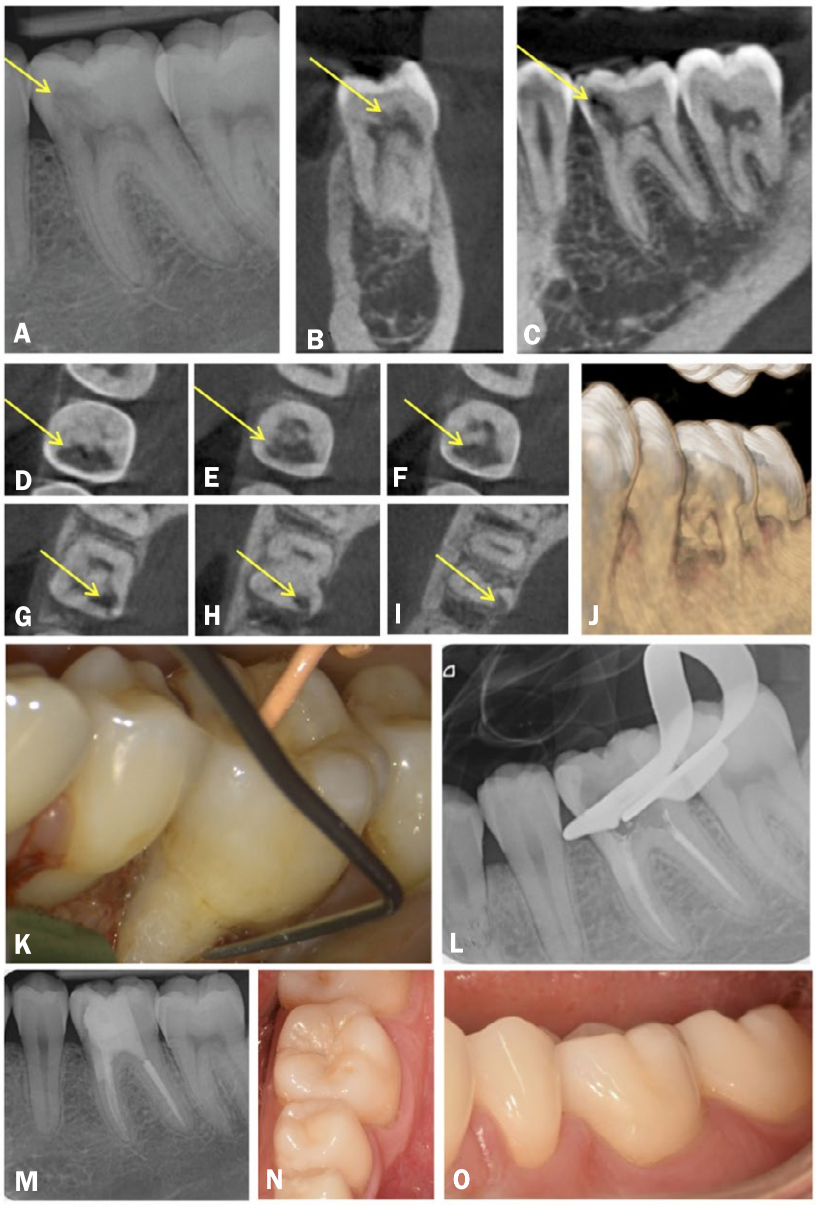

Figure 7 (video 6).
ECR case 6. **A)** Preoperative radiograph of a 3.6 showed ECR (yellow arrow) on the mesial aspect of the tooth. **B)** Frontal, **C)** sagittal and **D, E, F, G, H, I)** axial CBCT slices let know the real extension of ECR (yellow arrows) that reached the endodontic system.**J)** 3D rendering of the tooth. **K)** External repair of the defect with a bulk-fill composite. **L)** Intraoperative radiographic control. **M)** Postoperative radiographic control. **N, O)** Conservative restoration.

tion, the resorption was classified as class 2 following Heithersay classification.
The medical anamnesis was negative; the patient reported no history of trauma, orthodontic treatment or any other predisposing factor.
An antibiotic therapy was prescribed and a small FOV CBCT was planned. Seven days later, the abscess had reduced itself and the patient was asymptomatic, so we performed both the CBCT and the tooth treatment. Sagittal (Figure 7B), frontal (Figure 7C) and axial (Figure 7D-I) slices confirmed that the ECR coronal-apical extension had stopped just above the coronal third of the root, but it had developed mainly into the coronal dentine and it had reached the endodontic system, despite we had assessed the pulp vitality. The lesion is an 1Bp stage following Patel classification. The overall extension could be well understood with a 3D rendering image (Figure 7J), thanks to the volume rendering tool (39).
Considering the 3D rendering image, we performed a further clinical investigation and we proposed to the patient a root canal treatment, as the endodontic system was involved, combined with an external root repair in the same appointment.
We performed the first step of the stamp technique, saving the occlusal anatomy (30, 31). A conservative cavity access let us remove the pulp and carry out a preflaring. We temporary put a K-file into the buccal mesial canal in order to localize it also from an external view. We opened a surgical flap, we removed the reparative tissue with a bur, we put a gutta-percha point into the canal in order to maintain the patency during the subsequent excavation of ECR defect and we performed a composite resin restoration (19). A new generation bulk-fill composite (Beautifil-Bulk, Shofu) (Figure 7K) was chosen, as it has got a low polymerization shrinkage and let us reduce the time needed (36, 40). We underwent all the finishing and polishing steps under water: a starting diamond bur delivered a deeper and more aggressive cut; flexible abrasive points and coarse, medium, fine and super fine discs produced a smooth and glossy surface finish (37). Then we closed the flap, we put the rubber dam around the tooth and finished the endodontic treatment with warm gutta-percha (Figure 7L). We used the same bulk-fill composite for direct coronal reconstruction, except for a capping layer of conventional hybrid composite (40). 7 days later, a postoperative radi-

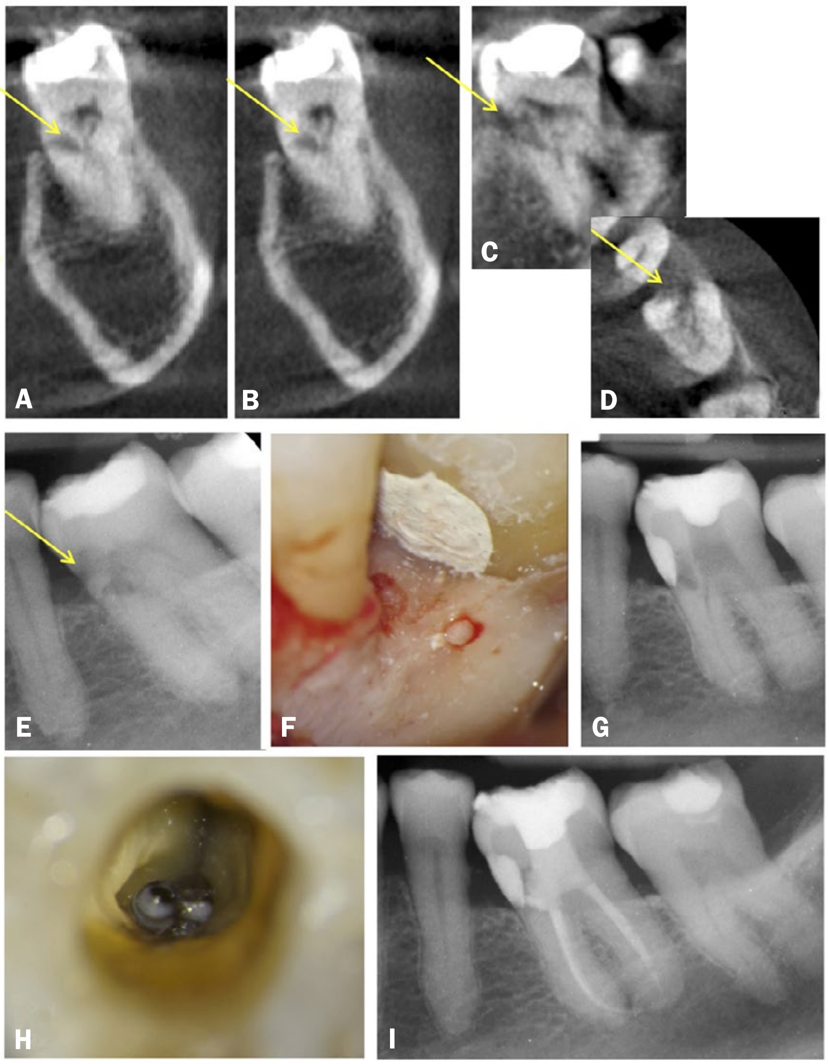

Figure 8 (**video 7**).
ECR case 7. **A, B)** Frontal, **C)** sagittal and **D)** axial CBCT slices let diagnose an ECR of 3.7 (yellow arrows). **E)** Two-dimensional X-ray radiograph of 3.7 showed the resorption (yellow arrow) on the mesial aspect of the tooth. **F)** External defect restoration using SuperEBA® Cement. **G)** Two-dimensional X-ray of external defect restoration. **H)** Internal defect restoration using MTA. **I)** Postoperative radiographic control.

ographic control was taken (Figure 7M), followed by a further polishing step of coronal restoration with abrasive polishing paste compounds, to reduce and smooth any roughness and surface scratch created by finishing instruments (Figure 7N, O). All the steps are shown in video 6. The patient has always been asymptomatic until today and the last mesial buccal periodontal probing depth is 3 mm, without bleeding and suppuration on probing.

#Case 7
The seventh case was detected in October 2019.
A 52-year-old male patient in good general health and no oral symptoms had undergone some extractions some years ago because of carious processes. He presented again requiring an implant treatment. Therefore we performed a new clinical analysis of the mouth without notice any pathological signs and took a CBCT in order to plan a guided surgery: here a radiolucent area was noticed in element 3.7 (Figure 8A-D). It was clearly a 3Bp stage of ECR by Patel classification.
In this case we directly had a definitive diagnosis of ECR, referring to the diagram in Figure 1. A clinical reinvestigation with a periapical X-ray (Figure 8E) followed. A normal response to both cold and percussion test was tested on element 3.7. The endodontic diagnosis was normal pulp and normal periapex. The tooth was mesially inclined as 3.6 was lost in youth and never replaced: the 3.6 extraction might have worked as predisposing factor. We proposed external debridement and restoration because of a direct mesial accessibility.
In the following appointment, a flap was opened and the bulk of reparative tissue was removed with a bur, exposing the pulp tissue, and replaced with SuperEBA® Cement (Figure 8F, G). As the initial debridement caused the pulp exposure, an orthograde endodontic treatment was initiated: we removed the pulp and put a temporary restoration with metacresylacetate. Subsequently, we performed also an internal reparation with MTA (Figure 8H), a classical canal therapy with warm gutta-percha and a composite restoration with a stamp technique (30, 31) (Figure 8I). All the main steps of clinical management are shown in video 7. Controls were planned and the patient has been asymptomatic until today.

Discussion

ECR is a relatively uncommon form of external tooth resorption, often undiagnosed or misdiagnosed (4, 5). It invades the tooth from the PDL, apical to the epithelial attach-

ment, and develops through both resorptive and reparative stages (3, 6).
Aetiology is still rather unclear: orthodontic treatment, iatrogenic or accidental dental trauma and poor oral health have been the most frequent predisposing factors detected until today and ECR itself has been proposed to be mainly multifactorial (7).
The lesion could be asymptomatic until later stages, therefore an early diagnosis is difficult and the process is often accidentally found with a radiographic control, where it appears as a barely up to a highly discernable radiolucency (11).
Heithersay developed the first classification system that divides the condition into 4 classes according to the extent of the lesion in periapical X-rays (12). But the true nature and real extension of an ECR defect cannot be assessed on conventional radiographs: a three-dimensional analysis is mandatory. CBCT scans clarify the exact location of the entry point of granulation tissue into the root, the dimension of the resorption and the presence of dentin surrounding the resorption tissue. Besides, it can diagnose also starting lesions undetectable with periapical X-rays (16, 17). Nowadays a three-dimensional classification by Patel is available: it considers height (coronal-apical extent) of the lesion, its circumferential spread and proximity to the root canal and it is easily applicable (18).
Different management approaches have been proposed too, also linked with the three-dimensional classification (19).
However, a common standardized workflow has not been published yet. Therefore we tried to developed it and test its clinical applicability: it start from a preliminary ECR diagnosis, but then a small FOV CBCT is mandatory to confirm it, to study the real extension of the lesion and to choose, with a clinical reinvestigation, the more appropriate treatment planning. When the restorability is assessed, external and internal repair are the most recommended clinical management options.
We used our protocol in 7 consecutive ECR cases (Table 1). Only in case 7 the ECR lesion was directly diagnosed on CBCT imaging, nevertheless the workflow has been applicable from that step: it has proven to be flexible.
Six of the seven cases of ECR were detected in the post-lateral lower jaw and only in case 3 an anterior element was affected, in contrast with previous findings (8). Both male and female patients presented to our attention: ECR does not seem to be linked to the sex of the patient, as already established (7). These patients were of different ages, even young people: case 3 was diagnosed in a 12-years-old female; which agrees with the new literature data (7). Predisposing factors have not always been found; when verified they were: a previous (case 2) or contextual (case 3) orthodontic treatment, occlusal trauma and poor oral health (case 4) or a previous surgery in the same oral area - extraction of a neighboring tooth - (case 7) (7). 3 cases were symptomatic on a late stage (case 2, 5, 6): as a matter of fact, these patients were seen in urgency; whereas the other 4 ECR (case 1, 3, 4, 7) were casually found (11).
Everyone accepted a CBCT study which turned out to be useful especially for pulp involvement investigation and management choice (19): fortunately, in all cases the elements were maintainable with an external or internal repair, associated with an endodontic treatment except for case 3, where an external approach associated with a pulp capping was mandatory, especially considering the age of the patient (35).
Three main rules were followed: we tried to remove reparative tissue, as its incomplete removal is likely to result in the recurrence of ECR (19), we apply the most conservative approach for each case and we perform the management within one or two appointments. Stamp technique succeed in maintaining most of the coronal tissue and allowing a fast and anatomically precise restoration (case 1, 5, 6, 7) (30, 31). Different materials, such as MTA (case 1, 4, 5, 7), SuperEBA® Cement (case 2, 5, 7) and composite (case 3, 7), were already proposed for clinical ECR management because of their sealing ability and biocompatibility (19) and turned out to be useful in our clinical practice. In cases 5 and 7, a combination of techniques and of materials

Table 1

Overview of the 7 ECR clinical cases

CASE	TOOTH	PATIENT SEX	PATIENT AGE (years old)	PREDISPOSING FACTOR(S)	MANAGEMENT	HEALING	FIGURE	VIDEO
1	3.7	male	17	not revealed	Internal repair with MTA + root canal therapy	yes	2	1
2	3.3	female	17	previous orthodontic treatment	External repair with SuperEBA® Cement + root canal therapy	yes	3	2
3	1.2	female	12	contemporary orthodontic treatment	External repair with resin composite + pulp capping	yes	4	3
4	3.3	female	52	occlusal trauma and poor oral health	Internal repair with MTA + root canal therapy (sandwich technique)	yes	5	4
5	3.6	male	40	not revealed	Internal repair with MTA + external repair with SuperEBA® Cement + root canal therapy	yes	6	5
6	3.6	female	21	not revealed	External repair with bulk-fill composite + root canal therapy	yes	7	6
7	3.7	male	52	previous surgery in the same oral area: extraction of element 3.6	External repair with SuperEBA® Cement + internal repair with MTA + root canal therapy	yes	8	7

was even used, in order to fill the irregular form of the lesions.
All patients were extremely motivated to follow-up visits, with both clinical and radiographic checks in order to evaluate the treatment over time: it is an extremely important aspect. The main outcome is the survival of the element, the secondary outcomes are the absence of resorption progression, no symptoms and a healthy periodontal depth. All the seven cases described have reached both the primary and secondary outcomes until today, however their follow-up must continue.

Additional supporting information: Video Legends

Video 1. ECR case 1 – element 3.7. The main steps of the treatment under operating microscope are here reported: reparative tissue removal, MTA location, irrigation, root canal filling.
Video 2. ECR case 2 – element 3.3. The consecutive axial CBCT slices are compiled as a video.
Video 3. ECR case 3 – element 1.2. 1 year recall probing: a light bleeding on probing matched with healthy periodontal probing values.
Video 4. ECR case 4 – element 3.3. The consecutive sagittal CBCT slices are compiled as a video.
Video 5. ECR case 5 – element 3.6. The main steps of the treatment under operating microscope are here reported: access cavity, pulp removal, reparative tissue removal, irrigation, root canal filling with gutta-percha, internal MTA location, external SuperEBA® cement location, 10 months recall.
Video 6. ECR case 6 – element 3.6. The

main steps of the treatment under operating microscope are here reported: occlusal anatomy stamp, access cavity, pulp removal, preliminary shaping, surgical flap, reparative tissue removal, external defect repair with a bulk-fill composite, root canal treatment, adhesive restoration.
Video 7. ECR case 7 – element 3.7. The main steps of the treatment under operating microscope are here reported: reparative tissue removal, external SuperEBA® cement location and linked X-ray, internal MTA location, root canal therapy, adhesive restoration.

Conclusions

A correct and predictable management of ECR needs a standardized workflow (including a CBCT study), a quick and definitive conservative approach and a follow-up overtime. This case series has succeeded in applying the rules above for the treatment of 7 external cervical root resorptions. However, both long-term follow-up visits and other clinical cases are necessary to confirm the real usefulness of the method.

Clinical Relevance

The workflow above can help clinicians to have a correct diagnosis of ECR cases but also in the decision making process regarding their treatment plan.

Conflict of Interest

The authors deny any conflict of interest.

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References

1. *American Association of Endodontists. AAE glossary of endodontic terms, 10th edn Chicago: American Association of Endodontists; 2020.*
2. *Harokopakis-Hajishengallis E. Physiologic root resorption in primary teeth: molecular and histological events. J Oral Sci 2007;49:1-12.*
3. *Patel S, Mavridou AM, Lambrechts P. External cervical resorption-part 1: histopathology, distribution and presentation. Int Endod J 2018;51:1205-23.*
4. *Patel S, Saberi N. The ins and outs of root resorption. Br Dent J 2018;224:691-9.*
5. *Patel J, Beddis HP. How to assess and manage external cervical resorption. Br Dent J 2019;227:695-701.*
6. *Patel S, Lambrechts P, Shemesh H, Mavridou AM. European society of endodontology position statement: external cervical resorption. Int Endod J 2018;51:1323-26.*
7. *Mavridou AM, Bergmans L, Barendregt D, Lambrechts P. Descriptive analysis of factors associated with external cervical resorption. J Endod 2017;43:1602-10.*
8. *Jeng PY, Lin LD, Chang SH et al. Invasive cervical resorption-distribution, potential predisposing factors, and clinical characteristics. J Endod 2020;46:475-82.*
9. *Heithersay GS. Invasive cervical resorption: an analysis of potential predisposing factors. Quintessence Int 1999;30:83-95.*
10. *Kandalgaonkar SD, Gharat LA, Tupsakhare SD, Gabhane MH. Invasive cervical resorption: a review. J Int Oral Health 2013;5:124-30.*
11. *Heithersay GS. Clinical, radiologic, and histopathologic features of invasive cervical resorption. Quintessence Int 1999;30:27-37.*
12. *Heithersay GS. Invasive cervical resorption following trauma. Aust Endod J 1999;25:79-85.*
13. *Patel K, Mannocci F, Patel S. The assessment and management of external cervical resorption with periapical radiographs and cone-beam computed tomography: a clinical study. J Endod 2016;42:1435-40.*
14. *Gunst V, Mavridou A, Huybrechts B, et al. External cervical resorption: an analysis using cone beam and microfocus computed tomography and scanning electron microscopy. Int Endod J 2013;46:877-87.*
15. *Patel S, Brown J, Pimentel T, et al. Cone beam computed tomography in Endodontics - a review of the literature. Int Endod J 2019;52:1138-52.*
16. *Patel S, Durack C, Abella F et al. European society of endodontology position statement: the use of CBCT in endodontics. Int Endod J 2014;47:502-4.*
17. *Patel S, Brown J, Semper M, et al. European society of endodontology position statement: use of cone beam computed tomography in endodontics: european society of endodontology (ESE) developed by. Int Endod J 2019;52:1675-8.*
18. *Patel S, Foschi F, Mannocci F, Patel K. External cervical resorption: a three-dimensional classification. Int Endod J 2018;51:206-14.*
19. *Patel S, Foschi F, Condon R, et al. External cervical resorption: part 2 – management. Int Endod J 2018;51:1224-38.*
20. *Coyle M, Toner M, Barry H. Multiple teeth showing invasive cervical resorption - an entity with little known histologic features. J Oral Pathol Med 2006;35:55-57.*
21. *Pace R, Giuliani V, Pagavino G. Mineral trioxide aggregate in the treatment of external invasive resorption: a case report. Int Endod J 2008;41:258-66.*
22. *Schwartz RS, Robbins JW, Rindler E. Management*

of invasive cervical resorption: observations from three private practices and a report of three cases. J Endod 2010;36:1721-30.
23. *Yu VS, Messer HH, Tan KB. Multiple idiopathic cervical resorption: case report and discussion of management options. Int Endod J 2011;44:75-85.*
24. *Patel S, Saberi N. External cervical resorption associated with the use of bisphosphonates: a case series. J Endod 2015;41:742-8.*
25. *Salzano S, Tirone F. Conservative nonsurgical treatment of class 4 invasive cervical resorption: a case series. J Endod 2015;41:1907-12.*
26. *Shemesh A, Ben Itzhak J, Solomonov M. Minimally invasive treatment of class 4 invasive cervical resorption with internal approach: a case series. J Endod 2017;43:1901-08.*
27. *Alqedairi A. Non-invasive management of invasive cervical resorption associated with periodontal pocket: a case report. World J Clin Cases 2019;7:863-71.*
28. *Aminoshariae A, Kulild JC, Syed A. Cone-beam computed tomography compared with intraoral radiographic lesions in endodontic outcome studies: a systematic review. J Endod 2018;44:1626-31.*
29. *Tawil PZ, Duggan DJ, Galicia JC. Mineral trioxide aggregate (MTA): its history, composition, and clinical applications. Compend Contin Educ Dent 2015;36:247-52.*
30. *Alshehadat SA, Halim MS, Carmen K, Fung CS. The stamp technique for direct class II composite restorations: a case series. J Conserv Dent 2016;19:490-3.*
31. *Murashkin A. Direct posterior composite restorations using stamp technique-conventional and modified: a case series. Int J Dentistry Res 2017;2:3-7*
32. *Zanini M, Meyer E, Simon S. Pulp Inflammation Diagnosis from Clinical to Inflammatory Mediators: A Systematic Review. J Endod 2017;43:1033-52.*
33. *Roscoe MG, Meira JB, Cattaneo PM. Association of orthodontic force system and root resorption: a systematic review. Am J Orthod Dentofacial Orthop 2015;147:610-26.*
34. *Bovolato L, Tonini R, Boschi G et al. Novel hollow fiber sandwich composite post system: mechanical characteristics. Minerva Stomatol 2020;69:63-71.*
35. *Duncan HF, Galler KM, Tomson PL, et al. European Society of Endodontology position statement: Management of deep caries and the exposed pulp. Int Endod J 2019;52:923-34.*
36. *Bonfanti C, Barabanti N, Piccinelli G, et al. Microbiological characterization and effect of resin composites in cervical lesions. J Clin Exp Dent 2017;9:e40-e45.*
37. *Jefferies SR. Abrasive finishing and polishing in restorative dentistry: a state-of-the-art review. Dent Clin North Am. 2007;51:379-97.*
38. *Tonini R. Root canal repair with the MTA sandwich technology. Dental Tribune 2014;14.*
39. *Venkatesh E, Elluru SV. Cone beam computed tomography: basics and applications in dentistry. J Istanb Univ Fac Dent 2017;51:S102–S121.*
40. *Chesterman J, Jowett A, Gallacher A, Nixon P. Bulk-fill resin-based composite restorative materials: a review. Br Dent J. 2017;222:337-44.*

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CASE REPORTS

Endodontic management of maxillary permanent molar C-shaped morphology

ABSTRACT

Aim: *Report on the successful endodontic management of four permanent maxillary molars with a C-shape configuration of the root canal system.*
Summary: *Even though C-shaped anatomies are more commonly described in the mandibular molars, its occurrence in permanent maxillary molars has also been reported. Complexity of the root canal system may pose a difficulty in achieving proper chemo-mechanical debridement, and consequently jeopardize the success of endodontic therapy. Additional dedicated armamentarium can help the clinician recognize, assess, plan and better manage such complex configurations.*
Key learning points

- *Although of rare prevalence, clinicians must be aware and recognize the possible existence of a C-shaped root canal configuration in both first and second permanent maxillary molars.*
- *This anatomy can be attributed to an embryologic alteration that leads to the non-division of roots during the root formation stage of tooth formation.*
- *Further studies on the prevalence of the C-shaped configuration in permanent maxillary molars may contribute to a better understanding and, consequently, proper management of this morphology.*
- *Specific tools, such as the dental operating microscope and specific ultrasonic tips, as well as additional means of enhancing chemical debridement, are essential for the correct management of these intricate anatomies and appear to positively impact outcome of root canal treatment.*

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Introduction

A proper mechanical debridement and disinfection of the root canal system are two of the most important aspects when aiming for a successful endodontic treatment procedure (1). Prior knowledge of the most common configurations and possible variations of the teeth's internal anatomy is mandatory to optimize the cleaning and shaping procedures, which ultimately may increase the chance of positive outcomes (2). Although the C-shaped root canal configuration has been well documented for several groups of teeth, it is more commonly found in the mandibular second molar, in which it may reach a prevalence of 44.5% in East Asian populations (3). This morphology in maxillary permanent molars, although uncommon, has been described as far back as in 1984 (4). In these teeth, this anatomic configuration may present a proportion that may be as high as 1.1% and 3.8% for maxillary first and second molars (5), respectively. Complementary to the few prevalence studies on this anatomy (5, 6) a few case reports have also been documented (4, 7, 8) that are quite variable in their characteristics. The location, number and course of the main root canals in C-shaped permanent maxillary molars may prove a challenge for the instrumentation and disinfection of the canal system. More complex morphologies may lead to unidentified canals during root canal treatment procedures, which have been linked to persistence of periapical pathology on endodontically treated teeth (9). Moreover, canals with such morphologies exhibit large areas of untouched canal areas following preparation (10), which, especially in infected cases, may result in insufficient disruption of the bacterial load to levels compatible with periapical health.

The aim of the present case series is to present four cases of successful endodontic treatments performed on maxillary permanent molars presenting a C-shaped root canal configuration.

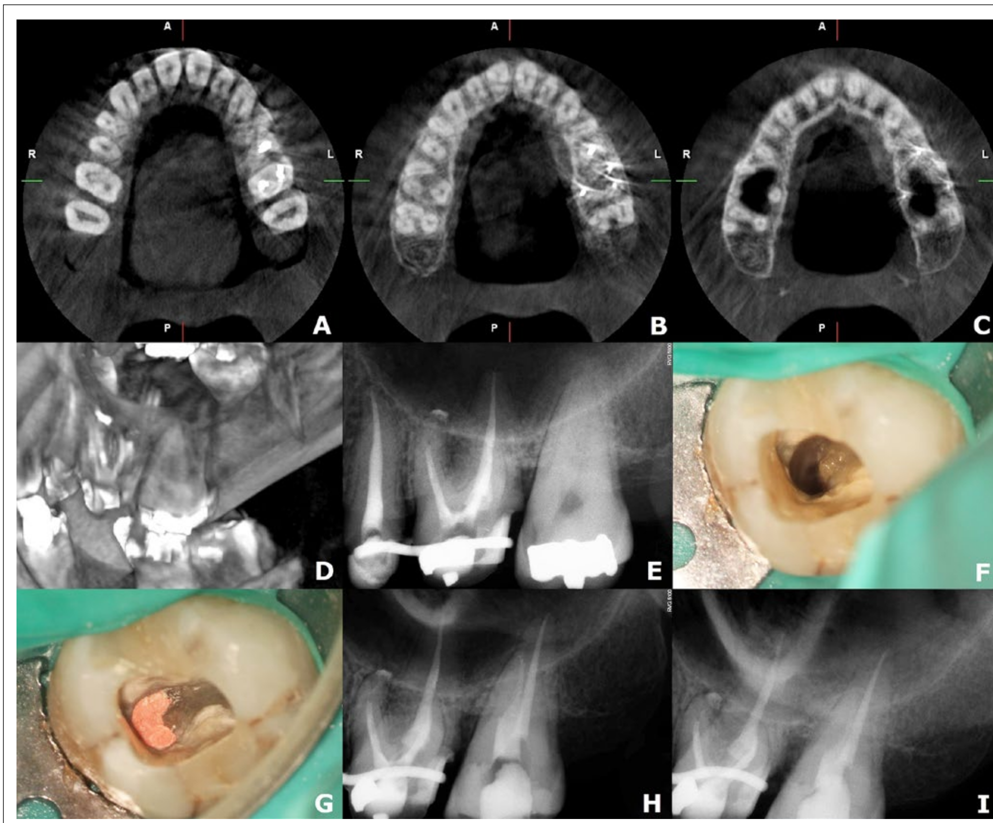

Figure 1.
A) CBCT axial view, coronal third. **B)** CBCT axial view, middle third of the root of tooth 27 shows a large buccal root canal. **C)** CBCT axial view, apical third. **D)** CBCT 3D reconstruction, a large buccal root can be observed instead of the typical two buccal roots. **E)** Initial periapical radiograph, where two superimposed conical roots can be observed. **F)** A buccal C-shaped canal orifice can be observed in the pulp chamber floor. **G)** Obturation of the C-shaped root canal. **H)** Final radiograph of the root canal treatment. **I)** Eight months recall.

Case reports

General clinical procedures

All patients were referred for endodontic evaluation at private practices. In all cases the medical and dental history was reviewed and considered non-contributory, and an intra-oral and radiographic examination was performed. Periodontal pockets and mobility were within the physiological limits for all teeth considered. Diagnosis of pulpal and periapical pathosis was made based on pulpal tests and periapical radiographs. Root canal therapy was recommended and accepted by all patients. After local infiltration (Artinibsa, Inibsa, Barcelona, Spain) and rubber dam placement, access was obtained under dental operating microscope magnification, using round burs (Komet Medical, Rösrath, Germany; R&S, Tremblay-en-France, France) or Start X ultrasonic tips (Dentsply Maillefer, Baillagues, Switzerland) or both. A full debridement was performed under copious irrigation with 5.25% sodium hypochlorite (Denta Flux, J. Ripoll SL, Madrid, Spain). Working length was established using electronic apex locators (Root ZX II, Morita, Komuro, Japan; or Propex Pixi, Dentsply Maillefer, Baillagues, Switzerland). In all cases, the final irrigation procedures included also a 17% EDTA (EDTA, Laboratorios Clarben S.A., Madrid, Spain), one-minute irrigation prior to a final sodium hypochlorite rinse under ultrasonic activation (Irri K, VDW GmbH, Munich, Germany). After canal obturation with a vertical condensation technique with gutta percha and a resin based root canal sealer (AH Plus, Dentsply DeTrey, Konstanz, Germany), intra-coronal sealing was performed with Ionoseal (VOCO GmbH, Cuxhaven, Germany) and the teeth were provisionally restored with Cavit (Cavit W, 3M ESPE, Seefeld, Germany). All cases were sent to the referring dentist for the direct definitive restoration of the access cavity after the endodontic treatment was completed. A posterior follow-up appointment was able to show an improvement of the clinical condition of all cases.

Case #1

A 39-year-old Caucasian female was referred for evaluation of tooth 27 (maxillary left second molar). Her chief complaint was spontaneous pain and increasing pain to temperature variations. The radiographic analysis revealed a secondary carious lesion on tooth 27. The diagnosis was irreversible pulpitis with normal periapical tissues. After the occlusal amalgam filling was removed and the carious lesion was excavated, access to the pulp chamber was achieved and a pre-endodontic resin restoration was built to avoid leakage. Upon examination of the pulp chamber floor two canals were easily identified; a semi-lunar shape buccal canal orifice, surrounded by developmental grooves, and a palatal canal (Figure 1F). A previously obtained CBCT scan confirmed the diagnosis of a maxillary second molar C-shaped B1 configuration (Figures 1A to 1D). Mechanical instrumentation was performed with Protaper Universal NiTi rotary files (ProTaper Universal, Dentsply Maillefer, Baillagues, Switzerland) using recommendations by the manufacturer. The developmental groove and the coronal portion of the C-shaped root canal was also cleaned using a #3 ProUltra ultrasonic tip (ProUltra, Dentspy Maillefer, Baillagues, Switzerland) and obturated (Figure 1G and 1H). At the 8 months recall the tooth showed no clinical or radiographic findings (Figure 1I).

Case #2

A 31-year-old Caucasian female was referred for endodontic treatment with a chief complaint of spontaneous pain and pain upon mastication in the maxillary right quadrant. Clinical examination revealed tenderness to percussion on tooth 17 (maxillary right second molar). The radiographic examination showed a previous single root canal filling in what appeared to be a single root (Figure 2F). A diagnosis of previously treated tooth with symptomatic apical periodontitis was made. After access, a large C-shaped distopalatal canal connected by a developmental groove to an independent

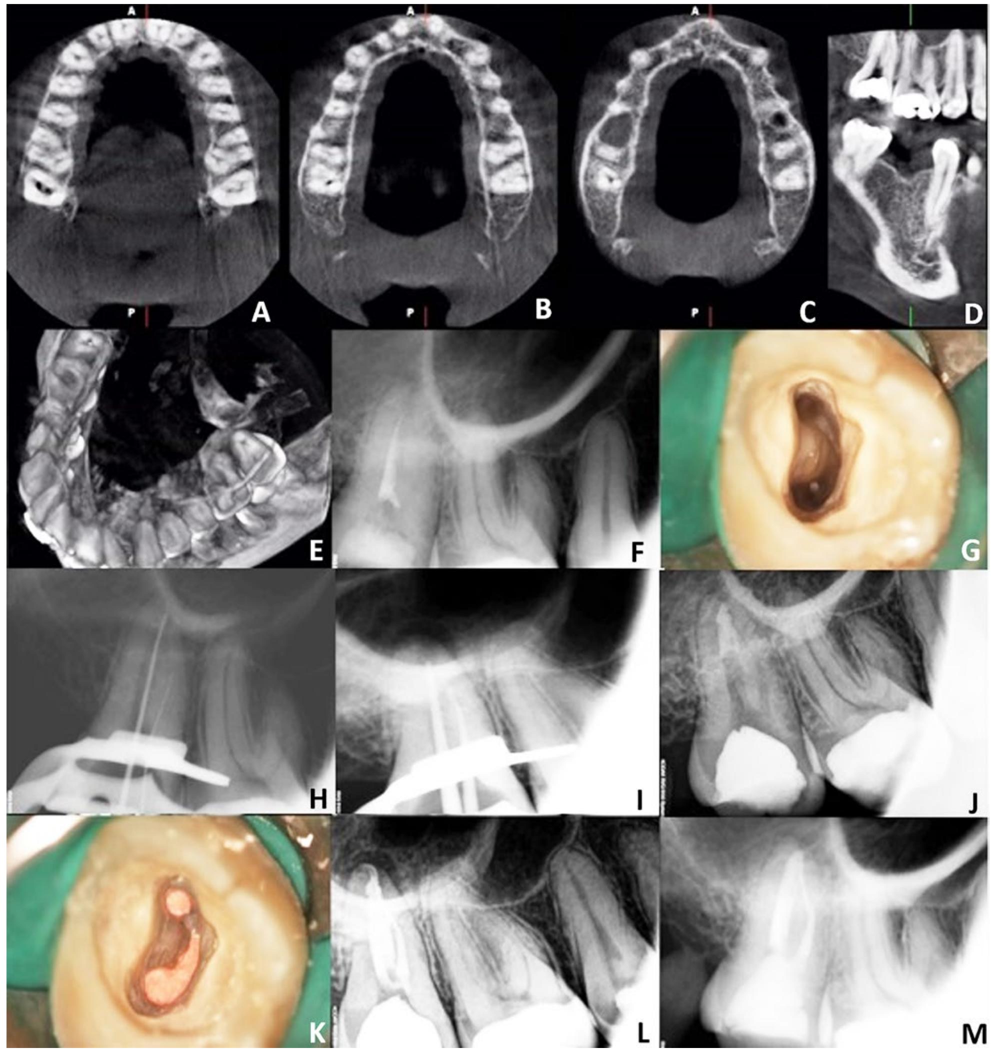

Figure 2.
A) CBCT axial view, coronal third. **B)** CBCT axial view, middle third of the root. A complete fusion of the roots of tooth 17, with two root canals can be observed. **C)** CBCT axial view, apical third. Both canals merge together. **D)** CBCT sagittal view. **E)** CBCT 3D reconstruction, where fusion of the three roots of tooth 17 is observed. **F)** Initial periapical radiograph. A large single root is displayed in the tooth 17. **G)** C-shaped root canal configuration. **H)** Working length radiograph of distopalatal canal and ledge of the mesiobuccal canal. **I)** Cone fit radiograph after successfully managing the ledge in the mesiobuccal canal. **J)** Radiographic assessment of MTA plug in distoplatal canal. **K)** Obturation of the C-shaped root canal. **L)** Final radiograph of the root canal treatment. **M)** Ten months recall.

mesiobuccal canal (Figure 2G) was observed, which corresponds to an E2 morphology. The prior treatment had created a large ledge in the mesiobuccal root canal. A diagnostic CBCT scan was taken in between visits which revealed that this mesiobuccal canal merged apically with the C-shaped distopalatal canal (Figures 2A to 2E). The pre existing ledge was successfully negotiated, but created a significant platform that can be seen in the final obturation radiograph (Figures 2H, 2I and 2L). Canals were mechanically instrumented with Protaper Universal NiTi rotary files (ProTaper Universal, Dentsply Maillefer, Baillagues, Switzerland) according to the manufacturer's recommendations. The C-shaped distopalatal root canal was sealed with a ProRoot MTA apical barrier (ProRoot MTA, Dentsply Tulsa, Tulsa, USA) (Figure 2J) and subsequently obturated with gutta-percha and sealer (Figure 2K). The 10 months recall radiograph showed complete periapical healing (Figure 2M).

Case #3

A 53-year-old Caucasian male was referred with a chief complaint of spontaneous pain and increasing pain to temperature variations in the maxillary left quadrant. A clinical observation detected an amalgam filling on the occlusal surface of tooth 27 (maxillary left second molar). The reaction to the ice sensibility test was intense pain that lingered for

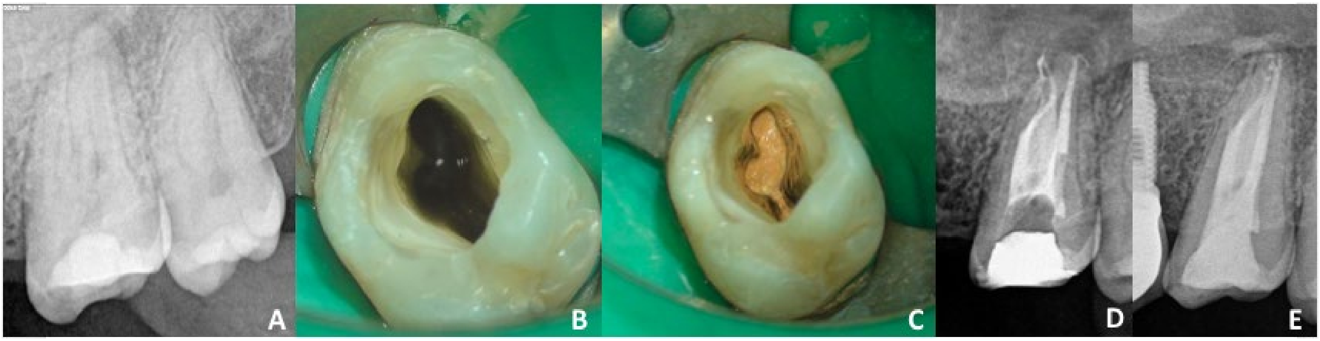

Figure 3.
A) Initial periapical radiograph of tooth 27, with periapical lesion and what appear to be two roots.
B) Pulp Chamber floor after endondontic access, C-shaped root canal configuration. Two canals could be identified: one palatal canal, and fused distobuccal and mesiobuccal canals, forming a buccally faced semi-lunar shape.
C) Obturation of the C-shaped root canal.
D) Final radiograph of the root canal treatment.
E) Nine years recall.

several minutes, with no alterations to percussion nor palpation. The periapical radiography (Figure 3A) showed alteration of the periapical tissues. The diagnosis was irreversible pulpitis with asymptomatic apical periodontitis. After removing the occlusal filling and achieving access to the pulp chamber, two canals could be identified: one palatal canal, and fused distobuccal and mesiobuccal canals, forming a buccally faced semi-lunar shape, corresponding to a B2 configuration (Figure 3B). Mechanical instrumentation was performed with Protaper Next NiTi rotary files (ProTaper, Dentsply Maillefer, Baillagues, Switzerland) using recommendations by the manufacturer. The developmental groove and the coronal portion of the C-shaped root canal was also cleaned using a #3 Start-X ultrasonic tip (Start-X, Dentspy Maillefer, Baillagues, Switzerland) and filled (Fig. 3C and D). A recall appointment 9 years after treatment revealed absence of symptoms and the periapical radiography showed resolution of periapical pathosis (Figure 3E).

Case #4

A 54-year-old Caucasian female was referred for endodontic treatment of tooth 16 (maxillary right first molar). There was no response to the cold sensibility test, and there was tenderness to vertical percussion. Periapical radiography showed apical radiolucency (Figure 4A), and the diagnosis of necrotic pulp and symptomatic apical periodontitis was established. Access was done through the existing rehabilitation, and two large distopalatal and mesiobuccal canals were identified (Figure 4B). A CBCT scan performed before for a surgical procedure was accessed to further understand this unusual anatomy, and used to confirm the diagnosis of a maxillary first molar

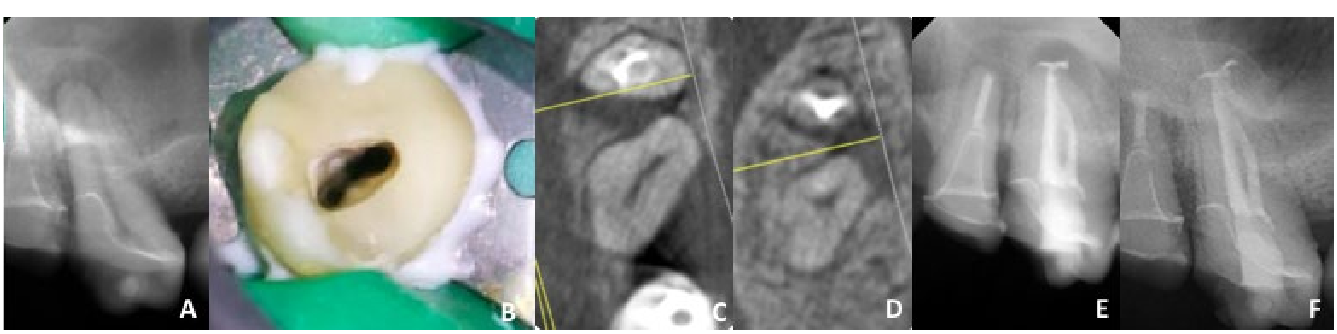

Figure 4.
A) Initial periapical radiograph of tooth 17, with periapical lesion and what appears to be a single root. **B)** Pulp chamber floor, with identifiable distopalatal and mesiobuccal canals. **C)** CBCT axial view, middle third of the root. A complete fusion of the roots of tooth 17, with two root canals can be observed. **D)** CBCT axial view, apical third. Both canals merge together. **E)** Final radiograph of the root canal treatment. **F**) Six months recall.

E2 C-shaped configuration (Figure 4C and 4D). Mechanical instrumentation was performed with Reciproc blue 25 and 40 files (Reciproc, VDW GmbH, Munich, Germany) and the canals filled (Figure 4E). At the 6 months recall inspection, the tooth showed no clinical findings, and an improvement of the periapical condition (Figure 4F).

Discussion

C-shaped root canal system morphologies have been presented as one of the most complex variations of the root canal system anatomy (11). This anatomic configuration is characterized by presenting a root canal system which may present several morphological features such as root canal merging, fusions and fins (6, 11). Although the higher prevalence of mandibular C-shaped molars has been associated with certain ethnic groups (12, 13), the same has not been proved for other groups of teeth, such as mandibular premolars and maxillary molars (14). The proportions of this morphology for both permanent maxillary molars are low, so any true variation between ethnic groups may go unnoticed. The prevalence on maxillary first molars may be as high as 1.1% (5), although several studies have reported not a single finding (15-17). As for the second molars, the percentages of cases may reach 3.8% (5) in a Portuguese sub-population, with other studies reporting even lower proportions 1.9% (16) and 0.9% (18) in Polish and Thai sub-populations, respectively. Just as in first molars, no findings have also been reported (17, 19).

As opposed to the mandibular C-shaped molar, in which the internal root canal system morphology is dependent of the fusion between two roots (mesial and distal) (20), leading to some predictability of the C-shaped configuration, the maxillary C-shaped configuration is dependent on the fusion of two or three roots, which leads to a superior variability of this inner anatomic configuration in these group of teeth (5). Moreover, and as has already been noted in a previous study (8), the fusion between the roots in a maxillary C-shaped molar may not be a true fusion. Instead, it may be described as non-division of roots because of inadequate development during the embryological phase of root formation due to what might have been an incorrect invagination of the epithelium of the Hertwig's horizontal root sheath. Since it can be described as inadequate root development, it may be difficult to classify different types of C-shaped configurations. However, with the information available from the published studies (6, 8), it is possible to understand that five types (and two sub-types) of configurations can be described depending on the roots that are fused (5).

The cases reported in this study represent three different anatomic configurations for the maxillary first and second molar. The first and third cases morphologies represent two possible variations of the buccal C-shaped canal (connecting both mesiobuccal and distobuccal root canals) depending to which side the convexity of the C is facing, while the second and fourth reported cases represent anatomies with fusions between the mesial and palatal root canals, which resemble the type C2 of Melton's classification for the mandibular molar (21). The root presents a semi-lunar cross-section in the middle and apical portion of the root due to the incomplete division of the three usual roots. The root canal system appears to be a complex system with a semi-lunar disto-palatal root canal that connects to an independent mesiobuccal root canal by an isthmus and then both canals converge together apically.

In the four cases presented, the use of a dental operating microscope, confirmation of the clinically observed C-shaped morphology through previously obtained CBCT images, and the use of specific ultrasonic tips allowed the clinicians to properly manage these uncommon configurations while eliminating the possibility of misguided and unnecessary elimination of dentin (22-26).

Mechanical instrumentation of C-shaped canals poses a challenge to the clinician.

The increased volume of the root canal space, the oval configuration and the existence of irregularities increase the occurrence of areas left untouched by endodontic files (27, 28), and the possibility of incomplete elimination of contaminated pulp tissue, dentine debris and adhered bacterial biofilm (29). Moreover, care must be taken not to overly enlarge the already thin dentinal walls (30). Both reciprocating and rotary files have been reported to perform equally in oval canals, with no difference in untouched canal areas and similar increase in overall preparation volume gain (31).
In these intricate variations of internal anatomy, the role of chemo-mechanical debridement becomes paramount (32). In the present study, 5.25% sodium hypochlorite (NaOCl) was used as an irrigant because of its strong disinfectant and antibacterial effects, as well as its ability to remove organic debris (33). In the final irrigation protocol, either manual dynamic agitation with a tapered gutta percha cone, or ultrassonic activation were applied, to further enhance the action of the employed irrigants (34). Such supplemental techniques of irrigation are essential to maximise the potential of the irrigants employed and play a decisive role in the outcome of treatment of C-shaped anatomies (29, 35).
Fused root morphology can result in root canal merging, and in some cases, multiple root canals can merge into a single portal of exit (36, 37). This anatomic particularity can contribute to a good quality apical seal, stated as a significant factor for the outcome of the endodontic treatment (38, 39), and hence minimize the effect of the complexity of a C-shaped configuration on the success of root canal therapy.
However, one of the cases required the application of an apical barrier of MTA since the gauged apical diameter would not allow an adequate apical seal with the thermoplastic technique alone. MTA is a biocompatible material that has been shown both *in vitro* an *in vivo* to have excellent adaptability to the canal walls (40).
Assessment of root canal treatment outcome should be made one year after the procedure, both clinically and radiographically (41). Even though CBCT is considered superior for diagnostic purposes, periapical radiographs also have good accuracy and are commonly employed for post-treatment evaluation in the absence of contradicting or non-specific signs and symptoms (42). Although in three of the presented cases follow-ups were made prior to the one year mark, there were already indications of resolution of periapical pathosis, and maintenance of periapical health in the case where no alteration was identified at the time of treatment, which may be considered as indicative of success of the endodontic treatment (43).
The actual impact of this uncommon root canal anatomy on the outcome of endodontic treatment needs further assessment.

Conclusions

The C-shaped anatomic configuration is an uncommon condition in both first and second maxillary molars. The available literature suggests the existence of five configuration types, depending on where the non-division of roots occurred in the tooth developmental phase. The use of the dental operating microscope and CBCT scan images leads to a better understanding of this rare anatomy, and ultrasonic tips assists in managing these root canal configurations. Optimization of irrigation protocols is essential to ensure a good outcome of endodontic therapy. As long as the biological principles prevail, the root canal treatment of these teeth appears to have a favorable outcome, as depicted in the four cases described.

Clinical Relevance

Knowledge of canal anatomy and its possible variations, including C-shaped anatomies in maxillary permanent molars, is paramount to ensure the success of endodontic treatment.

Conflict of Interest

Nothing to declare.

Acknowledgments

Nothing to declare.

References

1. Orstavik D, Qvist V, Stoltze K. A multivariate analysis of the outcome of endodontic treatment. Eur J Oral Sci 2004;112:224-30.
2. Estrela C, Bueno MR, Couto GS et al. Study of root canal anatomy in human permanent teeth in a subpopulation of brazil's center region using cone-beam computed tomography - Part 1. Braz Dent J 2015;26:530-6.
3. von Zuben M, Martins JNR, Berti L et al. Worldwide prevalence of mandibular second molar c-shaped morphologies evaluated by cone-beam computed tomography. J Endod 2017;43:1442-7.
4. Newton CW, McDonald S. A c-shaped canal configuration in a maxillary first molar. J Endod 1984;10:397-9.
5. Martins JN, Mata A, Marques D et al. Prevalence and characteristics of the maxillary c-shaped molar. J Endod 2016;42:383-9.
6. Jo HH, Min JB, Hwang HK. Analysis of c-shaped root canal configuration in maxillary molars in a korean population using cone-beam computed tomography. Restor Dent Endod 2016;41:55-62.
7. De Moor R. C-shaped root canal configuration in maxillary first molar. Int Endod J 2002;35:200-8.
8. Martins JNR, Quaresma S, Quaresma MC, Frisbie-Teel J. C-shaped maxillary permanent first molar – a case report and literature review. J Endod 2013;39:1649-53.
9. Baruwa AO, Martins JNR, Meirinhos J et al. The influence of missed canals on the prevalence of periapical lesions in endodontically treated teeth: a cross-sectional study. J Endod 2020;46:34-9.e1.
10. Siqueira JF, Pérez AR, Marceliano-Alves MF et al. What happens to unprepared root canal walls: a correlative analysis using micro-computed tomography and histology/scanning electron microscopy. Int Endod J 2018;51: 501-8.
11. Ordinola-Zapata R, Martins JNR, Bramante CM et al. Morphological evaluation of maxillary second molars with fused roots: a micro-ct study. Int Endo J 2017;50:1192-1200.
12. Jafarzadeh H, Wu YN. The c-shaped root canal configuration: a review. J Endod 2007;33:517-23.
13. Martins JNR, Gu Y, Marques D et al. Differences on the root and root canal morphologies between asian and white ethnic groups analyzed by cone-beam computed tomography. J Endod 2018;44:1096-104.
14. Martins JNR, Marques D, Silva E et al. Prevalence of c-shaped canal morphology using cone beam computed tomography - a systematic review with meta-analysis. Int Endod J 2019;52:1556-72.
15. Kim Y, Lee SJ, Woo J. Morphology of maxillary first and second molars analyzed by cone-beam computed tomography in a korean population: variations in the number of roots and canals and the incidence of fusion. J Endod 2012;38:1063-8.
16. Olczak K, Pawlicka H. The morphology of maxillary first and second molars analyzed by cone-beam computed tomography in a polish population. BMC Med Imaging 2017;17:68.
17. Silva EJ, Nejaim Y, Silva AI et al. Evaluation of root canal configuration of maxillary molars in a brazilian population using cone-beam computed tomographic imaging: an in vivo study. J Endod 2014;40:173-6.
18. Ratanajirasut R, Panichuttra A, Panmekiate S. A cone-beam computed tomographic study of root and canal morphology of maxillary first and second permanent molars in a thai population. J Endod 2018;44:56-61.
19. Zhang R, Yang H, Yu X et al. Use of cbct to identify the morphology of maxillary permanent molar teeth in a chinese subpopulation. Int Endod J 2011;44:162-9.
20. Fan B, Cheung GS, Fan M et al. C-shaped canal system in mandibular second molars: part I-anatomical features. J Endod 2004;30:899-903.
21. Melton DC, Krell KV, Fuller MW. Anatomical and histological features of c-shaped canals in mandibular second molars. J Endod 1991;17:384-8.
22. Arens D. Introduction to magnification in endodontics. J Esthet Restor Dent 2003;15:426-39
23. Low JF, Mohd Dom TN, Baharin SA, Magnification in endodontics: a review of its application and acceptance among dental practitioners. Eur J Dent 2018;12:610-6.
24. AAE and AAOMR joint position statement: use of cone beam computed tomography in Endodontics 2015 update. J Endod 2015;41:1393-6
25. Torabinejad M, Rice D, Maktabi O et al. Prevalence and size of periapical radiolucencies using cone-beam computed tomography in teeth without apparent intraoral radiographic lesions: a new periapical index with a clinical recommendation. J Endod 2018;44:389-94.
26. Weller RN, Hartwell GR. The impact of improved access and searching techniques on detection of the mesiolingual canal in maxillary molars. J Endod 1989;15:82-3.
27. Raisingani D, Gupta S, Mital P, Khullar P. Anatomic and diagnostic challenges of c-shaped root canal system. Int J Clin Pediatr Dent 2014;7:35-9.
28. Amoroso-Silva PA, Ordinola-Zapata R, Duarte MA, et al. Micro-computed tomographic analysis of mandibular second molars with c-shaped root canals. J Endod 2015;41:890-5.
29. Zhao Y, Fan W, Xu T et al. Evaluation of several instrumentation techniques and irrigation methods on the percentage of untouched canal wall and accumulated dentine debris in c-shaped canals. Int Endod J 2019;52:1354-1365.
30. Kim Y, Lee D, Kim DV, Kim SY. Analysis of cause of endodontic failure of c-shaped root canals. Scanning 2018;2516832.
31. Versiani MA, Leoni GB, Steier L, et al. Micro-computed tomography study of oval-shaped canals prepared with the self-adjusting file, reciproc, waveone, and protaper universal systems. J Endod 2013;39:1060-6.
32. Byström A, Sundqvist G: Bacteriologic evaluation

of the efficacy of mechanical root canal instrumentation in endodontic therapy. Scand J Dent Res 1981;89:321-8
33. Zou L, Shen Y, Li W, Haapasalo M. Penetration of sodium hypochlorite into dentin. J Endod 2010; 36:793-6.
34. Jiang LM, Lak B, Eijsvogels LM el al. Comparison of the cleaning efficacy of different final irrigation techniques. J Endod 2012;38:838-41.
35. Pereira B, Martins JNR, Baruwa AO, et al. Association between endodontically treated maxillary and mandibular molars with fused roots and periapical lesions: a cone-beam computed tomography cross-sectional study. J Endod 2020;46:771-7.e1.
36. Martins JN, Mata A, Marques D, Caramês J. Prevalence of root fusions and main root canal merging in human upper and lower molars: a cone-beam computed tomography in vivo study. J Endod 2016;42:900-8.
37. Kartal N, Yanikoğlu FC. Root canal morphology of mandibular incisors. J Endod 1992;18:562-4.
38. Ng YL, Mann V, Rahbaran S et al. Outcome of primary root canal treatment: systematic review ofthe literature - Part 2. Influence of clinical factors. Int Endod J 2008;41:6-31.
39. Ng YL, Mann V, Gulabivala K. Outcome of secondary root canal treatment: a systematic review of the literature. Int Endod J 2008;41:1026-46.
40. Gundam S, Patil J, Venigalla BS et al. Comparison of marginal adaptation of mineral trioxide aggregate, glass ionomer cement and intermediate restorative material as root-end filling materials, using scanning electron microscope: An in vitro study. J Conserv Dent 2014;17:566-70.
41. European Society of Endodontology. Quality guidelines for endodontic treatment: consensus report of the European Society of Endodontology. Int Endod J 2006;39:921-30.
42. Dutra KL, Haas L, Porporatti AL et al. Diagnostic accuracy of cone-beam computed tomography and conventional radiography on apical periodontitis: a systematic review and meta-analysis. J Endod 2016;42:356-64.
43. Ørstavik D, Qvist V, Stoltze K. A multivariate analysis of the outcome of endodontic treatment. Eur J Oral Sci 2004;112:224-30.

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CASE REPORT

Management of an unusual foreign body in periapical tissues of immature permanent maxillary central incisor

ABSTRACT

Aim: *The present report describes a clinical case of apical surgery with a 3-year clinical and radiographic follow-up in an immature permanent maxillary central incisor with persistent apical periodontitis caused by a foreign body in the periapical tissues.*
Summary*: The traumatic injuries of immature permanent teeth may have harmful influence on physiological pulp and root development. Root canal treatment of an immature tooth is a significant challenge because of the size of the canal, the thin and fragile dentin walls, and the large open apex. Even though endodontic failure is usually associated with the persistence of bacteria within the root canal system another less common cause of endodontic failure is the presence of a foreign body in the periapical tissues.*

Key Learning Points
- *Foreign object in the periapical tissues act as a source of pain and infection, cause difficulty in elimination of infection.*
- *Surgical endodontic treatment should be seen as the best choice for treating persistent apical periodontitis.*
- *This treatment can manage the infection source and provide a direct hermetic seal of the apical area.*

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Introduction

The traumatic injuries of immature permanent teeth may have harmful influence on physiological pulp and root development. Such injuries may lead to the loss of pulp vitality, arrested or interrupted root development, and inflammatory or replacement root resorption (1). Root canal treatment, at this time, stands a significant challenge, due to the large diameter of the root canal, in combination with large open apex and thin dentin walls (2). Consequently, the instrumentation of root canal in order to achieve chemomechanical debridement cannot be performed in such conditions, due to fragile root canal structure.

Apexification had been the most indicated procedure to treat necrotic immature permanent teeth. Through this technique, a calcified apical barrier is formed, allowing subsequent conventional root canal filling1. Although $Ca(OH)_2$ high antimicrobial activity, and ability to induce hard-tissue deposition (3), the traditional $Ca(OH)_2$ apexification presents disadvantages, such as a long, up to 24 months treatment, in which the patient needs to attend multiple sessions. Besides, the alkalinity of Ca(OH)2 affects the links between hydroxyapatite crystals and collagen, weakening the flexural strength of dentine (4) which increases the risk of root fracture (5).

The mineral trioxide aggregate (MTA) apical plug offered an improvement in terms of timing (6) with similar apical barrier formation rates in comparison to calcium hydroxide apexification. The clinical procedures for the MTA apical plug technique comprise a first session in which $Ca(OH)_2$ paste is placed for at least one week into the root canal; and in the second appointment, MTA is condensed into the apical part of the canal until a 3-4 mm plug is formed. To promote the setting of the material, a moist cotton pellet is applied into the root canal, the tooth is temporarily sealed, and after 72 h, a gutta-percha and sealer obturation can be performed (7).

Since both, calcium hydroxide and MTA apexification and the presents the limitations of an interrupted root development, leaving the tooth with a fragile root structure and a poor crown-to-root ratio (8-12), the regenerative endodontic procedures (REP) have been advocated as an alternative treatment for immature permanent teeth with necrotic pulp (13). This method induces further root development and eventually strengthening of the tooth (14), however much is still not known about clinical and biological aspects of regenerative endodontics (15). However, case selection is of extreme importance as choosing a patient that does not correctly apply for this procedure may result in an unsuccessful outcome.

Even though endodontic failure is usually associated with the persistence of bacteria within the root canal system (16) another less common cause of endodontic failure is the presence of a foreign body in the periapical tissues. The apical periodontium can encapsulate a foreign object. And an intense inflammatory response characterized by abundance of macrophages and giant cells and a subsequent foreign body reaction can also be observed (17). In these cases, surgical endodontic treatment can be the treatment of choice for persistent apical periodontitis because it can manage the infection source and provide a hermetic seal of the apical area directly (18). The results for outcomes of apical root resection presented in the literature mostly rely on a time span of 1 or 2 years, and some even up to 5 years (19, 20). This case report describes surgical endodontic treatment in an immature permanent maxillary central incisor with persistent apical periodontitis caused by a foreign body in the periapical tissues.

Case report

A 15-year-old boy with frequent pain, discomfort and swelling was referred to the dental clinic due to persistent apical periodontitis. The dental history reported that when the patient was 10 years old, he suffered a trauma of lateral luxation

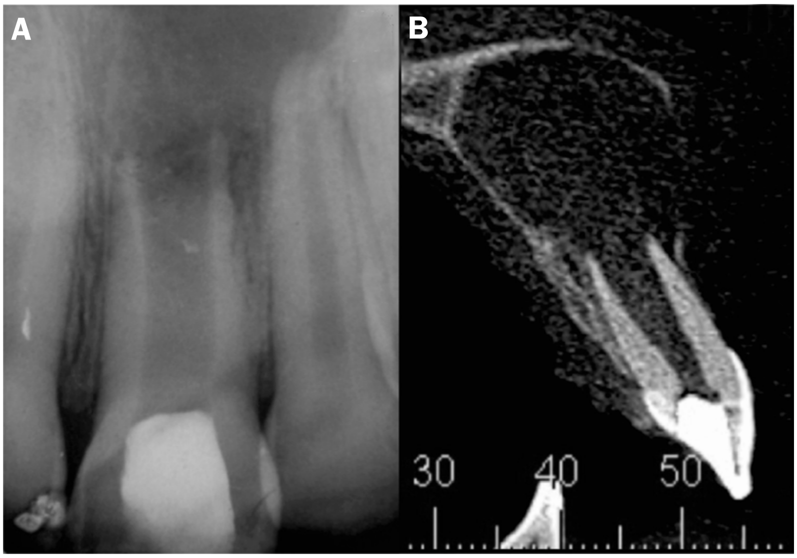

Figure 1.
A) Radiographic image at initial diagnosis. Maxillary left central incisor with open apex associated with chronic apical periodontitis.
B) Computed tomography showing extensive bone resorption associated with the maxillary left central incisor.

of immature maxillary left central incisor, which led to tooth necrosis.
At that time, the treatment of choice was conventional apexification, with calcium hydroxide multiple-visit. This approach, however, was unsuccessful, since there was a constant drainage through the root canal, which did not allow the Endodontic treatment. It was also reported that during agudization events, an incision and drainage was necessary to decompress the periapical tissue with the installation of a drain. The radiographic examination revealed a wide root canal with open apex, associated with chronic apical periodontitis (Figure 1A).
Taking into consideration the persisting apical periodontitis, an apical surgery was suggested as the treatment of choice. The planned treatment was discussed with the patient and their parents and an informed consent was signed by them. A cone bean tomographic examination of the region was performed for the surgery planning (Figure 1B). Following local anesthesia with 2% lidocaine with 1:80000 epinephrine, a full mucoperiosteal flap was raised. The internal surface of the flap was found impregnated with small pieces of rubber, suggesting that a rubber dam was used as a drain during the period of acute apical abscess (Figure 2A). These rubber remnants were removed by the use of surgical curettes. With the flap folded, the lesion was completely removed. With the defect bone cleaned and the flap folded, the root canal was submitted to antisepsis with 2% chlorhexidine and saline solution, dried with paper points and filled with white MTA (Ângelus, Londrina, Paraná, Brasil) (Figure 2B). The flap was readapted and the palatine suture was performed to maintain the flap position and minimize gingival retraction.
The patient was first recalled 7 days after the surgical procedure. Clinical assessments indicated no sensitivity to palpation and percussion, and the absence of clinical signs. The 3-year follow-up revealed no signs or symptoms, with radiographic evidence of periapical bone healing (Figure 3) and healthy periodontal tissue.

Discussion

Although the primary etiological factor of periapical lesions is microbial, there are other independent factors that can adversely affect the outcome of endodontic treatment (21).
In this case report, we present morphological evidence in support of the role of a foreign body reaction of periapical tissue to rubber dam used as a drain during drainage of an acute endodontic infection.
Various foreign objects have been reported to be lodged in the pulp chamber and root canal of teeth (22, 23). However, there are few case reports of foreign bodies in the periapical tissues, persisting the lack of direct scientific evidence for nonmicrobial causes of persistent apical periodontitis (24, 25). As well as reported in another clinical report, according to the dental history of the patient, the rubber dam fragment may have been left iatrogenically inside the periapical tissue. Running sutures should be placed to prevent impaction of rubber drains in the tissue (24).
Remaining foreign bodies in periapical tissue have been reported to act as a focus of infection (26) and have been associated with Endodontic failure. Consequent-

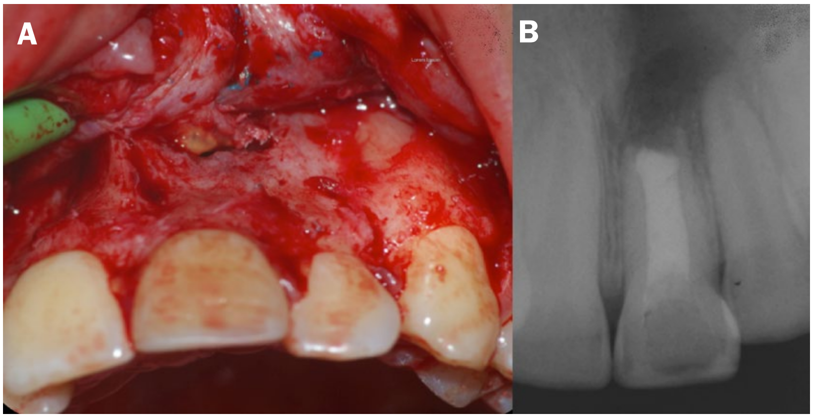

Figure 2.
A) The internal surface of the flap was impregnated with small pieces of rubber.
B) Final Radiographic image.

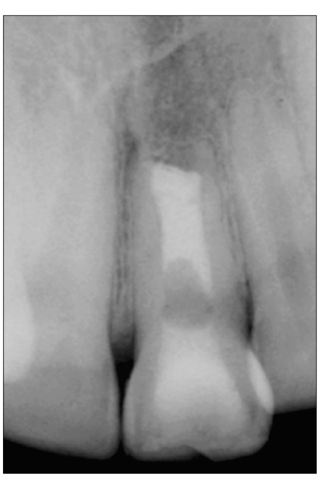

Figure 3.
Radiographic image at the 3-year follow-up, showing the complete healing of apical periodontitis.

ly, even it is not a common event, in persistent root canal infections, the existence of a foreign body should be suspected. The apical periodontal tissues can encapsulate a foreign object and an intense inflammatory response characterized by the abundance of macrophages and giant cells and a subsequent foreign body reaction can be developed (18). Furthermore, if a patient has a record of emergency assistance, with incision and drainage procedures, the risk of drain displacement in the periapical tissue due to improper suturing must be strongly considered. Being aware of this possibility aids in avoiding the erroneous diagnosis of periapical granulomas and repeated changes of intracanal medication (24). In this clinical report, after numerous dental appointments, in which conventional apexification was considerate, the apical surgery was assumed to be the treatment of choice, due to persistent infection and acute events. Apical surgery is a procedure aimed at diagnosing and treating endodontic lesions (apical periodontitis) that are not responsive of or do not improve with conventional endodontic procedures (27). Success rates for endodontic microsurgery have been reported to be between 88.9% and 100% (28), and these high levels of healing corroborated with the successful treatment reported in this article. Apical surgery has advanced to become a modern technique which has good results with regard to treatment of endodontic lesions with predictable healing patterns (29).

After the removal of the foreign object from the periapical tissues of the tooth presenting an open apex, the closure of the apex is of paramount consideration. In this report, MTA was used for retrograde filling material. In reason that Mineral trioxide aggregate (MTA), provides good clinical and histological results, which makes it the retrograde filling material of choice, largely due to its physical/chemical/biological properties (2, 30). Histologic analysis of periapical regions showed deposition of new cementum, not only onto the resection plane (cut dentinal surface) but also directly onto MTA (30). Furthermore, a 10-year follow-up study of teeth treated with apical surgery and MTA as root-end filling material showed an acceptable rate of healed cases (27).

This case presents a successful outcome of surgical endodontic treatment with immediate MTA barrier in an immature tooth with persistent apical periodontitis caused by a foreign body in the periapical tissues. This clinical report helps to avoid erroneous diagnosis and unsuccessful repeated changes in intracanal medication.

Conclusions

Even with all the attempts of endodontic treatment, in this clinical case it was not possible to reduce the clinical signs of persistent infection. Thus, surgical endodontic treatment was the treatment of choice, which permitted the periapical healing of an immature tooth with persistent apical periodontitis caused by a foreign body in the periapical tissues.

Clinical Relevance

This case report describes surgical endodontic treatment in an immature permanent maxillary central incisor with persistent apical periodontitis caused by a foreign body in the periapical tissues.

Conflict of Interest

The authors have no conflict of interest to declare.

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References

1. Hecova H, Tzigkounakis V, Merglova V, Netolicky J. A retrospective study of 889 injured permanent teeth. Dent Traumatol 2010; 26: 466-75.
2. Chala S, Abouqal R, Rida S. Apexification of immature teeth with calcium hydroxide or mineral trioxide aggregate: systematic review and meta-analysis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011; 112: e36-42.
3. Mohammadi Z, Dummer PM. Properties and applications of calcium hydroxide in endodontics and dental traumatology. Int Endod J 2011; 44: 697-730.
4. Andreasen JO, Farik B, Munksgaard EC. Long-term calcium hydroxide as a root canal dressing may increase risk of root fracture. Dent Traumatol 2002; 18: 134-7.
5. Cvek M. Prognosis of luxated non-vital maxillary incisors treated with calcium hydroxide and filled with gutta-percha. A retrospective clinical study. Endod Dent Traumatol 1992; 8: 45-55.
6. Witherspoon DE, Ham K. One-visit apexification: technique for inducing root-end barrier formation in apical closures. Pract Proced Aesthet Dent 2001; 13:455-60.
7. Torabinejad M, Chivian N. Clinical applications of mineral trioxide aggregate. J Endod 1999; 25: 197-205.
8. Katebzadeh N, Dalton BC, Trope M. Strengthening immature teeth during and after apexification. J Endod 1998; 24: 256-9.
9. Jeeruphan T, Jantarat J, Yanpiset K, Suwannapan L, Khewsawai P, Hargreaves KM. Mahidol study 1: comparison of radiographic and survival outcomes of immature teeth treated with either regenerative endodontic or apexification methods: a retrospective study. J Endod 2012; 38: 1330-6.
10. Wigler R, Kaufman AY, Lin S, Steinbock N, Hazan-Molina H, Torneck CD. Revascularization: a treatment for permanent teeth with necrotic pulp and incomplete root development. J Endod 2013; 39: 319-26.
11. Kahler B, Rossi-Fedele G, Chugal N, Lin LM. An Evidence-based Review of the Efficacy of Treatment Approaches for Immature Permanent Teeth with Pulp Necrosis. J Endod 2017; 43: 1052-7.
12. He L, Kim SG, Gong Q et al. Regenerative Endodontics for Adult Patients. J Endod 2017; 43: S57-S64.
13. Silujjai J, Linsuwanont P. Treatment Outcomes of Apexification or Revascularization in Nonvital Immature Permanent Teeth: A Retrospective Study. J Endod 2017; 43: 238-45.
14. Hargreaves KM, Diogenes A, Teixeira FB. Treatment options: biological basis of regenerative endodontic procedures. J Endod 2013; 39: S30-43.
15. Kim SG, Malek M, Sigurdsson A, Lin LM, Kahler B. Regenerative endodontics: a comprehensive review. Int Endod J 2018; 51: 1367-88.
16. Nair PN, Henry S, Cano V, Vera J. Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after "one-visit" endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005; 99: 231-52.
17. Nair PN. On the causes of persistent apical periodontitis: a review. Int Endod J 2006; 39: 249-81.
18. Tsesis I, Shoshani Y, Givol N, Yahalom R, Fuss Z, Taicher S. Comparison of quality of life after surgical endodontic treatment using two techniques: a prospective study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005; 99: 367-71.
19. Gagliani MM, Gorni FG, Strohmenger L. Periapical resurgery versus periapical surgery: a 5-year longitudinal comparison. Int Endod J 2005; 38: 320-7.
20. Wang H, Li D, Tian Y, Yu Q. [A retrospective study of 180 cases of apical microsurgery]. Zhonghua Kou Qiang Yi Xue Za Zhi 2014; 49: 421-7.
21. Nair PN, Sjogren U, Krey G, Sundqvist G. Therapy-resistant foreign body giant cell granuloma at the periapex of a root-filled human tooth. J Endod 1990; 16: 589-95.
22. Chand K, Joesph S, Varughese JM, Nair MG, Prasanth S. Endodontic management of an unusual foreign body in a maxillary central incisor. J Conserv Dent 2013; 16: 474-6.
23. Ramugade MM, Sapkale KD, Metkari SS. Patient induced unusual metallic obturation of the root canal of permanent maxillary central incisor with an immature apex - A rare case report. Singapore Dent J 2014; 35: 77-82.
24. Kim HJ, Min KS. Cystic lesion caused by latex material. Quintessence Int 2019; 50: 316-8.
25. Plascencia H, Cruz A, Solis R, Diaz M, Vazquez J. Iatrogenic displacement of a foreign body into the periapical tissues. Case Rep Dent 2014; 2014: 698538.
26. Costa F, Robiony M, Toro C, Sembronio S, Politi M. Endoscopically assisted procedure for removal of a foreign body from the maxillary sinus and contemporary endodontic surgical treatment of the tooth. Head Face Med 2006; 2: 37.
27. von Arx T, Jensen SS, Hanni S, Friedman S. Five-year longitudinal assessment of the prognosis of apical microsurgery. J Endod 2012; 38: 570-9.
28. Setzer FC, Shah SB, Kohli MR, Karabucak B, Kim S. Outcome of endodontic surgery: a meta-analysis of the literature–part 1: Comparison of traditional root-end surgery and endodontic microsurgery. J Endod 2010; 36: 1757-65.
29. Serrano-Gimenez M, Sanchez-Torres A, Gay-Escoda C. Prognostic factors on periapical surgery: A systematic review. Med Oral Patol Oral Cir Bucal 2015; 20: e715-22.
30. Katsamakis S, Slot DE, Van der Sluis LW, Van der Weijden F. Histological responses of the periodontium to MTA: a systematic review. J Clin Periodontol 2013; 40: 334-44.

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CASE REPORT

3D Diagnosis and management of external cervical resorption

ABSTRACT

Aim: *This article reports the different clinical approaches of three external cervical resorptions (ECR) cases, based on a three-dimensional (3D) classification.*
Summary: *Treatment planning was based on a 3D diagnosis and the cone beam computed tomography (CBCT) was fundamental to do an accurate assessment of the extension of the lesions. The three cases presented different degrees of complexity, related to the extension of the resorption. In two cases, root canal treatment was performed and internal management of the resorptive lesion was accomplished. In a third case, with a more severe extension of ECR, surgical approach with flap elevation was done to access the resorptive defect. Minimum period of 6-months radiographic and clinical follow-up showed periapical and periodontal healing. This case report presents the importance of a correct 3D diagnosis and treatment planning in maintaining teeth with ECR.*
Key learning points:
- *ECR is a pathologic root resorptive process with an unclear aetiology. Beginning in a point of entry in the cervical area of the root, in later stages, the resorptive process can reach the pulpal space.*
- *Management of ECR lesions should be based on a 3D diagnosis, considering height, circumferential spread and proximity/involvement of the root canal.*
- *The use of CBCT enables the clinician to establish a proper diagnosis and evaluate the restorability of the tooth in question.*

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Introduction

Resorption of permanent teeth, unlike in the primary dentition, is pathologic and unwanted (1-3). Based on its location, root resorption can be classified as internal or external, and this latter may be further subclassified into surface resorption, external inflammatory resorption, external cervical resorption, external replacement resorption and transient apical resorption (4).

External cervical resorption (ECR) can be defined as a dynamic process arising from the periodontal root surface as a result of the activity of clastic cells. This process is characterized by the destruction of dental hard tissue which, in advanced stages, might involve pulpal tissues and the periapical area, leading to a complex restorative and endodontic management (4-7).

Even though the aetiology of ECR is not yet fully understood, it is assumed that there must be damage to the periodontal ligament (PDL) and cementum, that can either be related to an anomaly during development, resulting in a gap at the cemento-enamel junction, or to damage induced by trauma (6).

Concerning the ECR histopathogenesis, three main stages are present separately or coexist in the same areas of the lesion: resorption initiation, resorption progression and reparative (remodelling) (6, 8).

Some potential initiating factors could be involved, including previous orthodontic treatment, traumatic injury, internal bleaching, surgery and restorative treatment. More factors described in recent studies, have been associated to the initiation of this resorptive process, as extraction of a neighbouring tooth, malocclusion, playing wind instruments, periodontitis and autotransplantation (6). Moreover, a stimulating factor such as infection (bacteria), continuous mechanical force on the PDL (e.g. during orthodontic treatment), discontinuous mechanical unloading caused by chewing, parafunction or a combination, must be present to maintain the activity of the clastic cells (6).

The stage, degree of progression and location are important factors that define a highly variable clinical presentation of this type of lesion, making it a challenge to a proper diagnosis. Most ECR are incidental findings, eliciting no symptoms. Radiographic presentation may become a challenge too, as this lesion might present themselves as symmetrical or asymmetrical, with margins varying from being well defined to poorly defined with no clear delineation between ECR and healthy root structure (4). The existence of distinct patterns with respect to resorptive activity and formation of mineralized substitution tissue can affect the radiographic diagnosis (5).

Heithersay's classification (9), which is based on two dimensional (2D) imaging, does not describe the resorptive and reparative pattern of the ECR, especially concerning the circumferential spread of the lesion (4). Consequently, a three-dimensional (3D) classification based on periapical radiographs and cone beam computed tomography (CBCT), ensuring a more accurate diagnosis and aid communication between clinicians has been proposed (4).

The literature is replete with case (series) reports confirming that periapical radiographs do not consistently reveal the true nature of ECR compared to CBCT, but only a limited number adopts the newest 3D classification since its publication, hence, presenting complex multidisciplinary approaches (4, 10).

The objective of this article is to describe three case reports of advanced stages of ECR with pulpal involvement based on a 3D classification and management.

Case report

#Case 1

A 21-year-old male patient was referred to the Endodontics Postgraduation clinic at University of Lisbon School of Dentistry (FMDUL) for assessment of tooth 46 (mandibular right first molar). The referring dentist had detected an apparent carious lesion on a bitewing radiography, and started excavation with high speed burs,

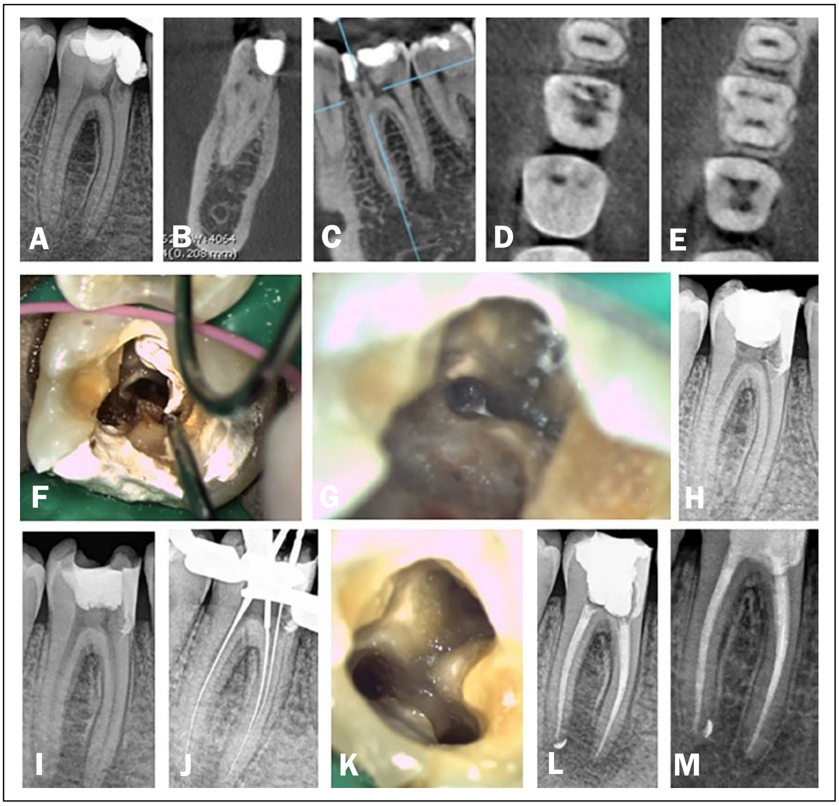

Figure 1.
A) Initial periapical radiograph; **B)** CBCT Sagittal view; **C)** CBCT Coronal view **D)** and **E)** CBCT Axial views; **F)** Removal of granulation tissue. Root canals orifices were covered with Teflon; **G)** Cleaned resorptive lesion; **H)** Periapical radiograph showing the adaptation of the pre-endodontic restoration; **I)** Periapical radiograph taken two months after initial session showing extensive periapical rarefaction; **J)** Working length determination radiograph; **K)** Root canals after the cleaning and shaping; **L)** Final periapical radiograph; **M)** 20-months radiograph follow-up.

stopping when detecting “odd looking dentin”. Clinically, a mesio-occlusal provisional filling was present, and probing was within normal depths in all surfaces. The patient reported a dull and constant pain that irradiated over the angle of the mandible and upwards, which he had been managing with over-the-counter non-steroidal anti-inflammatory medication. A discrete facial edema was also noted. Tooth 46 was tender to vertical percussion, with no increase in mobility. There was no response to the ice sensibility test (Endo cold spray, Henry Schein, Langen, Germany), and periapical radiography showed apical bone rarefaction, and a mesial lesion compatible with ECR (Figure 1A). A diagnosis of necrotic pulp and symptomatic apical periodontitis was made. A CBCT was requested to further assess the extension of the ECR lesion and for treatment planning (Figures 1B-E). The lesion was classified as 2Ap (2 [height], subcrestal; A [circunferencial spread], ≤90°; p [proximity to the root canal], probable pulp involvement) and the proposed treatment was root canal treatment with non-surgical management of the resorptive lesion, which was suggested and accepted. The treatment was performed under a Leica M320 dental operating microscope (M320, Leica, Wetzlar, Germany), in three appointments. In the first appointment, after proper anesthesia using 4% articaine with 1:200.000 epinephrine (Artinibsa, Inibsa, Barcelona, Spain) and rubber dam isolation, the access cavity was established with round burs and improved with Start-X ultrasonic tips (Start-X, Dentsply Tulsa Dental, Tulsa, USA). The orifices of the root canals were covered with teflon, and the granulation tissue associated with the resorptive lesion was removed with high speed round burs, and with dentin excavators (Figure 1F). After removal of all the resorptive tissue, visually confirmed under the dental operating microscope (Figure 1G), a universal matrix band was adapted to the tooth with an Ivory retainer and a pre-endodontic restoration was made with composite resin (Tetric EvoCeram, Ivoclar Vivadent, Schaan, Liechtenstein) to restore the mesial margin and allow absolute isolation with rubber dam (Figure 1H). A calcium hydroxide paste was applied over the mesial margin of the tooth, and the tooth was provisionally restored with Cavit (Ultracal, Ultradent, South Jordan, USA). In the following appointment, two months after the initial session, the patient had developed an acute abscess and had been prescribed systemic antibiotics by the referring dentist. A periapical radiograph showed extensive periapical rarefaction (Figure 1I). After achieving anesthesia and absolute isolation with rubber dam, access was re-established and a purulent discharge from the canals provided immediate relieve of symptoms. The root canals were negotiated with 0.10 and 0.15 stainless-steel K-files (K-File, Dentsply, Ballaigues, Switzerland) and the determination of the working length was performed with a Root ZX electronic apex locator (Root Zx, Morita, Komuro, Japan) and confirmed radiographically (Figure 1J). All root canals were shaped with WaveOne Gold reciprocating files (WaveOne Gold, Dentsply Maillefer, Ballaigues, Switzerland)

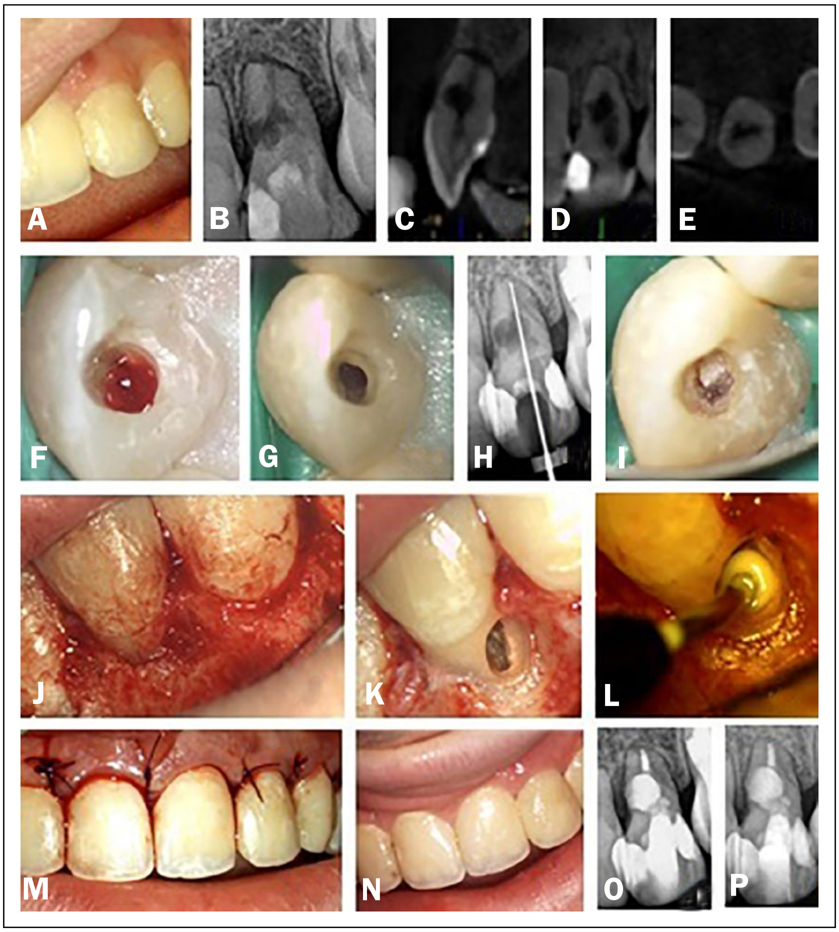

Figure 2.
A) Extraoral photograph-sinus tract associated with tooth 22; **B)** Initial periapical radiograph; **C)** CBCT Sagittal view; **D)** CBCT Coronal view; **E)** CBCT Axial view; **F)** Granulation tissue; **G)** Cleaned resorptive lesion; **H)** Working length determination radiograph; **I)** Reparation of the defect with MTA; **J)** Full thickness flap **K)** Cleaned resorptive lesion; **L)** Sealing root defect with glass ionomer; **M)** Repositioning of the flap with sutures; **N)** Extraoral photograph - complete healing of the sinus tract; **O)** Final radiograph; **P)** 7-months follow-up radiograph.

according to the manufacturer's instructions, with Medium file in the distal canal and Primary in both mesial canals (Figure 1K). Copious irrigation with 5.25% sodium hypochlorite (Denta Flux, J. Ripoll SL, Murcia, Spain) using a 5 ml syringe and a 27G notched needle (CanalPro Slotted-End Tips, Coltene, Lezzenes, France) was performed throughout all the root canal treatment. In the last appointment the patient was free of symptoms. A final irrigation protocol included one-minute irrigation with 10% citric acid prior to a final 5.25% sodium hypochlorite rinse under manual dynamic agitation with pre-fitted 4% tapered gutta-percha points (Zipperer, VDW, Munich, Germany), after which the root canals were dried with 4% tapered paper points (Zipperer, VDW, Munich, Germany) and filled with gutta-percha and an epoxy resin-based sealer (AH Plus, Dentsply Tulsa Dental, Tulsa, USA) with a continuous wave of obturation technique with the B&L system (Biotech, Seoul, Republic of Korea) (Figure 1L). The canals were sealed with Ionoseal (VOCO GmbH, Cuxhaven, Germany) and the tooth provisionally restored with Cavit (Ultracal, Ultradent, South Jordan, USA) before the prosthodontics department makes the direct composite resin restoration. At 20 months follow-up appointment, the patient maintained absence of symptoms, and normal bone trabeculation could be seen (Figure 1M).

#Cases 2 and 3

A 24-year-old male patient was referred to the Endodontics Postgraduation clinic at FMDUL for assessment of teeth 22 (Figure 2) and 23 (Figure 3, maxillary left lateral incisor and canine). Medical history was non-contributory and there was no previous trauma but the patient had undergone orthodontic treatment as a child. There were no symptoms, and no pain on percussion nor palpation. Tooth 22 had a mesiopalatal restoration in composite resin, with no apparent secondary caries, and tooth 23 presented neither carious lesion nor previous restoration. Probing was within normal values in all surfaces of both teeth, which also maintained normal mobility. There was a fistula associated with tooth 22 (Figure 2A). There was no response to the ice sensibility test (Endo cold spray, Henry Schein, Langen, Germany) in either teeth. Periapical radiography showed apical bone rarefaction associated with tooth 22, with considerable loss of root length, in a pattern consistent with orthodontic forces, and a mid-root alteration compatible with ECR (Figure 2B). ECR compatible lesion was also identifiable in tooth 23, with maintenance of periapical tissues health (Figure 3A). Both teeth were diagnosed as necrotic, with chronic periapical abscess and normal periapical tissues for teeth 22 and 23, respectively. A CBCT was requested to further assess the extension of the ECR lesions and for treatment planning. The ECR lesion of tooth 22 was classified as 3Bp (3 [height], extends into mid 1/3; B [circunferencial spread], >90° to≤180°; p [proximity to the root canal], probable pulp involvement)

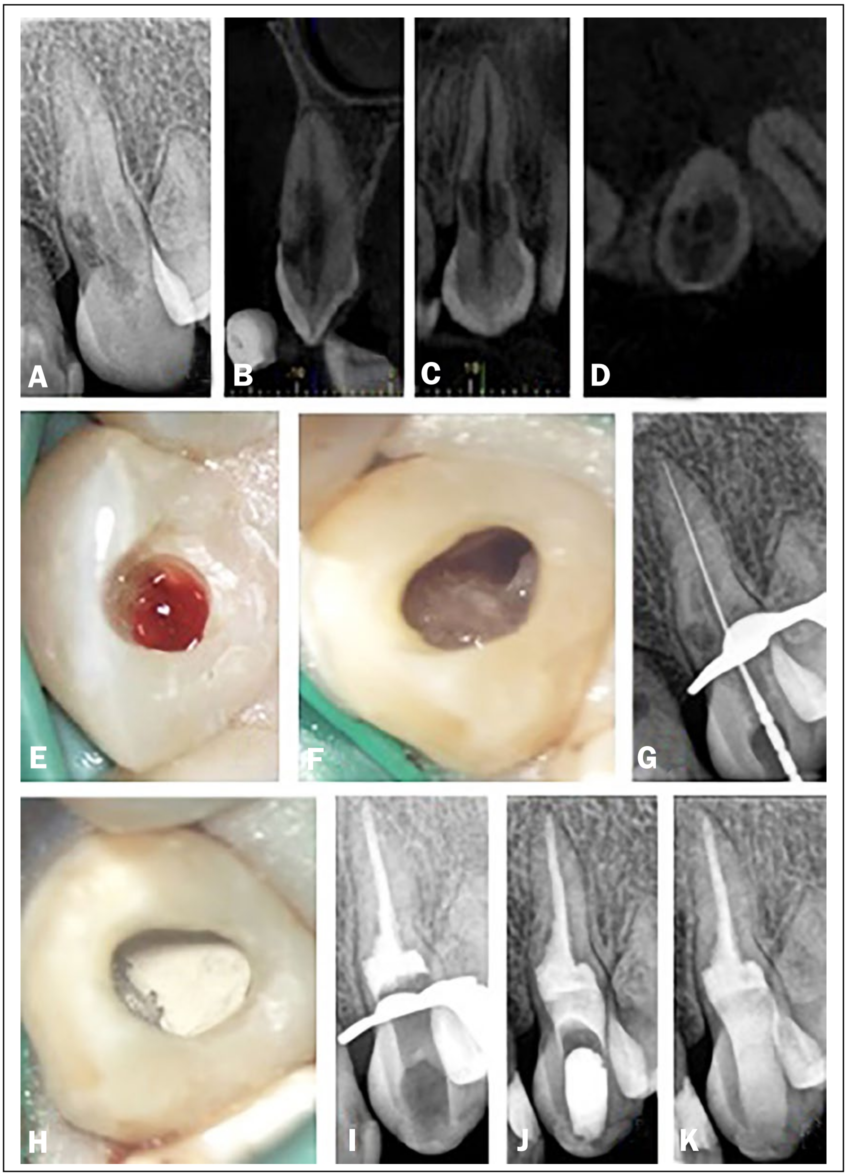

Figure 3.
A) Initial periapical radiograph; **B)** CBCT Sagittal view; **C)** CBCT Coronal view **D)** CBCT Axial view; **E)** Granulation tissue; **F)** Cleaned resorptive lesion; **G)** Working length determination radiograph; **H)** Reparation of the defect with MTA; **I)** Confirmation of defect reparation radiograph **J)** Final radiograph; **K)** 9-months follow-up radiograph.

(Figures 2C-E) and the lesion of tooth 23 as 3Cp (3 [height], extends into mid 1/3; C [circunferencial spread], >180º to≤270º; p [proximity to the root canal], probable pulp involvement) (figures 3B-D). The proposed treatment was root canal treatment with non-surgical management of the resorptive lesion, which was suggested and accepted. The treatments were performed under a Leica M320 dental operating microscope (M320, Leica, Wetzlar, Germany). After proper anesthesia using 4% articaine with 1:200.000 epinephrine (Artinibsa, Inibsa, Barcelona, Spain) and rubber dam isolation, the access cavities were established with round burs and improved with Start-X 1 ultrasonic tips (Start-X, Dentsply Tulsa Dental, Tulsa, USA). The granulation tissue associated with the resorptive lesions (Figures 2F, 3E) was removed with high speed round burs, and with dentin excavators. An aqueous solution of 90% tricloroacetic acid was also applied for 3 minutes to neutralize undetectable resorptive cells and clean the resorptive cavity (Figures 2G, 3F). The root canals were negotiated with 0.10 and 0.15 stainless-steel K-files (K-File, Dentsply, Ballaigues, Switzerland) and the determination of the working length was performed with a Root ZX electronic apex locator (Root Zx, Morita, Komuro, Japan) and confirmed radiographically (figures 2H, 3G). The canals were shaped with Protaper Next rotary files up to X5 (Protaper, Dentsply Maillefer, Ballaigues, Switzerland) according to the manufacturer's instructions. Copious irrigation with 5.25% sodium hypochlorite (Denta Flux, J. Ripoll SL, Murcia, Spain) using a 5 ml syringe and a 27G notched needle (CanalPro Slotted-End Tips, Coltene, Lezzenes, France) was performed throughout all the root canal treatment. In the last appointment the patient was free of symptoms. A final irrigation protocol included one-minute irrigation with 17% EDTA prior to a final 5.25% sodium hypochlorite rinse. After drying the canals with 4% tapered paper points (Zipperer, VDW, Munich, Germany), the canals were sealed with Mineral Trioxide Aggregate (MTA) (ProRoot MTA, Dentsply Tulsa Dental, Tulsa, USA). In both teeth, MTA was packed just short of the cemen-to-enamel junction (Figures 2I, 3H, 3I), sealed with glass ionomer Ionoseal (VOCO GmbH, Cuxhaven, Germany) and provisionally restored with Cavit (Ultracal, Ultradent, South Jordan, USA) (Figure 2J); in the lateral incisor, although a plug of MTA was applied, the extension of the resorption proved to be an obstacle for the correct sealing of the entire resorptive defect, and a surgical approach to correctly seal the space and margins was advised and accepted. Completion of the treatment of tooth 22 was done in a subsequent appointment, with elevation of a full thickness flap (Figure 2J), sealing of the entire root defect (Figure 2K) with Ionoseal (VOCO GmbH, Cuxhaven, Germany, Figure 2L) and the flap was repositioned with suture (Figure 2M). Two weeks after the surgical procedure, the fistula had disappeared (Figure 2N) and both teeth were sent for definitive restoration with composite resin (Figure 2O). At a follow-up appointment of teeth 22 and 23, 7 and 9 months after treatment, respectively, the patient maintained absence of symptoms, and normal bone trabeculation could be seen (Figures 2P, 3K).

Discussion

This article presents three cases of ECR in which classification and treatment plan was based on the most recently proposed 3D classification (4).

To properly classify and diagnose an ECR, the clinician must be aware of the different types of root resorption and their clinical findings. Depending on the location in relation to the root surface, a root resorption can be classified as internal or external (4). ECR lesions are included in the external resorption category.

The ECR can be challenging to diagnose, as it is usually asymptomatic in the early stages because pulpal and/or periodontal involvement may only appear at the later stage of disease progression (4). A previous histological and scanning electron microscopy (SEM) analysis and 3D nano focus CT imaging study allowed to understand why, in the progression phase, ECR has difficulty to progress into the pulpal space. A higher mineralized tissue composed by predentin, dentin, and reparative (bone-like) tissue, denominated pericanalar resorption-resistant sheet (PRRS), seems to be the pulp's response to this noxious stimulus. However, the structure of this sheet may not remain constant during the progression of the lesion, presenting loss of thickness in some areas and with occasional disruptions, associated with modification of the pulp tissue consistency in these regions (8). Moreover, ECR is capable of destroying PPRS layer and reach the pulp space, creating interconnections between the resorption cavity and the pulp, and pathways for opportunistic bacteria (8). These findings may explain the development of pulpal necrosis and symptomatic apical periodontitis associated with tooth 46 and chronic apical abscess associated with tooth 22.

The aetiology of ECR lesions remains unclear. According to previous studies, orthodontic forces have been established as one of the major etiological factors for ECR. Considering the potential predisposing factors for ECR presented in the literature, a study involving 337 teeth showed that orthodontic treatment was the factor most frequently associated (45.7%) with ECR cases (6). These forces may have been strongly implicated in the development of ECR in tooth 22 and 23, once the patient had a dental history of orthodontic treatment, which also resulted in loss of cortical bone in the vestibular plate as can be seen on the CBCT scan. Despite all the other listed predisposing factors in the literature, it is still not possible to establish a cause-effect relationship for the development of ECR in tooth 46.

Nowadays, 3D imaging, such as CBCT, brought to discussion the well-known shortcomings of 2D imaging that can result in a deficient evaluation and diagnosis of the resorption process and its true dimensions, consequently leading to a poor management of the ECR (7). CBCT is a very important tool to establish a good diagnosis and to assist the clinician to plan the management of several complex endodontic problems (1).

Therefore, clinicians with the aid of CBCT, are able to evaluate the extension of the resorptive lesion, and concerning its location in the root, circumferential spread and proximity to the root canal (4). In ECR lesions, the decision-making process is based on symptoms, dimension of the resorptive defect and tooth restorability (1). The proper approach depends on the severity and accessibility and restorability of the lesion, as it is of most importance to accomplish a complete removal of the resorptive tissue, to seal the resultant defect and portal of entry, thus preventing recurrence (10).

The most recent developed 3D classification considers the height of the lesion establishing four levels, being the number 1 at the cemento-enamel junction level or coronal to the bone crest (supracrestal), and number 2, 3 and 4 extending apical to the bone crest, within each third of the root, respectively. The circumferential spread is measured in degrees, being A≤90°, B>90° to≤180°, C>180° to≤270° and D>270°. The proximity to the root canal contemplates d: lesion confine to dentine and p, probable pulpal involvement (4). Considering the advantages stated, the approach of these cases was based in their 3D classification: tooth 46 2Ap; tooth 22 3Bp; tooth 23 3Cp. Tooth 46 lesion extends into coronal third of the root and apical to the bone crest (subcrestal), with a circumferential spread inferior to 90°, whilst both lesions on teeth 22 and 23 had an extension into mid-third of the spot, concerning height. Tooth 22 lesion had a circumferential spread between 90° and 180°, while tooth 23 lesion was classified having the biggest circumferential spread (between 180° and 270°). A probable pulp involvement was considered for every case.A complete diagnosis was accomplished with sensibility tests and clinical evaluation, as we should take in consideration that this 3D classification is to be applied to CBCT scans only and does not contemplate information regarding the pulpal vitality.

Considering the extent and accessibility of the resorptive lesion, various treatment options are presented in the literature: external repair of the resorptive defect; internal repair and root canal treatment; intentional replantation; periodic review; and extraction (7, 10). The main purpose when managing an ECR case, is to arrest the resorptive process by removing all the resorptive tissue, restore the defect with an aesthetic biocompatible material and prevent recurrence of the process (10).

After treatment plans were presented, patients were aware of the prognosis, especially for tooth 22, a borderline case with a questionable prognosis, concerning the loss of root length due to external inflammatory resorption. The absence of tooth mobility and probing depths played a major role in the decision-making. With the patient's informed consent and acceptance of treatment plan, root canal treatments were performed with internal repair of tooth 23, external repair of tooth 46 with a composite resin and external repair with surgical approach in tooth 22, and the defect sealed with glass ionomer. The literature suggests internal approach as a treatment option when ECR is close to or has invaded the pulp space, when it is difficult to access surgically the resorptive defect or when excessive removal of sound tooth structure is needed (10). Decision-making for the approach of tooth 23 was based on the literature, also considering that, from CBCT imaging evaluation, small points of entry were involved. Inaccessible and small entry points may expand circumferentially and apicocoronally and are best managed with an internal approach (10). On the contrary, a greater destruction of the root surface was evaluated on tooth 22, impossible to repair with an internal approach. A mucoperiosteal flap was needed to allow adequate access to the ECR for curettage of the granulomatous tissue from both root and periodontium. Despite the fact that tooth 46 already presented considerable tooth structure loss due to previous attempt of restauration after a misdiagnosed decay, a supraosseous portal of entry was identified, which, according to literature, is best managed by an external approach (10).

There are several products in the literature recommended to accomplish the better removal of the resorptive tissue. Trichloracetic acid (TCA) at 90%, applied for a period of 3-4 minutes, calcium hydroxide and even sodium hypochlorite enable tissue within the resorption cavity to become progressively avascular due to a process of coagulation necrosis. TCA as also a proven action of infiltrating the small channels and recesses of ECR that are difficult to reach by mechanical preparation (11-13). Consequently, under magnification, the tissue is easily removed by curettage and a complete visualization of the resorption defect is accomplished, one of the most important steps to prevent ECR recurrence (11, 12, 14). For tooth 23, with internal and nonsurgical treatment, the

difficulty in eliminating all the entry point of the lesion must be considered. This case relied in the action of TCA to cauterize any resorptive tissue that remained (14).
The same protocol with TCA was applied to the surgical management of tooth 22, but based on the knowledge that this aqueous solution severely demineralizes dentin, the clinician opted for the use of calcium hydroxide on tooth 46 not to compromise the bonding strength when restoring the tooth with composite resin (14). Calcium hydroxide is a strong alkaline substance, with a pH of approximately 12.5, which is widely known by its antimicrobial properties. Nonetheless, several authors have confirmed its use for several clinical situations according to its properties of dissolving tissues and inhibition of tooth resorption (15). To restore the root surface, the location of the root defect must be considered. Several materials such as amalgam, MTA, which may be used with adhesive bonding agents (16), and others bioactive cements, resin composite, glass ionomer cements have been proposed as resorptive lesion reparation materials (10, 17). As for the supracrestal lesions, communicating with the oral environment, as performed in tooth 46, composite resin or glass ionomer cements have been recommended (10). Chemical interaction is the primary bonding mechanism for resin modified glass ionomer cements (RMGICs), thus hybridizing the dentin (18). Therefore, these materials depend on the ionic bonding to the calcium present in hydroxyapatite (14). TCA is application will result in elimination the hydroxyapatite in the dentine surface, contributing to a severely demineralized tissue. On tooth 22, a round bur was used to eliminate the deprived dentin surface from hydroxyapatite and to refresh the surface, enabling the calcium to be present for glass ionomer ionic bonding (14). In case composite resin is used, a flowable resin may be advisable to be used between the adhesive layer and the composite resin in order to improve the cementum-restoration interface (19).
Bioactive materials have also been described in the literature as a good material choice for application in subgingival ECR cavities. Among their antimicrobial properties and biocompatibility, a good sealing ability and capacity to promote periodontal reattachment are the most important to consider in this subgingival cavities (10). A study aiming to compare the biological interaction of human osteoblasts and periodontal ligament cells with different materials (MTA, Biodentine, amalgam and composite) reported the highest survival and proliferation rate when the cells where in contact with Biodentine (Septodont, Lancaster, PA, USA) (20). Despite these findings, and even considering its lower setting time (10-12 minutes) when compared to other bioactive cements, and also its reasonable aesthetic appearance (21), a different material was chosen for the repair o tooth 22.
Because dentin bonding is extremely difficult due to its humidity and organic nature (22), a composite resin technique was not considered for the sealing of the defect of tooth 22. Ionoseal (VOCO GmbH, Germany), a light curable RMGIC, was chosen because of its adequate restorative mechanical properties and also its ability to provide a good sealing and remineralization (18). RMGIC cements were added hydrophilic monomers and photoinitiators to improve the mechanical and physical properties of conventional GI cements, and also to reduce its early moisture sensitivity (17). Thus, making these materials a great material option to seal resorptive defects.
Ideally the 12-months follow-up period should be performed (7). A total remission of symptoms was observed before that period, and all teeth remained functional, even in the short term follow-up.
Overall, comprehending the course of this pathology and its aetiology is fundamental to allow timely diagnosis of this condition in routine treatments. Proper diagnosis and adequate treatment plan is only accomplished with a 3D evaluation of the resorptive defect.

Conclusions

The correct assessment and treatment planning of these three ECR cases resorting to a 3D classification and CBCT analysis, lead to successful approaches with remission of symptoms. Nonetheless, with the informed consent of patients, borderline cases should be followed with a long period of time to ensure the maintenance of normal periapical tissues and function.

Clinical Relevance

Management of external cervical resorption lesions should be based on a 3D diagnosis, considering height, circumferential spread and proximity/involvement of the root canal. The use of CBCT enables the clinician to establish the proper 3D diagnosis maximizing the outcomes of the treatments under way.

Conflict of Interest

None.

Acknowledgements

None.

References

1. Patel S, Saberi N. The ins and outs of root resorption. Br Dent J. 2018; 224(9):691-9.
2. Jebril A, Aljamani S, Jarad F. The surgical management of external cervical resorption: a retrospective observational study of treatment outcomes and classifications J Endod. 2020; 46(6):778-85.
3. Matny LE, Ruparel NB, Levin MD, Noujeim M, Diogenes A. A volumetric assessment of external cervical resorption cases and its correlation to classification, treatment planning, and expected prognosis. J Endod. 2020; 46(8): 1052-8.
4. Patel S, Mavridou AM, Lambrechts P, Saberi N. External cervical resorption - part 1: histopathology, distribution and presentation. Int Endod J. 2018; 51(11):1205-23.
5. Luso S, Luder HU. Resorption pattern and radiographic diagnosis of invasive cervical resorption. Schweizer Monatsschrift für Zahnmedizin. 2012; 122(10):914-22.
6. Mavridou AM, Bergmans L, Barendregt D, Lambrechts P. Descriptive analysis of factors associated with external cervical resorption. J Endod. 2017; 43(10):1602-10.
7. Patel S, Lambrechts P, Shemesh H, Mavridou A. European society of endodontology position statement: external cervical resorption. Int Endod J. 2018; 51(12):1323-26.
8. Mavridou AM, Hauben E, Wevers M, et al. Understanding external cervical resorption in vital teeth. J Endod. 2016; 42(12):1737-51.
9. Heithersay GS. Invasive cervical resorption: an analysis of potential predisposing factors. Quintessence Int. 1999; 30(2):83-95.
10. Patel S, Foschi F, Condon R, et al. External cervical resorption: part 2 – management. Int Endod J. 2018; 51(11):1224-38.
11. Heithersay GS. Invasive cervical resorption. Endod Topics. 2004; 7:73-92.
12. Patel S, Kanagasingam S, Pitt Ford T. External cervical resorption: a review. J Endod. 2009; 35(5):616-25.
13. Espona J, Roig E, Durán-Sindreu F, et al. Invasive cervical resorption: clinical management in the anterior zone. J Endod. 2018; 44(11):1749-54.
14. Schwartz RS, Robbins JW, Rindler E. Management of invasive cervical resorption: observations from three private practices and a report of three cases. J Endod. 2010; 36(10):1721-30.
15. Siqueira JF, Lopes HP. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. Int Endod J. 1999; 32(5):361-9.
16. Cervino G, Fiorillo L, Spagnuolo G, Bramanti E, Laino L, Lauritano F, Cicciù M. Interface between MTA and dental bonding agents: scanning electron microscope evaluation. J Int Soc Prev Community Dent. 2017; 7(1):64-8.
17. Tavares WLF, Lopes RCP, Oliveira RR, et al. Surgical management of invasive cervical resorption using resin-modified glass ionomer cement. Gen Dent. 2013; 61(7):16-8.
18. Toledano M, Osorio R, Cabello I, et al. Oral function improves interfacial integrity and sealing ability between conventional glass ionomer cements and dentin. Microsc Microanal. 2017; 23(1):131-44.
19. Lo Giudice G, Cicciù M, Cervino G, Lizio A, Visco AM. Flowable resin and marginal gap on tooth third medial cavity involving enamel and radicular cementum: a SEM evaluation of two restoration techniques. Indian J Dent Res. 2012; 23(6):763-9.
20. Jung S, Mielert J, Kleinheinz J, Dammaschke T. Human oral cells' response to different endodontic restorative materials: an in vitro study. Head Face Med. 2014; 10:55.
21. Wang Z. Bioceramic materials in Endodontics. Endod Topics. 2015; 32:3–30.
22. Perdigao J, Reis A, Loguercio AD. Dentin Adhesion and MMPs: A Comprehensive Review. J Esthet Restor Dent. 2013; 25(4):219-41.

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ORIGINAL ARTICLE

Using the Periapical Index to evaluate the healing of periapical lesions after root canal treatment

ABSTRACT

Aim: *Root canal treatment serves to prevent or cure periapical periodontitis. The aim of our study was to evaluate the remission of periapical lesions radiographically in patients who had undergone root canal treatment.*
Materials and Methods: *We conducted an observational, longitudinal study of patients in the endodontics clinic of the Autonomous University of the State of Mexico. Using an ambispective (retrospective and prospective), comparative (before and after) approach, we analyzed 19 patients. We monitored the progress of their treatment from February to June 2017 by means of periapical x-rays, and compared the results of the final vs. the initial radiographs. To evaluate the periapical root status of patients, we employed the Periapical Index (PAI) created by Ørstavik et al. in 1986.*
Results: *The mean age of our study sample was 40.31 ± 12.75 years, and 63.2% of participants were female. The mean interval between the initial and control radiographs was 618.42 ± 102.38 days. A comparison between the initial and final periapical states of all teeth yielded favorable results, with positive outcomes observed in the periapical lesions of all participants (p=0.0001).*
Conclusions: *In our study sample, root canal treatment proved highly successful in reducing apical periodontitis; it secured the full recovery of the periradicular tissues in the dental organs. In developing countries such as Mexico, root canal treatment demonstrates effectiveness at two years. Its use is recommended as an optimal means of preserving teeth among the Mexican population.*

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Introduction

The purpose of root canal treatment is the preservation of teeth while maintaining their full function in the oral cavity. As the choice of last resort for this purpose, it seeks to prevent, or failing that, to cure periapical periodontitis (1). Periapical lesions, the most common pathology in the alveolar bone, expose the dental pulp to bacteria and their byproducts. These, in turn, act as antigens; they produce both nonspecific inflammatory and specific immune responses in the periradicular tissue, ultimately causing periapical lesions (2). In the root canal system, pulpal tissue infection caused by caries or other pathways is the main cause of apical periodontitis (3). It has been demonstrated that necrosis and periradicular inflammation develop in the pulps of rats when they are exposed to oral microorganisms, contrary to laboratory pulps kept free from microorganisms (4).
Clinical diagnoses of periapical inflammatory diseases are based primarily on clinical signs and symptoms, the duration of the disease, pulpal sensitivity tests, percussion, palpation and a radiological study. By contrast, histological diagnoses rely on morphological and biological profiles of the cells and the extracellular matrix of diseased tissue. However, these can only be performed after the organ has been extracted from the oral cavity. While clinical diagnoses are provisional and are based on symptoms, signs, and test results, histological diagnoses are definitive and relate to diseased tissue. The absence of clinical symptoms and periapical signs on radiographs does not necessarily indicate the absence of apical periodontitis. Similarly, the clinical success of endodontics – that is, the absence of either signs and symptoms or radiological periapical signs after nonsurgical treatment of the root canal system – does not necessarily imply that a periapical lesion has been histologically cured. It has thus been recognized that the clinical diagnostic methods commonly employed such as percussion, palpation and pulp tests (based on cold, heat and electricity) are not sufficiently sensitive to either pinpoint the stage or provide a histological diagnosis of inflammatory periapical diseases (5).
In 1986, Ørstavik et al. (6) created an index for the radiographic evaluation of the periapical state of roots (PAI) which has proven useful and reliable. Based on radiographic evidence, the PAI has been validated through histological diagnoses. It uses an ordinal scale of 1 to 5, where 1 denotes optimal health and 5 evident periapical disease. Studies conducted in numerous countries have used the PAI (7-10).
Our work was aimed at evaluating the remission of periapical lesions radiographically in patients having received root canal treatment.

Materials and Methods

Our study used an observational, longitudinal approach with an ambispective (retrospective and prospective) and comparative (before and after) design. Based on convenience sampling, we analyzed a group of patients from the Center for Research and Advanced Studies in Dentistry of the Faculty of Dentistry in the Autonomous University of the State of Mexico. To this end, we retrospectively reviewed the clinical records of all endodontics patients treated between February and June 2015 (n=73). A sample size of 22 participants was established according to the following criteria: a proportion of 98% for estimates, a 95% confidence interval and 5% precision. A total of 19 patients with periapical lesions met the following inclusion criteria: they (1) were of both sexes, (2) had undergone root canal treatment at the endodontics clinic between February and June 2015, (3) had submitted a radiograph showing a periapical lesion, and (4) signed an informed consent letter. Exclusion criteria referred to patients who (1) submitted an initial radiograph that was defective or could not be evaluated, (2) were unreachable, (3) refused to undergo radiographic follow-up, (4) were pregnant, or (5) suffered from a systemic disease. We conducted the follow-up evaluations between February and June 2017. Our dependent variable included the final PAI scores of the patients, while the independent variables pertained to age, sex, type of tooth, the initial PAI scores, and the number of days that had elapsed after treatment.

Radiographic Material
All radiographs – both initial and follow-up – were performed with the same X-ray equipment, using 70 kV (X-mind ac, Satelec, Italy). Exposure was 0.40 s for anterior teeth, 0.50 s for premolars and 0.60 s for molars. We utilized the long cone paralleling technique with XCP positioners (Rinn Co., Elgin, IL, USA). The periapical radiographs were size #2 for adults (IP21 Insight, Kodak/Carestream Health Inc. NY, USA). All radiographs were revealed manually in a dark room using liquid developer (Kodak Dental READYMATIC, NY, USA).

Radiographic evaluation
Periapical root condition was evaluated using the Periapical Index (PAI) created by Ørstavik et al. (1986). This approach involves the use of reference radiographs which have been corroborated by histological diagnosis. The PAI includes five categories of disease progression represented on an ordinal scale as follows: (1) normal periapical structures; (2) small changes in the periapical bone or bone structure; (3) changes in the periapical bone structure with mineral loss, characteristic of apical periodontitis; (4) demineralization of the periapical bone within a well-defined radiolucent area; and (5) demineralization of the periapical bone with exacerbations and expansion in bone structure.
The radiographs were interpreted by a previously trained and standardized evaluator: a second-year resident in endodontics at the Autonomous University of the State of Mexico. The evaluator analyzed the radiographs using a standardized method involving work in a dark room, using a lightbox and magnification loupes (3.5×).
For purposes of statistical analysis, we established the following cutoff points to categorize the PAI values: 1 denoted no disease and values from 2 to 5 indicated the presence of periapical disease. We termed this variable "health-disease".

Statistical analysis
We created a database using the Excel program. Univariate analysis consisted of calculating the frequencies and percentages of qualitative variables as well as the mean and standard deviations for the quantitative variables. For the bivariate analysis, we calculated chi-square statistics and ran the Wilcoxon test (with related samples) to explore the differences between the initial and final radiographic measurements. All estimates were performed with the Stata 11 statistical package.

Ethical considerations
We conducted all procedures in accordance with both the institutional and national chapters of the corresponding committee on human experimentation and the Declaration of Helsinki 1975, as revised in 2008. The study protocol was approved at the Dr. Keisaburo Miyata Center for Research and Advanced Studies in Dentistry of the Faculty of Dentistry at the Autonomous University of the State of Mexico. Prior informed consent was obtained from all patients included in the study.

Results

Table 1 shows the results of our univariate analysis. We examined 19 patients, focusing specifically on ten molars, six premolars and three anterior teeth each. The average age of participants was 40.31 ±12.75 years, and 63.2% were women. The average number of days between the initial and final radiographs was 618.42 ±102.39. In the initial PAI distribution, most patients (47.4%) exhibited stage 4 periapical lesions. The final PAI evaluation yielded the following results: eight teeth (42.1%) obtained a value of 1, ten teeth (52.6%) a value of 2, and one tooth (5.3%) a value of 3. For purposes of statistical analysis, we dichotomized the health-disease variable

Table 1
Descriptive characteristics of participants

	Mean ± SD	Min–Max
Age	40.31 ± 12.75	18–64
Days between initial and control radiographs	618.42 ± 102.39	476–771
	Frequency	**%**
Sex		
Men	7	36.8
Women	12	63.2
Type of tooth		
Anterior	3	15.8
Premolar	6	31.6
Molar	10	52.6
Initial periapical evaluation		
Stage 3	7	36.8
Stage 4	9	47.4
Stage 5	3	15.8
Final periapical evaluation		
Stage 1	8	42.1
Stage 2	10	52.6
Stage 3	1	5.3
Final periapical status		
Healthy	8	42.11
Diseased	11	57.89

n=19, SD: Standard Deviation

and found that, after root canal treatment, 42.1% of teeth were healthy. In analyzing the final periapical status, we found no significant differences by type of tooth (Table 2). Broken down by sex, women showed a slightly higher percentage of periapical improvement than did men ($p=0.061$). Wilcoxon testing for related samples (Table 3) indicated a positive change between the initial and final periapical conditions of all participants ($p<0.001$), demonstrating that root canal treatment was consistently successful.

Discussion

Our study, performed at a university clinic, found that all periapical lesions showed positive radiographic changes following root canal treatment. In developing countries such as Mexico, where tooth extraction in the population is a treatment of choice for dental caries (11, 12), root canal treatment demonstrates effectiveness two years after it is performed; it thus offers patients the possibility of keeping their teeth for a longer period. Previous studies have reported success rates ranging from 88% to 97% (13,14) in the absence of apical periodontitis prior to root canal treatment. When apical periodontitis *does* exist, the success rate varies between 73% and 90% (15, 16). As suggested by Prati in 2018, it would be worthwhile extending the follow-up period beyond that used in this study (less than two years). In a cohort study at 20 years follow-up, Prati reported that 80% of teeth with root canal treatment survived in the oral cavity, while 20% were lost for non-endodontic reasons such as periodontal disease or caries.
During Phase III of the famous Toronto study, it was observed that only 43% of teeth with periapical lesions showed im-

Table 2
Results of bivariate analysis: periapical health status by sex and type of tooth

Variable	Healthy	Diseased	p-value
Sex			
Men	1 (14.3)	6 (85.7)	
Women	7 (58.3)	5 (41.7)	0.061*
Type of tooth			
Anterior	2 (66.7)	1 (33.3)	
Premolar	2 (33.3)	4 (66.7)	
Molar	4 (40.0)	6 (60.0)	0.622*

*Chi-square test

Table 3
Changes in stage of periapical lesions

	Observed	Expected	
Positive	19	95	
Negative	0	95	
Without change	0	0	**0.0001****

**Wilcoxon test

provement or reduction in lesion size at 4 and 6 years follow-up (24). This contrasts with the results of our study, in which 100% showed improvement in less than two years (618 days on average). In the Toronto study, treatments were performed by graduate students supervised by qualified specialists; in our study, treatments were carried out by graduate students in endodontics. However, it should be noted that the concentration of sodium hypochlorite used was 2.5% in the Toronto study vs 5.25% in the university clinic where we conducted our study.

The design of this study differed from that of previous studies on periapical status (17-19). According to the European Society of Endodontics (20), four years of observation are required for evaluating periapical status. However, in our study, an average of only 618 days proved sufficient. Other similar studies have been conducted at two years follow-up (21-23). The Toronto study, performed in several phases, supported these conclusions: it found that the attrition rate for patients was greater with longer follow-up (24, 25).

As regards methodology, conventional periapical radiographs were used for radiographic evaluation both initially and at follow-up, as has been done in other studies (26, 27). Orthopantomography has also been used in previous research initiatives (28-30). However, those studies have employed this technology to evaluate other aspects in addition to periapical status, thus avoiding unnecessary patient exposure to additional radiation. Another important feature of our study was using the PAI for evaluation of periapical radiographs instead of orthopantomographies. It is important to note that cone beam computed tomography (CBCT) offers greater sensitivity in the diagnosis of apical periodontitis compared to conventional radiographs, but the latter has shown excellent efficacy in advanced periapical pathological processes (8). Future research projects should consider additional variables. These could include the type of root canal treatment used to shape the root canal, the obturation and restoration techniques selected, and the number of appointments held with patients. This complementary information would enhance our understanding of the outcomes of root canal procedures and provide an indication of the difficulties encountered in the various cases treated.

Our study had a number of limitations. Among these were the lack of standardization in clincal procedures such as those utilized in the instrumentation and obturation techniques. The lack of standardization also applied to the irrigation protocols and the sealant, as root canal treatments were performed by different professionals. It must be underlined that these variables can affect the prognosis for the treated teeth. For example, as Chiara reported in 2018, using a thermoplasticized filling technique yielded a success rate of 85% at ten years evaluation. Another limitation concerned the fact that our PAI cutoff point for health differed from the cutoffs used in previous studies. For final evaluation purposes, we dichotomized the health-disease variable such that 1 denoted healthy teeth and higher values indicated the presence of disease, whereas other authors (10, 11) have generally set cutoff for health at 2. Kirklevang reported in 2014 that using the conventional PAI cutoffs offered a higher diagnostic value. Our study provided no prognoses for the teeth in our sample of participants. One final limitation was that we were unable to contact several patients, significantly reducing our sample size and thus directly affecting our analysis.

Conclusions

In conclusion, we found that the techniques for root canal treatment aimed at preventing or reducing apical periodontitis were effective in all cases evaluated in our sample of Mexican adults.
Additional studies and larger samples involving follow-up treatments are required to obtain definitive results. Expanding the study to include multiple clinics would also represent an improvement on our study design.

Clinical Relevance

In conjunction with clinical parameters, PAI is an important tool in evaluating the success of root canal treatment.

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Conflict of Interest

The authors declare no competing interests.

References

1. *Trope M. The vital tooth–its importance in the study and practice of endodontics. Endod Topics 2003;5:1-1.*
2. *García-Rubio A, Al BD, Rodríguez-Archilla. Lesiones periapicales. Diagnóstico y tratamiento. Av. Odontoestomatol. 2015;31:31-42.*
3. *Molander A, Reit C, Dahlen G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. Int Endod J. 1998;31:1-7.*
4. *Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol. 1965;20:340-349.*
5. *Paula-Silva FWG de, Wu MK, Leonardo MR, Bezerra da Silva LA, Wesselink PR. Accuracy of Periapical Radiography and Cone-Beam Computed Tomography Scans in Diagnosing Apical Periodontitis Using Histopathological Findings as a Gold Standard. J Endod. 1986;2:20-34.*
6. *Ørstavik D, Kerekes K, Eriksen HM. The periapical index: a scoring system for radiographic assessment of apical periodontitis. Dental Traumatology 1986;2:20-34.*
7. *Trope M, Delano EO, Orstavik D. Endodontic treatment of teeth with apical periodontitis: single vs. multivisit treatment. J Endod. 1999;25:345-50.*
8. *Estrela C, Bueno MR, Leles CR, Azevedo B, Azevedo JR. Accuracy of cone beam computed tomography and panoramic and periapical radiography for detection of apical periodontitis. J Endod. 2008;34:273-9.*
9. *Chevigny C, Dao TT, Basrani BR, Marquis V, Farzaneh M, Abitbol S, Friedman S. Treatment outcome in endodontics: the Toronto study-phase 4: initial treatment. J Endod. 2008;34:258-63.*
10. *Boucher Y, Matossian L, Rilliard F, Machtou P. Radiographic evaluation of the prevalence and echnical quality of root canal treatment in a French subpopulation. Int Endod J. 2002;35:229-38.*
11. *Medina-Solís CE, Pontigo-Loyola AP, Pérez-Campos E, Hernández-Cruz P, De la Rosa-Santillana R, Navarrete-Hernandez JJ, et al. Principales razones de extracción de dientes permanentes en una muestra de adultos mexicanos. Rev Invest Clín. 2013;65:141-149.*
12. *Delgado-Perez VJ, De La Rosa-Santillana R, Medina-Solís CE, Pontigo-Loyola AP, Navarrete-Hernández JJ, Casanova-Rosado JF, et al. Principales razones de extracción de dientes permanentes de adultos mexicanos en un Centro de Salud. Revista CES Salud Pública 2017;8:1-9.*
13. *Ørstavik D, Kerekes K, Eriksen HM. Clinical performance of three endodontic sealers. Endod Dent Traumatol. 1987;3:178-186.*
14. *Sjögren U, Hagglund B, Sundqvist G, Wing K. Factors affecting the long-term results of endodontic treatment. J Endod. 1990;16:498-504.*
15. *Strindberg, L. The dependence of the results of pulp therapy on certain factors. An analytic study based on radiographic and clinical follow-up examination. Acta Odont Scand 1956;14:21.*
16. *Peters OA, Barbakow F, Peters CI. An analysis of endodontic treatment with three nickel-titanium rotary root canal preparation techniques. Int Endod J. 2004;37:849-859.*
17. *Dugas NN, Lawrence HP, Teplitsky PE, Pharoah MJ, Friedman S. Periapical health and treatment quality assessment of root-filled teeth in two Canadian populations. Int Endod J. 2003;36:181-192.*
18. *Kirkevang LL, Vaeth M, Hörsted-Bindslev P, Bahrami G, Wenzel A. Risk factors for developing apical periodontitis in a general population. Int Endod J. 2007;40:290-299.*
19. *Eriksen HM, Bjertness E. Prevalence of apical periodontitis and results of endodontic treatment in middle-aged adults in Norway. Endod Dent Traumatol. 1991;7:1-4.*
20. *Löst C. Quality guidelines for endodontic treatment: Consensus report of the European Society of Endodontology. Int Endod J 2006;39(12):921-30.*

21. Sunay H, Tanalp J, Dikbas I, Bayirli G. Cross-sectional evaluation of the periapical status and quality of root canal treatment in a selected population of urban Turkish adults. Int Endod J. 2007;40: 139-45.
22. Lupi-Pegurier L, Bertrand MF, Muller-Bolla M, Rocca JP, Bolla M. Periapical status, prevalence and quality of endodontic treatment in an adult French population. Int Endod J. 2002; 35: 690-697.
23. Peršić, R, Kqiku, L, Brumini, G, Husetić M, Pezelj-Ribarić S, Brekalo Prso I, et al. Difference in the periapical status of endodontically treated teeth between the samples of Croatian and Austrian adult patients. Croat Med J. 2011;52:672-678.
24. Marquis VL, Dao T, Farzaneh M, Abitbol S, Friedman S. Treatment outcome in endodontics: the Toronto Study. Phase III: initial treatment. J Endod. 2006;32:299-306.
25. Chevigny C, Dao TT, Basrani BR, Marquis V, Farzaneh M, Abitbol S, et al. Treatment Outcome in Endodontics: The Toronto Study-Phase 4: Initial Treatment. J Endod. 2008;34:258–263.
26. Imfeld TN. Prevalence and quality of endodontic treatment in an elderly urban population of Switzerland. J Endod. 1991;17:604-607.
27. Tarim Ertas E, Ertas H, Sisman Y, Sagsen B, Er O. Radiographic assessment of the technical quality and periapical health of root-filled teeth performed by general practitioners in a Turkish subpopulation. Scientific World Journal 2013;2013:514841.
28. Asgary S, Shadman B, Ghalamkarpour Z, Shahravan A, Ghoddusi J, Bagherpour A. Periapical status and quality of root canal fillings and coronal restorations in iranian population. Iran Endod J. 2010;5:74-82.
29. Lupi-Pegurier L, Bertrand MF, Muller-Bolla M, Rocca JP, Bolla M. Periapical status, prevalence and quality of endodontic treatment in an adult French population. Int Endod J. 2002;35:690-697.
30. Loftus JJ, Keating AP, McCartan BE. Periapical status and quality of endodontic treatment in an adult Irish population. Int Endod J. 2005;38:81-86.
31. Kirkevang LL, Ørstavik D, Wenzel A, Væth M. Prognostic value of the full-scale Periapical Index. Int Endod J 2015;48(11):1051-8.
32. Prati C, Pirani C, Zamparini F, Gatto MR, Gandolfi MG. A 20-year historical prospective cohort study of root canal treatments. A Multilevel analysis. Int Endod J 2018;51(9):955-968.

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ORIGINAL ARTICLE

Surface integrity of root ends following apical resection with targeted trephine burs

ABSTRACT

Aim: *Targeted endodontic microsurgery may be performed utilizing trephine burs. The purpose of this study was to determine if guided resection using trephine burs produces cracks or fractures within the root.*
Methodology: *Twenty maxillary anterior and twenty mandibular molar mesial roots from extracted teeth were cleaned, shaped, and obturated. Roots were resected with either targeted trephine burs or multipurpose burs. Resected root ends were analyzed using light microscopy with a fluorescent filter and a dental operating microscope. Teeth were graded based on the presence, extent, and location of cracks.*
Results: *One (10%) anterior trephine-resected root demonstrated cracks, while three (30%) anterior multipurpose bur-resected roots had cracks. These findings were not statistically different (P=0.264, Chi-square=1.25). No molar teeth had detectable cracks. There was no significant difference between the groups with regards to crack formation.*
Conclusions: *Analysis of the samples supports the use of trephine burs in targeted endodontic microsurgery.*

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Introduction

The surgical removal of root ends, or root end resection, is a treatment for teeth that have undergone non-surgical root canal therapy without successful resolution of the apical pathosis. The goal is to eliminate periapical pathologic tissues and irritants in order to promote healthy tissue regeneration. By removing the last 3-4 mm of the root, one can eliminate the majority of accessory canals and apical ramifications where resistant bacteria may reside (1, 2).
In recent decades, the introduction of the dental operating microscope, piezoelectric ultrasonics, and more biocompatible root end filling materials has led to improved success rates in endodontic root end surgery. A meta-analysis of the literature found that endodontic microsurgery using modern techniques had a success rate of 91.6% (3). Despite these outcomes, anatomical challenges and restrictive access to the root end deter many skilled endodontists from tackling more challenging cases. Results of a web-based survey reported that 34.3% of responding endodontists were referring at least some endodontic surgeries to oral surgeons (4). Mandibular and maxillary posterior teeth present many of the greatest challenges due to anatomical considerations such as the thickness of the buccal plate, decreased visibility and access, shallow vestibules, and proximity to vital structures such as the inferior alveolar nerve, the greater palatine artery, and the maxillary sinuses (5).
With the advent of cone beam computed tomography, improved treatment planning can be accomplished in three dimensions. In the field of implant dentistry, CBCT has been used in combination with CAD/CAM software and 3-dimensional printing to guide precision implant placement, ensuring proper angulation while avoiding vital structures (6). These same technologies can likewise be used to guide precision endodontic surgeries. With the use of a CBCT scan and CAD/CAM software, a surgical guide can be created to aid in the osteotomy and root end resection while maintaining adequate access to the desired root, thereby overcoming many of the challenges of endodontic microsurgery.
Using these technologies, osteotomy size, bevel angulation, and the level of apical root end resection can be virtually planned. In a preclinical study, Pinsky et al. found that osteotomies performed utilizing CBCT and CAD/CAM surgical guides were able to localize the root apices more precisely and consistently than freehand osteotomies (7). Strbac et al. reported on the use of a surgical guide and piezoelectric ultrasonic instruments to remove cortical bone to gain access to the root apex (8). Ahn et al. presented a case report in which a CAD/CAM surgical template was utilized to guide an anchor drill, localizing the apex of a mandibular molar through thick cortical bone (9). Depending on the surgical site, the custom designed surgical guides can be supported by mucosa, teeth, bone, or with a combination technique which can lend additional support in soft tissue retraction.
Recently, Giacomino et al. described a technique they termed targeted endodontic microsurgery (TEMS) in which root end resection is performed utilizing a 3-dimensionally printed guide directing a surgical trephine bur (10). A recent study comparing TEMS with traditional endodontic microsurgery found that TEMS reduces surgical time and the amount of over-resection of the root (11). In addition, Popowicz et al. report on two cases in which they utilized the cortical plate harvested with the trephine bur during tissue grafting procedures (12). A case report by Antal et al. presented the use of custom trephine burs with a TEMS surgical guide (13). Unlike conventional trephine burs with somewhat wider cutting ends, the authors designed the custom burs with uniform diameters. Since the cutting action of trephine burs is different from currently utilized instruments, the mechanical effects of a TEMS resection technique on root ends are currently unknown. Such effects could include creation and/or propagation of dentinal cracks, which could affect the prognosis of such procedures. The purpose of this research was to evaluate any such effects.

Materials and Methods

Extracted human maxillary anterior and mandibular molar teeth were collected with patients' consent under a protocol approved by the Dwight D. Eisenhower Army Medical Center Institutional Review Board. All extracted teeth were stored in 0.1% NaOCl for no longer than two months. Mesial roots were sectioned from the mandibular molars using a diamond-coated disc. Microscopic evaluation of the roots was performed and those roots with suspected cracks or surface defects were excluded from the study. Forty roots were divided into two groups. Each group (n=20) consisted of 10 maxillary anterior roots and 10 mandibular molar mesial roots. Group 1 served as the control group in which the roots would be resected with a multipurpose bur (Dentsply Maillefer, Johnson City, TN, USA) while Group 2 was the experimental group in which roots would be resected with the guided 5.1 mm trephine burs (BIOMET 3i, Palm Beach Gardens, FL, USA).

Utilizing a dental operating microscope, non-surgical root canal therapy was completed on all roots. Roots were wrapped in saline-soaked gauze to keep them moist during the procedure. Accesses were made with a 557 bur in a high speed handpiece. The working lengths were recorded by visually placing a size 10 K-file until it was visible at the apical foramen and subtracting 1 mm. Root canals were cleaned and shaped using Vortex Blue (Dentsply Sirona, York, PA, USA) rotary files utilizing a crown-down technique to a maximum apical file size of 35/.04 (molar roots) or 50/.04 (anterior roots). Irrigation with 8.25% sodium hypochlorite between each successive file and a final rinse of 5 ml 17% EDTA and 5 ml 8.25% NaOCl was performed in each canal. Following cleaning and shaping, each canal was obturated using gutta-percha and AH Plus sealer (Dentsply Sirona, York, PA, USA) utilizing the warm vertical compaction technique. Following non-surgical treatment, the dental operating microscope and methylene blue dye were used to inspect the roots for any cracks or dentinal defects sustained during instrumentation and obturation.

A custom jig was designed to include a three-dimensionally printed surgical guide fabricated in conjunction with the Army Dental Laboratory at Fort Gordon, Georgia (Figure 1). Roots were secured within the guide using polyvinyl siloxane (PVS). The guide was manufactured to split along its vertical dimension, allowing the teeth to be removed easily. The two halves of the guide were fastened together with a benchtop vise.

Group 1 control roots were resected using a multipurpose bur with continuous water spray. Burs were premeasured based on parameters of the surgical guide to ensure a complete resection. The resection was completed with a single pass of the multi-purpose bur in a mesial-distal direction to create a level plane. No

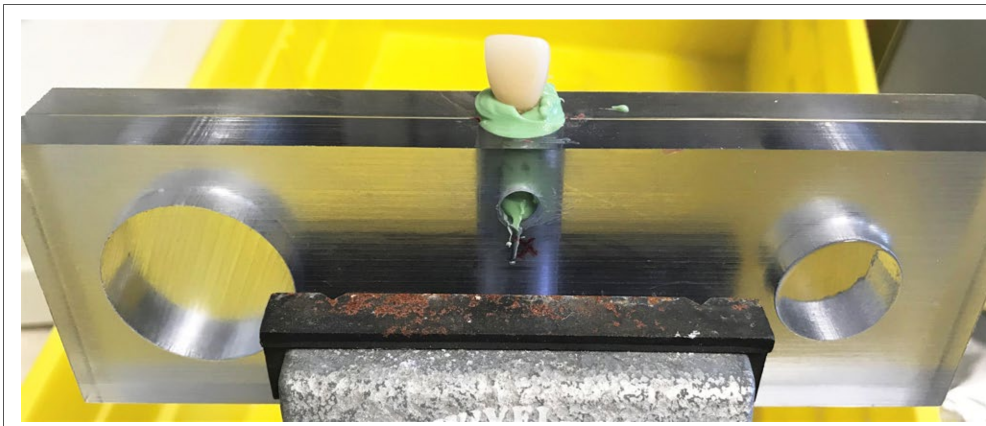
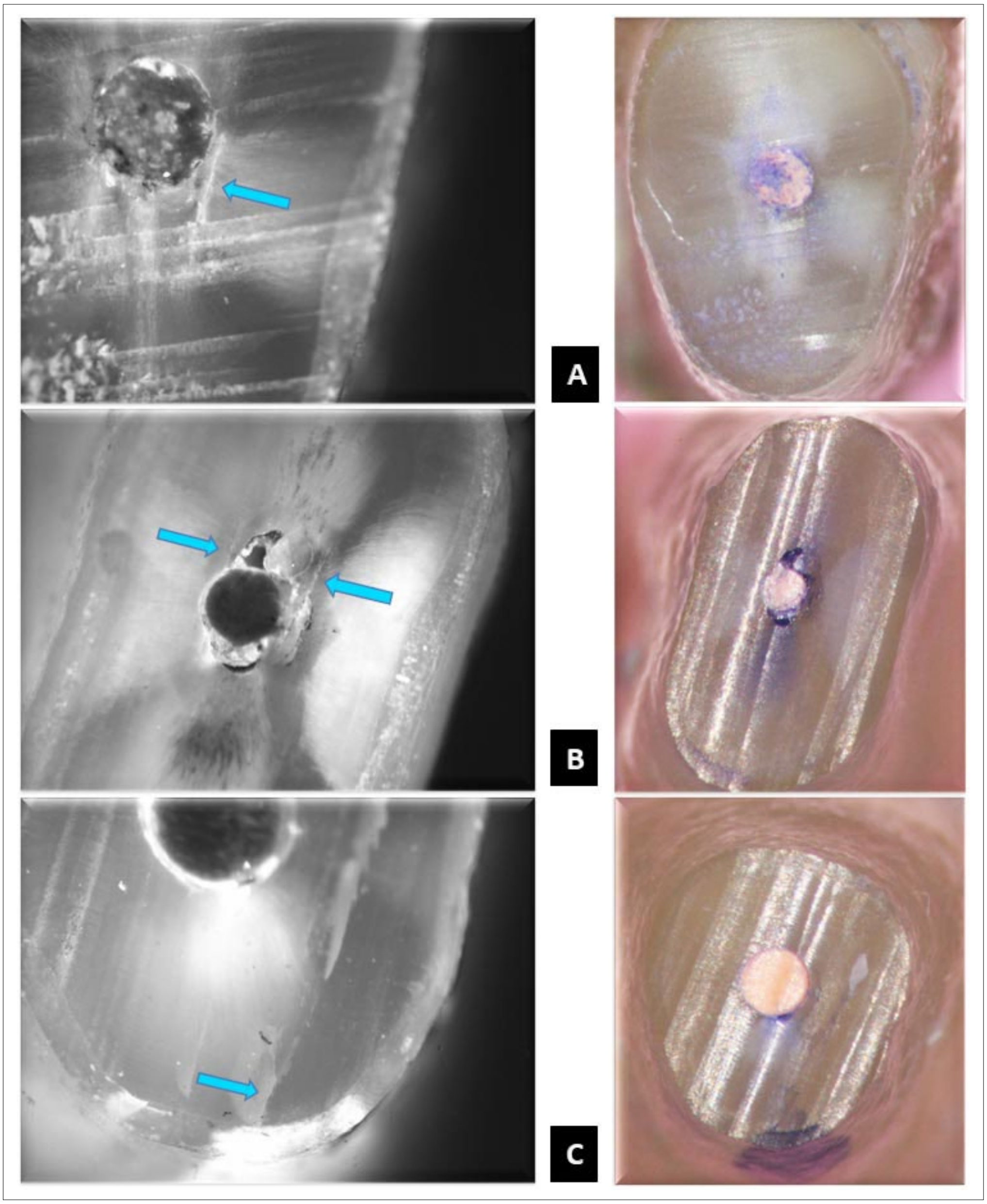


Figure 1. Custom-designed experimental jig with tooth in place.

Figure 2.
A) Microscopic image of a maxillary anterior root end following resection with a trephine bur showing transverse striations and an incomplete crack confined to the dentin, with corresponding photograph.
B) Microscopic image of a maxillary anterior root end following resection with a multipurpose bur showing characteristic striations and incomplete cracks radiating from the canal outward, with corresponding photograph.
C) Microscopic image of a maxillary anterior root end following resection with a multipurpose bur showing characteristic striations and an incomplete crack radiating from the external root surface inward, with corresponding photograph.



additional smoothing was performed. Group 2 experimental roots were resected using a 5.1 mm diameter trephine bur utilizing an electric handpiece (W&H, Dental Werk Bürmoos GmbH, Austria) rotating at the manufacturer's recommendation of 1000 rpm with maximum torque and continuous water irrigation. The root ends were resected with intermittent pressure from the bur from buccal to lingual. Following each resection, the root was removed from the PVS material and immediately placed in 100% humidity in appropriately labeled containers for future observation.

Root surfaces were observed with light microscopy and analyzed by four blinded observers who were calibrated to interpret the microscopic images. In cases of disagreement, consensus was achieved following a discussion categorizing each tooth. Analysis of the root surface was based on three categories, from least to most severe: 0-no cracks, 1-incomplete cracks (originating from the root canal and radiating into the dentin, originating

Table 1
Analysis of the root surface from least to most severe

Method of Resection	Tooth Type	Number of Teeth with Defects	Category of Defects
Multipurpose Bur	Anterior	3/10	1 (i), 1(e), 1 (id)
	Molar	1/10	d
Trephine Bur	Anterior	1/10	1 (c)
	Molar	1/10	d

0=No cracks, 1=incomplete cracks (originating from the root canal and radiating into the dentin or originating from the root surface radiating into the dentin, or confined to dentin), and 2=complete cracks (from the root canal to the root surface). Cracks and defects were further subcategorized as follows: (i) for internal, (e) for external, (c) for confined, and damage to cementum exposing dentin was identified with a (d).

from the root surface radiating into the dentin, or confined to dentin), and 2-complete cracks (from the root canal to the root surface or from the root surface to another root surface). Cracks were further subcategorized by location as follows: (i) internal, (e) external, and (c) confined. Damage to cementum that resulted in exposed circumferential dentin was identified with a (d). Chi-square analysis was performed to compare the groups.

Results

Inspection of the roots with methylene blue and the dental operating microscope did not reveal any cracks or defects resulting from the instrumentation and obturation of the teeth. Following apical root resection, the majority of teeth did not have any defects that could be identified by light microscopic analysis. Results are listed in Table 1. All cracks observed were category 1. Representative examples can be found in Figure 2. One (10%) anterior trephine-resected root demonstrated cracks, while three (30%) anterior multipurpose bur-resected roots had cracks, one of which also demonstrated damage to the cementum. These findings were not statistically different (P=0.264, Chi-square=1.25). No molar teeth in either group had detectable cracks. One molar from each group demonstrated damage to the cementum with no associated cracks. No significant difference in crack or defect prevalence between the trephine and multi-purpose bur groups was observed when combined molar and anterior teeth were analyzed (P=0.292, Chi-square=1.11).

Discussion

Surface integrity of the resected root end was evaluated based on crack production and external root surface damage. The trephine bur gave the most uniplanar resection, leaving only minor mesial-distal, transverse striations in some cases, while the multi-purpose bur produced more cracks as well as the characteristic buccal-lingual striations shown in Figure 2. Such striations from fissure bur resections have been described previously (11). In order to maintain consistency within the study, only one pass of the bur through the root end was allotted in each group. The favorable results of the trephine bur resection might be attributed to the surgical guide which limited bur movement.
Another factor which may have contributed to its smooth cutting and efficiency is the engineering of the bur with end cutting teeth. One distinct difference with the resection produced by the trephine bur was the concave surface that it created due to the bur's cylindrical shape. This may expose additional dentinal tubules at the periphery of the root surface. The significance of this difference and whether it poses a threat to long term success is unknown. Future studies may be performed to inspect these areas for residual biofilms. If desired, the root

surface can be smoothed following the initial resection. The term microcrack may be more suitable to describe the defects seen as they could not be stained with methylene blue dye or observed with a dental operating microscope. These defects could only be observed using a high powered light microscope at 40x magnification with the aid of a fluorescent light filter which reduced white light exposure. Since some in vitro studies have shown that instrumentation and obturation of root canals may cause cracks and induce stresses within root dentin (14-18), it is possible that, immediately prior to resection, the inspection of the roots with methylene blue and the dental operating microscope inappropriately identified the roots as intact. With this in mind, one possible explanation for the microcracks only occurring within anterior teeth in this study could be related to the larger diameter files resulting in excessive stresses being applied to the roots.

The custom jig used in this study attempted to replicate an in vivo environment where the roots would only have physiologic mobility while providing a surgical guide for the bur to cut with minimal deviation. The PVS material used in this study did not limit the cutting efficiency of the trephine bur and provided adequate stability to the root during resection. One limiting factor in the model was the absence of a lesion as would be common in most clinical scenarios. However, in order to stabilize the root within the guide, PVS material needed to fully encompass the root. This method was chosen to enable reproducibility within the study, minimizing variables such as bone loss and lesion size. The teeth in this experiment were stored in a 0.1% NaOCl solution since a previous study demonstrated that this concentration was safe for storing extracted teeth without affecting their hardness values (19), but it is still possible that the cracks identified in this study may have been affected by the ex vivo storage of the roots. Even with the confounding factors inherent in an extracted tooth model of this type, if significant differences were found between the resections with the trephine bur and resections with the multipurpose bur, it would have increased concerns when utilizing this promising surgical approach. While no significant differences were observed in this preliminary investigation, further studies utilizing a cadaver or in vivo model are appropriate. Many different methodologies have been employed to evaluate the creation or presence of radicular microcracks, including endoscopy, scanning electron microscopy, infrared thermography, and micro-computed tomography (20). In future studies, these additional techniques should be considered to evaluate crack formation during trephine bur resection.

Conclusions

Under the conditions of this study, there was no significant difference in crack production by root end resections utilizing either multi-purpose burs or trephine burs. The results of this study support the use of trephine burs for root end resections in targeted endodontic microsurgery.

Clinical Relevance

This study found that targeted endodontic microsurgery (TEMS) using trephine burs does not appear to increase the risk of dentinal microcracks or other defects compared to traditional techniques of root resection.

Conflict of Interest

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

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References

1. Kim S, Pecora G, Rubinstein R. Comparison of traditional and microsurgery in endodontics. In: Kim S, Pecora G, Rubinstein R, eds. Color atlas of microsurgery in endodontics. Philadelphia: W.B. Saunders, 2001: 5-11.
2. Degerness R, Bowles W. Anatomic determination of the mesiobuccal root resection level in maxillary molars. J Endod 2008; 34: 1182-1186.
3. Tsesis I, Faivishevsky V, Kfir A, Rosen E. Outcome of surgical endodontic treatment performed by a modern technique: A meta-analysis of literature. J Endod 2009; 35: 1505-1511.
4. Creasy J, Mines P, Sweet M. Surgical trends among endodontists: the results of a web-based survey. J Endod 2009; 35: 30-34.
5. Gutmann J, Harrison J. Posterior endodontic surgery: anatomic considerations and clinical techniques. Int Endod J 1985; 18: 8-34.
6. Kobayashi K, Shimoda S, Nakagawa Y, Yamamoto A. Accuracy in measurement of distance using limited cone-beam computerized tomography. Int J Oral Maxillofac Implants 2004; 19: 228–231.
7. Pinsky H, Champleboux G, Sarment D. Periapical surgery using CAD/CAM guidance: Preclinical results. J Endod 2007; 33: 148-151.
8. Strbac G, Schnappauf A, Giannis K, Moritz A, Ulm C. Guided modern endodontic surgery: A novel approach for guided osteotomy and root resection. J Endod 2017; 43: 496-501.
9. Ahn S, Kim N, Kim S, Karabucak B, Kim E. Computer-aided design/computer-aided manufacturing-guided endodontic surgery: Guided osteotomy and apex localization in a mandibular molar with a thick buccal bone plate. J Endod 2018; 44: 665-670.
10. Giacomino M, Ray J, Wealleans J. Targeted endodontic microsurgery: a novel approach to anatomically challenging scenarios using 3-dimensional-printed guides and trephine burs – a report of 3 cases. J Endod 2018; 44: 671-677.
11. Hawkins T, Wealleans J, Pratt A, Ray J. Targeted endodontic microsurgery and endodontic microsurgery: a surgical simulation comparison. Int Endod J 2020; 53: 715-722.
12. Popowicz W, Palatynska-Ulatowska A, Kohli R. Targeted endodontic microsurgery: computed tomography-based guided approach with platelet-rich fibrin graft: a report of 2 cases. J Endod 2019; 45: 1535-1542.
13. Antal M, Nagy E, Sanyo L, Braunitzer G. Digitally planned root end surgery with static guide and custom trephine burs: A case report. Int J Med Robotics Comput Assist Surg 2020; 16: e2115.
14. Morgan L, Marshall J. The topography of root ends resected with fissure burs and refined with two types of finishing burs. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998; 85: 585-591.
15. Meister F, Lommel T, Gerstein H. Diagnosis and possible causes of vertical root fractures. Oral Surg Oral Med Oral Pathol 1980; 49: 243–253.
16. Onnink P, Davis R, Wayman B. An in-vitro comparison of incomplete root fractures associated with three obturation techniques. J Endod 1994; 20: 32-7.
17. Wilcox L, Roskelley C, Sutton T. The relationship of root canal enlargement to finger-spreader induced vertical root fracture. J Endod 1997; 23: 533–534.
18. Kim H-C, Lee M-H, Yum J, Versluis A, Lee C-J, Kim B-M. Potential relationship between design of nickel–titanium rotary instruments and vertical root fracture. J Endod 2010; 36: 1195–1199.
19. Aydin B, Pamir T, Baltaci A, Orman M, Tugba T. Effect of storage solutions on microhardness of crown enamel and dentin. Eur J Dent 2015; 9: 262–266.
20. Versiani M, Souza E, De-Deus G. Critical appraisal of studies on dentinal radicular microcracks in endodontics: methodological issues, contemporary concepts, and future perspective. Endod Topics 2015; 33: 87-156.

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ORIGINAL ARTICLE

Analysis of the ability of XP Clean and Easy Clean files to remove Calcium Hydroxide paste from simulated internal tooth resorptions

ABSTRACT

Aim: This study compared the ability of instruments XP Clean and Easy Clean to remove the calcium hydroxide paste from simulated internal root resorptions.
Methodology: Thirty freshly extracted human lower incisors were sectioned through the long axis after biomechanical preparation with ProTaper files. On each half, a defect simulating an internal resorption was fabricated on the middle root canal third using a round bur. One week after insertion of calcium hydroxide paste, the teeth were divided into three groups according to the technique employed: G1-Conventional irrigation; G2-XP Clean and G3-Easy Clean. Each instrument was used in three cycles of 20 seconds, followed by renewal with 2 ml of irrigant. Thereafter, the specimens were analyzed on a stereoscopic magnifying glass to analyze the removal of dressing material.
Results: Similar performance was observed for both methods employed, even though the rates of absence of removal were higher for the group Easy Clean compared to the group XP Clean.
Conclusions: The XP Clean presented better results in removing the calcium hydroxide paste compared to Easy Clean, with statistically significant difference.

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Introduction

Root resorption in the permanent dentition is a pathology that causes loss of hard tissue as a result of osteoclastic cell activity, and may be classified as internal or external according to its location. Internal resorption initiates inside the root canal, tends to be asymptomatic, and is usually caused by chronic infection or trauma (1).

The treatment of choice to arrest this destructive process is non-surgical endodontic therapy. However, the irregular configuration of internal resorption lesions poses a technical difficulty for complete debridement, thus it is recommended to use an intracanal antibacterial drug to enhance the disinfection of defects produced by root resorption (1-3).

The calcium hydroxide paste has been the drug of choice for this purpose, since it presents a dissolution effect on the tissue, allowing removal of the remaining dental pulp in the internal root defect after the calcium hydroxide paste is kept in situ for several weeks (1, 4). However, the literature describes difficulty in removing this material from the irregularities of resorption by conventional irrigation procedures, which represents a disadvantage, since calcium hydroxide remnants on the root canal walls may affect the penetration of endodontic sealers into the dentinal tubules and chemically react with the sealer, affecting its physical properties (2, 3). This encouraged the search for different techniques for activation of irrigants inside the root canal system, to improve the effectiveness of irrigation (5, 6).

One such device is the Easy Clean file, composed of plastic (acrylonitrile butadiene styrene) with size of 25/.04, used as an irrigation aid for completion of endodontic treatment. These files are pre-sterilized, designed for single use to maximize the removal of debris removal and agitate the irrigants, offering advantages concerning time and cost saving. This material also provides flexibility to the instruments, allowing their utilization to the working length even in the presence of curvatures (7, 8). According to Prado et al. (7) the Easy Clean presents a particular shape, similar to that of an "airplane wing", characterized by a central matrix associated with two delicate edges throughout the instrument. These characteristic format generates two mechanisms of action, one represented by the agitation of the irrigating solution, dispersing forces within the canal, and the second determined by direct contact of the instrument with the root canal walls whose friction allows mechanical removal of debris and adhered biofilms, without causing damage, such as the formation of deviations in the root canal path.

Another device available in the market is the XP-Endo Finisher file, indicated for use as final irrigation protocol to improve the effectiveness of root canal disinfection. It is a 0.25 mm diameter cylindrical rotary instrument fabricated with a special NiTi alloy that allows change in shape according to the temperature. At room temperature the file is straight; when subjected to body temperatures it assumes a spoon shape with 1.5 mm depth in the final 10 mm of length. According to the manufacturer, due to its increased flexibility and capacity of expansion to adapt to the root canal three-dimensionally, it allows removal of accumulated hard tissue debris, smear layer and calcium hydroxide paste from the root canal system, without damaging dentin or altering the original root canal shape (9-11).

A finishing file recently introduced in Brazilian market, XP-Clean, has a similar design to XP-Endo Finisher yet without changing in shape according to the temperature variation. The XP-Clean is an asymmetrically shaped file made of nickel titanium alloy with thermal treatment of surface and triangular cross section, its tip is compatible with a file number 25 and taper 0.02. The speed of use recommended by the manufacturer is 800 to 900 rpm with a torque up to 1N, and it should penetrate 1mm below the working length in straight root canal and 2 to 3mm below curved root canals. This instrument is indicated for after the completion of the biomechanical preparation in order to achieve the same mechanisms described for Easy Clean, that is, promoting a swirling of the irrigating

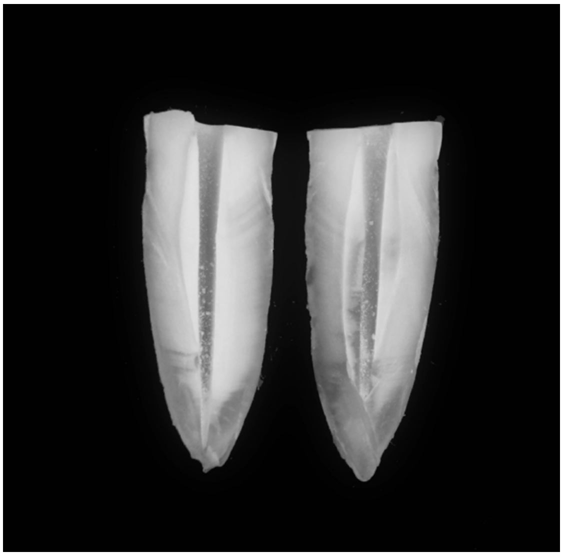

Figure 1. Lower incisor divided into two parts.

solution inside the root canal at the same time that its stem comes into mechanical contact with the internal walls providing a double cleaning action (11).
Thus, this study compared the ability of XP Clean and Easy Clean instruments to remove the calcium hydroxide paste from gaps inside the root canal simulating internal tooth resorption.

Materials and Methods

This study was conducted on 30 single-rooted human lower incisors freshly extracted at the Discipline of Oral and Maxillofacial Surgery of the Dental School of Unipar, Campus Sede, Umuarama, according to the reasons observed during the diagnosis and treatment plan previously elaborated with the assistance of a supervising professor, accepted by the patient, earlier submitted and approved by the Institutional Review Board (CEPEH), Paranaense University, Umuarama Campus, under protocol 99834118.9.0000.0109.
After extraction, the teeth were washed in running water and their surface was scraped in order to remove any type of residue, then they were radiographed in the mesio-distal direction to prove the existence of only one root canal and then they were kept in a flask with formalin until accomplishment of the procedures, which were carried out by a single operator so that there was a standardization in the execution of each step described below.
A perpendicular section was performed through the tooth long axis using stainless steel discs, removing the coronal portion to standardize a 15 mm remnant from the root apex. Next, exploration was performed with Kerr hand files n. 8, 10 and 15 to achieve patency of the apical foramen.
ProTaper rotary files (Dentsply Sirona, Ballaigues, Switzerland) were used for biomechanical preparation, with crosshead and torque defined according to the manufacturer's recommendations. The first instrument of the system, the SX file, had its penetration limit set at 10 mm to restrict its action to the cervical and middle thirds, while the other instruments, from file S1 to F3, were introduced at the standardized working length of 15 mm. Throughout the preparation, irrigation was performed with 2 ml of 1% sodium hypochlorite to remove debris after utilization of each instrument.
After instrumentation, the teeth were inserted into anesthetic tubes with putty silicone (Zetaplus, Zhermack, Badia Polesine, Rovigo, Italy). After setting of the impression material, the roots were removed from the mold and longitudinal grooves were fabricated in the buccal and lingual surfaces using a diamond disc connected to a low-speed adapter coupled to a straight handpiece under copious irrigation with water, avoiding penetration into the root canal. The roots were then separated into two halves using a chisel and hammer (Figure 1).
With the aid of a digital caliper, a mark was made on the middle third of each section at a distance of 7 mm from the most coronal portion of the tooth (Figure 2A), ensuring that the cavity simulating an internal root resorption was fabricated at the same site in both dental segments. The grindings made with a round diamond bur 1014 (KG Sorensen, Cotia, São Paulo, Brasil) had 0.5 mm depth and 1.6 mm diameter (Figure 2B). The two halves of the tooth were reconnected and repositioned in the mold previously made, and a McSpadden condenser (Dentsply Sirona, Ballaigues, Switzerland) adapted to a

Figure 2.
A) Determination of the middle third using a digital pachymeter.
B) Grinding with diamond burs simulating internal root resorption.

handpiece rotating in counterclockwise direction was used to apply a paste containing calcium hydroxide and iodoform, at a 3: 1 ratio, with sufficient saline as vehicle to achieve a pasty mixture. The complete filling of spaces was checked by images obtained by a radiographic sensor (Figure 3).
The specimens were kept in oven at 37 °C and 100% humidity for a period of one week. After this period, the lower incisors were divided into three groups with 10 teeth each and the calcium hydroxide paste was removed by the following protocol:

- Group 1: a 6 ml irrigation cycle with saline was performed using a 25X4 disposable needle calibrated at 15 mm with a duration of 60 seconds.
- Group 2: (Figure 4A) three application cycles were performed using the XP Clean file (MK Life, Porto Alegre, Rio Grande do Sul, Brazil) calibrated at 15 mm in a rotary movement driven by the Elements motor (KerrHawe SA, Bioggio, Switzerland) with a speed of 800 rpm and torque of 1 Ncm with duration of 20 seconds each, followed by irrigation with 2 ml of saline.
- Group 3: (Figure 4B) three application cycles of the Easy Clean file (Easy Equipamentos Odontológicos, Belo Horizonte, Minas Gerais, Brazil) calibrated at 15 mm were performed in reciprocating movements, activated through the Reciproc Direct contra-angle (VDW GmbH, Munich, Germany) coupled to the Elements motor (KerrHawe SA, Bioggio, Switzerland) with a speed of 20,000 rpm and torque of 1 Ncm with an angle of 150º counterclockwise and 30º clockwise for 20 seconds each, followed by irrigation with 2 ml of saline.

In all groups, concomitantly with utilization of the instrument to be tested, a back and forth movement was applied to produce greater turbidity of the irrigant inside the canal, thus enhancing the action of dressing removal.
Immediately after dressing removal, the teeth were removed from the molds and

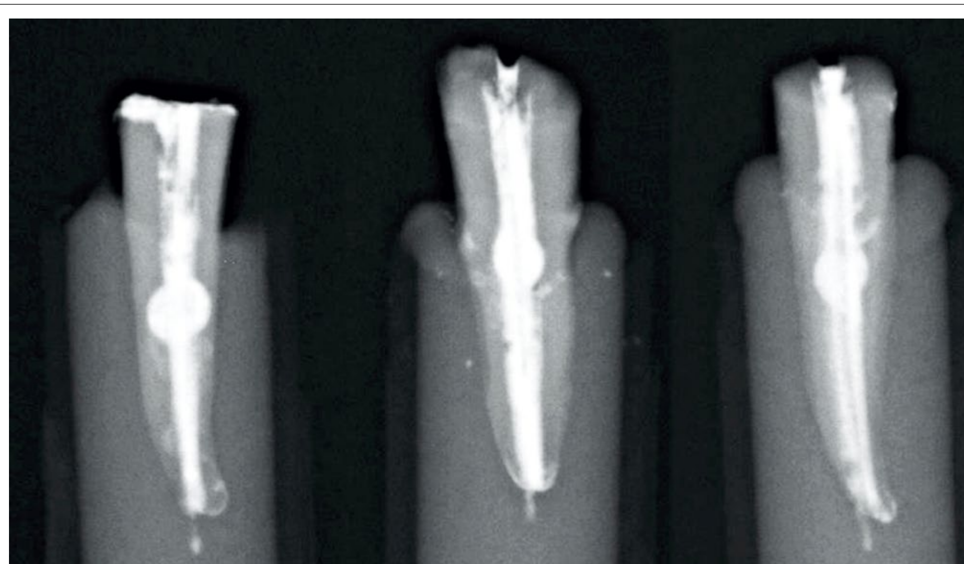

Figure 3.
Digital radiographic images to analyze the filling with calcium hydroxide paste throughout the root canal extent and in the simulated lesion.

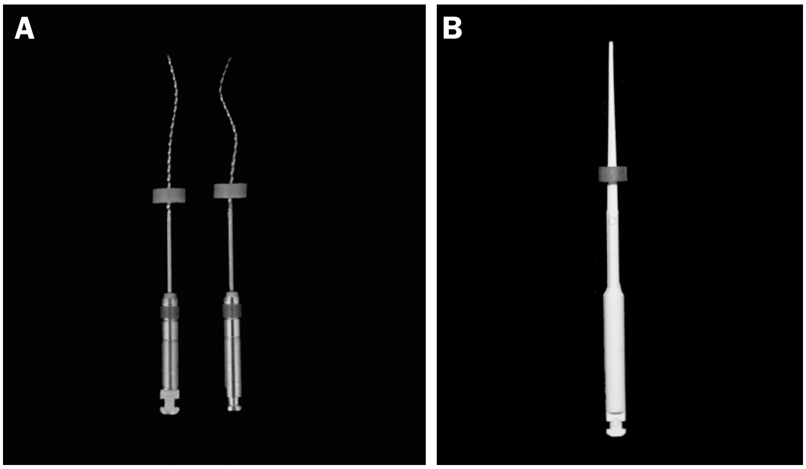

Figure 4.
A) XP Clean files.
B) Easy Clean file.

reopened to observe the simulated internal resorption in a 40x stereoscopic magnifying glass with 40x magnification. Three independent and previously calibrated examiners analyzed each root half and assigned one of the following scores according to the cleanliness of cavities:
-Score 0: indicating no removal of dressing (Figure 5A)
-Score 1: indicating partial removal of calcium hydroxide paste (Figure 5B)
-Score 2: indicating complete removal (Figure 5C)
The most frequent score for each simulated resorption was adopted in case of discrepancy between examiners.

Results

The specimens were analyzed according to the scores assigned by the three examiners. After conventional irrigation with 25x4 mm disposable needles, ten segments presented score 0; eight had score 1 and two segments had score 2. The files XP Clean achieved three segments with score 0; seven segments with score 1 and ten segments with scores 2. The Easy Clean files exhibited nine segments with score 0; ten segments with score 1 and one segment with score 2 (Table 1).
Table 2 presents the comparison of scores between groups. It evidences significant difference in scores between the techniques XP Clean and 25x4 mm needle (p value=0.003) and XP Clean and Easy Clean (p value=0.002), in which the XP Clean achieved higher percentage of score 2 (50.0%), while the techniques Easy Clean and 25x4 mm needle presented higher percentages of score 0 and 1. Also, figure 6 evidences that there was no significant difference in scores between the techniques Easy Clean and 25x4 mm needle (p value=1,000).

Discussion

Internal root resorption is a condition that affects permanent teeth affected by trauma or infections, in which demineralization of the pulp cavity contour produces an irregular anatomy that impairs the achievement of effective root canal cleaning and obturation (3, 10).
Currently, the most appropriate treatment for cases of internal root resorption is the application of a long-term dressing with calcium hydroxide paste after biomechanical preparation. However, complete removal of this paste before root canal filling is an obstacle, since the traditional manner to eliminate it is using a spray of irrigant applied using a syringe and needle (1, 12). This technique provides insufficient dressing

Figure 5.
A) Score 0: without removal of calcium hydroxide.
B) Score 1: partial removal of calcium hydroxide.
C) Score 2: total removal of calcium hydroxide.

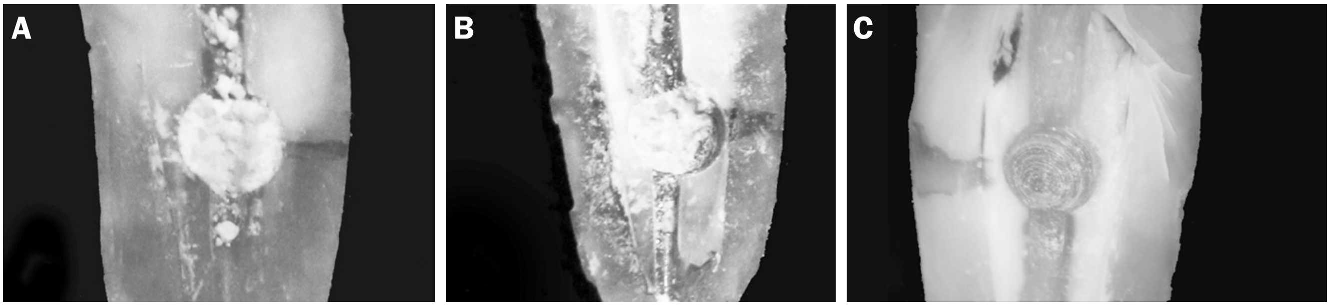

Table 1
Scores assigned by the examiners to each segment in the study groups

Side 1	Side 2	Side 1	Side 2	Side 1	Side 2
1	0	2	1	0	0
1	2	2	1	2	1
0	0	2	2	0	0
1	1	2	2	0	0
0	0	1	2	1	1
0	1	2	1	1	1
0	1	0	0	0	0
0	2	1	0	0	1
0	1	2	1	1	1
0	1	2	1	1	1
Disposable needle		XP Clean		Easy Clean	

Table 2
Comparison of scores between groups

Tecnic	Disposable needle		XP Clean		Easy Clean	
	N	%	N	%	N	%
0	10	50,0%	3	15,0%	9	45,0%
1	8	40,0%	7	35,0%	10	50,0%
2	2	10,0%	10	50,0%	1	5,0%

Cochran Armitage Test (p-Value): Disposable needle x XP=0.003; Disposable needle x Easy=1,000; XP x Easy=0.002.

removal from the irregular resorption regions, as demonstrated in the present study by the achievement of higher percentages of scores indicating absence of cleaning, a similar result as that presented by Topçuoğlu, et al. (1), in which the control group presented the lowest removal of calcium hydroxide.

Thus, several instruments and techniques that can promote the activation of the irrigant have been proposed to enhance the removal of calcium hydroxide paste from the root canal system.

One of these systems, introduced in the Brazilian market, is the XP-Clean file, which was designed to perform a similar effect as that presented by the XP-Endo Finisher endodontic file. As there are few studies in the literature regarding the performance of XP-Clean, we evaluate it legitimate to consider the results presented by the XP-Endo Finisher file as a bibliographic reference to support this discussion. Vaz-Garcia et al. (11) compared the roughness and cyclic fatigue of XP-Endo Finisher and XP-Clean, concluding that the XP-Endo Finisher instruments performed better, showing less roughness and greater resistance to cyclic fatigue than XP Clean. Despite this finding, there was no fracture of any of XP Clean files used in this study; however, it was difficult to introduce them into the root canal due to their pre curvature. Lack of standardization between the files used was also observed, in which one had greater curvature than the other, which led to difference in performance between both, and it was possible to obtain greater removal of calcium hydroxide paste using the file with greater curvature.

Notwithstanding, the XP Clean files presented the best results in this study, reaching 50% of scores representing total removal of calcium hydroxide paste and 35% of scores scored as partial removal. Similar result was observed by Ulusoy et al. (10), who observed greater effectiveness of XP-Endo Finisher files compared to ultrasound inserts in removing organic tissues from the simulated internal root resorptions. Donnermeyer et al. (4) obtained significantly better results with the use of sonic and ultrasonic inserts to remove calcium hydroxide paste from grooves made in the apical portion of root canals.

Easy Clean is another system available in the national market. In this study, the results obtained showed no difference when compared with conventional irrigation, reaching higher percentage of scores with partial removal (50%) or no elimination (45%). A recent study by Silva et al. (6), observed similar performance between Easy Clean and passive ultrasonic irrigation to remove calcium hydroxide paste inserted in simulated lateral canals in resin blocks, and none was able to completely remove the dressing. Conversely, Prado et al. (7) demonstrated improved cleaning capacity of QMix when using ultrasound inserts and Easy Clean compared to conventional irrigation over the same period. In the same study, the authors tested the effectiveness of Easy Clean file in removing smear layer by shaking the

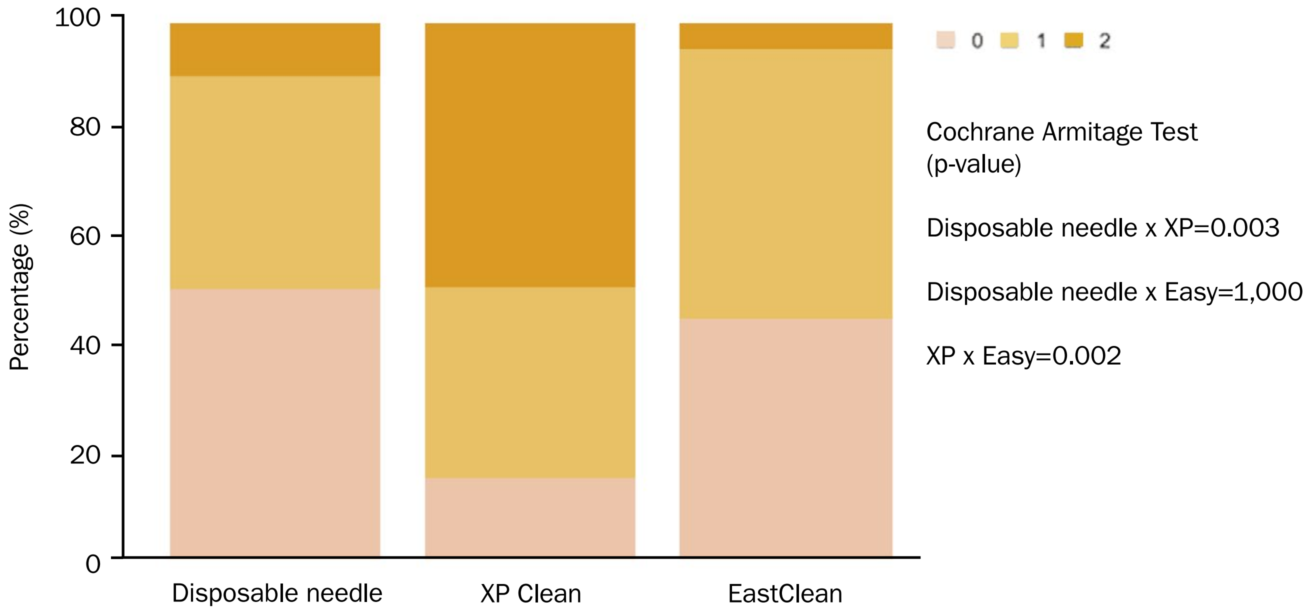

Figure 6.
Table of percentages of instruments employed.

QMix solution in rotational and reciprocating movements, without statistically significant difference between groups. Duque, et al. (13) compared the use of Easy Clean in continuous and reciprocating motion, besides other devices, to verify the effectiveness in removing debris from the root canals and isthmus through scanning electron microscopes obtained after the first, second and third activation of the irrigating solution in cuts made at three different levels, 2, 4 and 6 mm away from the root apex of 50 mesial roots of lower molars. The results showed that the activations of the irrigating solution provided a better cleaning of the canal and isthmus in relation to the conventional irrigation made with a Navitip 30G needle, in addition it was possible to verify that although Easy Clean in continuous rotation had lower percentages of remaining debris, there was no statistically significant difference compared to the use of Easy Clean in reciprocating rotation. Thus, this study used this device in reciprocating motion, as indicated by the manufacturer, and also considering a previous test which revealed that the rotation movement could cause greater instrument deformity.
This study used round diamond burs for preparation of simulated internal root resorption to create uniform and well-characterized cavities, allowing standardization of defects and thus equating the difficulty imposed on instruments to be analyzed (1). A similar approach was adopted regarding definition of the vehicle to be used to manipulate the calcium hydroxide paste, choosing saline to create more obstacle in dressing removal, since the resulting mixture presents drier consistency over time.

Conclusions

Based on the present results, it was concluded that Easy Clean file and conventional irrigation were unable to remove calcium hydroxide paste from simulated internal root resorptions, while XP Clean file showed the best results, evidencing higher capacity in reaching the irregularities fabricated on the root canal walls, with statistically significant difference.

Clinical Relevance

It is important for the dental surgeon to know the result of the comparison of instruments that promote the activation of irrigating solutions such as XP Clean and Easy Clean in order to verify whether they are effectively capable of providing a better cleaning of the root canal, allowing a more efficient removal satisfactory use of the calcium hydroxide paste used during the treatment of internal tooth resorption, especially close to the areas excavated in the dentin.

Conflict of Interest

The authors declare that there is no conflict of interest.

Acknowlegments

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References

1. Topçuoğlu HS, Düzgün S, Ceyhanli KT, et al. Efficacy of different irrigation techniques in the removal of calcium hydroxide from a simulated internal root resorption cavity. Int Endod J. 2015; 48:309-16.
2. Arslan H, Akcay M, Capar ID, et al. An in vitro comparison of irrigation using photon-initiated photoacoustic streaming, ultrasonic, sonic and needle techniques in removing calcium hydroxide. Int Endod J. 2015; 48:246-51.
3. Küçükkaya Eren SK, Aksel H, Parashos P. A novel model for testing the efficiency of removal of calcium hydroxide from complex root canal anatomies. Aust Endod J. 2017; 43:5-10.
4. Lambrianidis T, Kosti E, Boutsioukis C, Mazinis M. Removal efficacy of various calcium hydroxide/ chlorhexidine medicaments from the root canal. Int Endod J. 2006; 39:55-61.
5. Silva RM, Rezende GS, Lino IG, et al. Avaliação da eficiência de diferentes protocolos de irrigação na remoção de pasta de hidróxido de cálcio em canais laterais simulados. Revista Faipe, 2018; 8:1-10.
6. Donnermeyer D, Wyrsch H, Bürklein S, Schäfer E. Removal of calcium hydroxide from artificial grooves in straight root canals: Sonic activation using EDDY versus passive ultrasonic irrigation and XP Endo Finisher. J Endod. 2019; 45:322-6.
7. Prado MC, Leal F, Simão RA, et al. Effectiveness of a plastic endodontic file to remove smear layer in rotary or reciprocating motion. Int J of Clin Dent. 2017; 3:143-151.
8. Prado MC, Leal F, Simão RA, et al. The use of auxiliary devices during irrigation to increase the cleaning ability of a chelating agent. Restor Dent Endod. 2017; 42:105-10.
9. Keskin C, Sariyilmaz E, Sariyilmaz Ö. Efficacy of XP-endo Finisher file in removing calcium hydroxide from simulated internal resorption cavity. J Endod. 2017; 43:126-130.
10. Ulusoy Öi, Savur Ig, Alaçam T, Çelik B. The effectiveness of various irrigation protocols on organic tissue removal from simulated internal resorption defects. Int Endod J2018; 51:1030-6.
11. Vaz-Garcia ES, Vieira VTL, Petitet NPSF, et al. Mechanical properties of anatomic finishing files: XP Endo Finisher and XP-Clean. Braz Dent J. 2018; 29:208-13.
12. Kfir A, Blau-Venezia N, Goldberger T, et al. Efficacy of self-adjusting file, XP-endo finisher and passive ultrasonic irrigation on the removal of calcium hydroxide paste from an artificial standardized groove. Aust Endod J. 2018; 44:26-31.
13. Duque JA, Duarte MAH, Canali LCF, et al. Comparative effectiveness of new mechanical irrigant agitating devices for debris removal from the canal and isthmus of mesial roots of mandibular molars. J Endod. 2017; 43:326-31.

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ORIGINAL ARTICLE

Correlation between root surface strain and apical micro-cracks produced during canal preparation with thermally treated single or multi-file NiTi systems

ABSTRACT

Aim: *To determine the apical root surface strain (RSS) generated during shaping with thermally treated file NiTi systems, and its association with the development of apical microcracks.*
Methodology: *Twenty extracted human mandibular molars with severely curved mesial roots having two separate canals were prepared using XP-Endo Shaper files (Group A: XPS in mesio-buccal canals) and Hyflex CM files (Group B: HCM in mesio-lingual canals). Pre-instrumentation images of apical surface of each root were obtained with Environmental Scanning Electron Microscope (ESEM). Root surface strain (RSS) generated during canal preparation was measured as micro-strain (ìstrain) using electrical strain gauges fixed on apical third. Strain output was digitally recorded to analyze both instantaneous RSS and the maximum RSS. Mean maximum RSS values produced during canal preparation with both systems were tested for statistical significance using independent t-test. Post-instrumentation images were acquired to evaluate the presence/absence of apical microcracks. Examination was performed twice by three blinded examiners at 2-week intervals. Inter- and intra-evaluator reliability was analyzed using the Kappa statistic test. Association between the mean maximum RSS and development of apical microcracks was evaluated by linear regression.*
Results: *Increased baseline RSS from strain accumulation during canal shaping was observed in both groups. The mean ± SD maximum RSS recorded with XPS and HCM were 165.71 ± 86.57, and 132.14 ± 97.26 respectively with no statistical difference between them (p>0.05). Post-instrumentation microcracks were observed in only two canals prepared by XPS (10%) versus one canal prepared by HCM (5%), and this difference was also statistically non-significant (P>0.05). The inter-evaluator reliability for microcrack detection using ESEM had a Kappa value of 0.98 (p<0.001), while the intra-evaluator reliability had a Kappa value of 0.99 (p<0.001). The maximum RSS obtained during canal shaping was poorly correlated with the number of microcracks found (R2=0.090).*
Conclusions: *Within the limitations of this study, canal shaping by the two systems was confirmed to strain the tooth structure. However, the measured RSS was poorly correlated with the development of apical microcracks. Hence, from the clinical context, both systems can be safely used to prepare severely curved root canals.*

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Introduction

Biomechanical preparation of root canals is the most essential step in achieving endodontic success due to enabling bacterial elimination, removal of debris, and facilitating obturation (1, 2). The use of Nickel-Titanium (NiTi) engine-driven instruments in the preparation of the root canal space reduces operator fatigue, the time required for shaping, and the risk of procedural preparation errors (3). NiTi was firstly introduced in Endodontics in 1988, over 30 years of research and clinical use have led to advancements in NiTi rotary instruments including various design concepts and new techniques for root canal preparation. Firstly and foremost, NiTi endodontic instruments were found to be more flexible with an increased torsional fracture resistance when compared to stainless steel instruments (4). These enhanced characteristics allowed a major improvement in engine driven endodontic instruments (5). NiTi endodontic instruments contain approximately 56 wt% nickel and 44 wt% titanium resulting in a nearly one-to-one atomic ratio. This equiatomic NiTi alloy can exist in two different temperature-dependent crystal structures named austenite (high-temperature or parent phase, with a cubic B2 crystal structure) and martensite phase (low-temperature phase, with a monoclinic B19'crystal structure) and possesses two major characteristics which are superelasticity and shape memory effect (6).

The use of recently introduced martensitic alloys result in improvement of the mechanical properties, leading to more flexible instruments, with an increased cyclic fatigue resistance when compared to austenitic alloys (7). Because of these variations in the properties of the NiTi instruments, manufacturers have introduced several proprietary manufacturing procedures including thermal, mechanical and surface treatments. A change in the transformation temperatures (Ms, Mf, As, Af) of the utilized NiTi alloy, is the most important tool for manufacturers to alter the phase composition and consequently themomechanical properties of the NiTi alloy (7). Differently from austenitic NiTi files, martensitic instruments do not straighten during the preparation of curved root canals, thus resulting in more centered preparations (7).

Over time, advancements in NiTi instruments have introduced new generations of NiTi files with different working motions and design features such as the NiTi core diameter, cross-sectional shape, flute depth, and rake angle (1). During canal shaping, the development of forces at or near the tip of the instrument is inevitable and the file design is one of the features that can affect apical stress and strain concentrations during root canal instrumentation (8). The induced strain on the canal wall may lead to the development of dentinal microcracks that can originate apically and propagate coronally (9), mainly in the buccolingual direction (10), ultimately resulting in vertical root fractures (VRF). Vertical root fracture is one of the most frustrating complications of root canal treatment, often resulting in tooth extraction.

Attempts have been made to modify and improve the metallurgical and mechanical characteristics of NiTi instruments. Currently, there are many instruments with innovative metallurgical properties including XP-Endo Shaper files (XPS; FKG Dentaire, La Chaux-de-Fonds, Switzerland), which is a single-file system made with MaxWire (Martensite-Austenite-electropolish-fileX) alloy, instruments acquire higher flexibility, super elasticity, and the ability to progress within the canals, expanding or contracting according to the canal morphology.

HyFlex CM rotary instruments (Coltene-Whaledent, Allstetten, Switzerland) are made from CM wire, a thermally treated NiTi wire with controlled memory. CM-wire is a nearly equiatomic alloy produced by a peculiar thermomechanical process consisting in a specific sequence of heating and cooling methods during their grinding manufacturing process and leading to extremely flexible instruments (11, 12). These instruments are characterized by a symmetrical cross-sectional

design showing three cutting edges, with the exception of the instruments with size 25, .04 taper, which have a square cross section with four flutes.
Many studies have been performed on root surface strain development during canal shaping (8, 10, 13, 14). However, whether single-file or multi-file instruments may produce similar apical strain has not been examined yet. Therefore, observing the apical root surface strain generated during root canal preparation with XP-Endo Shaper and HyFlex CM instruments would provide relevant data to be utilized clinically. The null hypothesis was that there is no difference between XP-Endo Shaper and HyFlex CM in terms of strain generated development during canal instrumentation.

Materials and Methods

Selection of the teeth
The study design was reviewed and approved by the Ethics Committee and the University Institutional Review Board. Sound human mandibular first molars atraumatically extracted for periodontal reasons were collected from local clinics without demographic data. All superficial tissues were gently removed using a hand scaler, and the teeth were kept hydrated by immediate storage in distilled water. Teeth were examined under a digital microscope (OMS2350 Zumax, Suzhou New District, China) at a 6.9X magnification to confirm absence of cracks, fractures, resorptions or anatomic aberrations.
Coronal access was achieved using diamond burs, and the mesial canals were controlled for apical patency with a size 10 K-file (Mani, Tochigi, Japan). Teeth that did not allow the insertion of a size 10 K-file to the major foramen or the passive placement of a size 15 K-file to within 1 mm of the foramen were discarded. Slight occlusal reduction was done using diamond stone to standardize the working length of all mesial canals to 18.5 mm. This was done by measuring the length of the initial instrument (K-file #10) at the apical foramen minus 1 mm.
Digital radiographs (EzSensor classic, Vatech, Gyeonggi-do, Korea) with the files in place were obtained from the proximal view to identify mesial canals morphology. The acquired digital radiographs were transferred to AutoCAD 2008 (Autodesk, San Rafael, CA, USA), and the angle and radius of curvature of the mesio-buccal and mesio-lingual root canals were determined as described previously (15).
Finally, twenty molars with mesial roots having two separate canals from the orifice to the apex along the entire length, and whose radii of curvature ranged between 4 and 7 mm and whose angles of curvature ranged between 25° and 35° were selected for use in the study.

Preparation of the teeth
To simulate the periodontal ligament, each tooth was wrapped with a single layer of 0.3 mm aluminium foil (BMJ METAL, Zhejiang, China) and embedded in regular body addition silicon impression material (Zhermack silicone Elite HD, Badia Polesine, Italy) to set in an acrylic tube (12 mm tall and 20 mm in diameter), ensuring that each tooth was centrally positioned with the long axis of the roots aligned parallel to the sides of the tube. The tooth was then removed from the tube, and the aluminium foil peeled off, then super light body impression material (Zhermack silicone Elite HD, Badia Polesine, Italy) was injected in the socket and the tooth was immediately replaced. Finally, the apical end of the acrylic tube was cut to a level that exposed 4 mm of the root apex to allow strain gauge placement and intraoperative image recordings. The teeth were kept hydrated by immersion in distilled water until root canal instrumentation.

Pre-instrumentation imaging
Baseline pre-instrumentation images of the apical surface surrounding the apical foramen (AF) of each root canal were obtained with an Environmental Scanning Electron Microscope (ESEM) Model Quanta FEG-250 (ThermoFisher Scientific, Oregon, USA) under a magnification of 500X. Subsequently, the samples were kept hydrated by immersion in distilled water until root canal instrumentation.

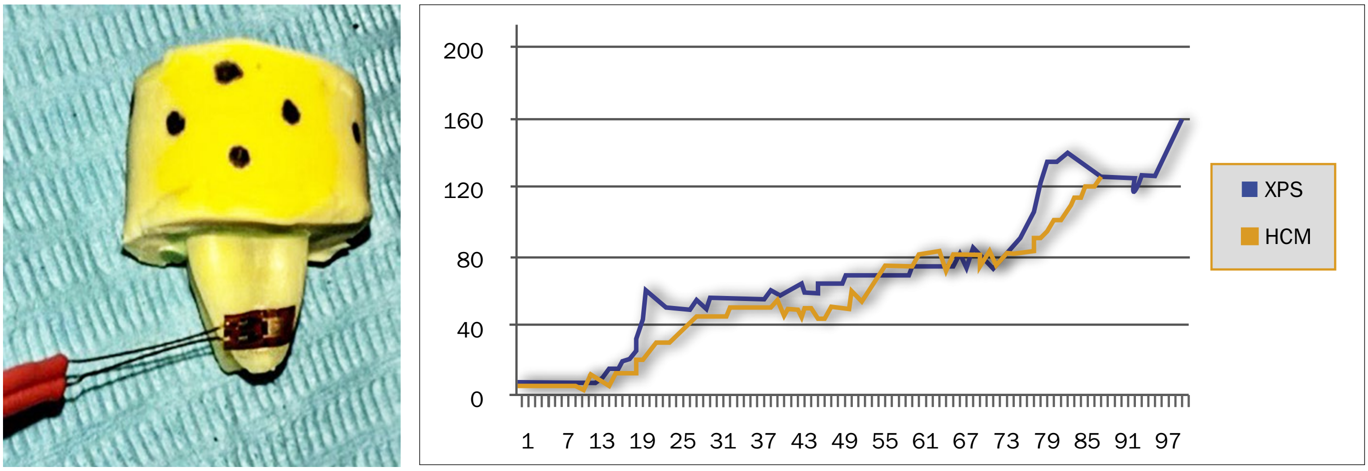

Figure 1. Instantaneous RSS (μstrain) recorded over time (seconds) by the strain gauge attached to the apical third of the mesial root during instrumentation of MB and ML canals by XPS and HCM files respectively.

Strain gauge mounting

An electrical strain gauge (KFG02-120-C1-16, Kyowa, Tokyo, Japan) was fixed on the exposed apical third of the proximal root surface 1mm from the apex before instrumentation as described previously (Figure 1) (10).

The root surface was first cleaned with acetone in order to bond the strain gauge with a cyanoacrylate adhesive (Super glue, Ontario, Canada).

The strain gauge was connected to a strain amplifier (EDX-200A Universal Recorder, Kyowa, Tokyo, Japan) via a bridge box (JM3812; JingMing Technology, Hangzhou, China) to measure the root surface strain (RSS) values that were induced by canal instrumentation.

Root canal shaping

Before shaping, the canals were irrigated with distilled water and agitated with K-file #10, and a glide-path was established using the same file in watch-winding motion until stable RSS readings appeared, then the amplifier was adjusted to zero to start the procedure. During canal shaping, all samples were fixed.

For each molar (n=20); the mesio-buccal canal was prepared using XP-Endo Shaper files (Group A: XPS) while the mesio-lingual canal was prepared using HyFlex CM files (Group B: HCM).

For group A the XPS file was first placed in 35 °C water for 1 minute to allow for phase transformation followed by immediate placement in the root canal at 800 rpm and 1 Ncm torque. According to the manufacturer instructions, the Booster Tip permits the XPS instrument to begin shaping and to steadily increase its working field to achieve a size 30 with one instrument.

For group B the HCM instrumentation began with size 20/0.04, then size 25/0.04, and finally ended up by size 30/0.04. Hence, the final apical preparation was set to size 30 in both groups. All HCM instruments were used at a rotational speed of 500 rpm, and the torque of 2.5 Ncm according to the manufacturers' instructions.

All instruments were used to the full length of the canals (single-length technique). The rubber stoppers of the files were fixed by cyanoacrylate at 18.5 mm. Instrumentation was performed by a single operator in strict accordance with the manufacturers' recommendations. All instruments were advanced in the canal with a vertical amplitude of about 3 mm. The flutes of the instruments were cleaned after three in-and-out movements (pecks) and the root canal was flushed with 3 mL of a distilled water solution using a 30-gauge needle (NaviTip; Ultradent, South Jordan, UT, USA) that was inserted as deeply as possible into the canal without binding. Apical patency was maintained using a size 10 K-file. Once the rotary instrument had negotiated to the end of the canal and had rotated freely, it was removed. Each instrument was used to prepare four canals only and the same operator performed all the instrumentation procedures.

RSS recording
During canal shaping, the strain output of the amplifier was digitally recorded by a multi-input data logger (NR-1000, Keyence, Osaka, Japan) and saved as data files, which contained the strain values (in micro-strain) induced by the canal shaping on the external surface of the root and converted to Excel files (Excel, Microsoft Corp., Redmond, WA, USA). The instantaneous RSS induced by each instrument, and the maximum RSSs were determined.

Post-instrumentation imaging
Once the root canal shaping was completed, post-instrumentation images were acquired under the same parameters to evaluate the presence/absence of apical microcracks. Any microcrack that originated from the root canal was assumed to have been produced by the shaping procedure and was annotated (Figure 2).
Examination was done twice by three blinded examiners at 2-week intervals. Inter-evaluator and intra-evaluator reliability were analyzed using the Kappa statistic test. In the event of differences of opinions, a consensus was reached after discussion amongst the three evaluators. To avoid artifacts by dehydration, the teeth were kept in distilled water.

Statistical analysis
RSS numerical data were explored for normality by checking the data distribution, calculating the mean and median values using Kolmogorov-Smirnov and Shapiro-Wilk tests. Data showed parametric distribution so; it was represented by mean and standard deviation (SD) values. Intergroup comparisons were done using independent t-test. The significance level was set at P≤0.05. Statistical analysis was performed with IBM® SPSS® Statistics Version 25 for Windows.

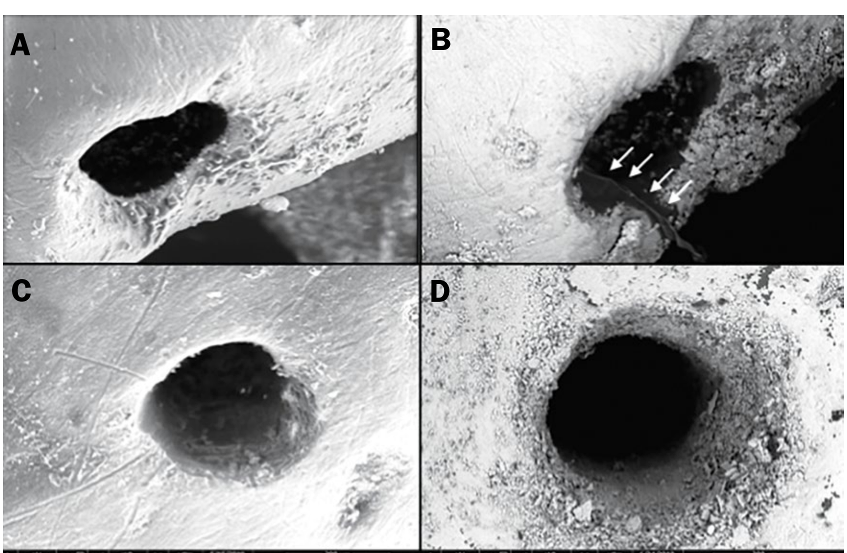

Figure 2.
Pre- **(A, C)** and post-instrumentation **(B, D)** ESEM micrographs for samples prepared with XPS showing presence (**B** white arrows) and absence **(D)** of post-instrumentation apical microcracks (magnification 500x).

Results

None of the XPS OR HCM instruments fractured during canal shaping. The Mean±SD value of the maximum RSS recorded and number/percentage of apical microcracks in the experimental groups are presented in Table 1. Increased baseline RSS from strain accumulation during canal shaping was observed in both groups. The mean ± SD maximum RSS recorded with XPS and HCM were 165.71±86.57, and 132.14± 97.26 respectively with no statistical difference between them (p>0.05). Regarding the incidence of post-instrumentation microcracks; this was observed in only two canals prepared by XPS (10%) versus one canal prepared by HCM (5%), and this difference (Figure 3) was statistically non-significant (P>0.05). The inter-evaluator reliability for microcrack detection using ESEM had a kappa value of 0.98 (p<0.001), while the intra-evaluator reliability had a kappa value of 0.99 (p<0.001). The maximum RSS obtained during canal shaping was poorly correlated with the number of microcracks found ($R2$=0.090).

Discussion

New thermally treated NiTi alloys are characterized by the appearance of martensite under clinical conditions, leading to an enhanced resistance to cyclic fatigue and a higher flexibility (16). However, preparing highly tortuous or curved root canals with NiTi rotary files often results in inadequate debridement or asymmetric canal shaping, leading to many adverse effects such as generation of abnormal root

Table 1

	Maximum RSS (mstrain)	Number of apical microcracks (%)
XPS	165.71 ± 86.57	2/20 (10%)
HCM	132.14 ± 97.26	1/20 (5%)
P value	0.344 ns	0.225 ns

Table 1.
Mean ± SD maximum RSS recorded and number/percentage of apical microcracks in the experimental groups.

surface strain, due to the contact between instruments and dentin that creates many momentary stress concentrations in dentin (8). These stresses are transmitted through the root to the surface, where they might also overcome the bonds holding dentin together hence induce dentinal defects such as microcracks (3). Despite the Literature provides controversial results (17), these dentinal microcracks may have the potential to propagate into root fractures, which usually lead to tooth loss as microorganisms can proliferate in crack lines leading to secondary bacterial infections (18). These complications necessitate a better understanding of the factors that initiate microcracks (19).
This laboratory study was designed to measure the apical root surface strain (RSS) during canal shaping with new NiTi thermally treated single and multi-file NiTi systems.
In the present study XP-Endo Shaper, and HyFlex CM files were selected for root canal preparation. To the best of our knowledge, there are no current data concerning the measurement of the apical RSS with these instruments. XP-Endo Shaper is the first endodontic NiTi alloy single-file system manufactured in MaxWire that combines both shape memory effect and superelasticity in clinical application (7). HyFlex CM files, are manufactured using a unique process in which the crystallographic phase transitions from austenite to martensite at room temperature in contrast to conventional NiTi files, making the files extremely flexible and fracture resistant (12).
Root canal irrigation with sodium hypochlorite irrigating solution could significantly affect strain, elastic modulus and flexural strength of the dentin, potentially predisposing teeth to fracture (20). In the present research, distilled water was used in order to avoid the irrigant effect on RSS as previously recommended for investigations of human dentine because it causes the least amount of changes in dentine over time (21).
External reinforcement was avoided using a thin layer of silicone as a simulated periodontal ligament (19). Because an "exposed" apex is common in teeth with chronic apical periodontitis or periapical cysts, the apical 4 mm portion of the root was exposed to allow for intraoperative strain recordings (22). Therefore, present results may not be extrapolated in teeth completely surrounded by bone tissue or with periapical lesion smaller than those simulated.
Canal shaping was performed by a single experienced operator who attempted to maintain a consistent pressure on the instruments. However, it was difficult to standardize the vertical load during shaping. In addition, because the direction and magnitude of internal strain on the root canal wall surfaces could not be measured, the outer circumferential RSS values (10) were evaluated in this study. The present laboratory study was conducted at room temperature, and this is a bias of the research. Especially for thermally treated files in fact, it is well

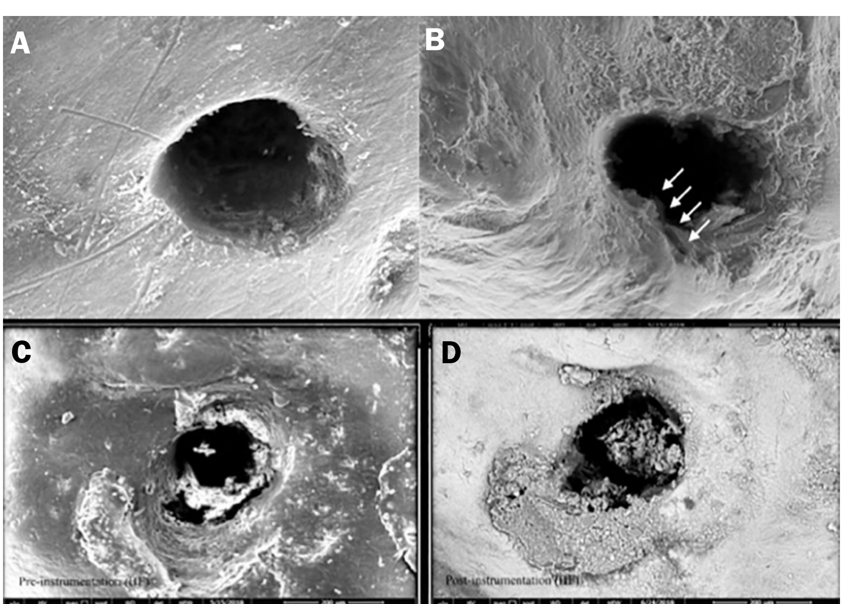

Figure 3.
Pre- **(A, C)** and post-instrumentation **(B, D)** ESEM micrographs for samples prepared with HCM showing presence (**B** white arrows) and absence **(D)** of post-instrumentation apical microcracks

known that the body temperature significantly affects the mechanical behavior with a better arrangement of the crystal structure (23). Results of the present results showed that canal shaping strained the tooth structure as previously reported (10, 18, 24). Nevertheless, the mean maximum RSS of XPS was found not to be different from that of HCM. Conservation of the dentin adjacent to the apical root canal is crucial to maintain strength and fracture resistance of the tooth structure. Another factor is working length calibration, which was 1 mm short of the apical foramen, as previously reported (9). This agrees with Versluis et al. (24) who found that stresses resulting from canal preparation 1 mm short of the apical foramen were one-third of the stresses recorded at more coronal levels.
The instantaneous root surface strain reflects the amount of strain that each instrument generates during canal shaping and on the other hand, the maximum RSS reflects the total amount of strain applied after completion of canal shaping. The measured RSS was poorly correlated with the development of apical microcracks in the canal wall. Apical microcrack formation has been a very controversial topic in the past (25). Results of our study validates recent evidence that microckacks in in vitro studies are not related to instrumentation, nor other conditions but are mainly related to in vitro conditions. This suggests that microcracks are not a direct result of an increased maximum RSS, but might be caused by other factors, such as extraction process and/or the post-extraction storage conditions (17). Thus, the null hypothesis is accepted.

Conclusions

Within the limitations of this study, canal shaping by the two systems was confirmed to strain the tooth structure. However, the measured RSS was poorly correlated with the development of apical microcracks. Hence, from the clinical context, both systems can be safely used to prepare severely curved root canals.

Clinical Relevance

Apical microcracks are not a direct result of an increased maximum root surface strain exerted during root canal instrumentation.

Conflict of Interest

Authors deny any conflict of interest related to this research.

Acknowledgements

The present research was self-founded.

References

1. *Hülsmann M, Peters OA, Dummer PMH. Mechanical preparation of root canals: shaping goals, techniques and means. Endodontic Topics 2005; 10, 30-76.*
2. *Schilder H. Cleaning and shaping the root canal. Dent Clin North Am 1974; 18, 269-96.*
3. *Peters O A. Current challenges and concepts in the preparation of root canal systems: a review. J Endod 2004; 30, 559-67.*
4. *Walia H, Brantley W A, Gerstein H. An initial investigation of the bending and torsional properties of nitinol root canal files. J Endod 1988; 14, 346-51.*
5. *Thompson S A, Dummer P M H. Shaping ability of ProFile.04 Taper Series 29 rotary nickel-titanium instruments in simulated root canals. Part 1. Int Endod J 1997; 30, 1-7.*
6. *Thompson S A. An overview of nickel-titanium alloys used in dentistry. Int Endod J 2000; 33, 297-310.*
7. *Zupanc J, Vahdat-Pajouh N, Schäfer E. New thermomechanically treated NiTi alloys – a review. Int Endod J 2018; 51, 1088-103.*
8. *Kim H C, Lee M H, Yum J et al. Potential relationship between design of nickel-titanium rotary instruments and vertical root fracture. J Endod 2010; 36, 1195-9.*
9. *Liu R, Hou B X, Wesselink P R et al. The incidence of root microcracks caused by 3 different single-file systems versus the protaper system. J Endod 2013; 39, 1054-6.*
10. *Jamleh A, Komabayashi T, Ebihara A et al. Root surface strain during canal shaping and its influence on apical microcrack development: A preliminary investigation. Int Endod J 2015; 48, 1103-11.*
11. *Pirani C, Iacono F, Generali L et al. HyFlex EDM: superficial features, metallurgical analysis and fatigue resistance of innovative electro discharge machined NiTi rotary instruments. Int Endod J 2016; 49, 483-93.*
12. *Iacono F, Pirani C, Generali L et al. Structural analysis of HyFlex EDM instruments. Int Endod J 2017; 50, 303-13.*
13. *Asundi A, Kishen A A strain gauge and photoelastic analysis of in vivo strain and in vitro stress distribution in human dental supporting structures. Arch Oral Biol 2000; 45, 543-50.*

14. *Pedullà E, Genovesi F, Rapisarda S et al. Effects of 6 Single-File Systems on Dentinal Crack Formation. J Endod 2017; 43, 456-61.*
15. *Schäfer E, Diez C, Hoppe W, Tepel J. Roentgenographic investigation of frequency and degree of canal curvatures in human permanent teeth. J Endod 2002; 28, 211-6.*
16. *Shen Y, Zhou H M, Zheng Y F et al. Current challenges and concepts of the thermomechanical treatment of nickel-titanium instruments. J Endod 2013; 39, 163-72.*
17. *De-Deus G, Cavalcante DM, Belladonna FG et al. Root dentinal microcracks: a post-extraction experimental phenomenon? Int Endod J 2019; 52, 857-65.*
18. *Shemesh H, Bier CAS, Wu MK et al. The effects of canal preparation and filling on the incidence of dentinal defects. Int Endod J 2009; 42, 208-13.*
19. *Okitsu M, Takahashi H, Yoshioka T et al. Effective Factors Including Periodontal Ligament on Vertical Root Fractures. Dent Mater J 2012; 24, 66–9.*
20. *Rajasingham R, Ng Y L, Knowles J C, Gulabivala K. The effect of sodium hypochlorite and ethylenediaminetetraacetic acid irrigation, individually and in alternation, on tooth surface strain. Int Endod J 2010; 43, 31-40.*
21. *Saber S E D M, Schäfer E. Incidence of dentinal defects after preparation of severely curved root canals using the Reciproc single-file system with and without prior creation of a glide path. Int Endod J 2016; 49, 1057-64.*
22. *Adorno C G, Yoshioka T , Suda H. The Effect of Root Preparation Technique and Instrumentation Length on the Development of Apical Root Cracks. J Endod 2009; 35, 389-92.*
23. *de Vasconcelos RA, Murphy S, Carvalho CA et al. Evidence for Reduced Fatigue Resistance of Contemporary Rotary Instruments Exposed to Body Temperature. J Endod 2016; 42,782-7.*
24. *Versluis A, Messer HH, Pintado MR. Changes in compaction stress distributions in roots resulting from canal preparation. Int Endod J 2006; 39, 931-9.*
25. *Jamleh A, Adorno C G, Ebihara A, Suda H. Effect of nickel titanium file design on the root surface strain and apical microcracks. Aust Endod J 2016; 42, 25-31.*

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ORIGINAL ARTICLE

Analysis of dentinal erosion and removing smear layer of different irrigation protocols: an *in vitro* study

ABSTRACT

Aim: *A material with an acidic pH and desiccating action (HybenX) has been generated to destroy the dental biofilm. This study aims to investigate the effect of HybenX used as an irrigating solution, estimating its efficacy in the elimination of the smear layer and rating how it may influence dentinal erosion.*

Methodology: *One hundred extracted, single-rooted, human teeth were used. Five groups were made in a random way (n=20), considering the type of irrigant used at the end of the instrumentation with ProTaper Gold sequence SX F4: Group A (NaOCl), Group B (NaOCl - EDTA 17%), Group C (NaOCl - EDTA 17% - NaOCl), Group D (NaOCl - HybenX), Group E (NaOCl - HybenX - NaOCl). The amount of the smear layer and the erosion were evaluated according to the Torabinejad method using a scanning electron microscope. A Kruskal-Wallis test was performed at each portion (i.e. apical, middle, coronal) and overall for both smear layer removal and erosion variables. A multiple comparison analysis was implemented as well within each portion and overall for both variables.*

Results: *The difference in debris removal at all three levels of the canals was statistically significant, comparing the five treatment groups (p<.0001). The statistic test showed a statistically significant greater erosion overall between group A and the other four groups (p<.0001).*

Conclusions: *Under the conditions of the present study, the use of a combination of NaOCl and HybenX, efficiently removes smear layer and produces a lower degree of erosion if compared with 17% EDTA.*

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Introduction

The instrumentation of a root canal produces smear layer, a 1 to 2 μ amorphous structure coating the canal walls, formed by an organic and an inorganic component, that gets into the dentinal tubules (smear plug) as far as 40 μ (1-3).
Its presence impedes irrigants, medications and filling materials to penetrate into the dentinal tubules and also might prevent them from touching the canal walls.
To remove the smear layer, a number of chelating substances and acid solutions have been suggested.
The most recommended combination of irrigants, to efficiently remove the smear layer from the root canal wall, has resulted being EDTA and NaOCl; a careful evaluation of this combination has been analysed, concerning application time and volumes, irrigation mode and the alternation of these two solutions (4-8).
Some authors pointed out that the above mentioned irrigation protocol can significantly modify the dentin mechanical properties (9-11).
A different irrigating solution (HybenX®, EPIEN Medical, Saint Paul, MN, USA) has been generated to destroy the dental biofilm; its composition is a mixture of hydroxybenzenesulfonic acid (37%) and hydroxymethoxybenzene acids (23%), sulphuric acid (28%), and water (12%). The product is currently marketed by the producer both for use in Periodontics (HybenX Oral Tissue Decontaminant) and for Endodontics (HybenX Root Canal Cleanser). The two forms of the product, which have the same chemical composition, differ in consistency: more viscous gel for periodontal use and more liquid gel for endodontic use.
This study aims to investigate the effect of HybenX used as an irrigating solution, estimating its efficacy in the elimination of the smear layer and rating how it may influence dentinal erosion.

Materials and Methods

In this study, 100 maxillary and mandibular, no decay, single-rooted, human teeth extracted for periodontal reasons were used. The local Ethical Committee approved the study protocol. For all patients an informed consent was obtained in order to include their teeth in the study.
Teeth with coronal restorations or endodontic treatments were excluded. To be sure that all teeth had a single canal, no complicated anatomy and apical curves and no calcifications, each one was checked with digital X-rays (Gendex, Hatfield, PA, USA). The selected teeth were mechanically cleaned from soft tissue and debris, stored in saline water and at a temperature of +4 °C.
After cutting out the crowns at the cement-enamel junction using a high-speed bur, the roots were standardized to a 15 mm length using a diamond disc at low speed. Fine barbed instruments were used to remove any residual pulp tissue.
With the use of a water-cooled diamond disc, parallel grooves were traced along the buccal and lingual surfaces, without touching the inner face.
To establish the working length (WT), an ISO size #10 K-type file (Dentsply Sirona, Ballaigues, Switzerland) was introduced into the root canal until just visible at the apical foramen. The apex was left unsealed, to guarantee a communication of air and vapour with the external surroundings.
ProTaper Universal Rotary instruments (Dentsply Sirona), up to apical size (F4), were used to prepare the root canal and a size 5 Gates Glidden drill (Dentsply Sirona), in the coronal 5 mm, to create a reservoir for the irrigant solution.
Between each file, 2 ml of 5.25% NaOCl (Ogna, Lab Srl, Muggiò, MB, Italy) were utilized to irrigate the canals, with a 30-G syringe needle (Kerr Dental, Orange, CA, USA), moved back and forward and keeping the needle 1 mm shorter than the WL. The final irrigation, at the end of the shaping's procedures, was carried out with 5 ml of 5.25% NaOCl for 1 minute, followed by 5 ml of distilled water for 1 minute.
Five groups of 20 teeth each were made in a random way, considering the type of irrigant used at the end of the instrumentation.

- Group A: NaOCl

- Group B: NaOCl - EDTA 17% (Ogna, Lab Srl, Muggiò, MB, Italy)
- Group C: NaOCl - EDTA 17% - NaOCl
- Group D: NaOCl - HybenX
- Group E: NaOCl - HybenX - NaOCl

5 ml of each solution from groups A, B and C were applied to remove the smear layer from the surface of the root canals; the exposure time was approximately 2 minutes.

Groups D and E irrigation mode was slightly different, due to the tested solution's physical features. HybenX is indeed a thicker and more viscous liquid, so, while the exposure time remained the same (2 minutes), the product was put in the upper half of the canal and then spread to the remaining part of the canal by a fitting size sterile paper point (ProTaper F4, Dentsply Sirona), moving it up and down all the time.

A final irrigation with 10 ml of sterile distilled water, followed by the insertion of a paper point to dry the canal was the last step of the procedure for all groups.

Evaluation by Scanning Electron Microscopy

Parallel grooves were traced along the buccal and lingual surfaces of 100 roots split in two halves, along the longitudinal axis, obtaining 200 sections in total.

Each group, containing 40 sections, 20 of which had been randomly chosen and the other 20 discarded.

A scanning electron microscope (SEM) (FEI Quanta 200 © 2018 Thermo Fisher Scientific) operating at 8 kV at a magnification of 2,000 was used to observe the samples of each group.

Three random images of the apical (0-5 mm), middle (5-10 mm) and coronal (11-15 mm) portions of each sample, from the canal dentin wall surface, were acquired using a motorized specimen stage. The area to be analysed was inspected at a low magnification (200x). The magnification was then, increased (2,000x), without moving the microscope.

In total, 60 images for each experimental group were obtained.

Two trained and blinded evaluators independently rated each masked fragment. Evaluators had no prior knowledge of the cleaning/shaping procedures; the types of irrigant used at the end of the instrumentation, and were well acquainted with qualitative analysis of the SEM images. When evaluator scores disagreed, the lower score was taken.

A four-level scoring system based on the severity of smear layer retention was used to evaluate the efficacy of smear layer removal (12). This scoring system' s criteria were:

1. no smear layer. Absence of smear layer on the surface of the root canals; all tubules open and clean;
2. moderate smear layer. Absence of smear layer on the surface of root canal, but tubules contained debris;
3. heavy smear layer. Smear layer covered the root canal surface and the tubules.

Then the degree of erosion of dentinal tubules was also scored as follows:

1. no erosion. All tubules normal in size and appearance;
2. moderate erosion. The peritubular dentin was eroded;
3. severe erosion. The intertubular dentin was damaged and tubules were connected with each other.

Statistical Analysis

The analysis was produced using SAS® version 9.4 in a secure and validated environment. The procedure "NPAR1WAY" was used.

A one-way ANOVA, which for Wilcoxon score is known as the Kruskal-Wallis test, with group as fixed factor was performed at each portion (i.e. apical, middle, coronal) and overall for both smear layer removal and erosion variables.

Additionally, Dwass, Steel, Critchlow-Fligner (DSCF) multiple comparison analysis, which is based on pairwise two-sample Wilcoxon comparisons was implemented as well within each portion and overall for both variables. A multiplicity correction for 10 simultaneous comparisons was applied within each portion and overall by using the Šidák alpha adjustment (i.e. $1-(1-\alpha)^{\frac{1}{m}}$ with m representing the number of pairwise contrasts).

Table 1
Descriptive statistics
(Median [interquartile range])

		Group A	Group B	Group C	Group D	Group E
		N=20	N=20	N=20	N=20	N=20
Erosion	Overall	1 [0]	2 [1]	3 [0]	2 [1]	2 [1]
	Coronal	1 [0]	2 [0]	3 [0]	2 [0]	2 [0.5]
	Middle	1 [0]	2 [2]	3 [1]	2 [0.5]	2 [1]
	Apical	1 [0]	1 [0]	3 [1]	1 [0]	1 [0]
Smear Layer	Overall	3 [0]	1 [1]	1 [0]	1 [1]	1 [1]
	Coronal	3 [0]	1 [0]	1 [0]	1 [0]	1 [0]
	Middle	3 [0]	1 [0]	1 [0]	1 [1]	2 [1]
	Apical	3 [0]	2 [1]	1 [1]	2 [0.5]	2 [0]

Table 2
Kruskal-Wallis test

		Chi-Square	Pr>Chi-Square
Erosion	Overall	142.0893	<.0001
	Coronal	74.505	<.0001
	Middle	46.0793	<.0001
	Apical	58.5154	<.0001
Smear Layer	Overall	159.5796	<.0001
	Coronal	92.0204	<.0001
	Middle	51.567	<.0001
	Apical	142.0893	<.0001

Results

The descriptive statistics is shown in Table 1.
The Kruskal-Wallis test resulted highly statistically significant ((p<.0001) at each portion and overall indicating a difference between groups for both Erosion and Smear Layer variables (Table 2).
In group A the root canals showed heavy smear layer along its whole length (Figure 1).
The difference in debris removing at all three levels of the canals and overall was highly statistically significant, comparing group A vs. groups B, C, D and E (p<.0001).
Additionally, at apical third the smear layer removal is significantly higher in group C (p=0.002) and E (p=0.004) than in group B.
Considering the multiple pairwise contrasts for the erosion, groups B, C, D and E presented a significantly greater erosion than in group A overall, and at apical and middle third.
At the coronal third exactly the same situation such as above was observed (Figure 2).
The lower erosion of group A with respect to the other four treatment groups was endorsed also at the middle third (p<.0001). Group C confirmed to have significantly greater erosion than group D and E (p< 0.05) while, if compared to group B, only a trend is shown (p=0.0647) (Figure 2).
The greater erosion in group C compared to groups A, B, D and E at the apical third was also confirmed (p<.0001).
The multiple comparisons both for erosion and smear layer are fully shown in Table 3.

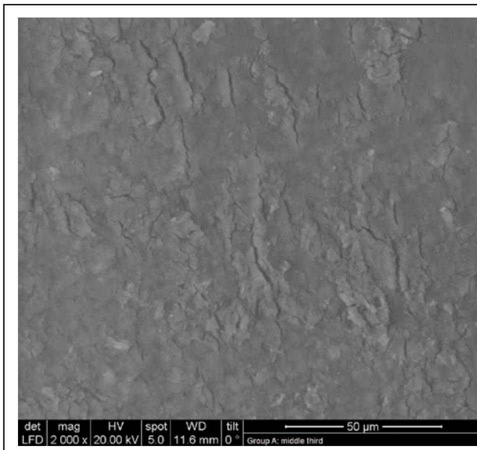

Figure 1.
Heavy smear layer in group A (middle third).

Discussion

Based on the results observed in this study, the use of NaOCl only didn't remove the smear layer as efficiently as in the samples in group B, C, D and E, in which the root canal surface and the dentinal tubules appeared to be much cleaner.
This study's results confirm that substances containing EDTA in groups B and C, and HybenX, in groups D and E are fundamental to remove smear layer efficiently.
In literature, on the other hand, is well documented how the use of a chelating agent to dissolve the inorganic components of the smear layer, can lead to a different levels of erosion (13-19). In addition some

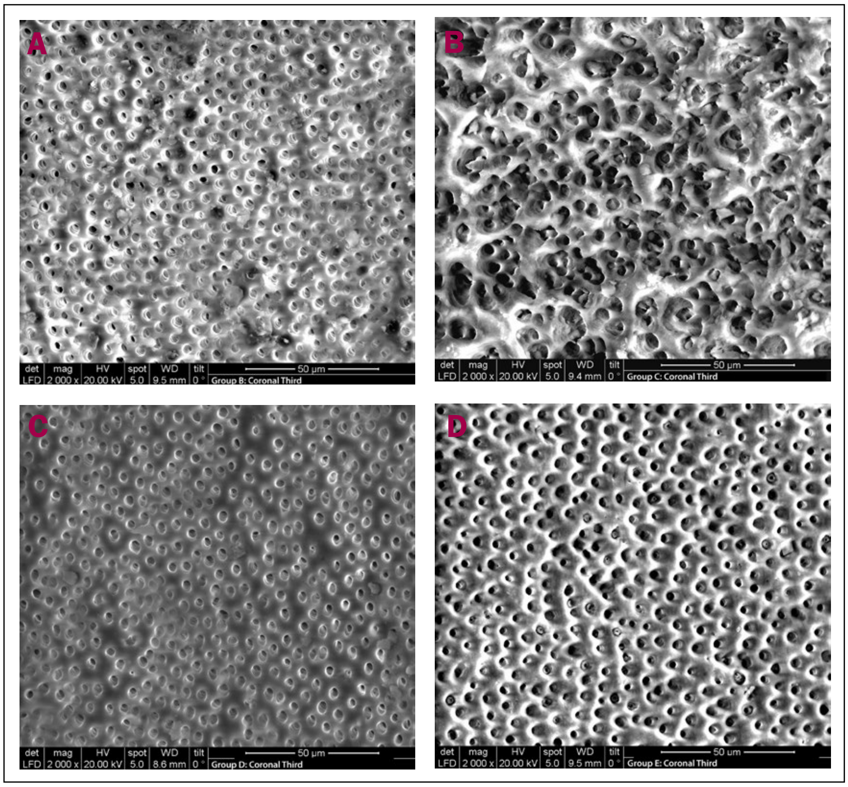

Figure 2 A-D.
Aspects of erosion in groups **B (A)**, **C (B)**, **D (C)** and **E (D)** respectively.

Figure 3 A-C.
Aspects of the root canal walls in group E at the apical third **(A)**, middle third **(B)** and coronal third **(C)** respectively.

studies demonstrate that irrigants can alter the structure of collagen and mineral content of the dentin, changing the proportion of phosphate and calcium. This results in a reduction of microhardness and flexural strength of the dentin, which could be a potential risk factor for vertical root fractures (20-23).
HybenX ability in removing the smear layer is not due to a chelating action but is likely produced by its chemical features: it is a mixture of sulphonic/sulphuric acids, so it has a strong affinity to water. Its acidic pH is active in dissolving the inorganic debris. Beyond that, the sulphate group exhibit oxygen atoms that provide it with a large negative charge; this attracts water molecules since their hydrogen atoms have a positive charge. Consequently, the determined chemical effect is dehydration and disintegration of organic biofilms.
From our observations it's clear that the higher level of erosion is shown in the irrigation sequence of group C: NaOCl - EDTA 17% - NaOCl. Especially at the coronal and middle third, the dentin is seriously eroded, the dentinal tubules are widened, their entrances look irregular and the peritubular dentin has totally disappeared. In some sites, the intratubular dentin has completely collapsed and has created pits.
In view of all these considerations it can be observed how in group B the erosion is definitively less and mainly confined to the peritubular dentin. In group D the grade of erosion is insignificant, the dentinal surface is homogeneous, smooth, with tubules free of debris. In group E the dentinal surface is smooth, with slight pits at the tubule's entrance (Figure 3). In group E, a dissolving action is shown, due to the NaOCl final rinse: this event is much lighter in group E than in group C, because HybenX's eroding action is definitively lower than EDTA's.
The results of the present study are based on laboratory SEM experiments in according to the Oxford Centre for Evidence Based Medicine are of grade 2 level of evidence (24).

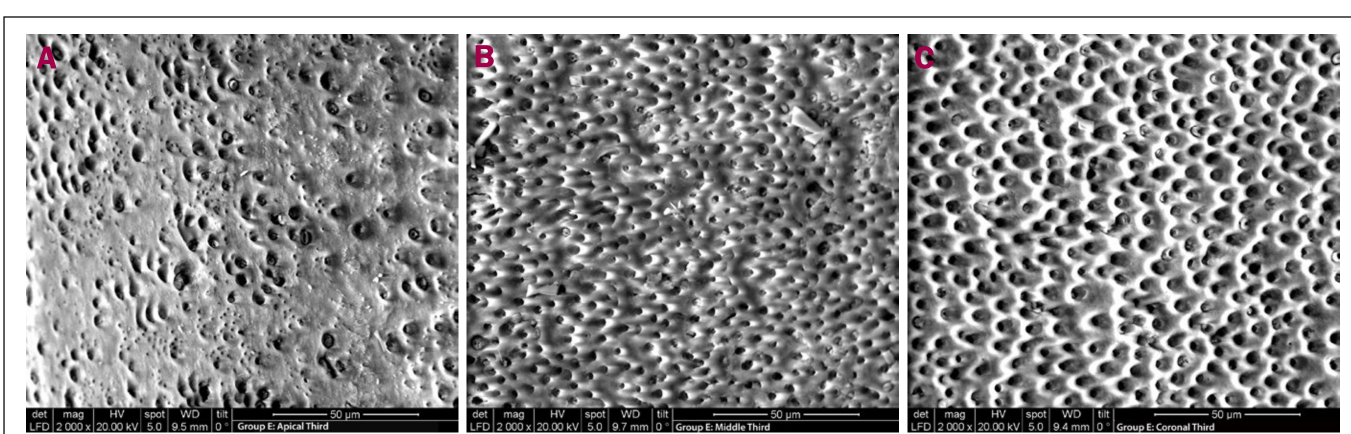

Table 3

Multiple Comparison

		Comparison	DSCF Value	Pvalue	Significance considering Sidak alpha adjustment
EROSION	Overall	Group A vs. Group B	9.40	<.0001	Yes
		Group A vs. Group C	14.43	<.0001	Yes
		Group A vs. Group D	10.21	<.0001	Yes
		Group A vs. Group E	10.55	<.0001	Yes
		Group B vs. Group C	9.91	<.0001	Yes
		Group B vs. Group D	0.28	1.00	No
		Group B vs. Group E	1.21	0.91	No
		Group C vs. Group D	10.60	<.0001	Yes
		Group C vs. Group E	9.64	<.0001	Yes
		Group D vs. Group E	1.05	0.95	No
	Coronal	Group A vs. Group B	7.79	<.0001	Yes
		Group A vs. Group C	8.73	<.0001	Yes
		Group A vs. Group D	7.91	<.0001	Yes
		Group A vs. Group E	8.44	<.0001	Yes
		Group B vs. Group C	7.02	<.0001	Yes
		Group B vs. Group D	0.99	0.96	No
		Group B vs. Group E	1.77	0.72	No
		Group C vs. Group D	7.82	<.0001	Yes
		Group C vs. Group E	6.31	<.0001	Yes
		Group D vs. Group E	3.02	0.20	No
	Middle	Group A vs. Group B	6.33	<.0001	Yes
		Group A vs. Group C	8.40	<.0001	Yes
		Group A vs. Group D	6.72	<.0001	Yes
		Group A vs. Group E	6.68	<.0001	Yes
		Group B vs. Group C	3.72	0.06	No
		Group B vs. Group D	0.89	0.97	No
		Group B vs. Group E	0.31	1.00	No
		Group C vs. Group D	5.25	0.002	Yes
		Group C vs. Group E	4.42	0.02	No
		Group D vs. Group E	0.64	0.99	No
	Apical	Group A vs. Group B	1.41	0.86	No
		Group A vs. Group C	7.75	<.0001	Yes
		Group A vs. Group D	2.94	0.23	No
		Group A vs. Group E	2.94	0.23	No
		Group B vs. Group C	7.52	<.0001	Yes
		Group B vs. Group D	2.07	0.59	No
		Group B vs. Group E	2.00	0.62	No
		Group C vs. Group D	6.25	<.0001	Yes
		Group C vs. Group E	6.94	<.0001	Yes
		Group D vs. Group E	0.22	1.00	No

		Comparison	DSCF Value	Pvalue	Significance considering Sidak alpha adjustment
SMEAR LAYER	Overall	Group A vs. Group B	12.88	<.0001	Yes
		Group A vs. Group C	14.43	<.0001	Yes
		Group A vs. Group D	13.83	<.0001	Yes
		Group A vs. Group E	14.55	<.0001	Yes
		Group B vs. Group C	4.15	0.03	No
		Group B vs. Group D	0.48	1.00	No
		Group B vs. Group E	0.24	1.00	No
		Group C vs. Group D	3.96	0.04	No
		Group C vs. Group E	4.97	0.002	Yes
		Group D vs. Group E	0.83	0.98	No
	Coronal	Group A vs. Group B	8.73	<.0001	Yes
		Group A vs. Group C	8.83	<.0001	Yes
		Group A vs. Group D	8.73	<.0001	Yes
		Group A vs. Group E	8.83	<.0001	Yes
		Group B vs. Group C	1.41	0.86	No
		Group B vs. Group D	0.00	1.00	No
		Group B vs. Group E	1.41	0.86	No
		Group C vs. Group D	1.41	0.86	No
		Group C vs. Group E	0.00	1.00	No
		Group D vs. Group E	1.41	0.86	No
	Middle	Group A vs. Group B	8.40	<.0001	Yes
		Group A vs. Group C	8.73	<.0001	Yes
		Group A vs. Group D	8.07	<.0001	Yes
		Group A vs. Group E	8.33	<.0001	Yes
		Group B vs. Group C	0.05	1.00	No
		Group B vs. Group D	2.75	0.29	No
		Group B vs. Group E	4.53	0.01	No
		Group C vs. Group D	2.93	0.23	No
		Group C vs. Group E	4.82	0.01	No
		Group D vs. Group E	1.97	0.63	No
	Apical	Group A vs. Group B	5.78	0.003	Yes
		Group A vs. Group C	7.39	<.0001	Yes
		Group A vs. Group D	7.36	<.0001	Yes
		Group A vs. Group E	8.50	<.0001	Yes
		Group B vs. Group C	5.22	0.002	Yes
		Group B vs. Group D	3.51	0.10	No
		Group B vs. Group E	4.94	0.004	Yes
		Group C vs. Group D	2.83	0.26	No
		Group C vs. Group E	2.83	0.27	No
		Group D vs. Group E	0.67	0.99	No

Some authors (25) pointed out how conventional scanning electron microscopy can produce considerable distortions: for examples they don't allow the observation of wet areas since the sample-chamber operates under high vacuum.
In this study an environmental electron microscope (ESEM) that allows the visualization of fresh dentinal preparations without having to subject them to dehydration and metallization processes was used, minimizing this bias.
Regarding the quantification of the results, the subjective nature of scoring systems required preliminary training to reduce interexaminer differences; two evaluators (VG and DL) were trained to read SEM images: a calibration kit of 100 original images not associated with the study and representing a wide range dentinal aspect was used. Agreement between and within examiners was determined by using the intra-class correlation coefficient.
An ideal model for evaluating intracanal cleanliness does not yet exist (26). For that reason it was possible to detect the following limitations of the present study.
The preparation of samples was standardized with a high apical diameter (size 40) which, generally, does not represent a common clinical situation; this was done to evaluate exclusively the action of the irrigating solutions in a standard experimental situation, unbound from intrinsic anatomical variables naturally present in clinical situations.
Furthermore, increased apical size and taper allowed enhanced irrigation in all areas of the root canal system and larger instruments may be employed to improve contact with canal walls, thereby producing more efficacious cleaning (27-29).
The evaluation of the erosion degree was carried out only through a surface score and therefore it is not possible, with this kind of study, to define the extent of the phenomenon along the thickness of the dentinal wall nor to determine any variations in the dentin microhardness.
Clearly, these *in vitro* preliminary data should be followed by further *in vitro* and *in vivo* investigations.

Conclusions

Our observations showed that, under these experimental conditions, the use of HybenX, was able to effectively remove the smear layer after irrigating the canal with 5% sodium hypochlorite.
Furthermore this irrigant produces a significantly lower degree of dentinal erosion than EDTA both if it was used alone and when its action was followed by irrigation with sodium hypochlorite (Figure 3).

Clinical Relevance

The present study showed a valid irrigation protocol that can successfully remove the debris on the root canal wall and, at the same time, reduce the erosion of the dentine, if compared with chelating agents, such as 17% EDTA.

Conflict of Interest

The authors deny any conflicts of interest.

Acknowledgements

None.

References

1. *Goldman LB, Goldman M, Kronman JH, Lin PS. The efficacy of several endodontic irrigating solutions: a scanning electron microscopic study. Oral Surg Oral Med Oral Pathol 1981;52:199-204.*
2. *Mader CL, Baumgartner JC, Peters DD. Scanning electron microscopic investigation of the smeared layer on root canal walls. J Endod 1984;10:477-83.*
3. *Sen BH, Wesselink PR, Türkün M. The smear layer: a phenomenon in root canal therapy. Int Endod J 1995;28:141-8.*
4. *Teixeira CS, Felippe MC, Felippe WT. The effect of application time of EDTA and NaOCl on intracanal smear layer removal: an SEM analysis. Int Endod J 2005;38:285-90.*
5. *Zehnder M, Schmidlin P, Sener B, Waltimo T. Chelation in root canal therapy reconsidered. J Endod 2005;31:817-20.*
6. *Mello I, Robazza CRC, Antoniazzi JH, Coil J. Influence of different volumes of EDTA for final rinse on smear layer removal. Oral Surg Oral Med Oral Pathol 2008;106:40-3.*
7. *Mello I, Coil J, Antoniazzi JH. Does a final rinse to remove smear layer interfere on dentin permeability of root canals? Oral Surg Oral Med Oral Pathol 2009;107:47–51.*
8. *Silveira LFM, Folgearini Silveira C, Martosa J, Suita de Castrob LA. Evaluation of the different irrigation*

regimens with sodium hypochlorite and EDTA in removing the smear layer during root canal preparation. J Microsc Ultrastruc 2013;1:51-6.
9. Eldeniz AU, Erdemir A, Belli S. Effect of EDTA and citric acid solutions on the micro- hardness and the roughness of human root canal dentin. J Endod 2005;31:107-10.
10. Marending M, Luder HU, Brunner TJ, Knecht S, Stark WJ, Zehnder M. (2007) Effect of sodium hypochlorite on human root dentine-mechanical, chemical and structural evaluation. Int Endod J 2007;40:786-93.
11. Marending M, Paque F, Fischer J, Zehnder M. Impact of irrigant sequence on mechanical properties of human root dentin. J Endod 2007;33:13-25.
12. Torabinejad M, Khademi AA et al. A new solution for the removal of the smear layer. J Endod 2003;29:170-5.
13. Dai L, Khechen K, Khan S, et al. The effect of QMix, an experimental antibacterial root canal irrigant, on removal of canal wall smear layer and debris. J Endod 2011;37:80-4.
14. Çalt S, Serper A. Time-dependent effects of EDTA on dentin structures. J Endod 2002;28:17-9.
15. Qian et al. Quantitative Analysis of the Effect of Irrigant Solution Sequences on Dentin Erosion. J Endod 2011;37:1437-41.
16. De Deus G, Paciornik S, Pinho Mauricio MH, et al. Real-time atomic force micro- scopy of root dentine during demineralization when subjected to chelating agents. Int Endod J 2006;39:683-92.
17. Eldeniz AU, Erdemir A, Belli S. Effect of EDTA and citric acid solutions on the microhardness and the roughness of human root canal dentin. J Endod 2005;31:107-10.
18. Zhang K, Tay FR, Kim YK, et al. The effect of initial irrigation with two different sodium hypochlorite concentrations on the erosion of instrumented radicular dentin. Dent Mater 2010;26:514-23.
19. Saghiri MA, Delvarani A, Mehrvarzfar P, et al. A study of the relation between erosion and microhardness of root canal dentin. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;108:29-34.
20. Uzunoglu E, Yilmaz Z, Erdogan O, Görduysus M. Final irrigation regimens affect fracture resistance values of root-filled teeth. J Endod 2016;42:493-5.
21. Ghisi AC, Kopper PM, Baldasso FE, et al. Effect of superoxidized water and sodium hypochlorite, associated or not with EDTA, on organic and inorganic components of bovine root dentin. J Endod 2015;41:925-30.
22. Aranda-Garcia AJ, Kuga MC, Chavez-Andrade GM, et al. Effect of final irrigation protocols on microhardness and erosion of root canal dentin. Microsc Res Tech 2013;76:1079-83.
23. Pascon FM, Kantovitz KR, Soares LE, et al. Morphological and chemical changes in dentin after using endodontic agents: Fourier transform Raman spectroscopy, energy-dispersive x-ray fluorescence spectrometry, and scanning electron microscopy study. J Biomed Opt 2012;17:1-6.
24. Phillips B, Ball C, Sackett D et al. Oxford Centre for Evidence-based Medicine Levels of Evidence in Level of Evidence and Grades of Recommendation. 2009 URL: http://www.cebm.net/oxford-centre-evidence-based-medicine-levels-evidence-march-2009.
25. De Deus G, Reis C, Paciornik S. Critical appraisal of published smear layer removal studies: methodological issues. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112:531-43.
26. Virdee SS et al. Efficacy of irrigant activation techniques in removing intracanal smear layer and debris from mature permanent teeth: a systematic review and meta-analysis. Int Endod J 2018;51:605-21.
27. Fornari VJ et al. Histological evaluation of the effectiveness of increased apical enlargement for cleaning the apical third of curved canals. Int Endod J 2010;43:988-94.
28. Huang TY, Gulabivala K, Ng YL. A bio-molecular film ex-vivo model to evaluate the influence of canal dimensions and irrigation variables on the efficacy of irrigation. Int Endod J 2008;41:60-71.
29. Butcher S, Mansour A, Ibrahim M. Influence of Apical Preparation Size on Effective Conventional Irrigation in the Apical Third: A Scanning Electron Microscopic Study. Eur Endod J 2019;4:9-14.

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ORIGINAL ARTICLE

Histological and histochemical aspects of the dentin-pulp complex in offsprings of rats treated with fluoxetine during pregnancy and lactation

ABSTRACT

Aim: *This study aimed to analyze histological aspects of the dentin-pulp complex in rats neonates treated with fluoxetine during pregnancy and lactation.*
Methodology: *For this purpose were used first molars of 24 Wistar rat pups, 25 days old-aged, divided into four groups. PCG and PCGL whose mothers positive controls received 0.9% NaCl (oral) during gestation and gestacion/lactation, respectively; FG and FGL both mothers received fluoxetine at 20 mg/kg (oral) during pregnancy and gestation/lactation, respectively. The animals were anesthetized, maxillaries removed and fixed in 4% formaldehyde, decalcified in EDTA and processed conventionally for light microscopy. Paraffin sections were stained by Hematoxylin and Eosin, Masson's Trichromatic and Pricosirius Red.*
Results*: There was no evidence of structural and histochemical changes in the pulp-dentin complex in the groups studied.*
Conclusions: *Therefore, we could conclude that the fluoxetine in 20 mg/kg dosage, administrated during pregnancy and lactation, did not interfered in the dentin-pulp complex development in offsprings of rats when analyzed in the age of 25 days.*

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Introdution

Depression is a mental disorder characterized by persistent sadness and loss of interest in normally pleasant activities, accompanied by an inability to carry out daily activities for at least two weeks. Women are considered to be a risk group for this kind of disorder, whether in pregnancy or in the postpartum period. During pregnancy, the female organism produces placenta hormones that can bring about significant organic and behavioral changes (3). Currently, 10-13% of pregnant women suffer from depressive episodes associated with pregnancy (2).

The depression treatment during the gestational and postpartum period is important to prevent obstetric complications, such as the birth of low weight preterm infants (1). The advance of pharmacology has promoted the emergence of highly selective chemicals that act specifically on the neurotransmitter systems. For the pharmacological treatment of mood and anxiety disorders, the most widely prescribed antidepressives worldwide are SSRIs such as fluoxetine (4, 5).

Fluoxetine is the antidepressive of choice for administration in women who are gestating and breast fed. Its metabolite, norfluoxetine, is able to cross the placental barrier and be excreted in breast milk, resulting in high therapeutic levels in plasma. Fluoxetine is able to induce long-term changes in the serotonin system. However, the precise effects are not yet clear. Details, including underlying neural mechanisms, are still being outlined (6).

There is a shortage of data on the direct action of SSRIs in differentiating human bone cells; however, some studies have shown that they have a deleterious effect on the formation and proliferation of osteoblasts from the defective signaling of the 5-HT receptors, causing their apoptosis.

The tooth and periodontium development process has been the object of a number of studies, which attempt to clarify the phenomenon of the histogenesis, induction and histodifferentiation of the tissues before its functional activity in the post-birth life. However, very little has been described in the literature about the influence of SSRIs on odontogenesis and the development of periodontal tissues and the basal bone of the jaw.

Based on the evidence that the serotoninergic system is associated with developing different human body tissues, mediating important epithelium mesenchymal interactions, this research was justified in order to evaluate fluoxetine's influence in developing and growing the dentin-pulp complex during gestation and lactation.

Materials and Methods

This research was approved by the Ethics Committee on the Use of Animals at the Federal University of Pernambuco (CEUA-UFPE), process n° 23076.017680/2011-83. All measures have been taken to minimize the pain or discomfort of mothers and offsprings. Were used 24 Wistar rats (Rattus norvegicus albinus, Rodentia, Mammalia) with 25 days of life, coming from the Bioterium of the Department of Nutrition of the UFPE. Six albino rats of the Wistar strain were used, kept in a room with a temperature of 23 ± 2 ºC and a light and dark cycle of 12/12 hours (light from 6 am to 6 pm and dark from 6 pm to 6 am), receiving the standard diet of vivarium (Presença Ratos e Camudongos - Presença, Paulínia, SP, Brazil) and water ad libitum. Mating between adult animals and vaginal smear tests were performed to prove pregnancy. The day of proof of pregnancy was the first day of pregnancy and the beginning of the animals' procedures (10).

Two groups comprised the research: the control group (Group C) and the group treated with fluoxetine (Group F). The groups were further divided into two subgroups: animals that were treated only during pregnancy (PCG and FG) and animals that were treated during pregnancy and lactation (PCGL and FGL). One pregnant rat was used for each subgroup of the control groups (PCG and PCGL) and, from each litter, 6 offsprings were used, of both sexes (n=6). For the group treated with fluoxetine, two pregnant rats were used for each subgroup and from each litter three offsprings were used, of both sexes, totaling six (n=6) animals per subgroup, totaling a sample of 24 offsprings. In the PCG group, the animals came from rats that received 0.9% sodium chloride at a dose of 10 µl/g orally (gavage) throughout the gestation period; in the PCGL group the animals came from rats that received 0.9% sodium chloride at a dose of 10 µl/g orally (gavage) during pregnancy and lactation and in the FG and FL groups the animals came from rats that were treated with fluoxetine hydrochloride (Pharma Nostra, Rio de Janeiro, Rio de Janeiro, Brazil) at a dose of 20 mg/kg of animal weight orally (gavage) administered until the end of pregnancy (group FG) and in group FGL the animals were treated with fluoxetine during pregnancy until the end of breastfeeding, that is, 21 days after birth (table 1).

When they reach 25 days, the animals were anesthetized by intraperitoneal injection with an association of ketamine (CEVA, Paulínia, São Paulo, Brasil) at a dose of 50 mg/kg animal weight and xylazine (CEVA, Paulínia, São Paulo, Brasil) at a dose of 20 mg/kg animal weight. The offisprings were perfused intracardiacally with 4% paraformaldehyde (Sigma Aldrich, São Paulo, Brasil) solution in sodium phosphate buffer. The heads were sectioned frontally so as to obtain transverse cuts of the maxilla mesially to the first molars and were immersed in solution of the same fixative for 24 hours at room temperature. The pieces were decalcified in a solution of 5% ethylenediaminetetraacetic acid (EDTA) (Vetec Química Fina Ltda, Duque de Caxias, Rio de Janeiro, Brasil) for 15 days.

After the decalcified, were inclusion in paraffin (Michalany, 1980) and sectioned in a hand microtome (LEICA RM 2125 RT, Leica Biosystems Nussloch GmbH, Nussloch, Alemanha), adjusted to 5 µm-thick each. Serial cuts were made of all specimens that were stained with Hematoxylin and Eosin (HE), Masson's Trichrome (MT) and Picrossirius Red, mounted with Entellan® (Merck KGaA, Darmstadt, Alemanha).

For histological analysis of the central zone of the coronary and root pulp, HE stained preparations were selected and a LEICA ICC50 HD light microscope (Leica Biosystems Nussloch GmbH, Nussloch, Germany) was used coupled to a microcamera, connected to a computer containing a plate image capture.

For the histochemical analysis of collagen, preparations stained by HE, TM and Picrossirius Red were used using a LEICA ICC50 HD light microscope (Leica Biosystems Nussloch GmbH, Nussloch, Germany), coupled to a microcamera, connected to a computer containing a plate image capture.

No statistical test was performed because the study design has a qualitative and histomorphological approach.

Table 1
Study groups

	Control (n=12)		**Experimental (n=12)**	
Mothers	PCG (n:1)	PCGL (n:1)	FG (n:2)	FGL (n:2)
Offsprings	n:6	n:6	n:3 n:3	n:3 n:3
Total offsprings				**n:24**

(*)Source: Own data
PCG: Positive control gestacion
PCGL: Positive control gestacion and lactacion
FG: Fluoxetine gestacion
FGL: Fluoxetine gestacion and lactacion

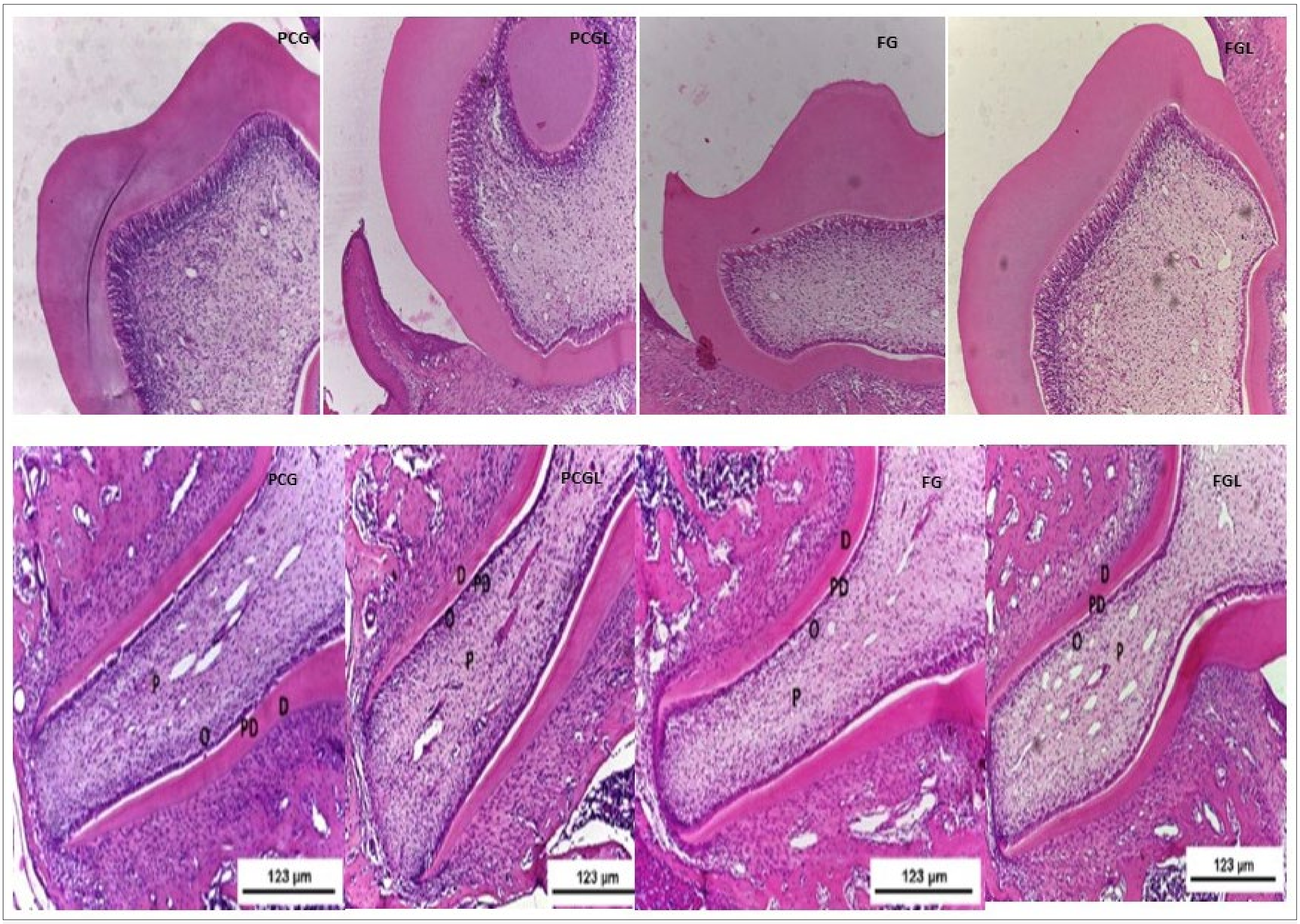

Figure 1.
Photomicrographs of part of the first upper molar crown and root of the rat with 25 days of life, corresponding to the Positive Control Gestation (PCG), Positive Control Gestation and Lactation (PCGL), Fluoxetine Gestation (FG) and Fluoxetine Gestation and Lactation (FGL). Observe the odontoblasts (O) in the region of dental crown with high prismatic aspect and with the nucleus polarized toward the dental pulp, dentin (D) thicker than the dentin (PD). P: Dental Pulp. VS: Blood Vessels. HE: coloration. In the root portion, the odontoblasts (O) present a low prismatic aspect and in a single layer with a polarized nucleus toward the dental pulp, dentin (D) thicker than pre-dentin (PD). P: Dental Pulp. VS: Blood Vessels. HE: coloration.

Results

It was observed that at 25 days of life the dental element of the first upper molars of rats was in phase of root development, and the apical portion was in formation, so the foremen was not closed. Comparing the coronary and radicular dentin-pulp structures between the groups NC, PCG, PCGL, FG e FGL, no differences were found in histological arrangement.

Analysis of the preparations stained with HE

Histologically, in all analyzed groups, the dentine presented thicker in the crown than in the root containing homogeneous matrix, acidophilic, with evidence of the Tomes fibers in its interior. These fibrils were arranged perpendicular to the pulp surface, distributed in a centripetal way. Below the dentin, it was evidenced a thin layer of pre-dentin, lighter than the surrounding dentin, exhibiting less pronounced acidophilia than the first, stained in light pink. Inside of pre-dentin, it was observed the layer of odontoblasts, distributed in palisade, with high prismatic aspect in the crown and low prismatic in the regular region.

In the cuspid portion, they exhibited a similar aspect to a pseudostratified epi-

thelium, which characteristic was not evident in the crown areas and radicular region. The odontoblasts showed an elongate nucleus, in the proximal region, with strongly acidophilous cytoplasm with basophilic tones in the basal region.
The dental pulp showed a subodontoblast layer containing mesenchymal cells densely clustered below the odontoblastic layer, interspersed by small-caliber blood vessels. The central zone of the pulp showed less concentrated fibroblasts than in the peripheral region, with weakly acidophilic blood vessels and weakly acidophilic collagenous matrix being evidenced between them. The root pulp had a conical aspect, with a forming foramen. No accessory channels or collateral foramina were observed between the studied groups (Figure 1).

Histochemical analysis of preparations stained with TM
When treated with TM, the dentin and pre-dentin of all analyzed groups were stained in blue, presenting a positive reaction for the presence of collagen, with pre-dentin being less stained than dentin. The pulp exhibited odontoblasts and fibroblasts with nuclei dyed black. The intercellular matrix of the coronary and radicular pulp showed a weakly positive reaction to the collagen, staining in light blue, being slightly more concentrated in the root (Figure 2).

Histochemical analysis of preparations stained with Picrossirius Red
The analyzed samples showed no differences regarding the type and disposition of the collagen in the dentin, pre-dentin and dental pulp of the animals subjected or not to fluoxetine during its development. The preparations showed positive reaction to Picrossirius Red in the dentin and pre-dentin of all animals from the studied groups, with the dentin stained in intense red and the pre-dentin in light red. On the other hand, the pulp expressed weakly reactive acquiring the green or yellow shade in the odontogenic region and a light red in the central region, being more intense in the radiular portion than in the crown (Figure 3).

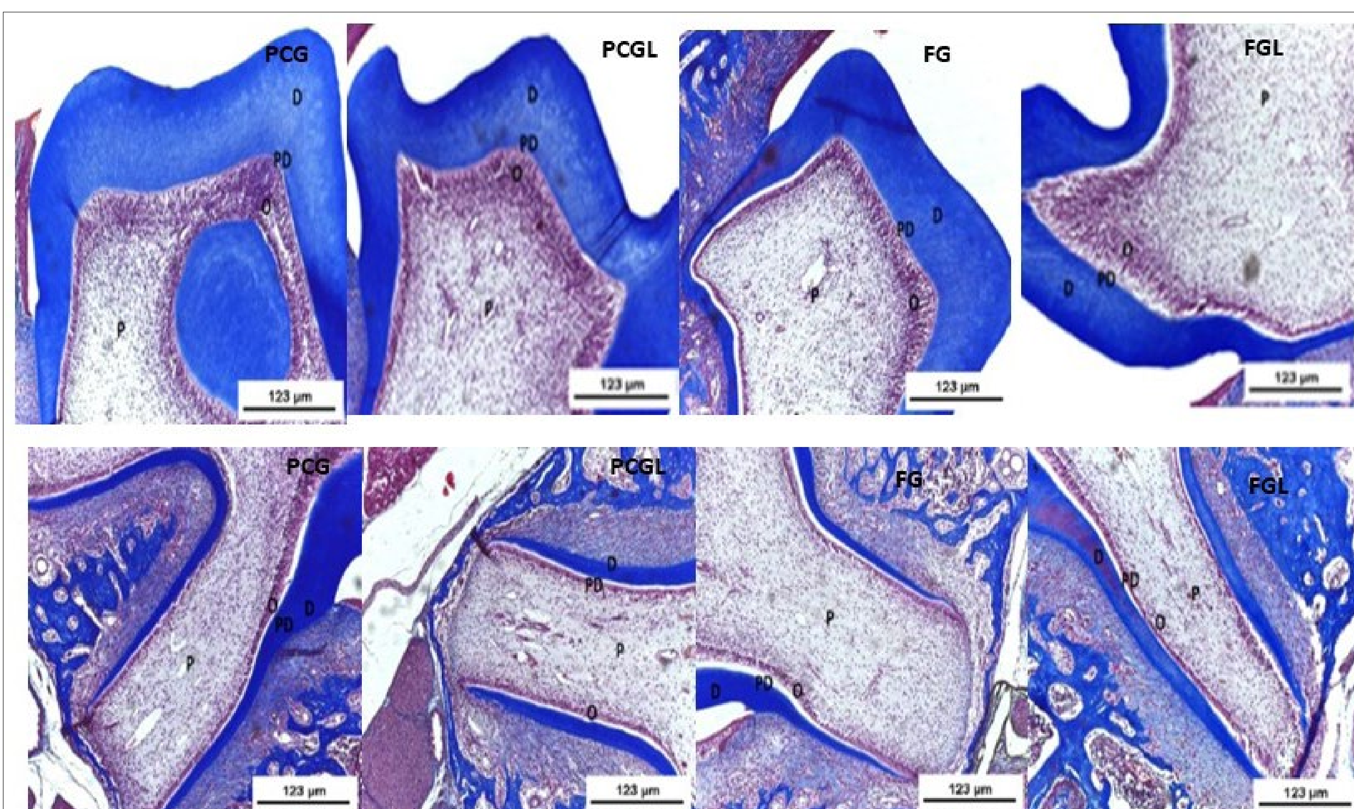

Figure 2. Photomicrographs of part of the first upper molar crown and root of the rat with 25 days old, corresponding to the Positive Control Gestation (PCG), Positive Control Gestation and Lactation (PCGL), Fluoxetine Gestation (FG) and Fluoxetine Gestation and Lactation (FGL). Pay attention to the dentin and pre-dentin with a strongly positive reaction for Masson's trichrome. Odontoblasts (O); dental pulp (P). Observe a positive reaction to the Masson's trichrome showing collagen fibers with bluish coloration. P: Dental Pulp. VS: Blood Vessels.

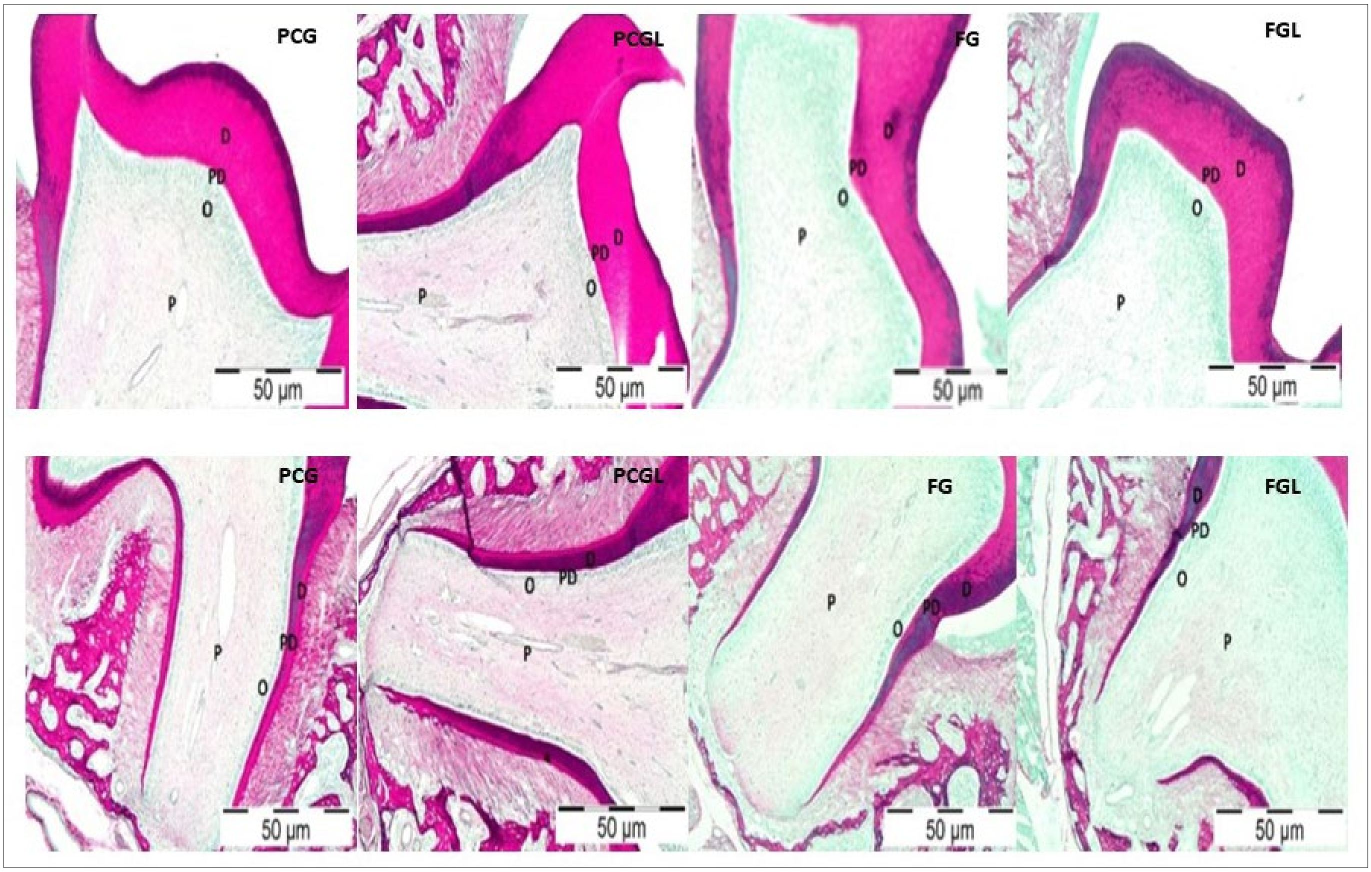

Figure 3. Photomicrographs of the upper first molar crown and root of the rat with 25 days old, corresponding to the Positive Control Gestation (PCG), Positive Control Gestation and Lactation (PCGL), Fluoxetine Gestation (FG) and Fluoxetine Gestation and Lactation (FGL). Observe positive reaction for Picrosirius Red, showing dentin (D) and pre-dentin (PD) in reddish coloration. P: Dental Pulp. VS: Blood Vessels. Observe positive reaction for Picrosirius Red showing collagen "I" in reddish color. P: Dental Pulp. VS: Blood Vessels. Picrosirius Red coloration.

Discussion

In the present study, it was observed that the chronology of the development of the upper first molar of the animals of 25 days of life showed in a similar way in all groups, in other words, it was in the phase of rhizogenesis, with half of root grown and the apex not closed yet. The crown had already erupted and the odontoblasts were in juxtaposed position with the pre-dentin layer and in greater number by area in nearest portions of the cusps, corroborating with data already showed in the literature (11).
Histologically, when stained with HE, it was not observed structural changes in the crown or radicular dentin-pulp complex in the experimental groups when compared with control groups following the same structural pattern written in publications (12).
As dentinogenesis progressed, the odontoblasts were synthesizing the organic matrix of the dentin, retreating and leaving a cellular extension within the dentin. A narrow band of pre-dentin between the odontoblasts and dentin was easily distinguishable in 25-day-old animals. These findings are in accordance with data reported in the literature where the presence of a richly sulfuric and less acidophilic matrix layer of the dentin organic matrix (pre-dentin) in the vicinity of the odontoblastic (13-15).
We observed that the dental pulp presented itself as a loose connective tissue still young and imature, with great number of cells, more evident in the root portions. These data also are reported in 2006 when they say that the presence of undifferentiated mesenchymal cells in dental pulp of the rat show that this tissue is capable of growth and reparation (16). The collagen fiber plays an important role keeping shape and structural role of the connective tissues. Changes in its molecular organization, amount and special distribution may provoke a series of pathologies (17). In the dentin-pulp complex, the collagen is the most abundant protein of the matrix and plays several functions as support, associ-

ation to the molecules of the amorphous substance and nucleation of hydroxyapatite crystals in dentin (18).

Two histochemical methods were used for detection of the collagen: Trichrome (TM) and Picrosirius Red. For the evidence of collagen fibers, the Masson's trichrome, is widely used to evidence collagen fibers and stained them in light green or blue-green (19). In an article from 2002, Kiernan describes the principle of the method characterizing a trichrome as a technique which uses one or more anionic dyes used in conjunction with phosphotungstic or phosphomolybdic acid. Its principle of action is not clear yet, but it is believed that these acids would bind to collagen as a mordant giving this molecule a negative charge which would attract the anionic dyes, in this case the aniline blue (20).

The TM technique allowed to observe the presence of collagen, which was abundantly distributed in a homogeneous way, independently of the growth phases of the tooth, corroborating with the findings (16). However, it was not possible to identify types of collagen present, since the technique does not have specify for differentiate types of collagen. Beyond that, the fibrils can be associated in bundles of different thickness, present different directions depending on the type of amino acid present and the function of the tissue. For this purpose, we use coloring with Picrosírius. The Picrosírius Red has been widely applied for detection of the collagen. It is a strongly acidic azo dye that presents six sulfonic groups, which allows detecting small amounts of collagen. This dyeing makes strong bonds between its acidic sulfonic groups and the basic groups of the collagen molecule, making it easily identifiable as collagen I, II or III by different colorations (21).

In the preparations stained with the Picrosírius Red under light microscope, there were thick reddish type I collagen fibers. However, the dental pulp showed weak reactivity to Picrosírius in the odontogenic zone, and the light green field tintorial characteristic indicates the presence of immature collagen fibers, which was also described in the literature [8]. The central zone shows a smooth red reaction, which has a thicker fibrillar arrangement than in the previous layer.

A lot studies describe that the collagen I is the predominant component in the dentin matrix. Besides that, the presence of collagen type I in the dentin, pre-dentin and dentin pulps has been classically reported as fibers stained red or yellow by the Picrossírius Red method (12, 16).

Previous studies, observed neonatal repercussions on craniofacial development in in the offspring of the Wistar rats treated with fluoxetine only during gestation at a dosage of 10 mg/ kg. In these studies, possible morphological changes during dentinogenesis, amelogenesis and formation of the temporomandibular joints (TMJs) were analyzed, in which, similarly, no significant alterations were observed (22-25).

In our study, when we analyzed the group treated with the control group, we found that the administration of 20 mg/kg shows similarity in color nuances, with no morphological and structural development of dentin-pulp tissues in both groups. Thus, we observed that the signaling of serotonin receptors was not sufficient to generate reduced and morphological changes in the present study.

Conclusions

Thus, our results allow us to conclude that fluoxetine at a dosage of 20 mg/kg of animal weight, administrated during pregnancy and lactation, did not interfere with the tissue arrangement as well as the process of formation of the intercellular collagen matrix during the development of the dentin-pulp complex in the offspring of rats when analyzed at 25 days of age.Studies using long-term administration of fluoxetine are required to confirm the safety of drug use during pregnancy and lactation, especially regards to the formation of mineralized tissues and odontogenesis.

Ethic Statement

Research approved by the Ethics Committee on Animal Use (CEUA) of the Center for Biological Sciences of the Federal

University of Pernambuco (CCB-UFPE) under the number 23076.017680/2011-83.

Clinical Relevance

The present study is important because it adds information to what is still little described: about the role of serotonin in the development of mineralized and non-mineralized dental tissues.

Conflict of Interest

All authors declare no conflict of interest

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References

1. *Hendrick V & Altshuler L. Management of major depression during pregnancy. Am J Psichiatry. 2002. Oct; 159(10): 1667-73. doi: 10.1176/appi.ajp.159.10.1667.*
2. *Hein A et al. Socioeconomic status and depression during and after pregnancy in the Franconian Maternal Health Evaluation Studies (FRAMES). Arch Gynecol Obstet. 2014. Apr; 289 (4): 755-63. doi: 10.1007 / s00404-013-3046-y.*
3. *Hodge et al. Selective serotonin reuptake inhibitors inhibit human osteoclast and osteoblast formation and function. Biol Psychiatry. 2013. Jul; 74(1):32-9. doi: 10.1016/j.biopsych.2012.11.003.*
4. *Moreno RA et al. Psicofarmacologia de antidepressivos. Rev Bras Psiquiatr. 1999. May; 21(1):24-40. doi: 10.1590/S1516-44461999000500006.*
5. *Fontana CAP et al. Morfologia do ligamento da cabeça do fêmur de ovinos da raça Santa Inês. Ars Veterinaria. 2007. Dec; 23(3): 158-64.*
6. *Kiryanova V et al. Long-term outcomes of developmental exposure to fluoxetine: a review of the animal literature. Dev Neurosci. 2013. Dez; 35(6):437-49. doi: 10.1159/000355709.*
7. *Luo X et al. Serotonergic regulation of somatosensory cortical development: lessons from Genetic Mouse Models. Devel Neurosci. 2003 Mar-Ago; 25 (2-4):173-83. doi: 10.1159/issn.0378-5866.*
8. *Muller CL et al. Impact of maternal serotonin transporter genotype on placental serotonin, fetal forebrain serotonin and neurodevelopment. Neuropsychopharmacology. 2017 Jan; 42(2):427-36. doi: 10.1038/npp.2016.166.*
9. *Bliziotes MM et al. Neurotransmitter action in osteoblasts: expression of a functional system for serotonin receptor activation and reuptake. Bone. 2001 Nov; 29(5):477-86. doi: 10.1016/S8756-3282(01)00593-2.*
10. *Lankas GR et al. Effects of ivermectin on reproduction and neonatal toxicity in rats. Food Chem Toxicol. 1989. Ago; 27(8): 523-29. doi: 10.1016/0278-6915(89)90048-3.*
11. *Hao J et al. Temporal and spatial localization of the dentin matrix proteins during dentin biomineralization. J Histochem Cytochem. 2009. Mar; 57(3): 227–37. doi: 10.1369/jhc.2008.952119.*
12. *Linde A & Goldberg M. Dentinogenesis. Crit Rev Oral Biol Med.1993. Oct; 4(5):679-728. doi: 10.1177/2F10454411930040050301.*
13. *Reith EJ. Collagen formation in developing molar teeth of rats. J Ultrastruc Res. 1968. Dez; 21(5-6): 383-414. doi: 10.1016/S0022-5320(67)80148-5.*
14. *Wang J et al. The effect of scaffold architecture on odobtogenic differentiation of human dental pulp stem cells. Biomaterials. 2011. Nov; 32(31):7822-30. doi: 10.1016 / j.biomaterials.2011.04.034.*
15. *Goracci G at al. Terminal end of the human odontoblasts process: a study using SEM and confocal microscopy. Clin Oral Invest. 1999. Sep; 3(3): 126-132. doi: 10.1007/s007840050090.*
16. *Abrahão IJ et al. Collagen analysis in human tooth germ papillae. Braz Dent J. 2006. Sep; 17(3): 208-212. doi: /10.1590/S0103-64402006000300006.*
17. *Pickering JG et al. Evidence for rapid accumulation and persistently disordered architecture of fibrillary collagen in human coronary restenosis lesions. Am J Cardiol. 1996. Sep; 78(6):633-37. doi: 10.1016/s0002-9149 (96) 00384-0.*
18. *He G & George A. Dentin matrix protein I immobilized on type I collagen fibrils facilitate apatite deposition in vitro. J Biol Chem. 2004. Mar; 279(12):11649-56. doi: 10.1074/jbc.M309296200.*
19. *Tolosa EMC et al. Manual de Técnicas para Histologia Normal e Patológica. 1ª ed. São Paulo: São Paulo; 2003.*
20. *Kiernam, JA. Histological e Histochemical Methods: Theory and Practice. 3ª ed. London: England; 2002.*
21. *Junqueira LCU et al. Picrosirius staining plus polarization microscopy – a specific method for collagen detection in tissue sections. Histochemistry J. 1979. Jul; 11(4):447-55. doi: 10.1007/BF01002772.*
22. *Cavalcanti UDNT et al. Morphological aspects of the embryonic development of the TMJ in rats (Rattus norvegicus albinus) treated with fluoxetine. Int J Morphol. 2009. Sep; 27(3):899-903. doi: 10.4067/S0717-95022009000300041.*
23. *Silva IHM et al. Morphological analysis of the enamel organ in rats treated with fluoxetine. Clinics. 2010. Jan; 65(1):61-6. doi: 10.1590/S1807-59322010000100010.*
24. *Santiago IMA et al. Morphological aspects of dentin-pulp complex development in the offspring of rats treated with fluoxetine during pregnancy. Braz J Oral Sci. 2013. Jan-Mar; 12(1):30-6. doi: 10.1590/S1677-32252013000100007.*
25. *Santiago IMA et al. Efeitos da Utilização do Cloridrato de Fluoxetina Sobre o Desenvolvimento de Tecidos Mineralizados de Ratos: Revisão Sistemática. Rev Bras Ciên Saúde. 2013. Jun; 17(2):181-88. doi:10.4034/RBCS.2013.17.02.12.*

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ORIGINAL ARTICLE

Antibacterial potential of nano-particulate intracanal medications on a mature *E. faecalis* biofilm in an *ex vivo* model

ABSTRACT

Aim: *To investigate and compare the antibacterial and antibiofilm activity of Chlorhexidine Digluconate functionalized Hydroxyapatite Nanoparticles and silver Nanoparticles as intracanal medicaments on a mature E. faecalis biofilm model in root canals of extracted teeth.*

Methodology: *Sixty-eight human maxillary central incisors were mechanically prepared, sterilized, infected with E. faecalis, and then incubated for 28 days under anaerobic conditions to develop a mature E. faecalis biofilm. Eight teeth were used to monitor biofilm formation and maturation over the incubation period with field emission scanning electron microscopy (Fe SEM), while the other 60 teeth were divided into two experimental groups (n=20) in which 2% chlorhexidine digluconate functionalized hydroxyapatite nanoparticles (Group A) and 0.02% silver nanoparticles (group B) were used as intra canal medications, and two control groups (n=10). The positive control group (Group C) was used to check for bacterial viability throughout the experiment, while the negative control group (Group D) was used to check for sterility of the procedures. Finally, bacterial samples were collected and analyzed quantitively by culture counts and qualitatively by real time PCR (RTQ-PCR).*

Results: *RTQ-PCR detected E. faecalis DNA in all groups except for the negative control group. The mean values of E. faecalis DNA detected in groups A, B and C were 99.6 ng/mL, 67.1 ng/mL, 2797.4 ng/mL respectively. Statistical analysis of these results showed that both treatment groups presented statistically significantly lower mean values of E. faecalis DNA quantities compared to the positive control group. However, there was no statistically significant difference between them (P<.05).*

Conclusions: *Chlorhexidine Digluconate functionalized Hydroxyapatite Nanoparticles and silver nanoparticles demonstrated an effective antimicrobial activity against mature E. faecalis biofilms.*

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Introduction

Microorganisms harboring within the root canal system are the primary cause of apical periodontitis (1). They populate dentinal tubules, apical ramifications, lateral canals and isthmuses, so they are less accessible to chemo-mechanical approach of disinfection (2, 3). Several anti-bacterial strategies have been employed to reduce the number of microorganisms in the infected root canal systems, including the use of various irrigation regimens and intracanal medicaments in combination with root canal instrumentation (4).
Although systemic antibiotics appear to be an effective adjunct in certain surgical and non-surgical endodontic procedures, their administration is not without the potential risk of adverse systemic effects, such as allergic reactions, toxicity and the development of resistant strains of microbes (5). Furthermore, a necrotic root canal is a secluded cavity inaccessible to the local immune system (6), and the concentration of drug that reaches the canal space after systemic administration of antibiotics is minimal and unlikely to inhibit bacterial growth (7).
Intracanal medicaments in the Nano-form can achieve optimal therapeutic activity through its interaction with the human body at both the sub-cellular and molecular levels. Nanoparticles exclusive features include smaller sizes, increased surface area to volume ratio, and higher chemical reactivity, compared with their bulk counterparts (8).
Silver is among the historical antimicrobials used since Hippocrates' early suggestions (9). Silver nanoparticles have several antibacterial effects such as interaction with bacterial DNA sulfhydryl groups, unwinding bacterial DNA, interference with cell-wall synthesis/cell division and production of reactive oxygen species (8). These collective effects account for their broad-spectrum bactericidal activity and the rare existence of bacterial resistance to it (10).
Functionalized nanoparticles, refers to conjugation of chemicals including drugs or proteins on nanoparticle surfaces to enhance their biological properties (11). Hydroxyapatite [molecular formula: $Ca_{10}(PO4)_6(OH)_2$] is a calcium phosphate bioceramic used for drug delivery, due to its chemical ability to incorporate molecules and drugs. In addition to its osteoconductive potential they are chemically and structurally similar to the mineral phase of bones and teeth (12, 13). Chlorhexidine is a lipophilic cation that interacts with the negatively charged phosphate group of the bacterial cell wall, resulting in altered osmotic equilibrium and increased permeability of the bacterial cell (5). The aim of the current study was to investigate and compare the antibacterial potential of chlorhexidine digluconate functionalized hydroxyapatite nanoparticles and silver nanoparticles as intracanal medicaments on a mature *E. faecalis* biofilm model in root canals of extracted teeth.

Materials and Methods

Selection and preparation of the samples
Ethics committee approval (number 01062017) was granted for all procedures from the review board of Ain-Shams dental school, Cairo, Egypt. Sixty-eight recently extracted human maxillary central incisors with mature apices and single root canals were selected for use in this study. All teeth were stored in 0.5% thymol solution at 4 °C before use. Following periapical radiographs, the crowns were reduced using a safe sided diamond disc (NTI diamond disc, Axis Dental, USA) mounted on a high-speed contra-angle with water coolant, and the tooth length was standardized to 18 mm from the root apex to the coronal border. Cleaning and shaping of samples were performed using the Protaper rotary nickel titanium system (Dentsply Maillefer, Ballaigues, Switzerland) in the presence of 2.5%NaOCl (Calix, Miami, USA) irrigation until finishing F5 (50/0.4). Following preparation, all canals received a final irrigation sequence of 5ml of 17% EDTA (Calix, Miami, USA) followed by 5ml of 2.5% NaOCl, and 5ml of sterile water (Baxter sterile water, Dealmed Medical supplies, USA) to remove the smear layer.

The teeth were then air-dried and steam autoclaved at 121 °C for 30 minutes. The entire root surface, including the apical foramen of each sample, was coated with two layers of nail polish (Max Factor, Cosmetics and Fragrances, London, UK).

Grouping of the samples

Table 1 presents the distribution of the selected samples (n=68) among the groups. Samples were divided into the following groups.

Table 1

GROUP A	N=20	Medicated with nano-hydroxyapatite functionalized with 2% chlorhexidine digluconate gel
GROUP B	N=20	Medicated with 0.02% silver nanoparticles gel
GROUP C	N=10	The positive control group used to check for bacterial viability throughout the experiment
GROUP D	N=10	The negative control group used to check for sterility of the procedures
GROUP E	N=8	Used to monitor biofilm maturation by SEM

Development of E. faecalis *biofilm*

Under strict aseptic conditions, *E. faecalis* American type culture collection (ATCC 29212) reference strain was inoculated in brain heart infusion (BHI) broth (Oxoid, Basingstoke, UK) and adjusted to bacterial count concentration of 0.5 McFarland ($1.5x10^8$ cfu/mL). All samples, except for the negative control group, were each transferred to centrifuge tubes filled with 1.5 mL *E. faecalis* BHI suspension, and incubated under anaerobic conditions at 37 ˚C for 4 weeks. Broth was replenished by fresh BHI regularly every second day to clear dead cells and to ensure bacterial viability. Samples of the negative control group followed exactly similar procedures except for inoculation of sterile BHI broth.

Monitoring of E. faecalis *biofilm maturation*

During the incubation period, 8 samples were submitted for Field emission scanning electron microscope (Fe SEM: Model Quanta 250 FEG, Netherlands) examination to detect the progress of *E. faecalis* biofilm development and maturation after 3 days, 7 days and 28 days as described previously (14). Briefly samples were split with a hammer and chisel into two halves, gently rinsed with 5 mL sterile phosphate-buffered saline (PBS) to remove the culture medium and nonadherent bacteria. Then submerged in 4% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer, dehydrated through ascending grades of ethanol, dried by critical point dryer, and sputter-coated with gold in a vacuum evaporator (Emitech K550X sputter coater, England).

Preparation of nano-particulate intra canal medications

Nano-hydroxyapatite (Nanotech Egypt photo-electronics, Cairo, Egypt) was functionalized with chlorhexidine digluconate (Sigma Aldrich, St Louis, USA) according to the method described by Soriano-Souza *et al.* 2015 (13). Briefly, 100 mg of nano-hydroxyapatite were added to 4 ml solution of 20% chlorhexidine digluconate. The mixture was sonicated at 37 ˚C for 24 hours. Then centrifuged at 600 rpm for 5 minutes. This was followed by washing one time with water and finally drying to collect the purified material.

This preparation was verified by high resolution Transmission Electron Microscope (JEM-2100, JOEL, USA), as well as by Field Emission Scanning Electron Microscope (Fe SEM: Model Quanta 250 FEG, Netherlands) equipped with Energy Dispersive X-ray (EDX) unit. The EDX analysis was done to detect the elements in the loaded nanoparticles to confirm the incorporation of chlorhexidine digluconate compositional elements in the hydroxyapatite nanoparticles. Ultraviolet spectrophotometry (Ocean Optics USB 2000, Winter Park, USA) was also used for further confirmation of structural change via detecting shift of the absorbance band between nano-hydroxyapatite functionalized with chlorhexidine digluconate and its precursors; nano-hydroxyapatite and chlorhexidine digluconate.

A 2% concentration was prepared by mixing 2 grams of nano-chlorhexidine with 100 ml distilled water under very well stirring, then 0.72 grams sodium chloride (Sigma Aldrich, MO, USA) were added under stirring, then 2.7 grams methyl cellulose (Sigma Aldrich, MO, USA) was added portion-wise under stirring for 1 hour, till a gel consistency was obtained. In order to prepare silver nanoparticles gel, 100 ml of 0.02% silver nanoparticles (Nanotech Egypt photo-electronics, Cairo, Egypt) was added to 0.72 grams Sodium Chloride and stirred. Then, 2.7 gm of Methyl Cellulose was added portion-wise under stirring for 1 hour, till a gel consistency was obtained. A plain gel containing Sodium Chloride, Methyl Cellulose mixed with distilled water was also prepared for application into samples of the positive and negative control groups.

Application of intracanal medication in root canals

Following an incubation period of 28 days, a standard volume (15 µL) of intracanal medications were introduced into the samples using microbiologic micropipettes, Group A was medicated with nano-hydroxyapatite functionalized with 2% chlorhexidine digluconate gel, Group B was medicated with 0.02% silver nanoparticles gel, while Groups C and D which represent the positive and negative control groups respectively were medicated with a similar volume of the plain gel. The coronal access of each root canal was dressed with sterile cotton pellet then sealed with Cavit (3M ESPE, Germany). Finally, samples were incubated anaerobically at 37 °C for 7 days in a 100% humid environment.

Root canal re-entry

Following seven days of incubation, Cavit was removed aseptically by a sterile excavator. Each sample was washed with 5 mL sterile Phosphate Buffered Saline (Thermo Fisher Scientific, Waltham, MA, USA) to remove the root canal contents and then submitted for detection. Four samples from each group were assessed for biofilm disruption by Fe SEM. The remaining samples underwent bacterial quantification by RTQ-PCR supplemented with culture evaluation of bacterial counts.

Bacterial quantification

For each root canal, a size 40 sterile absorbent paper point (Diadent, South Korea) was placed inside the root canal for 1 minute, then size 40 H-file (Mani Inc, Japan) was used to vigorously cut circumferential dentin, followed by another size 40 paper point. Finally, the H-file and paper points used in each root canal were transferred aseptically to a labeled vial containing 1 ml sterile saline solution. The vials were vortexed for 15 seconds, then form each vial 0.5 mL was collected and freezed for RTQ-PCR analysis and 0.5 mL was collected for culture analysis.

Polymerase chain reaction (PCR) amplification assay

E. faecalis DNA was extracted by Qiagen DNA mini Kit (Qiagen, Hamburg, Germany) in accordance with manufacturer's instructions for Gram-positive bacteria DNA extraction (i.e. including a lysozyme bacterial wall lysis). DNA was eluted in 200 µL of the elution buffer included in the kit. DNA was stored at (-20 °C) until used for RTQ-PCR (15).

RTQ-PCR amplification and detection was done using Real Time PCR ViiA 7 system (Applied Biosystems, Foster city, USA) using a 96-well format. Reactions were set-up in a PCR-specific hood under aseptic laboratory conditions. To quantify *E. faecalis*, RTQ-PCR reactions were performed in a total volume of 20 µL. Briefly, 20 µL total reactions consisted of 10 µL SybrGreen PCR Mastermix, forward and reverse primers 1µL from each primer (10 moles/µL) and 8 µL of extracted DNA.

The primer sequences of *E. faecalis* have been used according to Williams *et al.* 2006 description (16).

The published sequences of the forward and reverse primer respectively were:

5=-CGCTTCTTTCCTCCCGAGT-3=

5=-GCCATGCGGCATAAACTG-3=

Temperature cycles included an initial

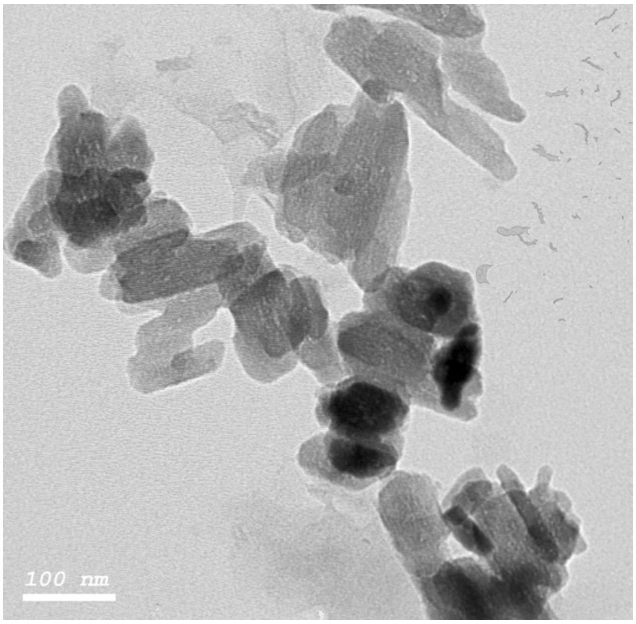


Figure 1.
HR-TEM image of functionalized hydroxyapatite nanoparticles showing dark highly electron-dense regions representing nano-hydroxyapatite, conjugated with light less electron-dense regions representing chlorhexidine digluconate.

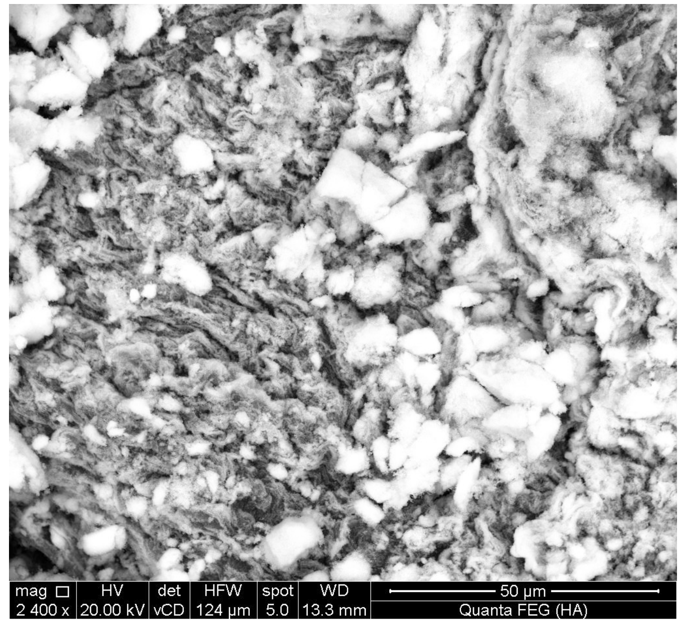


Figure 2.
Fe SEM image of functionalized hydroxyapatite nanoparticles showing white hydroxyapatite nanoparticles overlapping the grey chlorhexidine digluconate surface, indicating their successful conjugation.

enzyme activation and DNA denaturation step at 95 °C for 15 min, followed by 50 cycles of amplification including a denaturation step for 15 s at 94 °C, 60 °C annealing for 30 s and extension 30 seconds at 70 °C. Cycle threshold (CT) values were calculated using the RTQ-PCR Sequence Detection Software.
Melting curve profile was used to evaluate specifity of PCR reactions. Putative PCR artifacts (primer-dimmer) or contaminating nonspecific DNA will be presented as multiple peaks in melting curve profile representing artifacts (primer-dimmer).
RTQ-PCR sensitivity was verified via the construction of standard curve, the curve was derived via known quantities of *E. faecalis* reference strain, which was verified by Nanodrop weighing.

Culture based analysis
Each sample was serially diluted into suspensions equivalent to 10^2, 10^3, 10^4 and 10^5 CFU/mL. For detection of *E. faecalis*, 0.1 ml from every dilution was seeded on plates containing Bile Aesculin agar (Oxoid Basingstoke, UK). Plates were incubated for 24 hours at 37 ˚C. Following incubation, colonies were counted, and the number of colony-forming units (CFU/ml) were calculated.

Results

Hydroxyapatite functionalization with Chlorhexidine Digluconate characterization
High resolution TEM images of nano-hydroxyapatite functionalized with chlorhexidine digluconate (Figure 1) revealed highly electron-dense regions demarcated by dark colors representing nano-hydroxyapatite, overlapped with chlorhexidine digluconate that appeared as lighter regions due to its less electron-dense composition.
Fe SEM imaging (Figure 2) revealed white nano-metric plate-like particles representing hydroxyapatite nanoparticles overlapping the grey organic chlorhexidine digluconate surface, indicating their successful conjugation. Elemental analysis by EDX (Figure 3) revealed the presence of calcium and phosphate originating from hydroxy-

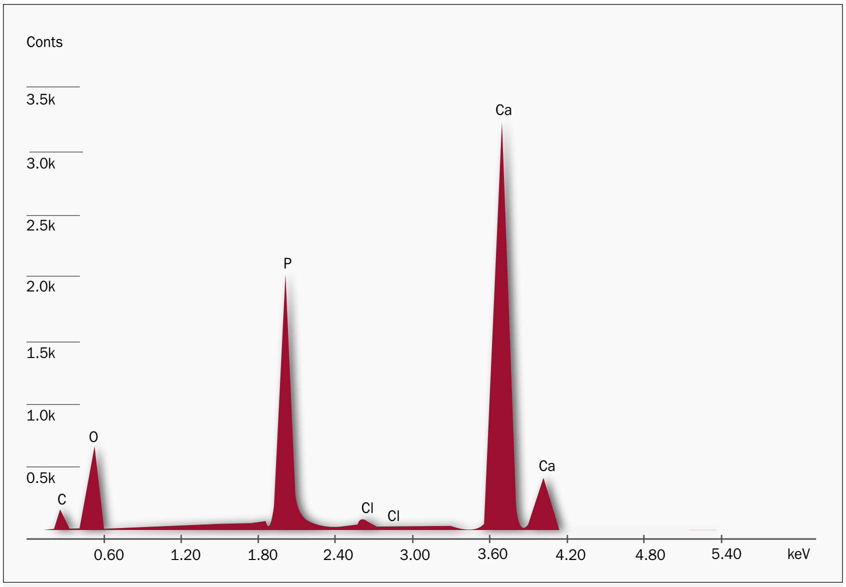


Figure 3.
Elemental EDX analysis for functionalized hydroxyapatite nanoparticles powder showing the presence of calcium and phosphate originating from hydroxyapatite nanoparticles (molecular formula $Ca_{10}(PO4)_6(OH)_2$) as well as Chlorine and Carbon originating from chlorhexidine digluconate ($C_{22}H_{30}Cl_2^{-N10}$).

apatite nanoparticles (molecular formula $Ca_{10}(PO4)_6(OH)_2$) as well as Chlorine and Carbon originating from chlorhexidine digluconate ($C_{22}H_{30}Cl_2^{-N10}$).

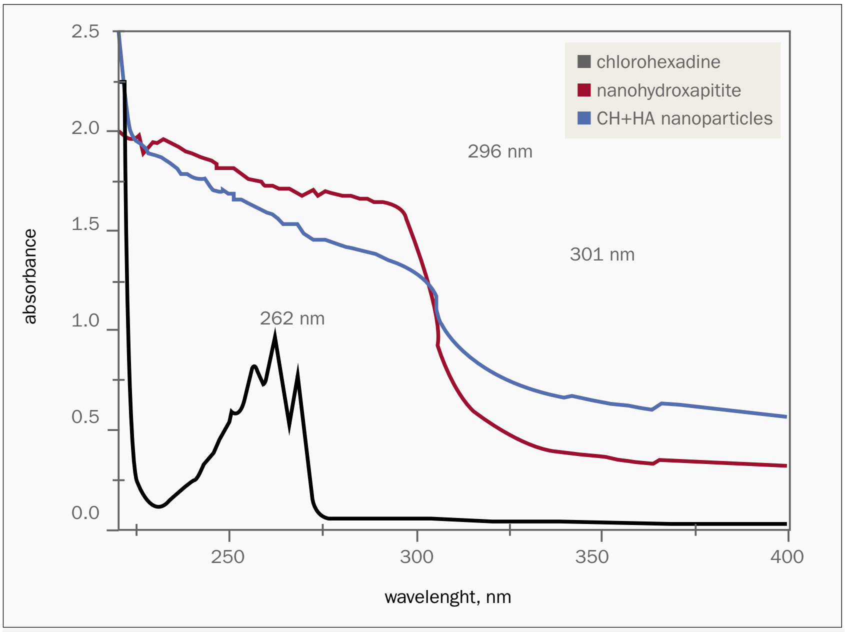


Figure 4.
UV spectroscopy showing the absorbance bands of the three different compounds; nano-hydroxyapatite functionalized with chlorhexidine digluconate (301 nm) in comparison with its precursors chlorhexidine digluconate and hydroxyapatite nanoparticles (262 nm) and (296 nm) respectively.

UV spectroscopy recorded a shift in the absorbance band of nano-hydroxyapatite functionalized with chlorhexidine digluconate to 301 nm in comparison with its precursors chlorhexidine digluconate and hydroxyapatite nanoparticles that recorded absorbance bands at 262 and 296 nm, respectively (Figure 4). Moreover, the high intensity of the absorbance recorded at 301 nm indicates that many CHX aggregates were successfully conjugated to the hydroxyapatite nanoparticles.
Fe SEM images of infected root canals (Figure 5A-D) showed that *E. faecalis* consistently adhered to collagen structures, colonized dentin surfaces, progressed toward the dentinal tubules and formed a thick mature biofilm at end of incubation period.

Bacterial Quantification
The specifity of RTQ-PCR was confirmed by the melting curve (Figure 6) which exhibited a single sharp peak at 80.9 °C that represents the target gene only, while the sensitivity of RTQ-PCR was confirmed by the standard curve (Figure 7) that displayed a linear regression of DNA mass after serial dilutions.
E. faecalis counts were expressed as *E. faecalis* weight in nano-grams per milliliter (ng/mL). RTQ-PCR detected *E. faecalis* DNA in all groups except for the negative control group.The mean values of *E. faecalis* DNA detected in groups A, B and C were 99.6 ng/mL, 67.1 ng/mL, 2797.4 ng/mL respectively (Table 2).
Statistical analysis of these results showed that both treatment groups presented statistically significantly lower mean values of *E. faecalis* DNA quantities compared to the positive control group ($p<0.001$). However, there was no statistically significant difference between them ($p>0.05$).

Culture results
All specimens exhibited CFU on culture plates except for the negative control group. Logarithmic transformation (Log_{10} transformation) of CFU count was performed due to the high range of bacterial counts. Mean values in groups A, B and C were 2.5 ± 0.3, 2.4 ± 0.3 and 4.2 ± 0.3 respectively (Table 3).

Table 2
Descriptive statistics and results of Kruskal-Wallis test for comparison between RTQ-PCR results in the three groups (nano-gram/mL)

Group	Mean	SD	Median	Min	Max	95% CI		P-value
						Lower bound	Upper bound	
A	99.6^{B}	16.7	96.5	80.0	129.0	87.7	111.5	<0.001*
B	67.1^{B}	25.0	65.5	32.0	119.0	49.2	85.0	
C	2797.4^{A}	1336.3	2565.5	1172.0	5424.0	1841.5	3753.3	

Significant at P≤0.05. Different superscripts in the same column are statistically significantly different.

Table 3
Descriptive statistics and results of one-way ANOVA test for comparison between Log10 CFU in the three groups

Group	Mean	SD	95% CI		P-value
			Lower bound	Upper bound	
A	2.5^{B}	0.3	2.2	2.7	<0.001*
B	2.4^{B}	0.6	2.0	2.8	
C	4.2^{A}	0.3	4.0	4.4	

Significant at P≤0.05. Different superscripts in the same column are statistically significantly different.

Statistical analysis of these results showed that both treatment groups showed statistically significantly lower CFU values of *E. faecalis* compared to the positive control group (p<0.001). However, there was no statistically significant difference between them (p>0.05). As regards to the negative control group, all samples showed no growth in culture plates.

Discussion

Nanoparticle medications are capable of reducing biofilm bacteria, disrupting biofilm constitution and furthermore, retaining a sustained antibacterial effect (10). This is provided through their higher surface area, charge density, and greater degree of interaction with cells (3, 17). Therefore, this study sought to investigate and compare the antibacterial potential of chlorhexidine digluconate functionalized hydroxyapatite nanoparticles and silver nanoparticles as intracanal medicaments on a mature *E. faecalis* biofilm model in root canals of extracted teeth.

Nano-hydroxyapatite functionalized with chlorhexidine digluconate and silver nanoparticles were selected as the test medications. The antibacterial potential of chlorhexidine and silver nanoparticles is well documented (8, 18, 19).

Adsorption of chlorhexidine digluconate onto the hydroxyapatite nanoparticles was confirmed by HR TEM, Fe SEM, EDX and UV Spectroscopy. This finding agreed with Soriano-Souza et al. 2015 (13).

Fe SEM monitoring for *E. faecalis* biofilm displayed that the bacteria formed the three configurations; single, pair and short chain (20). Fe SEM examination also presented some of *E. faecalis* virulence mechanisms such as aggregation, invasion of dentinal tubules and biofilm formation (21). At the end of the incubation period, it was evident that *E. faecalis* formed a mature endodontic biofilm that entirely covered the whole dentin surface as described by Saber and El-Hady 2012 (4).

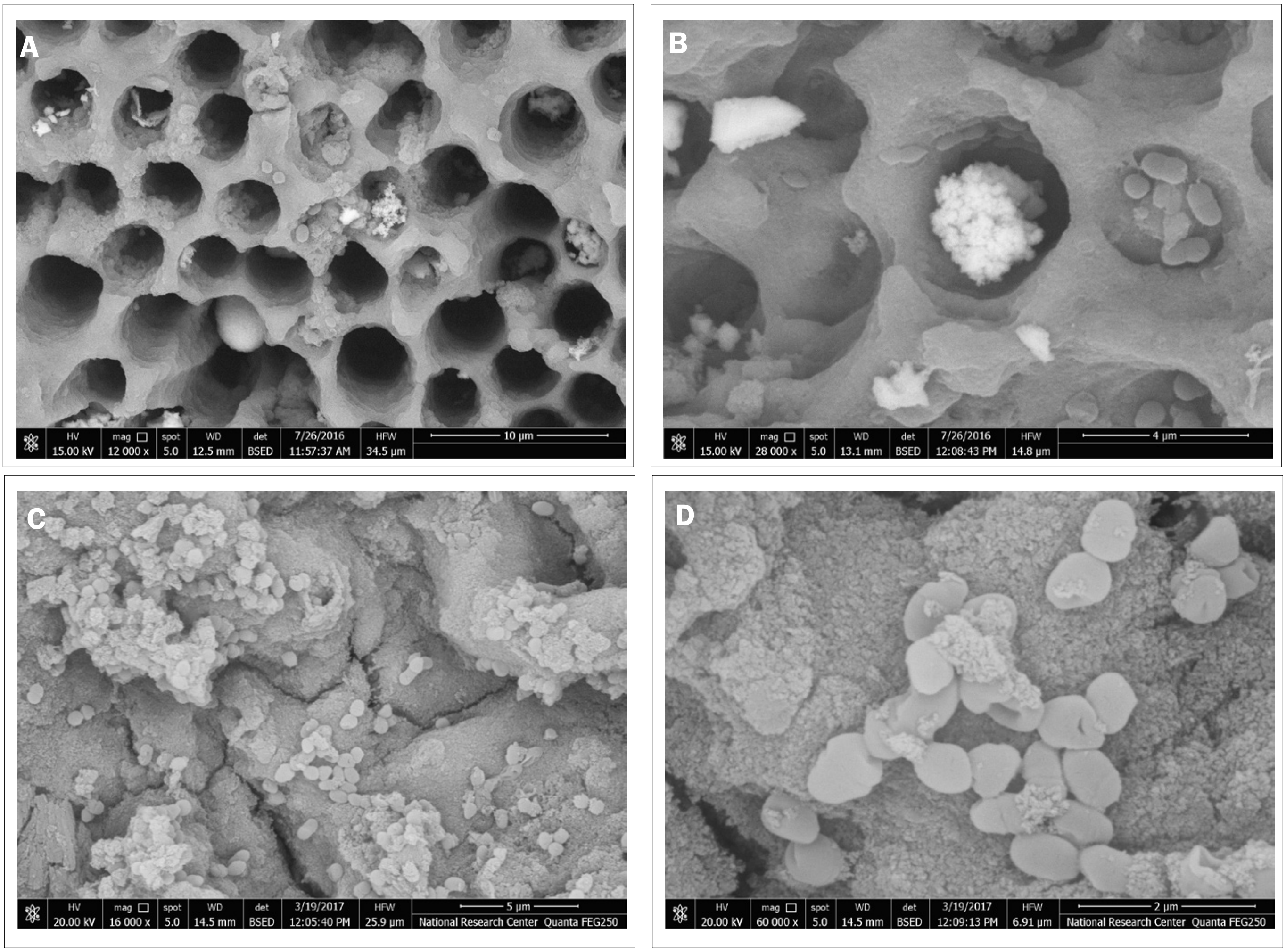


Figure 5.
A) 3-days old biofilm showing *E. faecalis* colonizing the dentin surface, and starting to invade the patent dentinal tubules.
B) Higher magnification showing the initial formation of extracellular polymeric matrix.
C, D) 7-days and 28-days old biofilm showing an increase in the density of the extracellular matrix and the number of microcolonies. *E. faecalis* demonstrated single, pair and short chain arrangements. precursors chlorhexidine digluconate and hydroxyapatite nanoparticles (262 nm) and (296 nm) respectively.

The current study employed specific closed end RTQ-PCR (Fourth generation) assay supplemented with culture-based analysis for microbiologic assessment. The rationale of using molecular methods are higher sensitivity for detecting endodontic pathogens, including cultivable species and as-yet uncultivated (VBNC) state of bacteria (22, 23). Pirani et al (24) detected, using PCR, the clinical presence of *E. faecalis* in root canal teeth affected by primary and secondary periapical lesions to be 7.6% and 39.1% respectively.
Bacterial quantification of medicated root canals by RTQ-PCR as well as by culture analysis showed a significant reduction in bacterial DNA counts after the application of nano-hydroxyapatite functionalized with chlorhexidine digluconate and silver nanoparticles ($p<0.001$) with no difference between them ($p>0.05$).
Results of the current study displayed the antibacterial and antibiofilm potentials of nanoparticles against mature *E. faecalis* biofilm. Previous studies described by Confocal laser scanning microscopy the potent antibiofilm action of functionalized hydroxyapatite nanoparticles against oral bacterial biofilms and that hydroxyapatite chlorhexidine association inhibited *E. faecalis* growth and adhesion for 6 days (12, 13). This anti-biofilm effect is attributed to the sustained release of chlorhexidine from functionalized hydroxyapatite nanoparticles during experimental assessment, in addition to the higher electrostatic surface charge of cationic chlorhexidine nanoparticles adhering to the negatively charged dentin surface (17).
According to results of the current study, canal medication with silver nanoparticles reduced *E. faecalis* counts and interrupted biofilm integrity. Results of the current

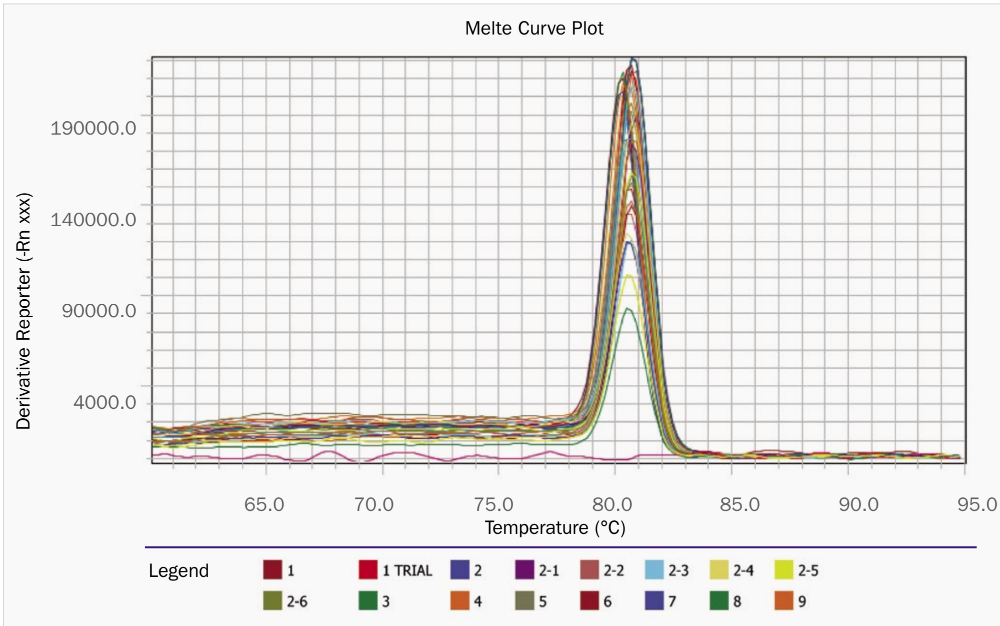
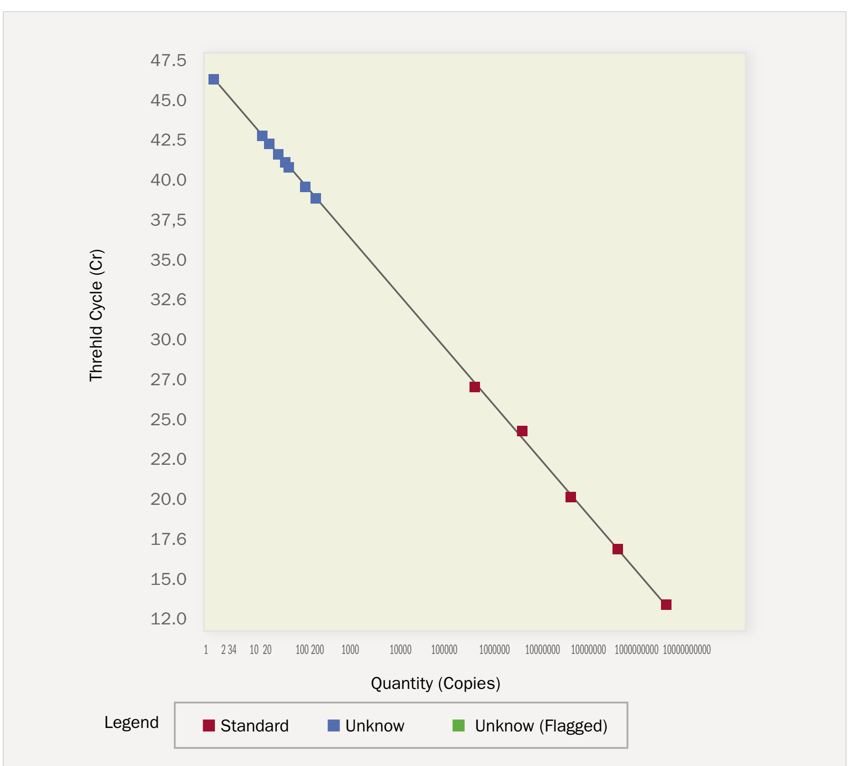


Figure 6.
Showing the melting curve profile used to evaluate the specifity of PCR reactions. A single sharp peak was recorded at 80.9 °C that represents the target gene only.

Figure 7.
Showing the standard curve used to evaluate the sensitivity.



study is in agreement with results of previous study reported that silver nanoparticles applied for 7 days exhibited an antibiofilm potential, that can be attributed to their positive charge which interact electrostatically with the negatively charged bacterial cells, resulting in leakage of intracellular bacterial components and lysis of bacteria, Moreover, the positively charged silver nanoparticles particles can bind to negatively charged dentin, and inhibit bacterial adherence (25).

Conclusions

Within the limitations of the current study, it can be concluded that nano-hydroxyapatite functionalized with chlorhexidine digluconate and silver nanoparticles demonstrated an effective antimicrobial activity against mature *E. faecalis* biofilms.

Clinical Relevance

Clinical use of nano-particulate intracanal medications should be considered in managing resistant cases of chronic apical periodontitis.

Ethics Statement

The current study was approved from the Research Ethics Committee of Ain Shams University (Cairo, Egypt).

Conflict of Interest

The authors deny any declarations of interest in this study.

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None.

References

1. Ricucci D, Siqueira JF Jr. Biofilms and apical periodontitis: study of prevalence and association with clinical and histopathologic findings. J Endod 2010;36:1277-88.
2. Siqueira JF Jr, Rôças IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. J Endod 2008;34:1291-1301.
3. Kishen A. Advanced therapeutic options for endodontic biofilms. Endod Topics 2012;22:99-123.
4. Saber SEDM, El-Hady SA. Development of an intracanal mature Enterococcus faecalis biofilm and its susceptibility to some antimicrobial intracanal medications; an in vitro study. Eur J Dent 2012;6:43-50.
5. Mohammadi Z, Abbott PV. The properties and applications of chlorhexidine in endodontics. Int Endod J 2009;42:288-302.
6. Stashenko P, Teles R, D'Souza R (1998). Periapical inflammatory responses and their modulation. Critical Reviews Oral Biol and Medicine 9, 498-521. 7. Abbott PV, Hume WR, Pearman JW (1990). Antibiotics and endodontics. Australian Dental Journal 35, 50-60.
8. Shrestha A, Kishen A (2016). Antibacterial Nanoparticles in Endodontics: A Review. Journal of Endodontics 42, 1417-26.
9. Chaloupka K, Yogeshkumar Malam Y and Seifalian AM (2010). Nanosilver as a new generation of nanoproduct in biomedical applications. Trends in Biotechnology 28, 580-8.
10. Shrestha A, Zhilong S, Gee NK, Kishen A (2010). Nanoparticulates for antibiofilm treatment and effect of aging on its antibacterial activity. Journal of Endodontics 36, 1030-35.
11. Thiruppathi R, Mishra S, Ganapathy M, Padmanabhan P, Gulyás B (2017). Nanoparticle Functionalization and Its Potentials for Molecular Imaging. Advanced Science 4, 1600279.
12. Soriano-Souza CA, Colombob APV, Soutob RM, Silva-Boghossianc CM, Granjeirod JM, Alvesd GG, Rossia AM, Rocha-Leaof MHM (2011). Adsorption of chlorhexidine on synthetic hydroxyapatite and in vitro biological activity. Colloids and Surfaces B: Biointerfaces 87, 310-8.
13. Soriano-Souza CA, Rossi AL, Mavropoulos E, Hausen MA, Tanaka MN, Calasans-Maia MD, Granjeiro JM, Rocha-Lea MHM, Rossi AM (2015). Chlorhexidine-loaded hydroxyapatite microspheres as an antimicrobial delivery system and its effect on in vivo osteo-conductive properties. Journal of Materials Science: Materials in Medicine 26, 166.
14. Sen BH, Piskin B, Demirci T (1995). Observation of bacteria and fungi in infected root canals and dentinal tubules by SEM. Endodontics and Dental Traumatology 11, 6-9.
15. Teles AM, Manso MC, Loureiro S, Silva R, Madeira IGC, Pina C, Cabeda JM (2014). Effectiveness of two intracanal dressings in adult Portuguese patients: a qPCR and anaerobic culture assessment. International Endodontic Journal 47, 32-40.
16. Williams JM, Trope M, Caplan DJ, Shugars DC (2006). Detection and quantitation of E. faecalis by real-time PCR (qPCR), reverse transcription-PCR (RT-PCR), and cultivation during endodontic treatment. Journal of Endodontics 32, 715-21.
17. Kishen A, Shi Z, Shrestha A, Neoh KG (2008). An investigation on the antibacterial and antibiofilm efficacy of cationic nanoparticulates for root canal disinfection. Journal of Endodontics 34, 1515-20.
18 Atila-Pektas B, Yurdakul P, Gulmez D, Gorduysus O (2013). Antimicrobial effects of root canal medicaments against Enterococcus faecalis and Streptococcus mutans. International Endodontic Journal 46, 413-8.
19. Wu D, Fan W, Kishen A, Gutmann JL, Fan B (2014). Evaluation of the antibacterial efficacy of silver nanoparticles against Enterococcus faecalis Biofilm. Journal of Endodontics 40, 285-90.
20. Portenier I, Waltimo TMT, Haapasalo M (2003). Enterococcus faecalis – the root canal survivor and 'star' in post-treatment disease. Endodontic Topics 6, 135-59.
21. Prabhakar J, Senthilkumar M, Priya MS, Mahalakshmi K, Sehgal PK, Sukumaran VG (2010). Evaluation of antimicrobial efficacy of herbal alternatives (triphala and green tea polyphenols), MTAD, and 5% sodium hypochlorite against Enterococcus faecalis biofilm formed on tooth substrate: An in vitro study. Journal of Endodontics 36, 83-6.
22. Kawashima N, Wadachi R, Suda H, Yeng T and Melbourne PP (2009). Root canal medicaments. International Dental Journal 59, 5-11.
23. Siqueira JF, Rocas IN (2014). Present status and future directions in endodontic microbiology. Endodontic Topics 30, 3-22.
24. Pirani C, Bertacci A, Cavrini F, Foschi F, Acquaviva GL, Prati C, Sambri V (2008). Recovery of Enterococcus faecalis in root canal lumen of patients with primary and secondary endodontic lesions. New Micribiologica 31,325-240.
25. Wu D, Fan W, Kishen A, Gutmann JL, Fan B (2014). Evaluation of the antibacterial efficacy of silver nanoparticles against Enterococcus faecalis Biofilm. Journal of Endodontics 40, 285-90.

ORIGINAL ARTICLE

Effect of different irrigation protocols and calcium hydroxide dressing on the microhardness of root canal dentin

ABSTRACT

Aim: *This study aimed to evaluate the effect of different irrigation protocols and calcium hydroxide dressing on the microhardness of root canal dentin.*
Methodology: *Thirty human teeth were decoronated and root segments were obtained from pre-established cuts made below the cemento-enamel junction. After specimen's preparation, the initial dentin microhardness (H0) was measured with a Vickers indenter, on the coronal surface, at 150 μm and 500 μm from the root canal lumen. Next, the specimens were randomly distributed into three groups (n=10), according to the different irrigation protocols: 1% NaOCl; 1% NaOCl + 17% EDTA; and 5% NaOCl. After irrigation, the hardness measurement was repeated (H1), in another quadrant. Then, Ca(OH)2 dressing was applied and left for 30 days, until it was removed and a new microhardness measurement (H2) was made, in a third quadrant. The data were statistically analyzed using the three-way analysis of variance (ANOVA) and Tukey tests, set at α=0.05.*
Results: *The factors analyzed (moment of VH, distance from the canal lumen and irrigation protocol) were statistically significant (p<0.05). There was a significant decrease of dentin microhardness after the irrigation protocols (H1<H0, p<0.05), however, with no significant difference after Ca(OH)2 dressing (H2~H1, p>0.05). The 5% NaOCl group shown the greatest difference between H0 and H1 measurements (p<0.05).*
Conclusions: *All irrigation protocols promoted significant decrease of the dentin microhardness. The Ca(OH)2 dressing for 30 days did not significantly affect the microhardness of the root canal dentin.*

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Introduction

The main objective of the chemomechanical preparation is to promote cleaning, shaping and disinfection of the root canal system (1). However, the complex anatomy of the root canal, which includes accessory canals, isthmuses, and apical ramifications, may hinders a proper disinfection (2). Therefore, the success of the endodontic treatment also depends on the chemical action of irrigating solutions (3) and an intracanal medication, especially in treatment of teeth with pulp necrosis (4).

Sodium hypochlorite (NaOCl) is the irrigating solution widely used in the root canal chemical preparation due to its antimicrobial properties (5) and the ability to dissolve organic tissues (3). This solution is used as a canal irrigant in several concentrations, which may range from 0.5% to 6% (6). At 1% concentration, NaOCl has shown to be efficient in dissolving organic matter and promote disinfection (7). Conversely, higher concentrations, such as 5.25%, may cause change in the dentin mechanical properties (8), as microhardness (9-11), because of the proteolytic action of NaOCl solution in the dentin collagen matrix (12).

However, NaOCl is not able to dissolve inorganic particles from the smear layer created from the instrumentation process (13), requiring the use of a chelating agent, as the ethylenediaminetetraacetic acid (EDTA) (14). The use of the EDTA increases the dentin permeability (15), which contributes to the intracanal medication diffusion (16), as well as the sealer penetration into dentinal tubules (17).

Even after an adequate chemomechanical preparation, studies have shown the permanence of opportunistic microorganisms inside of the root canal system (18, 19), which makes prudent the use of an intracanal medication (4). Due to its biological properties, calcium hydroxide [$Ca(OH)_2$] is widely used as intracanal medication (20, 21). Once introduced into the canal, $Ca(OH)_2$ dressing dissociates and releases calcium and hydroxyl ions, which are responsible for the dentin alkalization and, consequently, an antimicrobial effect (22). This ionic dissociation is also responsible for other properties of $Ca(OH)_2$, as the inhibition of phospholipase enzyme and stimulates the formation of mineralized tissue, which favors bone repair (23, 24). However, the alkalinity may reduce the organic matrix maintenance and interrupt the interaction between collagen fibrils and hydroxyapatite crystals (25), which negatively affects the dentin mechanical properties (26, 27).

Therefore, the present study aimed to evaluate the effect of different endodontic irrigation protocols and $Ca(OH)_2$ dressing on the microhardness of root canal dentin. The null hypothesis tested was that the different irrigating solutions and the subsequent use of intracanal medication would have no effect on dentin microhardness.

Materials and Methods

Sample Size Calculation

The sample size was estimated based on a previous study which assessed the effect of different irrigation protocols on the microhardness of the root dentin (28). Accordingly, for analysis with α=0.05 and 80% power, at least 10 teeth were allocated for each experimental group.

Sample Selection and Preparation

This study was previously approved by the local Human Research Ethics Committee (Protocol number 2083FR440212). Thirty freshly extracted human mandibular premolars teeth, with single root, straight, and fully formed roots were selected for the study. The teeth were extracted from young adult patients for orthodontic reasons. Initially, the teeth were cleaned with periodontal curettes (SM 17/18, Hu-Friedy, Rio de Janeiro, RJ, Brazil), followed by disinfection in 0.5% chloramine T solution at a temperature of 4 °C for 48 hours, and washing under running water for 24 hours. Next, a radiographic examination was performed, in both mesio-distal and buccal-lingual directions, in order to verify

the existence of a single and straight root canal; and fully formed roots. Also, the teeth were examined with stereoscopic lens under ×4 magnification (Illuminated Magnifying Glass, Tokyo, Japan) to exclude from the final sample teeth with caries, restorations and any signs of cracks.
A double-sided diamond disc (Brasseler Dental Products, Savannah, GA, USA), in low rpm and under air/water spray, was used to create perpendicular cuts along the teeth axis, 2 mm and 8 mm below the cemento-enamel junction, in order to obtain root segments with 6 mm length long. The remaining pulp tissue was removed with the aid of a size 30 K-type file (Dentsply-Maillefer, Tulsa, OK, USA). Next, the root segments were placed with the coronal surface down on a glass surface containing a double-sided adhesive tape (3M ESPE, St. Paul, MN, USA). Then, the specimens were individually mounted in a customized aluminum cylinder (6.0 mm height x 12.0 mm in diameter) and embedded in self-curing acrylic resin.
After the creation of the root segments, the root canals were initially scouted with a size 15 K-file (Dentsply-Maillefer). To standardize the size and taper, the root canals, they were prepared by using Gates-Glidden drills (no 1 to 4) (Dentsply-Maillefer), and with a no 2 drill from the DT Light-Post System (BISCO, Schaumburg, IL, USA). At each use of the drills, the root canals were rinsed with 2 mL of distilled water using a syringe with Navitip 30-gauge needle (Ultradent, South Jordan, UT, USA) inserted up the canal length. The root canal preparation was performed in order to standardized the coronal diameter (1.3 mm) and the apical size (1.1 mm).
Afterwards, the specimens were sequentially polished with silicon carbide sandpapers (3M ESPE) with progressively increasing grit sizes (#400, 600, 800 and 1200), under constant irrigation. The dentin and cement surface exposition, coronal and apical, were verified through a stereomicroscope (SteREO Discovery V12, Carl Zeiss, Jena, Germany) under ×100 magnification. A final polishing procedure was performed with alumina-based pastes in decreasing order of granulation (1.0, 0.5 and 0.03 μm) (Buehler, Ltda, Lake Bluff, IL, USA). At each change of abrasive paper or paste, the specimens were extensively rinsed in tap water, and at the end, they were washed in an ultrasonic tank (Cristófoli, Campo Mourão, PR, Brazil) for 10 minutes.

Irrigation Protocols

Before starting the irrigation protocols, an initial evaluation of the dentin microhardness of the specimens was performed (H0: hardness control). For this, the specimens were submitted to a microhardness test in a Shimadzu HMV2 hardness tester (Newage Testing Instruments, Inc., Southampton, PA, USA) with a Vicker's diamond indenter. In each specimen, the representative hardness value was obtained at two different lateral areas of the dentin, at the coronal surface, determined at distances of 150 μm and 500 μm in relation to the root canal lumen. At each lateral distance (150 μm and 500 μm), the microhardness was calculated as the average of three measurements, totaling six indentations in each specimen (Figure 1). The minimum distance between each indentation in the same line was established at 150 μm. Each indentation was made using a 50 g static load and dwell time of 10 seconds. Vickers microhardness number (VHN) was calculated based on the average of the diagonals measured under examination with an optical microscope (×400, Shimadzu HMV2) (Figure 2), with the aid of the Newage C.A.M.S software (Newage Testing Instruments, Inc., Southampton, PA, USA). Then, the specimens were randomly assigned to the following three experimental groups, according to the irrigation protocols performed (n=10): 1% NaOCl; 1% NaOCl + 17% EDTA; and 5% NaOCl.
The specimens had the coronal surface protected with a non-porous tape (3M ESPE) to allow exposition only of the root canal to the irrigating solutions. The irrigation was performed using an endodontic syringe with Navitip 30-gauge needle (Ultradent) containing the solution of each group. Simultaneously to the irrigation, the aspiration of the solution reflux was performed by the capillary tip (.014) (Ul-

Table 1

Analysis of variance based on the following factors: distance from the canal, irrigating solution and moment of VHN† evaluation (3-way ANOVA, F test, α=0.05)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Distance from canal	1	2338	2338	25.37	0.0000
Irrigating Solution	2	1222	611	6.63	0.0017
Moment of VHN	2	2664	1332	14.45	0.0000
Distance from canal*Irrigating Solution	2	10	5	0.05	>.05
Distance from canal*Moment of VH	2	252	126	1.37	>.05
Irrigating Solution*Moment of VH	4	1104	276	2.99	0.0203
Distance from canal*Irrigating Solution*Moment of VH	4	72	18	0.19	>.05
Residue	162	14930	92		

†VHN: Vickers Hardness Number

tradent), coupled in a Lüer cannula (Ultradent). The root canals were irrigated with 15 mL of the solution intended to each group, during 20 minutes, except for the group 1% NaOCl + 17% EDTA, which received initially 10 mL of 1% NaOCl for 15 minutes and, in the sequence, 3 mL of 17% EDTA for 3 minutes and 2 mL of 1% NaOCl for 2 minutes, also totalizing 15 mL of solution and 20 minutes of irrigating regimen. All the specimens were rinsed with 5 mL of distilled water to remove any residue of the chemical solutions.
Therefore, the specimens were dried with absorbent paper points (size 40, Dentsply-Maillefer) and submitted to a second microhardness evaluation (H1), in another quadrant of the coronal surface, in the same above-mentioned distances, as previously described.

$Ca(OH)_2$ Dressing

Next, the specimens received an intracanal medication with $Ca(OH)_2$ paste (Ultracal; Ultradent Products). In order to fill the root canal with the paste, each specimen was protected again with the non-porous tape on their coronal portion, exposing only the root canal entrance for the insertion of the needle with the medicament. The ready-to-use paste was introduced into the canal using the needle provided by manufacturer (Ultradent Products).
As soon as the quality of the root canal filling was confirmed, by radiographic examination, in both mesio-distal and buccal-lingual directions, the specimens had the root canal entrance totally sealed, with the same non-porous tape, and were stored in oven at 37 °C with 100% relative humidity, during 30 days.
Over the course of this period, the tape was removed, the specimens had their root canals irrigated with 2 mL of distilled water and they were also washed in an ultrasonic tank (Cristófoli). The final microhardness evaluation (H2) was performed in another quadrant of the coronal surface, in the same above-mentioned distances, as previously described.

Statistical Analysis

Data were analyzed using the SAEG statistical software (UFV, Viçosa, MG, Brazil). The mean values and standard deviations were calculated, and data homogeneity and normality were tested by the Levene's and the Kolmogorov Smirnov's tests, respectively. Three-way analysis of variance (ANOVA) was used to compare the following variables: A) moment of VH evaluation (H0. control of the initial dentin microhardness; H1, after irrigation protocol and H2, after $Ca(OH)_2$ dressing; B) distance from the canal lumen (at 150 µm or at 500 µm); and C) irrigating solution, as well as its interactions. The significance level was set at 5%.

Results

The results showed that all factors analyzed (moment of VH, distance from the canal

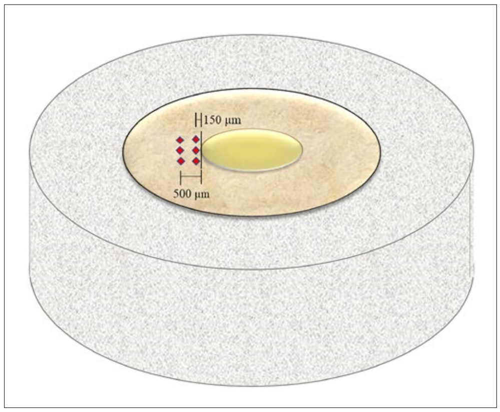

Figure 1. Schematic diagram showing a specimen with three indentations made for each lateral distance from the root canal lumen (150 µm and 500 µm). Six indentations were made at each moment of VH evaluation.

lumen and irrigation protocol) were statistically significant ($p<0.05$). However, the interaction of the factors showed significance only to the interaction between the irrigation protocol and moment of VH ($p<0.05$). The data are available in Table 1.

Overall, the dentin microhardness mean values at the distances of 150 µm and 500 µm from the canal lumen were 19.2 (±9.7) and 25.7 (±10.1), respectively, which were significantly different from each other ($p<0.05$). When analyzing the interaction between irrigation protocol and the moment of VH, it was noticed a significant decrease ($p<0.05$) in dentin microhardness after irrigation (H1), in relation to the initial hardness (H0) previously assessed in all groups (Table 2). When compared to the other irrigation protocols, the 5% NaOCl group had greater decrease of VH between H0 and H1. After $Ca(OH)_2$ dressing (H2), it was not noticed any significant change in the VH results ($p>0.05$). The VH values for the 1% NaOCl and 1% NaOCL +17% EDTA groups did not range significantly between them, regarding the moment of evaluation (H0, H1 and H2) (Table 2). Differences in dentin microhardness

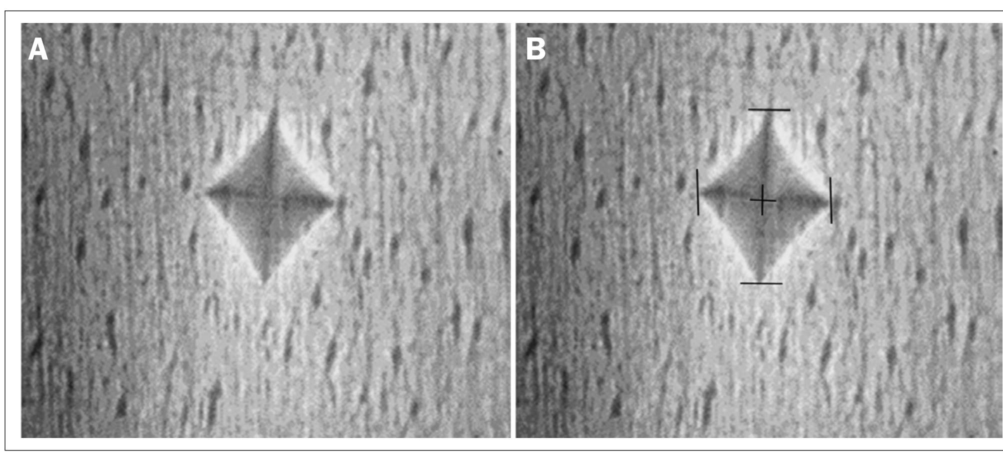

Figure 2. Representative optical microscopy image (400x) of the indentation performed during the hardness test in each experimental group **(A)**, and at the moment at which the diagonals used to calculate Vickers hardness were measured **(B)**.

Table 2

Comparison of the mean ± SD values of the VH into the factor's interaction: group/irrigation protocol × moment of VH evaluation*

Moment of VHN†	Group/Irrigation Protocol		
	1% NaOCl	1% NaOCl +17% EDTA	5% NaOCl
H0	23,5 ± 9,5^{Ba}	23,0 ± 9,8^{Ba}	35,5 ± 9,9^{Aa}
H1	18,1 ± 8,0^{Bb}	16,9 ± 7,1^{Bb}	22,3 ± 8,4^{Ab}
H2	19,5 ± 9,8^{Ab}	19,4 ± 9,9^{Aab}	18,8 ± 6,9^{Ab}

**Different uppercase letters in the lines indicate statistical difference among the irrigation protocols (Tukey's test, $p<0.05$).*
**Different lowercase letters in the columns indicate statistical difference among the VH values obtained at different moments (Tukey's test, $p<0.05$).*

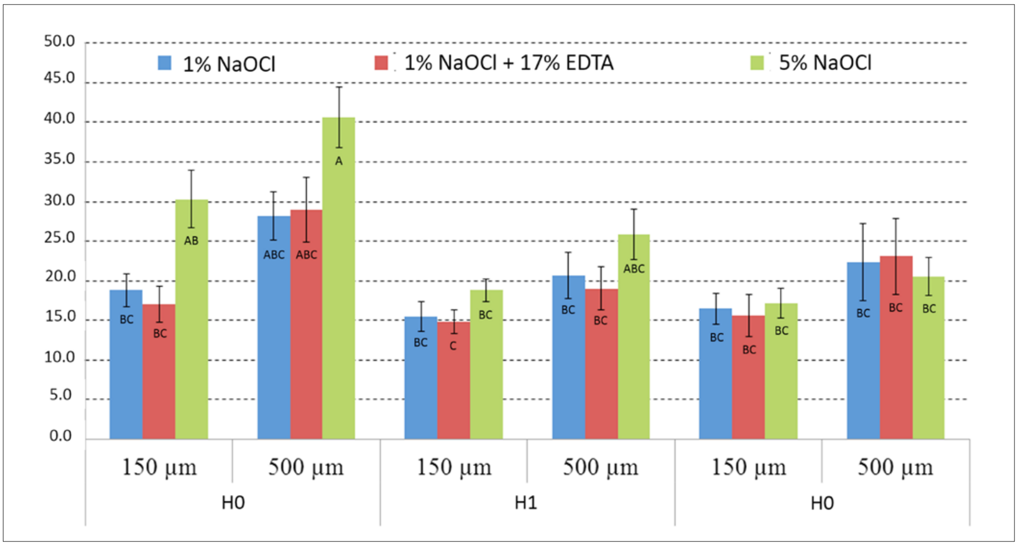

Figure 3.
Results for Tukey's multiple-comparison post hoc test according to the distance from the canal lumen (150 or 500 µm), moment of VH (H0, H1 and H2) and Group (irrigating solution used before H1). The same letters in the bars do not differ significantly Tukey's test ($p>0,05$). H0: control, initial dentin microhardness. H1: after irrigation protocol. H2: after $Ca(OH)_2$ dressing.

before and after specimen's treatment and among groups, in both distances evaluated (150 µm and 500 µm), are showed in Figure 3.

Discussion

The results of the present study demonstrated that, irrespective of the distance evaluated, a significant decrease in dentin microhardness for all groups was noticed after the irrigating protocols. Other studies have also noticed significant decrease on dentin microhardness when the NaOCl was used as irrigating solution in an isolated manner (9, 10, 28, 29) or even when combined with EDTA (30, 31). However, in the present study, the $Ca(OH)_2$ dressing for 30 days did not promote any significant effect to the dentin microhardness, in none of the groups. These findings may be explained by the following reasons: applied methodology in the study, dentin structural diversity, concentration of the irrigating solutions used and action time of $Ca(OH)_2$ dressing.
It is important to emphasize that, even with the specimen's standardization, it is possible to exist structural differences in the dentin evaluated (32). Considerable variations of the dentin microhardness values may occur in the same tooth (32). These structural variations usually occur in dentine, and may be related to genetic influence, atmosphere conditions, age, number of dentinal tubules, and root region evaluated (29, 30, 32). Closest areas from the canal lumen have lowest microhardness values (29), which may be justified by the higher quantity and bigger diameter of the dentinal tubules in this region (32). The results of the present study showed that the microhardness evaluated at 150 µm from the root canal lumen was significantly lower than the one evaluated at the distance of 500µm. This may also be explained by the mineralization degree variation and the amount of hydroxyapatite in intertubular dentin (33).
In addition, in the present study, all irrigation protocols promoted a significant decrease in dentin microhardness, which suggests direct and powerful effect of these solutions on the structural dentin components (28-30). The 5% NaOCl group presented the greatest decrease in the microhardness values after irrigation. Such results were also found by Ari et al. (9) and Slutzky-Goldberg et al. (10), which corroborates with this study.
Keine et al. (34) have reported greater reduction in dentin microhardness when the association between NaOCl and EDTA occurs. Conversely, in the present study, the additional irrigation with 17% EDTA did not intensified the decrease of dentin microhardness, since similar results were observed for 1% NaOCl and 1% NaOCl +17% EDTA groups. However, these results

corroborate with a previously study which demonstrated that the association between EDTA and NaOCl may reduce the NaOH/HClO release, inactivating the action of the NaOCl (35). NaOCl is a non-specific proteolytic agent that acts dissolving dentin organic components (11, 29). Dentin has a high percentage of organic content, mainly type I collagen, which sustain dentinal mineral content (32). The loss of organic support may cause collapse of the inorganic structures leading to the dentin weakening and lower hardness (8, 11). The use of EDTA associated to NaOCl did not significantly affect the dentin structure. It is possible that the amount of EDTA used (3 mL) was not enough to cause harmful effects to dentin, especially due to the small volume of NaOCl used in the sequence (only 2 mL).

According to Zou et al. (36), the temperature, concentration and time are factors that may potentially affect the NaOCl penetration into the dentin. These authors also reported the increase of 30 to 50% in the penetration of NaOCl when its concentration ranged from 1% to 6%. Some other researches showed that higher concentrations of NaOCl solution promote higher penetration capacity and, consequently, higher mechanical changes in the dentin, as the microhardness (11, 37). These results support the evidences found in this research, which the 5% NaOCl group had the highest dentine microhardness decrease, in both distances evaluated.

The use of 20 min in the irrigating protocol was based on the study of Zou et al. (36), in which was observed a higher penetration of the NaOCl into the dentinal tubules using the same time. Also, this time was selected in order to standardize the period in which the solutions kept in contact with the root canal walls, simulating what usually happens in a clinical situation during chemo-mechanical preparation. Likewise, 15 mL of irrigating solution was chosen taking into account the amount used during the chemo-mechanical (including final irrigation). During a conventional preparation, we usually use 2 to 3 mL of irrigating solution at each instrument change. At the end, a final irrigation is performed with amounts that may range from 5 mL to 10 mL, depending on the EDTA and NaOCl solutions used (2). The frequent NaOCl solution renewal inside the root canals is extremely important, because its solubilization capability is reduced in the presence of organic matter and most of its activity is lost 2 minutes after contact (38).

The volume of irrigating solution used in experimental studies varies widely from one study to another. We observed variations ranging from 0.1 mL (29, 34) to more than 40 mL (1, 37). In the study of Akay et al. (2012), for example, the specimens of each experimental group were soaked in 50 mL of the tested solutions. In other study (36), the authors used 5% NaOCl during root canal preparation, followed by a final irrigation with 6% NaOCl. Then, the authors have immersed the teeth for 20 minutes into 10 mL of NaOCl (at different concentrations), which resulted in an amount greater than 15 mL of irrigating solution delivered in root canal dentin. A recent study suggests that clinical studies must be performed using 15 mL to 20 mL during root canal preparation (39). According to this, the choice of using 15 mL of irrigating solution during root canal preparation appears to be a feasible option.

The results of the present study showed that the $Ca(OH)_2$ dressing for 30 days did not promote significant change in the dentin microhardness, in any of the distances evaluated, irrespective of the previous use of the irrigating solution. However, previous studies have reported that the use of intracanal dressing for 4 weeks reduced the dentin microhardness and promoted changes in the surface chemical structure (40, 41). Also, Yoldas et al. (41) reported a significant reduction in the dentin hardness after direct contact of specimens with $Ca(OH)_2$ for 3 and 7 days in 1 mm from the root lumen.

The moment of VH, in this research, performed in the mentioned distances, and considering a no direct dentine exposure and contact with the medicament, may partially explain the microhardness lack of changes. Thus, further studies are necessary to understand the exact mechanism of microhardness decrease after being ex-

posed to the $Ca(OH)_2$ dressing. It is important to emphasize that the results of the present study must be analysed with caution. Currently, due to the faster root canal instrumentation achieved with rotating and reciprocating systems, the contact time and the amount of irrigation solution used are becoming smaller (42). Therefore, new studies must be carried out to verify the performance of irrigation solutions in these scenarios.
The results found in this study suggest that, in clinical practice, irrigating substances such as NaOCl and EDTA would be used sparingly and safely, in order to achieve cleaning and disinfection objectives without, however, affecting dentin properties (3). Although, our study shown a reduction in the dentin microhardness with all irrigating protocols used, these findings may not be clinically relevant regarding to the longevity of the endodontically treated tooth. It is premature to state that the use of a smaller amount of irrigating solution may be beneficial for the treatment, as this might negatively interfere with other important outcomes, such as cleaning and disinfecting the root canal system.

Conclusions

The different irrigation protocols promoted significant decrease in the dentin microhardness. The subsequent $Ca(OH)_2$ dressing for 30 days did not significantly affect the microhardness of the root canal dentin, in none of the experimental groups.

Clinical Relevance

In clinical practice, irrigating substances, such as NaOCl and EDTA, would be sparingly and safely used, in order to achieve cleaning and disinfection of the root canal system, without affecting dentin properties.

Conflict of Interest

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

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References

1. *Akcay I, Sen BH. The effect of surfactant addition to EDTA on microhardness of root dentin. J Endod 2012;38:704-7.*
2. *Tuncer AK, Tuncer S. Effect of different final irrigation solutions on dentinal tubule penetration depth and percentage of root canal sealer. J Endod 2012;38:860-3.*
3. *Haapasalo M, Shen Y, Gian W, Gao Y. Irrigation in endodontics. Dent Clin North Am 2010;54:291-312.*
4. *Sirén EK, Kerosuo E, Lavonius E et al. CaOH2 application modes: in vitro alkalinity and clinical effect on bacteria. Int Endod J 2014;47:628-38.*
5. *Perdigão J, Lopes M, Geraldeli S et al. Effect of a sodium hypochlorite gel on dentin bonding. Dent Mater 2000;16:311-23.*
6. *Baumgartner JC, Cuenin PR. Efficacy of several concentrations of sodium hypochlorite for root canal irrigation. J Endod 1992;18:605-12.*
7. *Sirtes G, Waltimo T, Schaetzle M, Zehnder M. The effects of temperature on sodium hypochlorite short-term stability, pulp dissolution capacity, and antimicrobial efficacy. J Endod 2005;31:669-71.*
8. *Sim TP, Knowles JC, Ng YI et al. Effect of sodium hypochlorite on mechanical properties of dentine and tooth surface strain. Int Endod J 2001;34:120-32.*
9. *Ari H, Erdemir A, Belli S. Evaluation of the effect endodontic irrigation solutions on the microhardness and the roughness of root canal dentin. J Endod 2004;30:792-5.*
10. *Slutzky-Goldberg I, Liberman R, Heling I. Effect of sodium hypochlorite on dentin microhardness. J Endod 2004;30:880-2.*
11. *Oliveira ID, Carvalho CA, Nunes W et al. Effects of chlorhexidine and sodium hypochlorite on the microhardness of root canal dentin. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;104:e125-8.*
12. *Grigoratos D, Knowles J, Ng YI, Gulabivala K. Effect of exposing dentine to sodium hypochlorite and calcium hydroxide on its flexural strength and elastic modulus. Int Endod J 2001;34:113-9.*
13. *Ulusoy ÖI, Görgül G. Effects of different irrigation solutions on root dentine microhardness smear layer removal and erosion. Aust Endod J 2013;39:66-72.*
14. *Serper A, Calt S, Dogan AL et al. Comparison of the cytotoxic effects and smear layer removing capacity of oxidative potential water, NaOCl and EDTA. J Oral Sci 2001;43:233-8.*
15. *Soares JA, De Carvalho MAR, Santos SMC et al. Effectiveness of chemomechanical preparation with alternating use of sodium hypochlorite and EDTA in eliminating intracanal enterococcus faecalis biofilm. J Endod 2010;36:894-8.*
16. *Uzunoglu E, Aktemur S, Uyanik MO et al. Effect of ethylenediaminetetraacetic acid on root fracture with respect to concentration at different time exposures. J Endod 2012;38:1110-3.*

17. Machado R, Garcia LDFR, da Silva Neto UX et al. Evaluation of 17% EDTA and 10% citric acid in smear layer removal and tubular dentin sealer penetration. Microsc Res Tech 2018;81:275-82.
18. Siqueira Júnior J, Rôças IN, Oliveira JC. Prevalence of Prevotella intermedia and Prevotella nigrescens in infected root canals from a Brazilian population. Aust Endod J 2000;26:110-2.
19. Nair PN. On the causes of persistent apical periodontitis: a review. Int Endod J 2006;39:249-81.
20. Siqueira Jr JF, Lopes HP. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. Int Endod J 1999;32:361-9.
21. Rahimi S, Janani M, Lotfi M et al. A review of antibacterial agents in endodontic treatment. Iran Endod J 2014;9:161-8.
22. Sjogren U, Figdor D, Spangberg L, Sundqvist G. The antimicrobial effect of calcium hydroxide as a short-term intracanal dressing. Int Endod J 1991;24:119-25.
23. Lima RK, Guerreiro-Tanomaru JM, Faria-Junior NB, Tanomaru-Filho M. Effectiveness of calcium hydroxide-based intracanal medicaments against enterococcus faecalis. Int Endod J 2012;45:311-6.
24. Slutzky-Goldberg I, Hanut A, Matalon S et al. The effect of dentin on the pulp tissue dissolution capacity of sodium hypochlorite and calcium hydroxide. J Endod 2013;39:980-3.
25. Andreasen JO, Munksgaard EC, Bakland IK. Comparison of fracture resistance in root canals of immature sheep teeth after filling with calcium hydroxide or MTA. Dent Traumatol 2006;22:154-6.
26. Andreasen JO, Farik B, Munksgaard EC. Long-term calcium hydroxide as a root canal dressing may increase risk of root fracture. Dent Traumatol 2002;18:134-7.
27. Doyon GE, Dumsha T, VonFraunhofer JA. Fracture resistance of human root dentine exposed to intracanal calcium hydroxide. J Endod 2005;31:895-7.
28. Baldasso FER, Roleto L, Silva VDD et al. Effect of final irrigation protocols on microhardness reduction and erosion of root canal dentin. Braz Oral Res 2017;31:e40.
29. Garcia AJ, Kuga MC, Palma-Dibb RG et al. Effect of sodium hypochlorite under several formulations on root canal dentin microhardness. J Investig Clin Dent 2013;4:229-32.
30. Pedersen ND, Uzunoglu-Özyürek E, Dogan Buzoglu H. Influence of different irrigation protocols on microhardness and flexural strength values of young and aged crown dentin. Gerodontology 2020;37:53-8.
31. Saha SG, Sharma V, Bharadwaj A et al. Effectiveness of Various Endodontic Irrigants on the Micro-Hardness of the Root Canal Dentin: An in vitro Study. J Clin Diagn Res 2017;11:ZC01-ZC04.
32. Lopes MB, Sinhoreti MA, Gonine Júnior A et al. Comparative study of tubular diameter and quantity for human and bovine dentin at different depths. Braz Dent J 2009;20:279-83.
33. Panighi M, G'Sell C. Influence of calcium concentration on the dentin wettability by an adhesive. J Biomed Mater Res 1992;26:1081-9.
34. Keine KC, Kuga MC, Coaguila-Llerena H et al. Peracetic acid as a single endodontic irrigant: effects on microhardness, roughness and erosion of root canal dentin. Microsc Res Tech 2020;834:375-80.
35. Zehnder M, Schmidlin P, Sener B, Waltimo T. Chelation in root canal therapy reconsidered. J Endod 2005;31:817-20.
36. Zou L, Shen Y, Li W, Haapasalo M. Penetration of sodium hypochlorite into dentin. J Endod 2010;36:793-6.
37. Tartari T, de Almeida Rodrigues Silva E Souza P, Vila Nova de Almeida B et al. A new weak chelator in endodontics: effects of different irrigation regimens with etidronate on root dentin microhardness Int J Dent 2013;2013:743018.
38. Clarkson RM, Moule AJ, Podlich H et al. Dissolution of porcine incisor pulps in sodium hypochlorite solutions of varying compositions and concentrations. Aust Dent J 2006;51:245-51.
39. Arruda, JAA, Schuch, LF, Pereira, A et al. Investigation of different sodium hypochlorite volumes, concentrations and times of irrigation in endodontic therapy: a systematic review. Arch Health Invest 2019;8:185-191.
40. Naseri M, Eftekhar L, Gholami F et al. The Effect of Calcium Hydroxide and Nano-calcium Hydroxide on Microhardness and Superficial Chemical Structure of Root Canal Dentin: An Ex Vivo Study. J Endod 2019;45:1148-54.
41. Yoldaş O, Doğan C, Seydaoğlu G. The effect of two different calcium hydroxide combinations on root dentine microhardness. Int Endod J 2004;37:828-31.
42. Gazzaneo I, Vieira GCS, Pérez AR, et al. Root Canal Disinfection by Single- and Multiple-instrument Systems: Effects of Sodium Hypochlorite Volume, Concentration, and Retention Time. J Endod 2019;45:736-41.

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ORIGINAL ARTICLE

Comparison of antibacterial effectiveness between Sealapex and AH-plus sealer against *Enterococcus faecalis:* a systematic review of *in vitro* studies

ABSTRACT

Aim: *To summarize the outcome of in vitro studies comparing the antibacterial effectiveness of Sealapex and AH-plus sealer against E. faecalis.*
Methodology: *The research question was developed using the PICO methodology and studies were identified from three electronic databases in Medline, Scopus, and EBSCOhost (Dentistry; Oral Sciences Source) since inception up to November 2019. The title and abstract of the selected articles were independently reviewed by two reviewers based on the specified inclusion and exclusion criteria and extracted the data using the data extraction form. The quality of selected in vitro studies was appraised using revised Cochrane Risk of Bias tool.*
Results: *Sixteen studies satisfied the inclusion criteria and were included in this systematic review. Due to the lack of homogeneity in the data, meta-analysis could not be conducted. The quality of the evidence was "low", since every study had at least three questions related to high risk of bias. Different laboratory tests and protocols were used, their results were contradicting even for studies using the same laboratory tests and quality of evidence was found to be low. No study provided strong evidence, twelve studies provided moderate evidence, three studies provided limited evidence and one study provided conflicting evidence. The research question could not be meaningfully addressed.*
Conclusions: *No difference was observed in the antimicrobial efficacy of Sealapex and AH-plus root canal sealers against* Enterococcus faecalis. *There was an identification of poor quality relevant studies with contradicting results that indicates the need for development of standardized protocols for future in* vitro *studies.*

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Introduction

The main objective of the root canal treatment is to eliminate bacteria from the root canal system and prevent reinfection. However, due to complex root canal anatomy, bacteria can be found left in the root canal system even after thorough chemomechanical debridement. A number of microorganisms have been isolated from infected root canal system based on the type of infection and clinical manifestation of the disease (1). Endodontic infection can either be primary in nature due to the invasion of microorganisms or microbial by-products into the pulp tissue or secondary due to reinfection after root canal therapy or remnant or persistent infection. Although the occurrence of root canal failure is multifactorial, the persistence of microorganisms within the root canal system after treatment has found to be the most significant reason for endodontic failure (2). The microflora of primary infected canals with untreated apical periodontitis and secondary infected canals (failed endodontic treatment) differs in number and in phenotypes. In canals with secondary infection, facultative anaerobic and gram-positive bacteria predominate and comprised of one or two species per canal including *Enterococcus, Streptococcus, Peptostreptococcus*, *Actinomyces* species, *Fusobacterium nucleatum* and *Propionibacterium* (3).

Among these microorganisms, *Enterococcus faecalis (E. faecalis)* has been one of the most prevalent microorganisms isolated from failed root canal treated teeth (4, 5). Furthermore, *E. faecalis* has been more often associated with primary endodontic infections with asymptomatic chronic periradicular lesions than with acute periradicular periodontitis. Rôças et al. observed presence of *E. faecalis* nine times higher in endodontic cases with secondary infection than primary endodontic infection (6).

E. faecalis can survive harsh conditions as it can create biofilms and has the ability to penetrate into dentinal tubules (7). Bacteria in intracanal biofilms develop mechanisms to protect themselves against antibiotic medicaments, becoming 1,000 to 1,500 times more resistant compared to bacteria in planktonic form (8). In fact, calcium hydroxide, which is a very popular intracanal medicament used for elimination of the remaining bacteria after instrumentation and irrigation, can neither prevent *E. faecalis* from being organized in biofilms, nor eliminate these biofilms due to its adhering ability to the dentine that increases biofilms' resistance (9). The shaping and cleaning of the root canal system is followed by obturation using a core material and sealer necessary to establish a fluid tight seal of the root canal system (10). According to the Glossary of Endodontic terms which was developed by the American Association of Endodontists, a sealer is a radiopaque dental cement usually used in combination with a solid or semi-solid core material, to fill voids and seal root canals during obturation (11). The ideal properties of endodontic sealers were described by Grossman et al. (1988) (12). At this moment, there is no sealer that can fulfil all the above criteria and can be considered as the gold-standard although manufacturers may emphasize on various benefits of the sealer.

An informal market survey was conducted by compiling information from the sales teams from United Kingdom, European Union and Malaysian based dental distributors in order to distinguish the most popular sealers in the market. Dental suppliers in Scotland pointed to Sealapex as the most popular root canal sealer. AH-plus has also been a very well-studied sealer in the literature and found to be almost in every *in vitro* study investigating the antibacterial efficacy of sealers (13). Sealapex, a calcium hydroxide-based sealer produced by SybronEndo has shown to have antibacterial property that may facilitate quick periapical healing and hard tissue formation (14, 15). It is a catalyst/base system, introduced in two tubes or in a double barrel syringe (Sealapex Xpress). AH-plus, an epoxy-resin based sealer produced by

DENTSPLY DeTrey is a paste/paste system, introduced in two tubes or in a double barrel syringe (AH-plus Jet). According to manufacturers it has several advantages such as high radiopacity, high dimensional stability, good dentinal adherence and good sealing ability. Moreover, it does not release formaldehyde or cause tooth discoloration unlike its predecessor AH26 (16). Many *in vitro* studies have been done focusing on the antibacterial efficacy of sealers however, assessing the antimicrobial efficacy using *in vivo* studies could be difficult due to many confounding factors affecting the endodontic treatment outcome. As a result, the antimicrobial efficacy of the sealer type cannot be distinguished or separated on treatment outcome.

There has been only one unpublished systematic review registered in PROSPERO available comparing the antibacterial efficacy of bioceramic sealers with other root canal sealers against different types of bacteria including both *in vivo* and *in vitro* studies. However, bioceramic sealers are newly introduced and not widely used at the moment. Another published systematic review was found in the literature focussing on the antibacterial efficacy of various sealers against *E. faecalis* (17). However, this systematic review included studies which strictly used the direct contact test as a laboratory model and excluded studies that adopted other laboratory tests to assess antimicrobial efficacy. This could lead to loss of useful information which could ultimately lead to misleading conclusions concerning the antibacterial activities of sealers and potentially wrong clinical decisions. Besides, the authors carried out the initial search in 2015 and repeated the search in March 2016.

Therefore, the aim of this systematic review was to compare the antibacterial efficacy of Sealapex and AH-plus against *E. faecalis*. A scoping search was carried out by the authors of the current review which revealed additional relevant laboratory studies published in 2017, 2018 and 2019 were included in this review.

Materials and Methods

Review question

The research question was developed by using the Population, Intervention, Comparison, Outcome and study design (PICOS) framework. In the extracted permanent human teeth with *Enterococcus faecalis* (P), does Sealapex sealer (I) show better antibacterial property (O) compared to the AH-plus sealer (C) from *in vitro* studies (S). This systematic review was carried out in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines (18). This study was carried out at the University of Central Lancashire, United Kingdom in collaboration with researchers at the International Medical University, Malaysia.

Search strategy

In order to identify the existing relevant papers needed to answer the research question, a specific process was undertaken as described below. Literature search was performed comprehensively using three electronic databases: Medline, Scopus and EBSCOhost (Dentistry; Oral Sciences Source) from inception to November 2019. The search terms are summarized in Table 1. Additional literature search was performed from the reference list of the eligible studies. Based on the journals publishing the content relevant to the topic, *Journal of Endodontics, International Endodontic Journal, Journal of Dentistry, Australian Endodontic Journal and Journal of Conservative Dentistry* were hand searched to identify any relevant studies.

Inclusion criteria

Inclusion criteria for this review were as follows. i) Time period: no time restriction was applied. ii) Population: papers that used *E. faecalis* among the test microorganisms were included. iii) Intervention and comparator: studies that used both AH-plus and Sealapex. iv) Type of studies: only *in vitro* studies. v) Types of outcome: the antibacterial efficacy of the sealers was assessed by determining the remain-

Table 1
Search strategy terms logic grid

Electronic databases search strategies			
	Pubmed	**EBSCO Dentistry & Oral Sciences Source**	**Scopus**
	((((((((enterococcus faecalis) OR Enterococcus faecalis) OR e. faecalis) OR E faecalis) OR Biofilms) OR biofilm)) **AND** (((Sealapex) OR calcium hydroxide-based sealer) OR calcium hydroxide-based sealer cement)) **AND** (((((AH-plus) OR resin-based sealer) OR resin-based sealer cement) OR epoxy resin-based sealer) OR epoxy resin-based sealer cement)) **AND** ((antimicrobial) OR antibacterial)	((enterococcus faecalis) OR (Enterococcus faecalis) OR (e. faecalis) OR (E faecalis) OR (Biofilms) OR (biofilm)) **AND** ((Sealapex) OR (calcium hydroxide-based sealer) OR (calcium hydroxide-based sealer cement)) **AND** ((AH-plus) OR (resin-based sealer) OR (resin-based sealer cement) OR (epoxy resin-based sealer) OR (epoxy resin-based sealer cement)) **AND** ((antimicrobial) OR (antibacterial))	(enterococcus AND faecalis OR enterococcus AND faecalis OR e. AND faecalis OR e AND faecalis OR biofilms OR biofilm) **AND** (sealapex OR calcium AND hydroxide-based AND sealer OR calcium AND hydroxide-based AND sealer AND cement) **AND** (ah-plus OR resin-based AND sealer OR resin-based AND sealer AND cement OR epoxy AND resin-based AND sealer OR epoxy AND resin-based AND sealer AND cement) **AND** (antimicrobial OR antibacterial)
Total records	**19**	**87**	**53**

ing viable bacteria after the action/application of sealers, and always in relation to the laboratory method used. vi) Language: no language restrictions were applied. Applicable articles were included regardless of the language used, since support from translators was available.

Exclusion criteria
Exclusion criteria included, *in vitro* studies that did not include both AH plus and Sealapex assessing the antibacterial efficacy against *E. faecalis*, studies which assessed antibacterial efficacy against other species of bacteria, unpublished articles, review articles, *ex vivo* articles and systematic reviews.

Study selection and data extraction process
The title and abstract of the selected articles were independently reviewed by two reviewers (DN and SK) based on the specified inclusion and exclusion criteria.
The two reviewers had an almost perfect agreement with a Cohen's Kappa score of 0.9 (19). The reviewers independently read the selected articles for the review and extracted the data using the data extraction form exclusively developed for this study. This form consisted of following details: author, year, preparation of AH-plus and Sealapex, *E. faecalis* strain, control group, laboratory test, antibacterial evaluation, evaluation timing, statistical tests, findings and outcome. Any disagreement between the two reviewers was resolved by discussion with a third reviewer (AP).

Quality assessment of the included studies
The quality of each article was appraised using Cochrane Risk of Bias tool (RoB 2.0) (20). This tool was modified to include the contents based on the methodology employed in the included *in vitro* studies. The quality of included studies was assessed based on following domains: manufacture of materials, instructions of manufacturers followed, existence of control group, repetition of experiment, consistent measurements for repeated experiments, establishment of test methods, objective outcome measurement, direct outcome measurement, who undertook key parts of the experiment, blinding of assessors, use of appropriate statistical tests, report of variability. Two authors (DP and SK) independently evaluated and scored the articles based on the above domains. In case of disagreement, consensus was arrived in discussion with another reviewer (AP).
Haase (21) pointed the importance of quality assessment of the selected studies. Unfortunately, there was no validated tool

for bias assessment for *in vitro* studies. A decision was made to develop one by synthesizing the existing literature and applying necessary modifications to serve the individual purposes of this systematic review. More specific, a template questionnaire was developed by Neil Cook (2018), (Research Associate, School of Dentistry, University of Central Lancashire) based on six studies (22-27).
After individual bias assessment for each included study was completed, a traffic light system similar to the Cochrane Risk of Bias (RoB) tool was developed.

Results

Study Selection process
After completing the search of the three electronic databases, 19 articles were identified in Medline database, 87 articles in EBSCOHost (Dentistry, Oral Sciences Source), and 53 in Scopus database. In total 159 articles were identified after the electronic literature search.
After removing duplicates and abstract screening, 17 studies were found eligible for full text screening.
It was found that all of them met the inclusion criteria, however, two studies had three authors in common and used exactly the same numerical results for AH-plus and Sealapex (28, 29).
If both were included in the analysis the data would be double counted so it was decided that the most recent of the two studies should be excluded from analysis (28). In total 16 studies were included in this systematic review (29-44).
The process followed for study selection was presented via PRISMA Flowchart in Figure 1.
Due to the lack of homogeneity in the data, since studies used different bacteri-

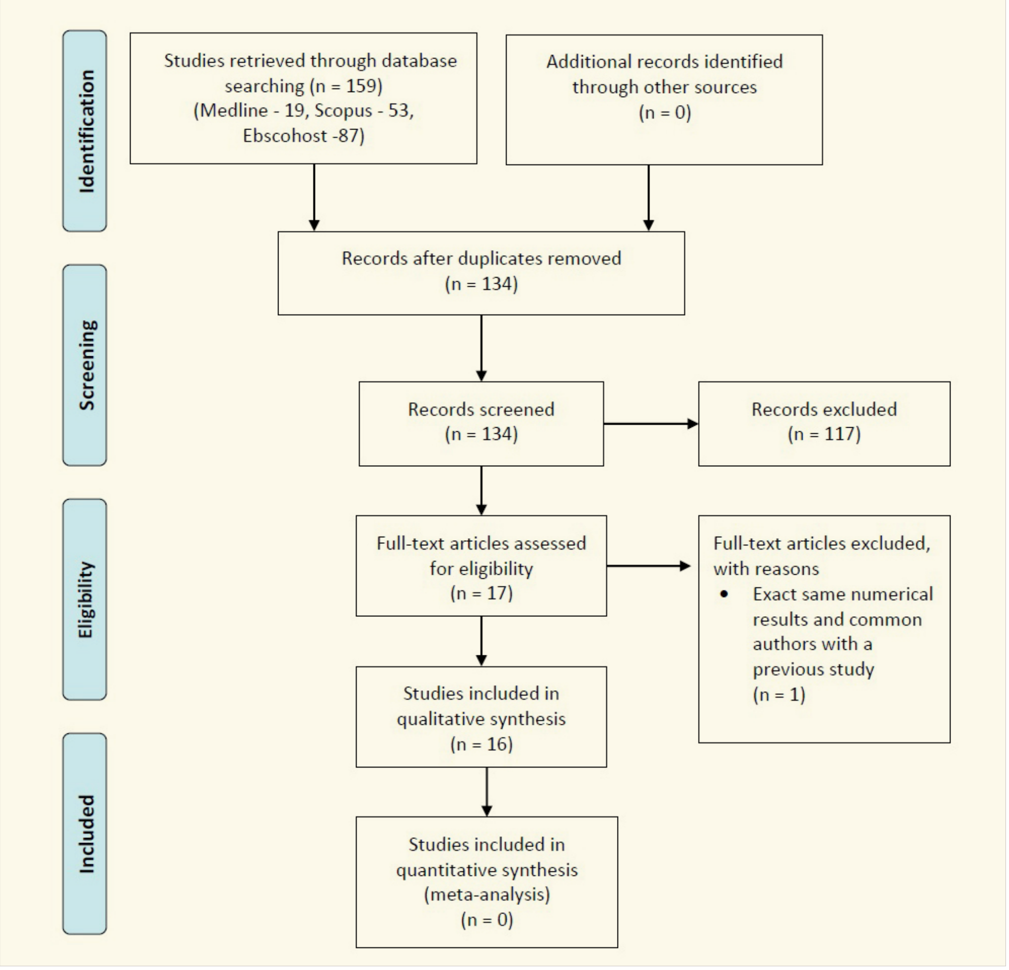

Figure 1.

al strains, different laboratory models to assess antibacterial efficacy and different evaluation times, a meta-analysis could not be conducted. Instead a narrative analysis was performed.

Characteristics of included studies

The studies included in this systematic review were published between 2000 and 2018. Six studies used the agar diffusion test as a laboratory method to assess the antibacterial efficacy of sealers against *E. faecalis* (31, 35-37, 42, 43), five studies used the direct contact test (32, 34, 38, 41, 44), three studies used both methods (29, 30, 33) and three studies used other methods such as membrane restricted contact test (34), time kill essay (39) and broth method (40). Following this, agar diffusion test and direct contact test can be considered as the most widely used laboratory method to assess the antibacterial efficacy of sealers.

Three studies have been found to use optical density and record results using spectrophotometer as antibacterial evaluation method (30, 33, 41). Whereas, six studies employed the method of determination of colony-forming units (CFU) (29, 32, 34, 38, 39, 44). Six studies evaluated the antibacterial activity thorough measurement of inhibition zones (31, 35-37, 42, 43) and one study measured *E. faecalis* growth as a function of sealers' concentration by broth method (40). Two studies employed both spectrophotometer and measurement of inhibition zones (30, 33) and one study determined by using CFU and measurement of inhibition zones (29).

The most commonly used strain of *E. faecalis* is the "ATCC 29212", which is used in nine studies (29, 30, 32, 35, 37, 39-42). Among the different evaluation times each study used, the most common evaluation time is 24 hours after sealers had been mixed and is included in ten studies (31, 33-37, 39, 42-44). Among the studies that used agar diffusion test as a laboratory method to assess the antibacterial effect of AH-plus and Sealapex against *E. faecalis*, three studies concluded that Sealapex has stronger antibacterial efficacy compared to AH-plus (31, 35, 36), and more specific two of them showed that the antibacterial efficacy of Sealapex is significantly higher (31, 36). On the other hand, three studies found that neither Sealapex nor AH-plus had any antibacterial effect against *E. faecalis* (33, 37, 42). Moreover, two studies found that AH-plus has stronger antimicrobial efficacy than Sealapex (29, 42). It must be pointed out though that one study showed that the difference found between the two sealers was not statistically significant (42), while on the contrary another study found a statistically significant difference between them (29). Finally, only one study found that there was no statistical difference between the antibacterial efficacy of the two sealers (30). Hence, contradictory results were shown from these three studies (29, 30, 32).

Among the studies that used the direct contact test as a laboratory method to assess the antibacterial efficacy of AH-plus and Sealapex, four studies showed that AH-plus had higher antibacterial efficacy compared to Sealapex (30, 33, 34, 41), and more specific, three of them showed that the antibacterial efficacy of AH-plus was significantly higher (33, 34, 41). It should be mentioned that one study showed that the difference was statistically significant for freshly mixed sealers only (41). On the other hand, three studies concluded that Sealapex had a statistically significant increased antibacterial efficacy compared to AH-plus (29, 32, 38).

Finally, only one study showed opposing results for different evaluation times (43). More specific, for freshly mixed sealers AH-plus showed statistically significant higher antimicrobial efficacy compared to Sealapex, but after 1, 3 and 7 days, Sealapex was more efficient. Total there were six studies measured the fresh mixed samples (33, 29, 39-41, 44), ten studies measured after 24 hours (31, 33-37, 39, 42-44), nine studies measured after 48 hours (29, 31-33, 36, 38, 41, 42, 44) and eight studies measured after 7 days (29, 30, 32, 38, 39, 41, 42, 44). The characteristics of the included *in vitro* studies were shown in Table 2.

Table 2
Characteristics of included studies in the systematic review

No.	Study	E. Faecalis strain	Control group	Laboratory test	Antibacterial evaluation	Evaluation timing	Statistical tests	Outcome
1	Poggio et al. [29]/2017/ Italy	ATCC 29212	ADT: Two plates without bacterial suspension, one without sealer DCT: Sealer-free saline suspension	ADT and DCT	ADT: Measurement of inhibition zones (mm) DCT: Determination of colony-forming units (CFU/ml)	ADT: After 48h DCT: After 6', 15' and 60' (set sealers-7 days)	Student's t- test	ADT: AH-plus showed significantly higher antimicrobial efficacy than Sealapex DCT: Sealapex showed significantly higher antimicrobial efficacy than AH-plus.
2	Cobankara et al. [30] /2004/ Turkey	ATCC 29212	NA	ADT, DCT	ADT: Measurement of inhibition zones (mm) DCT: Turbidometric measurement of bacterial outgrowth (optic density at 620nm)	ADT: After 7 days DCT: 12 measurements every 30mins for the first 6h and 12 measurements in the last 6h	ADT: Kolmogorov-Smirnov DCT: -	ADT: No statistical difference found between AH-plus and Sealapex DCT: AH-plus showed higher antibacterial efficacy than Sealapex.
3	Dalmia et al. [31]/2018/ India	MTCC 2093	NA	ADT	Measurement of inhibition zones (mm)	After 24h, 48h and 72h	ANOVA, Unpaired t-test	Sealapex showed statistically significant higher antimicrobial efficacy compared to AH-plus
4	Faria-Junior et al. [32]/2013/ Brazil	ATCC 29212 organised in biofilm	Biofilm not exposed to sealers	Modified DCT	Determination of colony-forming units (CFU/ml) in biofilm	After 5h, 10h and 15h (2 and 7 days after sealers had set)	Kruskal-Wallis and Dunn tests	Sealers set for 2 days, Sealapex showed statistically significant higher antimicrobial efficacy compared to AH-plus. Sealers set for 7 days, no statistical significance was detected between 0-5h, but Sealapex again showed statistically significant higher antimicrobial efficacy compared to AH-plus between 5-15h.
5	Heyder et al. [33]/2013/ Germany	DSMZ 20376	ADT: Chlorhexidine as positive control and distilled water as negative control DCT: culture medium as negative control and 100µl of bacterial suspension with baseline cell concentration mixed with 400µl Schaedler liquid medium as positive control	ADT and DCT only for sealers that showed good antibacterial effect in ADT	ADT: Measurement of inhibition zones (mm) DCT: Turbidometric measurement of bacterial outgrowth (optic density at 560nm)	ADT: After 48h (both for freshly mixed and set sealers) DCT: After 0, 2, 4, 6, 8, 12 and 24h	ADT: Mann-Whitney U-test DCT: two-tailed t-test	ADT: Neither AH-plus nor Sealapex showed antibacterial activity against E. faecalis (for fleshly mixed and set sealers) DCT: AH-plus significantly reduced E. faecalis, whereas Sealapex showed no antibacterial activity against E. faecalis.
6	Kayaoglou et al. [34] /2005/ Turkey	A197A	Teflon disc	DCT and membrane-restricted test	Determination of colony-forming units (CFU/ml)	After 24h	Student's t-test	AH-plus showed statistically significant higher antimicrobial efficacy compared to Sealapex
7	Leonardo et al. [35] /2000/ Brazil	ATCC 10541	NA	ADT	Measurement of inhibition zones (mm)	After 24h	NA	Sealapex showed stronger antibacterial efficacy than AH-plus

8	Mickel et al. [36]/2003/ USA	NA	amoxicillin disc (positive control)	ADT	Measurement of inhibition zones (mm)	After 24h and 48h	ANOVA, Tukey's test	Sealapex showed antibacterial effect against E. faecalis, while AH-plus did not show any antimicrobial effect. Difference is statistically significant.
9	Miyagak et al. [37]/2006/ Brazil	ATCC 29212	NA	ADT	Measurement of inhibition zones (mm)	After 24h	Non-parametrical test (without determining which)	Neither AH-plus nor Sealapex showed antibacterial effect towards E. faecalis
10	Rezende et al. [38]/2016/ Brazil	ATCC 51299	Dentine blocks with formed biofilm not exposed to sealers	DCT	Determination of colony-forming units (CFU/ml)	After 2, 7 and 14 days	Single-factor ANOVA model, Sapiro-Will, Kruskal-Wallis one-way test	Sealapex showed statistically significant higher antimicrobial activity than AH-plus in all the time periods
11	Sagsen et al. [39]/2009/ Turkey	ATCC 29212	A tube containing brain-heart infusion broth without bacteria, a tube containing brain-heart infusion broth with bacteria	Time kill essay	Determination of colony-forming units.	After 20', 24h, 7d and 9d	NA	AH plus is bactericidal at 20' and 24h and less bactericidal on 7th and 9th days. Sealapex is found bacteriostatic on the 7th and 9th days but without effect at 20' and 24h.
12	Shin et al. [40]/2018/ Republic of Korea	ATCC 29212	Cultures not treated with the spent culture medium	Broth method	Measurement of E. faecalis growth as a function of sealers' concentration	Before and after setting	Kruskal-Wallis and Mann-Whitney tests	Sealapex showed higher antibacterial activity than AH-plus.
13	Smadi et al. [41]/2008/ Jordan	ATCC 29212	Uncoated wells containing identical size inoculation, wells containing test materials without bacterial inoculation	DCT	Turbidometric measurement of bacterial outgrowth (optic density at 620nm)	After 20', 48h and 7d	Multiple t-tests	AH-plus showed statistically significant higher antibacterial efficacy than Sealapex when sealers were freshly mixed. No significant difference is found between them at 48h and one week tests.
14	Smadi et al. [42]/2008/ Jordan	ATCC 29212	Sterile saline	ADT	Measurement of inhibition zones (mm)	After 24h, 48h and 7 days	ANOVA, Tukey's test	Neither AH-plus nor Sealapex showed antibacterial effect against E. faecalis
15	Yasuda et al. [43]/2008/ Japan	ATCC 10541	Plate without sealers	ADT	Measurement of inhibition zones (mm)	After 24h	ANOVA, Tukey's test	AH-plus showed higher antibacterial efficacy than Sealapex, but difference is not statistically significant
16	Zhang et al. [44]/2009/ Canada	VP3-181	Bacterial suspensions on the wall of uncoated wells	Modified DCT	Determination of colony-forming units (CFU/ml)	Fresh sealers, set for 1, 2, 3 and 7 days	ANOVA, Tukey test	Fresh AH-plus significantly reduced E. faecalis numbers at 2' and eradicated them within 5'-20', whereas Sealapex started reducing E. faecalis significantly after 20'. Similar results were found after 1 day and 3 days of setting: Sealapex eradicated E. faecalis in 60' whereas AH-plus failed to kill E. faecalis in the same time. Seven days after mixing: Sealapex shows higher antibacterial efficacy eradicating E. faecalis at 20' and 60' whereas AH-plus shows slight antibacterial efficacy in 2', 5' and 20' and none in 60'.

Table 3
Risk of bias assessment of included studies

Author/Year/Country	Manufacture of materials	Instructions of manufacturers followed	Existence of control group	Repetition of experiments	Consistent measurements for repeated experiments	Establishment of test methods	Objective outcome measurement	Direct outcome measurement	Who undertook key parts of the experiment	Blinding of assessors	Use of appropriate statistical tests	Report of variability
Poggio et al. [29]/2017/Italy	+	+	+	+	–	+	–	+	–	–	+	+
Cobankara et al. [30]/2004/Turkey	+	+	–	+	?	+	–	+	–	–	+	–
Dalmia et al. [31]/2018/India	+	+	–	+	+	+	–	+	–	–	+	+
Faria-Junior et al. [32]/2013/Brazil	+	+	+	+	+	+	–	+	–	–	+	+
Heyder et al. [33]/2013/Germany	+	+	+	+	?	+	–	+	–	–	+	–
Kayaoglou et al. [34]/2005/Turkey	+	+	+	+	+	+	–	+	–	–	+	+
Leonardo et al. [35]/2000/Brazil	+	+	–	+	–	+	–	+	–	–	–	–
Mickel et al. [36]/2003/USA	+	+	+	+	?	+	–	+	–	–	+	–
Miyagak et al. [37]/2006/Brazil	+	+	–	+	?	+	–	+	–	–	+	–
Rezende et al. [38]/2016/Brazil	+	+	+	+	–	+	–	+	–	–	+	+
Sagsen et al. [39]/2009/Turkey	+	+	+	+	?	+	–	+	–	–	–	–
Shin et al. [40]/2018/Republic of Korea	+	+	+	+	+	+	–	+	–	–	+	+
Smadi et al. [41]/ 2008/Jordan	+	+	+	+	?	+	–	+	–	–	+	–
Smadi et al. [42]/2008/Jordan	+	+	+	+	?	+	–	+	–	–	+	+
Yasuda et al. [43]/2008/Japan	+	+	+	+	+	+	–	+	–	–	+	+
Zhang et al. [44]/2009/Canada	+	+	+	+	?	+	–	+	–	–	+	–

Quality of included studies

The studies were analysed using the modified Risk of Bias tool. A table with the traffic-light system was created to assess the overall quality of the included studies (Table 3).

After a thorough look of the traffic-light system it can be stated that the quality of the evidence was "low", since every study had at least three questions related to high risk of bias.

More specific, concerning how the sealers were manufactured, all studies reported that in detail, presenting tables with the ingredients of the sealers and preparing them according to the manufacturers' instructions. As a result, there was very low risk of bias in sealers' manufacturing and preparation.

This was expected as described in the background, sealers exist in the market in tubes that contain specific and standardized substances and were mixed in specific ratios which can be easily achieved.

As far as the existence of control group was concerned, only four studies did not

use a control group (30, 31, 35, 37), while twelve studies used a control group resulting in a relatively low risk of bias (29, 32-34, 36, 38-44). However, it should be mentioned that not all of them used both a positive and negative control group. The existence of control groups ensures the reliability of the results in experiments and the purpose of including them in the study design was to enhance the statistical validity of the dataset. The existence of both a positive and a negative control provides a reassurance that the experiment is designed and conducted properly. In addition, a very low risk of bias arises from repetition of experiments, since all studies repeated their experiments. Repetition of the experiments reduces the possibility that findings occurred by chance and as a result maximises their validity.
There is a higher risk of bias arising from consistency of measurements, since eight studies did not report the standard deviation when reporting their measurements and as a result it is unclear if their measurements were consistent (30, 33, 36, 37, 39, 41, 42, 44), three studies did not have consistent measurements (29, 35, 38), while only four studies had consistent measurements (31, 32, 34, 40, 42). Lack of consistency could mean that it is unlikely that results were significant. In other words, it minimises the validity of the findings.
There was a low risk of bias arising from the test methods used, since all studies used well established laboratory antimicrobial tests to assess the antimicrobial efficacy of the sealers. Each study described in detail the settings of the test and the methodology that is followed.
A low risk of bias was arising from direct outcome measurement, since all studies measured either absence of bacteria (inhibition zones) or presence of bacteria (colony forming units, optic density). Measurement of outcome in a direct way means that there were no cofounding factors that could possibly interfere and produce false results.
High risk of bias arises from objectivity in outcome measurement, blinding of assessors and the person or persons who undertook the key parts of the experiment.
None of the studies reported who conducted the experiments, how many people took part in the experiment, or if the assessors were blinded to the sealers. The importance of blinding lies in the concept of minimizing bias and consequently maximises the validity of the findings. However, it has to be mentioned that Sealapex has a slight grey appearance when mixed and AH-plus has a yellowish appearance when mixed, which arises the necessity of non-dentists or non-clinicians as assessors. Otherwise if assessors were dentists or clinicians, blinding is not possible since they could differentiate between the two sealers due to the different appearance in their shade.
There was a lower risk of bias arising from the use of appropriate statistical tests since only two studies did not perform any statistical analysis (35, 39), while all the rest did use statistical tests to identify any existing significant difference in their results (29-34, 36-38, 40-44). If a study identified a difference in the antimicrobial activity between the two sealers and did not perform statistical analysis, no conclusions can be drawn concerning whether this difference is real or occurred by chance. Therefore, the validity of the findings of such a study is minimised.
Finally, eight studies reported on variability of their measurements (29, 31, 32, 34, 38, 40, 42, 43), while the rest eight studies (30, 33, 35-37, 39, 41, 44) did not give any relevant information, resulting in a high risk of bias concerning this factor.
Without reporting variability, no conclusions can be drawn concerning how the data were spread. Hence, there was no evidence that measurements were repeatable and following this, results cannot be considered reliable. No study provided strong evidence, twelve studies provided moderate evidence (29, 31-34, 36, 38, 40-44), three studies provided limited evidence (30, 37, 39) and one study provided conflicting evidence (35).
Based on the assessment, none of the studies was of a high quality. Hence, it was decided not to exclude any study at this stage; instead an analysis including all studies was approached and the limitations arising from bias and low quality in relation to the interpretation of the results

and their implications for the clinical practice was discussed in this systematic review.

Discussion

This systematic review intended to give an answer to the question whether Sealapex had higher antibacterial efficacy against *E. faecalis* compared to AH-plus. Unfortunately, this question cannot be answered for the following reasons. Firstly, different laboratory tests gave contradicting results. Secondly, results were contradicting even for studies that used the same laboratory tests. Finally, the existing literature is constituted from studies of low quality, biased, that consequently produce untrustworthy findings. AH-plus and Sealapex are very well-studied sealers with respective advantages. AH-plus is slightly thixotropic, non-mutagenic, non-genotoxic, a weak sensitizer, easily removed if needed, and unlike its predecessor AH26, does not release formaldehyde or cause tooth discoloration (16, 45).

On the other hand, Sealapex also has its advantages by being biocompatible, ability to exhibit antibacterial effect immediately following manipulation and several days later and maintaining high pH in the medium (44, 46). The antimicrobial effect of epoxy resin-based sealers is due to the presence of bisphenol A diglycidyl ether or the release of formaldehyde during polymerisation. However, AH-plus does not release formaldehyde unlike AH-26 sealer (45, 47). In Sealapex, its antimicrobial property is attributed to the release of hydroxide ion which creates an alkaline environment (38). A pH level of more than 9 may reversibly or irreversibly inactivate cellular membrane enzymes of the microorganism resulting in loss of biological activity of the cytoplasmic membrane (48).

Agar diffusion test is a very commonly used laboratory method to assess sensitivity of bacterial species against antibiotic substances (49). During the test, cells of the bacterial species of interest were inoculated on nutrient agar petri dishes. Wafers containing the sample materials were placed in the centre of the petri dishes and were incubated at 37 °C for 18-24 hours. Evaluation is based on measurement of the inhibition zones, which were the areas around the wafers that bacteria have not grown enough to be visually detected. The larger the inhibition zones were, the more susceptible the bacterial species is to the test material.

This method has the advantages of being simple and easily conducted, without necessitating special equipment. However, several limitations have been attributed to this test method, which need to be taken into consideration. First of all, this test method follows a demanding procedure, which necessitates well controlled inoculum density, medium content, agar viscosity, agar plates' storage conditions, specimens' size and number (per plate), specimens' location and arrangement (on plate), incubation time and temperature, and adequate specimens' and agar contact. Secondly, it is insensitive and semiquantitative test method, which does not distinguish between bacteriostatic and bactericidal properties of the dental materials (9). Furthermore, the results produced by this method depend on the molecular size and diffusion constant of the antimicrobial component, the toxicity of the material against the bacterial species tested, the inoculum size, the incubation time and the degree of contact between the material and agar. Moreover, agar diffusion test can be applied only on water-soluble materials, because the antibacterial agent has to diffuse through agar which is in an aqueous form (50, 51). Solubility comes in contrast to the physical properties an endodontic sealer ideally should have. As a result, if a sealer contains an antimicrobial agent that it is insoluble it will not show inhibition zones and its antibacterial activity, although existing, will falsely be undetected.

Direct contact test is a laboratory method to assess antimicrobial efficacy of sealers and root canal filling materials, given the existing limitations of agar diffusion test and as an attempt to overcome some of them (9). According to Weiss et al. (1996), direct contact test is based on the turbidometric determination of bacterial growth in 96-well microtiter plates (9). The kinetics of the outgrowth in each well is monitored at 600 nm at 37 °C and recorded every 30 min using a temperature-controlled microplate spectrophotometer (9). This method is based on direct contact of the bacterial specie and the endodontic sealer and it has the advantage of being reproducible, quantitative method and uninfluenced by the size of the inoculum and the diffusion properties of the materials tested and the media used.

Other than agar diffusion and direct contact tests which were the most widely used and discussed methods, time kill assay, broth method and membrane restricted test were used as well. Time kill assay is a laboratory method used to assess the antibacterial efficacy of a material against a bacterial strain by determining the bactericidal or bacteriostatic activity of the test material in relation to time. It should be pointed out though, that the bacteriostatic effect may risk late failure as there is a risk of continued growth of surviving bacteria and potential loss of the antibacterial activity of endodontic sealers.

The broth method using the elute, is another method for determining susceptibility of bacterial species to antibiotic substances (17). However, the time kill assay and the broth method although validated as laboratory methods for assessment of antimicrobial activity, they have not been widely used for sealers. Their strengths and limitations associated to endodontic sealers have not been examined or discussed and there is no evidence to support their clinical relevance. Membrane restricted test is a non-contact test to assess antibacterial efficacy. It was developed and used for the first time for assessment of antibacterial efficacy of endodontic sealers in one of the studies (34). Further research is needed to validate this as a reliable method to assess the antibacterial activity of sealers and support its clinical relevance.

Having discussed the weaknesses of agar diffusion test in relation to endodontic sealers, even if the direct contact test can be considered a more reliable method to assess their antimicrobial efficacy, still results between studies were contradicting. Three studies showed that AH-plus had a significantly higher antibacterial activity compared to Sealapex (33, 34, 41) and out of these three, one study showed the result when freshly mixed (41) and two studies portrayed the results after 24 hours (33, 34). Moreover, in particular, this study showed no significant difference when tested after 48 hours and 7 days (41). The antimicrobial effect of AH-plus is due to the presence of bisphenol A diglycidyl ether (47). In contrary, three studies showed the exact opposite, namely that Sealapex had a significantly increased antimicrobial activity compared to AH-plus when tested after freshly mixed (29), from fifth to fifteenth hour (32) and during 2, 7 and 14 days (38). The possible reason to the contradiction is Sealapex has longer setting time and releases hydroxyl ions that is antimicrobial even up to 30 days as showed in a study comparing to Apexit plus (46, 52). Considering the above, there is no evidence to support the use of Sealapex against AH-plus, or vice versa in terms of their antibacterial efficacy against *E. faecalis*. Consequently, one cannot be considered superior to the other.

Concerning the different evaluation times most studies used, these times can be connected to the setting times of the sealers used. All studies in this review investigated multiple sealers, including the two sealers of interest. Sealapex takes 24 hours to set completely, which is longer time then the 8 hours AH-plus needs to set, so the evaluation after 24 hours which is the most popular evaluation time, can be considered important in terms of clinical practice. It is doubtful whether it is clinically relevant to evaluate the long-term antibacterial efficacy of the endodontic sealers. The reason why is connected to the physical properties an endodontic sealer should have. If a substance slowly releases antimicrobial agents, it will ultimately lose part of its initial mass, resulting in compromise of

its dimensional stability, strength, porosity and resistance to wear (9). This comes in contrast to the requirements an endodontic sealer should meet in order to fulfil its purpose during the process of obturation of the root canal system, which is to provide a tight-fluid seal, always in combination with the core filling material.
The standard strains that most studies used were usually preferred so that results can be reproducible. Nevertheless, bacterial susceptibility to antibacterial agents may differ between strains and clinical isolates (50). Moreover, assessing susceptibility of *E. faecalis* organised in biofilm can be considered more clinically relevant, since this bacterial species is usually found in biofilms in the root canal system (53). It is possible, that organisation of *E. faecalis* in a biofilm could further differentiate its antimicrobial susceptibility. This scenario is assessed only in one study though (32).
Hand searching did not reveal any other relevant studies in addition to the *in vitro* articles which were identified from the electronic searches. The quality assessment tool that was used has not been validated, but as previously mentioned there is no specific tool for bias assessment for *in vitro* studies. The fact that such a tool has not been developed yet, could be due to the different settings, methodologies and special characteristics of different types of *in vitro* studies, which makes it difficult to develop a single tool applicable for all types of *in vitro* studies. Nevertheless, an attempt was made to form a questionnaire including many questions and assessing as many aspects of the study design as possible so that any existing bias could be identified. The authors suggest that this tool will be adopted for future use when assessing the quality of *in vitro* antibacterial studies.
Generally, the most important disadvantage of the *in vitro* studies lies in the difficulty to extrapolate their results to the clinical situation or a randomised controlled trial. However, they were the first step in research for testing and assessing the properties of dental materials. In order to produce results that can be more easily related to clinical practice, laboratory methods need to simulate as much as practically possible the environment of the root canal system (54, 55). The reason why the antibacterial activity of root canal sealers against *E. faecalis* or any other bacterial species cannot be studied with clinical studies is the existence of confounding factors affecting treatment outcome. As confounding factors can be regarded for example the existence of the filling material, other surviving bacterial species in the root canal system that possibly interact with each other, the protocols used for disinfection of the root canal system and the complex anatomy of the root canal system. All these make it impossible to draw conclusions specifically about sealers' activity only. Nevertheless, high quality *in vitro* studies can provide the clinician with important information that can be taken into account when choosing between several available commercial sealers.
Microorganism in the root canal system are highly organized entities known as biofilms which is a form of protection for the planktonic bacteria towards antimicrobial agents (56, 57). Hence, studies using biofilm comprising of multiple microorganisms gives a higher impact factor in clinical relevance. When making a decision about the use of a specific sealer clinically, there are many aspects that need to be taken into consideration. All physical properties of the material need to be assessed to be as similar as possible to the ideal properties of an endodontic sealer as they have been described by Grossman et al. (12). Moreover, the antibacterial efficacy of the sealer against other bacterial species that can survive initial root canal treatment or were highly pathogenic should be taken into consideration, provided though that there were *in vitro* studies of high methodological quality and low risk of bias that can be used as evidence during clinical decision making. Furthermore, practical aspects of the daily clinical

practice such as easy and predictable placement of the sealer into the root canal system and cost can be considered essential when deciding which material is best to opt for.
All above aspects are important, and a balance needs to be found between properties and cost, and always in accordance to the individual challenges an endodontic case shows.

Conclusions

Due to the identification of poor quality relevant studies which also provided contradicting results, the above answer could not be addressed, and the two sealers of interest performed similarly against each other in terms of their antimicrobial efficacy against *E. faecalis*.

Clinical Relevance

Antibacterial effect of root canal sealers plays a crucial role in endodontic treatment. This systematic review provides an evidence of the antibacterial efficacy of Sealapex and AH-plus against *E. faecalis*. Based on the findings of this systematic review, freshly mixed AH-plus sealer showed higher antimicrobial efficacy compared to Sealapex but after 1, 3 and 7 days, Sealapex was found to be more efficient.
It also provides recommendation for future approach to carry out studies evaluating antibacterial efficacy of sealers.

Conflict of Interest

All authors declare that they have no conflicts of interest. In addition, all authors have read and approved the manuscript as submitted, are qualified for authorship, believe the submission represents honest work and take full responsibility for the reported findings. The study was self-funded.

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References

1. *Fouad AF. Endodontic Microbiology and Pathobiology: Current State of Knowledge. Dent Clin North Am. 2017;61(1):1-15.*
2. *Prada I, Micó-Muñoz P, Giner-Lluesma T, Micó-Martínez P, Collado-Castellano N, Manzano-Saiz A. Influence of microbiology on endodontic failure. Literature review. Med Oral Patol Oral Cir Bucal. 2019;24(3):e364-e372.*
3. *Anderson AC, Hellwig E, Vespermann R, Wittmer A, Schmid M, Karygianni L et al. Comprehensive analysis of secondary dental root canal infections: a combination of culture and culture-independent approaches reveals new insights. PLoS One. 2012;7(11):e49576. doi: 10.1371/journal.pone.0049576.*
4. *Siqueira JF Jr, I Rôças, Ricucci D. Biofilms in endodontic infection. Endodontic Topics 2012; 22:33-49. doi:10.1111/j.1601-1546.2012.00279.*
5. *Wang QQ, Zhang CF, Chu CH, Zhu XF. Prevalence of Enterococcus faecalis in saliva and filled root canals of teeth associated with apical periodontitis. Int J Oral Sci. 2012;4(1):19-23.*
6. *Rôças IN, Siqueira JF Jr, Santos KR. Association of Enterococcus faecalis with different forms of periradicular diseases. J Endod. 2004;30(5):315-320.*
7. *Love RM. Enterococcus faecalis-a mechanism for its role in endodontic failure. Int Endod J. 2001;34(5):399-405.*
8. *Yoo YJ, Perinpanayagam H, Oh S, Kim AR, Han SH, Kum KY. Endodontic biofilms: contemporary and future treatment options. Restor Dent Endod. 2019;44(1):e7. Published 2019 Jan 31. doi:10.5395/rde.2019.44.*
9. *Zhang C, Du J, Peng Z. Correlation between Enterococcus faecalis and Persistent Intraradicular Infection Compared with Primary Intraradicular Infection: A Systematic Review. J Endod. 2015;41(8):1.207-1.213.*
10. *Guide to clinical endodontics. (2004). Chicago, IL: American Association of Endodontists.*
11. *Glossary of endodontic terms. (2003). Chicago, Ill.: American Association of Endodontists.*
12. *Grossman, L., Oliet, S. and Rio, C. (1988). Endodontic practice. 11th ed. Philadelphia: Lea & Febiger.*
13. *Nawal RR, Parande M, Sehgal R, Naik A, Rao NR. A comparative evaluation of antimicrobial efficacy and flow properties for Epiphany, Guttaflow and AH-Plus sealer. Int Endod J. 2011;44(4):307-313.*
14. *Desai S, Chandler N. Calcium hydroxide-based root canal sealers: a review. J Endod. 2009;35(4):475-480.*
15. *Mohammadi Z, Karim Soltani M, Shalavi S, Yazdizadeh M, Jafarzadeh M. Calcium hydroxide-based root canal sealers: an updated literature review. Compend Contin Educ Dent. 2014;35(5):334-340.*
16. *Eldeniz AU, Mustafa K, Ørstavik D, Dahl JE. Cytotoxicity of new resin-, calcium hydroxide- and silicone-based root canal sealers on fibroblasts derived from human gingiva and L929 cell lines. Int Endod J. 2007;40(5):329-337.*
17. *AlShwaimi E, Bogari D, Ajaj R, Al-Shahrani S, Almas K, Majeed A. In Vitro Antimicrobial Effectiveness of Root Canal Sealers against Enterococcus faecalis: A Systematic Review. J Endod. 2016;42(11):1.588-1.597.*

18. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009;6(7):e1000097.
19. McHugh ML. Interrater reliability: the kappa statistic. Biochem Med (Zagreb). 2012;22(3):276-282.
20. Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). Cochrane Handbook for Systematic Reviews of Interventions version 6.0 (updated July 2019). Cochrane, 2019. Available from www.training.cochrane.org/handbook.
21. Haase, S.C., 2011. Systematic reviews and meta-analysis. Plast Reconstr Surg, 127(2), 955-966.
22. Krithikadatta J, Gopikrishna V, Datta M. CRIS Guidelines (Checklist for Reporting In-vitro Studies): A concept note on the need for standardized guidelines for improving quality and transparency in reporting in-vitro studies in experimental dental research. J Conserv Dent. 2014;17(4):301-304.
23. Kushman ME, Kraft AD, Guyton KZ, Chiu WA, Makris SL, Rusyn I. A systematic approach for identifying and presenting mechanistic evidence in human health assessments. Regul Toxicol Pharmacol. 2013;67(2):266–277.
24. Matono Y, Nakagawa M, Matsuya S, Ishikawa K, Terada Y. Corrosion behavior of pure titanium and titanium alloys in various concentrations of Acidulated Phosphate Fluoride (APF) solutions. Dent Mater J. 2006;25(1):104-112.
25. MiloševI, Kapun B, Selih VS. The effect of fluoride ions on the corrosion behaviour of Ti metal, and Ti6-Al-7Nb and Ti-6Al-4V alloys in artificial saliva. Acta Chim Slov. 2013;60(3):543-555.
26. Nakagawa M, Matsuya S, Udoh K. Corrosion behavior of pure titanium and titanium alloys in fluoride-containing solutions. Dent Mater J. 2001;20(4):305-314.
27. OHAT Risk of Bias Rating Tool for Human and Animal Studies (2015) https://ntp.niehs.nih.gov/ntp/ohat/pubs/riskofbiastool_508.pdf
28. Colombo M, Poggio C, Dagna A, Meravini MV, Riva P, Trovati F et al. Biological and physico-chemical properties of new root canal sealers. J Clin Exp Dent. 2018 Feb 1;10(2):e120-e126.
29. Poggio C, Trovati F, Ceci M, Colombo M, Pietrocola G. Antibacterial activity of different root canal sealers against Enterococcus faecalis. J Clin Exp Dent. 2017;9(6):e743–e748. Published 2017 Jun 1.
30. Cobankara FK, Altinöz HC, Ergani O, Kav K, Belli S. In vitro antibacterial activities of root-canal sealers by using two different methods. J Endod. 2004;30(1):57-60.
31. Dalmia S, Gaikwad A, Samuel R, Aher G, Gulve M, Kolhe S. Antimicrobial Efficacy of Different Endodontic Sealers against Enterococcus faecalis: An In vitro Study. J Int Soc Prev Community Dent. 2018;8(2):104-109.
32. Faria-Júnior NB, Tanomaru-Filho M, Berbert FL, Guerreiro-Tanomaru JM. Antibiofilm activity, pH and solubility of endodontic sealers. Int Endod J. 2013;46(8):755-762.
33. Heyder M, Kranz S, Völpel A, Pfister W, Watts DC, Jandt KD et al. Antibacterial effect of different root canal sealers on three bacterial species. Dent Mater. 2013 May;29(5):542-9.
34. Kayaoglu G, Erten H, Alaçam T, Ørstavik D. Short-term antibacterial activity of root canal sealers towards Enterococcus faecalis. Int Endod J. 2005;38(7):483-488.
35. Leonardo MR, da Silva LA, Tanomaru Filho M, Bonifácio KC, Ito IY. In vitro evaluation of antimicrobial activity of sealers and pastes used in endodontics. J Endod. 2000;26(7):391-394.
36. Mickel AK, Nguyen TH, Chogle S. Antimicrobial activity of endodontic sealers on Enterococcus faecalis. J Endod. 2003;29(4):257-258.
37. Miyagak DC, de Carvalho EM, Robazza CR, Chavasco JK, Levorato GL. In vitro evaluation of the antimicrobial activity of endodontic sealers (published correction appears in Braz Oral Res. 2007 Jan-Mar;21(1):9). Braz Oral Res. 2006;20(4):303-306.
38. Rezende GC, Massunari L, Queiroz IO, Gomes Filho JE, Jacinto RC, Lodi CS, et al. Antimicrobial action of calcium hydroxide-based endodontic sealers after setting, against E. faecalis biofilm. Braz Oral Res. 2016;30:S1806-83242016000100228.
39. Sagsen B, Er O, Esel D, Yagmur G, Altintop Y. In vitro pharmacodynamic activities of root canal sealers on Enterococcus faecalis. J Contemp Dent Pract. 2009;10(3):35–42. Published 2009 May 1.
40. Shin JH, Lee DY, Lee SH. Comparison of antimicrobial activity of traditional and new developed root sealers against pathogens related root canal. J Dent Sci. 2018;13(1):54-59.
41. Smadi L, Khraisat A, Al-Tarawneh SK, Mahafzah A, Salem A. In vitro evaluation of the antimicrobial activity of nine root canal sealers: direct contact test. Odontostomatol Trop. 2008;31(124):11-18.
42. Smadi L, Mahafzah A, Khraisat A. An in vitro evaluation of the antimicrobial activity of nine root canal sealers. J Contemp Dent Pract. 2008;9(5):60–67. Published 2008 Jul 1.
43. Yasuda Y, Kamaguchi A, Saito T. In vitro evaluation of the antimicrobial activity of a new resin-based endodontic sealer against endodontic pathogens. J Oral Sci. 2008;50(3):309-313.
44. Zhang H, Shen Y, Ruse ND, Haapasalo M. Antibacterial activity of endodontic sealers by modified direct contact test against Enterococcus faecalis. J Endod. 2009;35(7):1.051-1.055.
45. Leonardo MR, Bezerra da Silva LA, Filho MT, Santana da Silva R. Release of formaldehyde by 4 endodontic sealers. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1999;88(2):221.225.
46. Duarte MA, Demarchi AC, Giaxa MH, Kuga MC, Fraga SC, de Souza LC. Evaluation of pH and calcium ion release of three root canal sealers. J Endod. 2000;26(7):389.390.
47. Singh G, Gupta I, Elshamy FMM, Boreak N, Homeida HE. In vitro comparison of antibacterial properties of bioceramic-based sealer, resin-based sealer and zinc oxide eugenol based sealer and two mineral trioxide aggregates. Eur J Dent. 2016;10(3):366-369.
48. Estrela C, Sydney GB, Bammann LL, Felippe Júnior O. Mechanism of action of calcium and hydroxyl ions of calcium hydroxide on tissue and bacteria. Braz Dent J. 1995;6(2):85-90.
49. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966;45(4):493-496.
50. Fraga RC, Siqueira JF Jr, de Uzeda M. In vitro evaluation of antibacterial effects of photo-cured glass ionomer liners and dentin bonding agents during setting. J Prosthet Dent. 1996;76(5):483-486.

51. *Fuss Z, Weiss EI, Shalhav M. Antibacterial activity of calcium hydroxide-containing endodontic sealers on Enterococcus faecalis in vitro. Int Endod J. 1997;30(6):397-402.*
52. *Tagger M, Tagger E, Kfir A. Release of calcium and hydroxyl ions from set endodontic sealers containing calcium hydroxide. J Endod. 1988;14(12):588-591.*
53. *Ordinola-Zapata R, Bramante CM, Garcia RB, de Andrade FB, Bernardineli N, de Moraes IG et al. The antimicrobial effect of new and conventional endodontic irrigants on intra-orally infected dentin. Acta Odontol Scand. 2013 May-Jul;71(3-4):424-31.*
54. *de Gregorio C, Estevez R, Cisneros R, Heilborn C, Cohenca N. Effect of EDTA, sonic, and ultrasonic activation on the penetration of sodium hypochlorite into simulated lateral canals: an in vitro study. J Endod. 2009;35(6):891-895.*
55. *de Gregorio C, Estevez R, Cisneros R, Paranjpe A, Cohenca N. Efficacy of different irrigation and activation systems on the penetration of sodium hypochlorite into simulated lateral canals and up to working length: an in vitro study. J Endod. 2010;36(7):1.216-1.221.*
56. *Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science. 1999;284(5418):1.318-1.322.*
57. *Vieira AR, Siqueira JF Jr, Ricucci D, Lopes WS. Dentinal tubule infection as the cause of recurrent disease and late endodontic treatment failure: a case report. J Endod. 2012;38(2):250-4.*

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ORIGINAL ARTICLE

Apical extrusion of debris produced by the heat treated single-file systems XP-Endo Shaper, Reciproc Blue and ProDesign Logic

ABSTRACT

Aim: *This study evaluated the apical extrusion of debris during root canal preparation produced by the single-file systems XP-Endo Shaper (FKG Dentaire, La Chaux-de-Fonds, Switzerland), Reciproc Blue (VDW GmbH, Munich, Germany), and ProDesign Logic (Easy Equipamentos Odontológicos, Belo Horizonte, MG, Brazil).*
Methodology: *Thirty single straight root canals in mandibular premolar were prepared and randomly assigned to 3 groups (n=10), according to instrument used for root canal preparation: XP-Endo Shaper, Reciproc Blue, or ProDesign Logic. Root canal shaping technique was performed by root segments and the apically extruded debris were collected and quantified using a debris collection setup with Eppendorf tubes and agar gel. Mean values were obtained by subtracting the initial weight of the test apparatus without specimens from its weight after root canal preparation. Then, one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test were applied for comparison of values. The statistical significance level was set at 5%.*
Results: *No significant difference was observed among groups (P>0.05), but all the instruments produced debris extrusion from the apical foramen.*
Conclusions: *The heat treated single-file systems XP-Endo Shaper, Reciproc Blue, and ProDesign Logic produce similar amounts of apically extruded debris during root canal preparation. All single-file systems evaluated promoted apical extrusion of debris.*

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Introduction

Regardless of working length adopted, root canal cleaning and shaping may force the contents from the intrarradicular space into the periapical region – such as dentinal debris, irrigating solutions, microorganisms and their by-products (1, 2). These irritants are capable to stimulate immunologic and/or infectious processes, what may originate clinical signs of inflammation, postoperative pain, delay in the process of periapical repair or flare-ups (1-4). The intensity of the acute inflammation after extrusion is defined by the volume of irritants in periapical tissues and the bacteria virulence (1, 5).

The production of extruded debris is associated with the preparation techniques and the endodontic files selected for root canal shaping as regards design and the type of instruments movement (1). Mechanized instrumentation can reduce extrusion compared to the use of manual instruments due to advances in design, such as radial lands, flute depth, different tapers and cross sections, and the use of different operational principles (1, 6). Also, it has been demonstrated that extruded debris are not influenced by the number of files (1, 6). In this sense, systems designed to prepare root canals with only 1 instrument have been advocated since they present efficiency and reduce working time (7). As for the use of reciprocating or rotary kinematics, it is known that both are capable of generating apical extrusion and expression of neuropeptides (1, 4).

Currently, heat treated nickel titanium (NiTi) single-file systems presenting improved properties can be found in either reciprocating [e.g., Reciproc (VDW GmbH, Munich, Germany), WaveOne (Dentsply Sirona Endodontics, Ballaigues, Switzerland)] or rotary [e.g., XP-Endo Shaper (FKG Dentaire, La Chaux-de-Fonds, Switzerland), ProDesign Logic (Easy Equipamentos Odontológicos, Belo Horizonte, MG, Brazil)] kinematics. However, scarce literature has evaluated the capacity of producing apically extruded debris by these newly developed systems, which is a relevant aspect for endodontic treatment. Furthermore, results of previous studies hamper a conclusion whether which single-file system reaches better extrusion standards (1, 4).

XP-Endo Shaper is a rotary file that presents a thermomechanically treated NiTi alloy named MaxWire (Martensite-Austenite-electropolish-fileX), providing shape memory effect and superelasticity (8). Moreover, Reciproc Blue (VDW GmbH) presents a blue thermal processing that changes its molecular structure yielding greater flexibility, resistance to cyclic fatigue, and the visible color blue to the file (9). XP-Endo Shaper has shown similar amounts of hard tissue debris extrusion in comparison to Reciproc (8), but superior results when compared to heat treated Reciproc Blue (9). In contrast, Reciproc Blue has demonstrated significantly less debris extruded than Reciproc (10).

In addition to that, the ProDesign Logic file, also known as Bassi Logic, presents a different hybrid design and controlled memory heat treatment. This system demonstrated some positive features in respect of general systems such as ProTaper Next (Dentsply Sirona Endodontics, York, PA, USA) and WaveOne Gold – adequate root canal and apical enlargement (11, 12), centered preparations (13), reduced percentage of debris and untouched surfaces in dentinal walls (11, 12), cyclic fatigue resistance (14), torsional strength (15), and fast preparation time (14, 16). Nevertheless, no study has quantified the amounts of extruded debris with the use of ProDesign Logic system. In this context, the aim of this study was to evaluate the apical extrusion of debris during root canal preparation produced by the single-file systems XP-Endo Shaper, Reciproc Blue, and ProDesign Logic. The tested null hypothesis was: there is no difference in the levels of apically extruded debris produced by the single-file systems evaluated.

Materials and Methods

Teeth selection and preparation

This study was approved by the University Ethics Committee (Protocol: 3.017.382).

Based on previous study (17), a α=0.05 and a margin error of 0.00136 was selected for sample size. As a result, a total of 30 human single-rooted mandibular premolars consisted the sample of this study. Firstly, all teeth were analyzed using a digital radiographic system (Micro Imagem, São Paulo, Brazil) in buccal and proximal directions. Only premolars presenting one isolated canal (Type I of Vertucci's Root Canal Configuration) (18), which should be straight (canal curvature angle ≤5°, calculated using the methodology of Schneider (19), were selected. Teeth with incomplete root formation, apical foramen greater than 0.15 mm or any evidence of previous endodontic treatment, dental caries, dental cracks, calcifications and resorptions were excluded. After selection, specimens were cleaned by periodontal curettes, immersed in 5% sodium hypochlorite solution during 1 hour, and then maintained in saline solution at room temperature until use.
All teeth were decoronated at a root length of 17 mm from the anatomic apex, using a high-speed diamond bur with copious irrigation. Under a dental operating microscope (DF Vasconcelos, Rio de Janeiro, Brazil) at 20× magnification, the apical patency was confirmed when a size 10 K-file (MANI, Inc, Utsunomiya, Tochigi, Japan) exceeded the apex, and the root length was verified when the instrument reached the apical foramen. Following this, the diameter of apical foramen was standardized to the size of a 15 K-file (Dentsply Maillefer, Ballaigues, Switzerland).

Debris collection setup and evaluation
This test apparatus was based on the work of Uslu et al. (9), with modifications. Stoppers were separated from the total of 30 sterile Eppendorf tubes and a hole was created in the center of stoppers by the aid of sterile high-speed diamond burs. This stage aimed to reach the hole diameters similar to the cervical region diameter of each specimen. After that, roots were covered tightly with a Teflon band, leaving solely the apical foramen exposed (9). Specimens were then fixed in the prepared stoppers with apex down, up to 12 mm from the anatomic apex, using cyanoacrylate adhesive (9) followed by gingival barrier material. This leaved 5 mm of more coronal root region in the external area of Eppendorfs. The remaining 12 mm roots inside Eppendorffs tubes were divided into 3 regions, with 4 mm each: cervical, middle and apical. This setup of tooth-stoppers was individually weighed three times by an electronic balance (Sartorius Analytical, G€ottingen, Germany) with an accuracy of ±0.00001 g. The mean weight of each one was calculated (W1).
Following this measure, 2 mL of 1.5% agar gel was injected into Eppendorf tubes and the setup of roots-stoppers were reconnected to Eppendorfs. Then, tubes were inverted to immerse specimens in agar for 24 h, obtaining gelation of the agar. The complete setup of specimens associated with Eppendorfs containing agar were newly weighed as previously described. The mean weight of each specimen was calculated (W2). A third measure, representing the weight of isolated Eppendorfs containing agar (W3), was calculated by subtracting W1 of the samples from W2 values (19). This calculation was performed so that the Eppendorf tubes were only opened after root canal preparation, avoiding an agar displacement that could influence the final weighing.
Eppendorf tubes were then positioned in a glass vial using a rubber dam to hold the apparatus and to prevent the operator from observing the root apex during endodontic therapy. After completion of root canal shaping, Eppendorfs were removed from the glass vials, and the configuration of roots-stoppers were removed from these tubes. Eppendorf tubes containing agar after root canal preparation were weighed as previously described (W4). Finally, the amount of extruded debris was calculated by subtracting the weight value of the initial Eppendorf tubes containing agar (W3) from the postpreparation weight value (W4). The mean of each setup was recorded (W5).

Root canal preparation
Root canal preparation was performed by a single operator. All files had single use.

All systems were powered by the torque-controlled Silver Reciproc endodontic motor (VDW GmbH), set at the designated function according to the used system.
The 30 specimens were randomly assigned to three groups, according to the single-file system used (n=10): XP-Endo Shaper (G1), Reciproc Blue (G2) and ProDesign Logic (G3). Root preparation was not operator blinded due to visible differences in the endodontic files evaluated. The working length (WL) was established in the total root length (17 mm). Root canal preparation was accomplished in all groups by root segments, starting with cervical region (WL=9 mm) and followed by middle (WL=13 mm) and apical (final WL=17 mm) thirds. During the use of all instruments, when a resistance requiring more apical pressure was detected, the file was removed and the flutes were cleaned with sterile gauzes soaked in alcohol. Patency was constantly confirmed using a #10 C-Pilot file (VDW GmbH) after finishing the use of single-files in each root segment. The protocol of each system, which followed manufacturers' instructions, is described in Table 1.
Each specimen was irrigated in endodontic therapy with a total volume of 8 mL of 0.9% sterile saline solution (SS), delivered using 27-G x 25 mm Endo-Eze irrigator tip (Ultradent products, South Jordan, UT, USA) by means of a peristaltic pump (LAP-101-3; MS Tecnopon, Piracicaba, SP, Brazil), using a flow rate of 5 mL/min. Before irrigation of root canals, the solution reservoir was preheated at 35 ºC. During preparation, 1 mL of SS was used before and after the insertion of each NiTi file. The irrigant was delivered in the predetermined WL for cervical and middle segments, but 2 mm short from the apical foramen (15 mm) in the apical region. Lastly, after finishing preparation of specimens, a final irrigation with 2 mL of SS was performed.

Statistical analysis
Preliminary analysis of data normality was performed with the Shapiro-Wilk test, showing that data were normally distributed. The one-way analysis of variance (ANOVA) followed by Bonferroni *post hoc* test were applied for comparison of values using SPSS 20.0 software (IBM Corp., Armonk, NY, USA). The statistical significance level was set at 5%.

Results

Mean, minimum and maximum values of the amount of apically extruded debris for each single-file system are presented in

Table 1
Manufacturer, features and preparation protocols according to groups of single-file systems evaluated

Groups	Manufacturer	File (tip.taper)	Preparation protocol
G1 XP-Endo Shaper	FKG Dentaire	30.01 initial taper; during use, this instrument expands to a minimum taper of 0.04	XP-Endo Shaper file was activated in continuous rotation kinematics at 800 rpm and 1 Ncm. Long and light up-and-down movements were applied inside root canals until reaching the intended WL in the root segment.
G2 Reciproc Blue	VDW GmbH	25.08	Reciproc Blue was activated in reciprocating kinematics. The file was gently inserted with an up-and-down pecking motion with a maximum amplitude of 3 mm until reaching the intended WL in the root segment.
G3 ProDesign Logic	Easy Equipamentos Odontológicos	25.06	ProDesign Logic file 25.06 was activated in continuous rotation kinematics at 950 rpm and 4 Ncm. The file was applied with gentle pecking motion and gentle apical pressure to advance the instrument until reaching the intended WL in the root segment.

Table 2
Amount of apically extruded debris (g) according to single-file groups

Groups	Mean (standard deviation)	Minimum	Maximum	P value
XP-Endo Shaper	0.0104 (0.0184)	0.0059	0.0087	0.787*
Reciproc Blue	0.0059 (0.0087)	0.0088	0.0153	
ProDesign Logic	0.0088 (0.0153)	0.0104	0.0184	

No significant difference was observed among groups.

Table 2. No significant difference was observed among groups (P>0.05), but all the instruments produced debris extrusion from the apical foramen.

Discussion

Several single-file systems are routinely used. As avoiding or decreasing the apically extruded debris from the apex is a relevant aspect for endodontic success, the evaluation of files performance is an important factor to be undertaken. This study quantified the apically extruded debris promoted by three heat treated single-file systems.

Formerly, aiming to simulate the resistance of periapical tissues in debris collection setups, a piece of flower arrangement foam was attached to roots specimens (20, 21). However, this may lead to misinterpretation of results since this product may absorb debris and irrigants while acting as an apical barrier (21). For this reason, empty Eppendorf tubes have also been used in debris collection evaluations (22); but the absence of back-pressure, representing no periapical resistance, does not reflect clinical conditions. At the present study, 1.5% agar gel was used because it does not interfere in debris collection and it presents similar values of density to periapical tissues (17). As a result, covering roots with agar gel created a matrix to satisfactory collect the extruded material and also offered a slight resistance to apical extrusion in a more realistic form (9).

Another methodology point that should be addressed is the root canal irrigation. In the current study, solely sterile saline solution was used. The use of chemical solutions was avoided because the creation of residues could have caused alterations in the results. For instance, sodium crystals formed with the evaporation of sodium hypochlorite could be attached to the hard tissues debris and lead to erroneous values of extrusion (9, 23). Moreover, a peristaltic pump was used to standardize the total volume and flow rate of saline solution, ensuring equal irrigating conditions for all groups and preventing biases. Although it was not used in the present study, it should also be pointed that supplementary irrigation protocols influence the total removal of accumulated hard tissue debris (24, 25) and, as a result, possibly in debris extrusion (23).

As regards root canal preparation technique, it is important to emphasize that coronal flaring prior to root canal shaping was accomplished. It has been demonstrated that this procedure is a relevant step that allows superior irrigation and cleaning of root canals (26), besides reducing significantly the production of dentin defects (27), and the amounts of extruded debris (21, 27). Therefore, the segmented canal preparation technique may have permitted higher debris removal from intracanal space and thus low debris extrusion for all files tested

According to data obtained, regardless of differences in files design and movements, all single-file systems presented some degree of debris extrusion from the apex. This consists with the overall findings of previous studies, in which no instrument was capable of rendering root canal therapy completely free of debris extrusion (6, 17, 21, 27). XP-Endo Shaper is a rotary snake-shaped file that presents a triangular Booster Tip (8). This instrument is capable to adapt to the morphology of the root canal system, since it significantly expands its initial taper at 37 °C (9). Reciproc Blue file presents a S-shaped cross-section that cuts dentinal walls counterclockwise, allowing deeper cutting and also favoring the removal of dentin chips (9). Finally, ProDesign Logic file also presents a S-shaped cross-section, but it is used in centric rotary motion, what the manufacturer states to reduces the screw-in effect, and has variable helical angles with two cutting edges (11, 14).

Statistical analysis did not reveal a significant difference between the evaluated instruments. Therefore, the null hypothesis was accepted. The design and the type of files movement did not interfere in the results of the present study. Previous literature presents inconsistent finding regarding the performance of endodontic instruments and thus the best approach in debris extrusion (4, 21). This results from a wide range of testing conditions and methodologies in *in vitro* studies to compare the systems (4). For this reason, it seems that the choice of endodontic systems should be mainly based in further relevant features such as root canal shaping, dentinal defects, cyclic fatigue and torsional failure.

Most studies evaluating apical extrusion of debris perform root canal treatment at room temperature (1, 4, 9). However, Uslu et al. (9) attempted to the intrarradicular temperature, which is approximately 35°C (28), and emphasized the importance of simulating these conditions; mainly considering that heat treated NiTi files can be affected by the ambient temperature. For instance, XP-Endo Shaper maintains its martensite phase at room temperature (8). Nevertheless, as a result of its MaxWire alloy, at body temperature this instrument convert to austenite phase and expands its structure (8, 9). Therefore, it is clear that analysis of apical extrusion of debris should mimic clinical conditions to achieve reliable results.

Scarce literature compared the extruded debris promoted by the single-files evaluated in the present study. Even though this study was based on the methodology of Uslu et al. (9), contrasting results were find since the authors observed less debris values from XP-Endo Shaper compared to Reciproc Blue. In this study, the intrarradicular temperature of 35 °C was also simulated. However, this temperature was obtained by heating the reservoir of irrigating solution before its application through the peristaltic pump. This is a limitation of the present study since temperature of solutions rapidly decrease inside intrarradicular space to reach equilibrium in the apical part of the root canal (28). In the study of Uslu et al. (9), the test apparatus was placed inside a glass bottle and immersed into 35 °C water to mimic intracanal temperature. Therefore, the thermodynamic behaviors of irrigating solutions within root canals may explain the difference in results of the present study compared to that of Uslu et al. (9).

Additionally, in the present study, total volume and flux of solution was controlled by a peristaltic pulp. This could have also influenced the results.

It is relevant to point out that the results of an *in vitro* study should be interpreted with caution. Even with best efforts, it is not possible to obtain identical clinical conditions. Moreover, as regards apical extrusion, bacterial virulence and host immune defenses are as important as the amount of extruded debris (29).

Conclusions

The heat treated single-file systems XP-Endo Shaper, Reciproc Blue, and ProDesign Logic produce similar amounts of apically extruded debris during root canal preparation. All single-file systems evaluated promoted apical extrusion of debris.

Clinical Relevance

This study compared for the first time the apical extrusion of debris produced by the heat treated single-fyle systems XP-Endo Shaper, Reciproc Blue, and ProDesign Logic. Despite considerable differences in design and kinematics, the evaluated instruments presented similar results. Moreover, all NiTi single-file systems evaluated promoted apical extrusion of debris.

Conflict of Interest

The authors deny any conflicts of interest.

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References

1. Caviedes-Bucheli J, Castellanos F, Vasquez N et al. The influence of two reciprocating single-file and two rotary-file systems on the apical extrusion of debris and its biological relationship with symptomatic apical periodontitis. A systematic review and meta-analysis. Int Endod J 2001;49:255-70.
2. Gunes B, Yesildal Yeter K. Effects of Different Glide Path Files on Apical Debris Extrusion in Curved Root Canals. J Endod 2018;44:1191-4.
3. De-Deus G, Brandão MC, Barino B et al. Assessment of apically extruded debris produced by the single-file ProTaper F2 technique under reciprocating movement. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;110:390-4.
4. Pedrinha VF, Brandão JMDS, Pessoa OF, Rodrigues PA. Influence of File Motion on Shaping, Apical Debris Extrusion and Dentinal Defects: A Critical Review. Open Dent J 2018;28:189-201.
5. Mittal R, Singla MG, Garg A, Dhawan A. A Comparison of Apical Bacterial Extrusion in Manual, ProTaper Rotary, and One Shape Rotary Instrumentation Techniques. J Endod 2015;41:2040-4.
6. Koçak S, Koçak MM, Sağlam BC et al. Apical extrusion of debris using self-adjusting file, reciprocating single-file, and 2 rotary instrumentation systems. J Endod 2013;39:1278-80.
7. Küçükyilmaz E, Savas S, Saygili G, Uysal B. Assessment of apically extruded debris and irrigant produced by different nickel-titanium instrument systems. Braz Oral Res 2015;29:1-6.
8. Alves FRF, Paiva PL, Marceliano-Alves MF et al. Bacteria and Hard Tissue Debris Extrusion and Intracanal Bacterial Reduction Promoted by XP-endo Shaper and Reciproc Instruments. J Endod 2018;44:1173-8.
9. Uslu G, Özyürek T, Yılmaz K et al. Apically Extruded Debris during Root Canal Instrumentation with Reciproc Blue, HyFlex EDM, and XP-endo Shaper Nickel-titanium Files. J Endod 2018;44:856-9.
10. Doğanay Yıldız E, Arslan H. The effect of blue thermal treatment on endodontic instruments and apical debris extrusion during retreatment procedures. Int Endod J 2019;52:1629-34.
11. Pinto JC, Pivoto-João MMB, Espir CG et al. Micro-CT evaluation of apical enlargement of molar root canals using rotary or reciprocating heat-treated NiTi instruments. J Appl Oral Sci 2019;27:e20180689.
12. Stringheta CP, Bueno CES, Kato AS et al. Micro-computed tomographic evaluation of the shaping ability of four instrumentation systems in curved root canals. Int Endod J 2019;52:908-16.
13. Pinheiro SR, Alcalde MP, Vivacqua-Gomes N et al. Evaluation of apical transportation and centring ability of five thermally treated NiTi rotary systems. Int Endod J 2018;51:705-13.
14. Menezes SEAC, Batista SM, Lira JOP, de Melo Monteiro GQ. Cyclic Fatigue Resistance of WaveOne Gold, ProDesign R and ProDesign Logic Files in Curved Canals In Vitro. Iran Endod J 2017;12:468-73.
15. Alcalde MP, Duarte MAH, Bramante CM et al. Torsional fatigue resistance of pathfinding instruments manufactured from several nickel-titanium alloys. Int Endod J 2018;51:697-704.
16. Coelho BS, Amaral RO, Leonardi DP et al. Performance of Three Single Instrument Systems in the Preparation of Long Oval Canals. Braz Dent J 2016;27:217-22.
17. Lu Y, Wang R, Zhang L et al. Apically extruded debris and irrigant with two Ni-Ti systems and hand files when removing root fillings: a laboratory study. Int Endod J 2013;46:1125-30.
18. Vertucci FJ. Root canal anatomy of the human permanent teeth. Oral Surg Oral Med Oral Pathol 1984;58:589-99.
19. Schneider SW. A comparison of canal preparations in straight and curved root canals. OralSurg Oral Med Oral Pathol 1971;32:271-5.
20. Hachmeister DR, Schindler WG, Walker WA 3rd, Thomas DD. The sealing ability and retention characteristics of mineral trioxide aggregate in a model of apexification. J Endod 2002;28:386-90.
21. Topçuoğlu HS, Üstün Y, Akpek F et al. Effect of coronal flaring on apical extrusion of debris during root canal instrumentation using single-file systems. Int Endod J 2016;49:884-9.
22. Altundasar E, Nagas E, Uyanik O, Serper A. Debris and irrigant extrusion potential of 2 rotary systems and irrigation needles. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112:e31-5.
23. Tanalp J, Güngör T. Apical extrusion of debris: a literature review of an inherent occurrence during root canal treatment. Int Endod J 2014;47:211-21.
24. Chan R, Versiani MA, Friedman S et al. Efficacy of 3 Supplementary Irrigation Protocols in the Removal of Hard Tissue Debris from the Mesial Root Canal System of Mandibular Molars. J Endod 2019;45:923-9.
25. Barbosa-Ribeiro M, Arruda-Vasconcelos R, Fabretti FL et al. Evaluation of Apically Extruded Debris Using Positive and Negative Pressure Irrigation Systems in Association with Different Irrigants. Braz Dent J 2018;29:184-8.
26. Vanni JR, Santos R, Limongi O et al. Influence of cervical preflaring on determination of apical file size in maxillary molars: SEM analysis. Braz Dent J 2005;16:181-6.
27. Borges ÁH, Damião MS, Pereira TM et al. Influence of Cervical Preflaring on the Incidence of Root Dentin Defects. J Endod 2018;44:286-91.
28. de Hemptinne F, Slaus G, Vandendael M et al. In Vivo Intracanal Temperature Evolution During Endodontic Treatment After the Injection of Room Temperature or Preheated Sodium Hypochlorite. J Endod 2015;41:1112-5.
29. Elmsallati EA, Wadachi R, Suda H. Extrusion of debris after use of rotary nickel-titanium files with different pitch: a pilot study. Aust Endod J 2009; 35:65-9.

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ORIGINAL ARTICLE

Effect of a root dentin deproteinization protocol on self-adhesive cementation of fiber posts

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ABSTRACT

Aim: *This study evaluated the effect of a root dentin deproteinization protocol on the bond strength of fiber post cemented with self-adhesive resin cement.*
Materials and Methods: *Twenty-four single-rooted teeth were randomly divided into two groups (n=12), as follows: G1 - cementation with self-adhesive resin cement RelyX U200 (3M/ESPE) according to manufacturer's instructions; G2 - treatment of root dentin with phosphoric acid at 37% for 15 s followed by sodium hypochlorite (NaOCl) at 5% for 5 minutes, and post cementation according to manufacturer's instructions. A light-emitting diode (LED) unit was used for photoactivation and after 24h at 37 °C in absolute humidity, teeth were sectioned into slices of 1 mm divided by different root thirds (cervical, middle and apical) and submitted to the push-out bond strength test. Two-criteria ANOVA was used to determine the statistical significance between groups ($p<0.05$).*
Results: *No statistical difference in bond strength between group cemented as manufacturer's instructions and group submitted to deproteinization was detected ($p>0.05$). As well as there was no statistical difference between the different root thirds evaluated ($p>0.05$).*
Conclusions: *The deproteinization protocol proposed was not able to improve the bond strength of self-adhesive cement to root dentin.*

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KEYWORDS *dental cements, deproteinization, post technique, root canal, collagen*

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Introduction

Endodontically treated teeth often have little remaining of coronal structure and require intraradicular retainers prior to restoration (1, 2). Nowadays, there is a variety of intraradicular posts and cements for the rehabilitation of these teeth (3). Fiber posts have been widely used to restore endodontically treated teeth when the dental remnant cannot provide adequate support and retention for restoration, and are presented as an alternative to cast metal post and core (3), since their elasticity modulus are similar to dentine, as well as they have good aesthetic properties and absence of corrosion (4, 5). However, the lack of intimate adaptation between fiber post and the inner walls of the root canal requires an adhesive cementation for greater retention (6). Conventional or self-adhesive resin cements are indicated as the material of choice for performing post cementation, particularly because they provide good aesthetics and good mechanical strength, besides low solubility and good bonding to the root dentin (7).

Self-adhesive cements were introduced in order to simplify the adhesive technique, so they are used in a single application and theoretically do not require pretreatment of dental surfaces, as there is a chemical reaction between acidic phosphate methacrylates and dental hydroxyapatite (8, 9). However, they have limited infiltration in dental tissues and some studies have shown low bond strength to dentin (10, 11). Thus, it is a subject that still generates some discussions, mainly in relation to the adhesion between fiber post and root dentin, mainly because of the difficulty in removing dentin smear layer, drying the excess moisture from root canal dentin, in addition to the complete removal of gutta-percha and endodontic cement of the root canal walls after endodontic treatment (12).

The mechanical preparation of the root canal generates a smear layer that is deposited on the dentin walls and which consists mainly of mineral and organic residues. The presence of this residual layer impairs the adequate contact between root dentin and adhesive/cement during the cementation process, thus interfering in bond strength to dentin. In this way, partial or total removal of the smear layer prior to cementation may improve post retention (4). Studies have proposed root dentin pre-treatment with sodium hypochlorite (NaOCl) to remove the smear layer and improve the bond strength between resin cement and dentin (13-15). However, it is unclear whether this beneficial effect would occur for self-adhesive resin cements (4). NaOCl is a common antibacterial irrigant used in chemical-mechanical preparation of the root canals, on the other hand it is a potent antioxidant agent that can create an oxygen-rich layer on the dentin wall inhibiting the resin-based cement polymerization, thus reducing the bond strength between cement and root dentin (16). However, according to Sung et al. (17) independent of the irrigating agent used, the bond strength of a composite to dentin is much more dependent on the bonding agent used. In addition, a systematic review showed that the type of cement used strongly influences the retention of the fiber posts in the root canals, however there are other variables that also influence, such as cement application protocol, irrigation product and protocol, sample storage conditions, among others (18). Some studies have evaluated deproteinization protocols with different irrigants and bonding agents in an attempt to improve the bond strength between fiber posts and root dentin (5, 6, 19, 20), however the results were quite variable and dependent on the methodologies used in each study.

Thus, the aim of this study was to evaluate the effect of a root dentin deproteinization protocol in the bond strength of a fiber post cemented with a self-adhesive resin cement. In addition, the influence of the root thirds (cervical, middle and apical) in bond strength and the pattern of fracture of the specimens were assessed. The null hypothesis tested was that the deproteinization protocol is not capable of producing a higher bond strength when compared to conventional cementation protocol. As well as there is no difference between the bond strength obtained in the different root thirds evaluated.

Materials and Methods

This study was approved by the Institutional Research Ethical Committee, protocol number (PT/0439/11). Twenty-four single-rooted human teeth extracted for periodontal or orthodontic therapeutic reasons were selected. For inclusion, teeth should be free of coronal or root caries, absence of endodontic treatment, root length of at least 14 mm and straight single root with a single canal.

Preparation of the roots

The teeth were cleaned of soft tissue and calculus and sectioned below the enamel-cement junction perpendicular to the long axis with the aid of a diamond blade under water cooling. After, the teeth were stored in 0.1% thymol solution at 4 °C for no longer than two months. The coronal access was made with a K-file #15 (Dentsply Maillefer, Ballaigues, Switzerland) placed into the canal until it was visible at the apical foramen. Then, the working length was set 1 mm shorter than this length. K-files #15 to 40 were used for the preparation of 1 mm from the apical foramen opening. The canals were irrigated between instrumentation with 2 mL of NaOCl at 5% and 2 mL of EDTA at 17% and finally irrigated with 5 mL of distilled water and dried with paper points. Finally, in the obturation process, the single cone technique was used (Odous De Deus, Belo Horizonte, Brazil) together with the AH Plus™ endodontic cement (Dentsply DeTrey GmbH, Konstanz, Germany). The gutta-percha was cut with heated Paiva plugger and vertical condensation technique was performed at the entrance of the channel. After seven days, the root canal enlargement was performed with a Largo drill system (1, 2 and 3 respectively) at a distance of 4 mm from the apical foramen. After, the low speed drill provided by the manufacturer was used, under constant irrigation with distilled water. Then, the root canals were again gently dried with paper points and randomly divided into two groups.

Cementation protocols

Twelve specimens were assigned to group 1 (G1), where fiber posts were cemented according to the manufacturer's instructions, which consists of abundant irrigation of the root canal with distilled water and drying with absorbent paper cones. In group 2 (G2; n=12), fiber posts were cemented after deproteinization of the root dentin. Phosphoric acid at 35% was applied inside the root canal for 15 s and washed thoroughly with distilled water for 30 s. The root canal was dried with absorbent paper cones and flooded with NaOCl at 5% for 5 minutes. After, the root canal was again irrigated with 5 mL of distilled water, and again dried with absorbent paper cones.

Before the cementation protocol of the two groups each post was cleaned with alcohol, dried and covered with a silane layer (RelyX Ceramic Primer, 3M ESPE Dental Products, St Paul, MN, USA). The self-adhesive resin cement RelyX U200 (3M ESPE, St Paul, MN, USA) was taken to the interior of the root canal through the applicator and then inserted the fiber post. The excess of self-adhesive resin cement was removed and then light-activated using a light-emitting diode (LED) unit (irradiance 900 mW/cm^2 [Radii-cal LED; SDI, São Paulo, SP, Brazil]) through the post for 40s. The irradiance was measured with a digital power meter (Ophir Optronics, Danvers, MA, USA). The roots were stored at 37 ºC and 100% of humidity for 24h until the push-out bond strength test. All roots were prepared and restored by a single trained operator and following the manufacturers recommendations.

Push-out bond strength analysis

After 24h the roots were positioned and fixed in a block of acrylic resin and sectioned transversely. Twelve slices were made on each root generating 6 specimens of 1 mm per root with a diamond steel disc in a cutting machine (Isomet 2000, Bue-

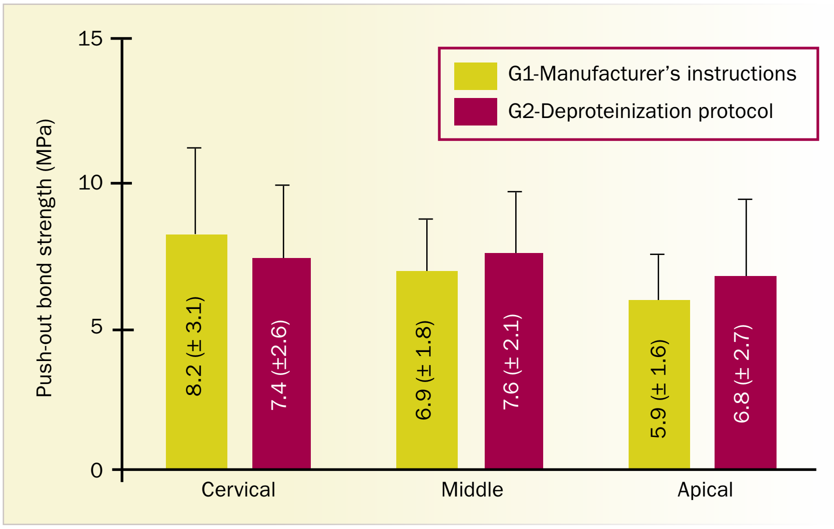

Figure 1.
Push-out bond strength (MPa) of the different groups tested (with or without root dentin deproteinization protocol prior to the application of self-adhesive resin cement RelyX U200) and the different root thirds evaluated. There was no statistical difference between the treatments proposed, as well as there was no difference between the root thirds evaluated.

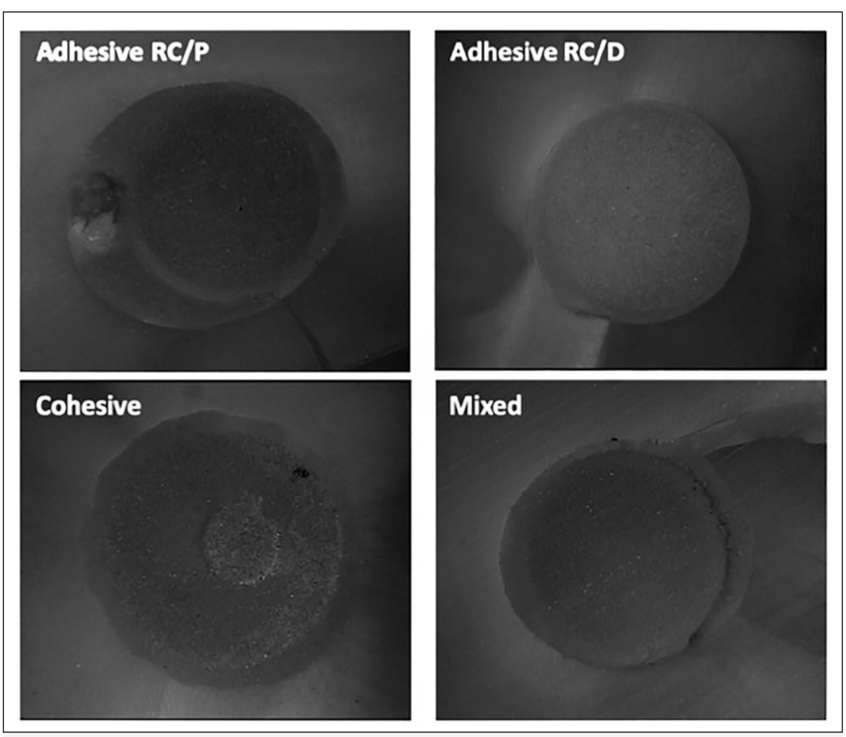

Figure 2.
Representative images of the different types of failures assessed under light optical microscope (40x).

hler, Lake Bluff, IL, USA) under water-cooling at 200 rpm. The first cut was made at 1 mm from the enamel-cement junction obtaining 6 slices per teeth respectively, and two specimens for root region: cervical, middle and apical. Each sample was placed in a metallic matrix with a central opening larger than the diameter of the post, with the coronary portion down, which was applied a cylindrical tip 0.6 mm in diameter connected to a universal testing machine (EMIC, São José dos Pinhais, PR, Brazil) at 1.0 mm/min. A digital caliper was used to measure the thickness of the slices (h), the largest (R) and smallest radius (r) of the dentin/post interface on each specimen. Then, the bonding surface was calculated using the formula of a frustoconical:

$$A = \pi (R+r) \sqrt{(R - r)^2 + h^2}$$

The bond strength (σ) was finally obtained by the formula σ=C/A, where C=load for rupture of the specimen (kgf) and A=interfacial area (mm^2).

Analysis of the failure mode
All debonded specimens were evaluated with a light optical microscope at a magnification of 40x. Two independent operators evaluated and classified the failure modes into four categories as follows: A) adhesive failure between fiber post and resin cement; B) adhesive failure between resin cement and root dentin, C) cohesive fracture of the fiber post and D) mixed failure (when more than one classification occurred in the same specimen).

Statistical analysis
The data of push-out bond strength (MPa) were analyzed using the GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). The statistical analysis was performed by two-way ANOVA (cementation protocol and root region). The level of significance was set at $p<0.05$. Data regarding failure mode was presented descriptively, with frequency distribution.

Results

Push-out bond strength test
The results indicated that the differences in values of push-out bond strength between G1 (RelyX U200 applied according to the manufacturer's instructions) and G2

Table 1
Distribution of type of failure occurred in each group in percentage

Type of failure	G1	G2
Adhesive RC/P*	5.6	0
Adhesive RC/D**	61.1	52.8
Cohesive***	0	2.8
Mixed****	33.3	44.4

**RC/P Adhesive failure between resin cement and fiber post.*
***RC/D Adhesive failure between resin cement and root dentin.*
****Cohesive failure of the post or root dentin.*
*****Mixed failure when more than one classification appeared in the same specimen.*

(deproteinization protocol) were not statistically significant. Also, no significant statistical difference was detected between push-out bond strength values for the different root thirds evaluated (Figure 1).

Failure pattern

Thirty-six specimens were evaluated for each group in relation to failure pattern, and a similar behavior was observed for both groups (Table 1). All debonded specimens were evaluated using a light optical microscope at 40x magnification. The adhesive failure pattern between cement and dentin was predominant in the two groups tested, followed by mixed fractures. Only two specimens presented adhesive fracture between cement and post and one specimen presented cohesive fracture in the post (Figure 2).

Discussion

In the present study, the application of phosphoric acid at 35% followed by NaOCl at 5% was used for remove the smear layer and to demineralize the root dentin, followed by the deproteinization of the collagen, respectively. Theoretically the deproteinization would promote a greater contact between the resin cement and the inorganic portion of the dentin, which could improve adhesion. However, the results demonstrated that dentin deproteinization did not produce any benefit to the bond strength. Similarly in a study using RelyX U100 self-adhesive resin cement, dentin deproteinization did not improve adhesion performance either (6).

Studies show that the bond strength values of self-adhesive cements are similar to the bond strength values of conventional cements (21, 22), however, for this to occur, the root canal must be previously irrigated and cleaned to remove the smear layer and debris after removing gutta percha and endodontic cement (23). However, some studies have shown that the different irrigation systems, whether activated or not, are not able to completely remove the root canal sealer remnants (24, 25), and interfere with the bond strength (26).

Some studies have demonstrated the limited ability of self-adhesive resin cements to demineralize and dissolve the smear layer to reach the underlying dentin (9, 27, 28). The high viscosity of this type of cement limits its capacity of demineralization (29). Since the adhesion of the self-adhesive cement occurs through the intimate contact between cement and dentin by the micromechanical retention and chemical bond, the dentin smear layer, together with the other debris, must be removed. And for this to occur satisfactorily, the irrigation protocol is essential (20). Several studies have already tested different irrigants and their properties in relation to adhesion with the dental structure, such as EDTA and citric acid (30), 2% chlorhexidine (20, 31), sodium hypochlorite and calcium hypochlorite (20), however the most widely used irrigants, which are EDTA and NaOCl, showed unsatisfactory results and may negatively affect the results of the endodontic treatments (30). Thus, there is a need for the development of new irrigators with bacterial action and that do not interfere in the bond strength. On the other hand, NaOCl is still the most tested irrigator as a deproteinizing agent, as it promotes changes in the structure of dentin, mainly in the protein content of dentin (32).

In one study, the authors concluded that the use of NaOCl as a pretreatment for root dentin also does not interfere in the immediate adhesion of fiber posts. However, the evaluation after 12 months showed that the bond strength values obtained with the NaOCl treatment were higher than those obtained with the conventional technique. On the other hand, the effect of coronal dentin deproteinization on the bond strength has also been studied and the results showed a reduction, or no beneficial effect in adhesion, independently of the adhesive system used (33-35). Perdigao et al. (36) demonstrated with transmission electron microscopy (TEM) an incomplete removal of the collagen fibers along the resin-dentin interface and in the walls of the dentinal tubules after treatment with NaOCl at 10% for 60s. Other authors, who also used TEM, reported that the use of NaOCl at 5% for 120s did not completely remove the collagen fibrils from dentin (37). Sauro et al. (38) reported that the application of NaOCl at 12% even for 10 minutes was not able to completely remove the collagen from the porous surface of the intra and intertubular dentin walls. Deproteinization with NaOCl is a recognized treatment for removing the organic matrix, leaving a clean and mineralized surface (5, 38). However, in some studies, NaOCl has been shown to be harmful when used as an irrigant (39), or as deproteinizing agent (6), this can occur because this effect on dentin is not always homogeneous. Thus, the concentrations and time of application play an important role in the process (38). In addition, NaOCl dissociates in sodium and oxygen and this can interfere with the polymerization of the cement, leading to reduced adhesion (40). However, a long-term *in vitro* study concluded that the use of NaOCl as a deproteinizer minimized the degradation of the conventional and self-adhesive cement interface (32). Nevertheless, Seballos et al. (20) concluded that NaOCl and CaOCl should not be used as deproteinizing agent, as they negatively affected the bond strength between fiber posts cemented with self-adhesive resin cement and root dentin. Thus, our findings could be better interpreted with the aid of complementary techniques to morphologically evaluate the deproteinization of the root dentin.

Regarding the adhesion at the different root thirds, no significant differences were found between the thirds in the two groups evaluated in this study. Other research has shown that the RelyX U100 self-adhesive cement showed the same performance in the three different root thirds evaluated (apical, middle and cervical), producing an uniform bond strength throughout the root (6). One hypotheses has been proposed to explain this, some authors suggest that this may occur due to the good adaptation promoted between the fiber post and the root canal after the introduction of the customized drilling systems by the manufacturers (41). This factor would cause a decrease in the thickness of the resin cement, which could consequently reduce the polymerization shrinkage as well as the polymerization stress (42).

Many methods have been used to measure the bond strength between endodontic posts and tooth structure (43). In the present study, the push-out test was used because it has the benefit of simulating clinical conditions as closely as possible. In our study, a predominance of adhesive failure between cement and dentin was observed, followed by the mixed failure type. No difference was observed between the fracture pattern of the groups cemented with or without deproteinization. Similar studies also demonstrate that the most prevalent failure type was adhesive between cement and dentine, followed by mixed failure (5, 44). As this is an *in vitro* study, the present study has the characteristic limitations of these methodologies. The attempt to increase the contact between the cement and the mineralized dentin tissue, by the total or partial removal of the collagen, was not able to produce better results of bond strength. Another limitation of the present study is that the surface of the root dentin has not been morphologically evaluated to observe the effects caused by NaOCl.

Conclusions

There was no difference in the performance of the self-adhesive resin cement tested when applied with or without root dentin deproteinization protocol proposed. Thus, despite not impairing adhesion, the proposed deproteinization protocol would only represent the addition of a clinical cementation step, which may increase technique sensitivity and chair time.

Clinical Relevance

Deproteinization protocol would only represent the addition of a clinical cementation step, which may increase technique sensitivity and chair time

Conflict of Interest

None.

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References

1. Maroulakos G, He J, Nagy WW. The Post–endodontic Adhesive Interface: Theoretical Perspectives and Potential Flaws [Internet]. Vol. 44, Journal of Endodontics. Elsevier Inc.; 2018 [cited 2020 Aug 2]. p. 363-71. Available from: https://pubmed.ncbi.nlm.nih.gov/29306531/.
2. Conde MCM, Chisini LA, Sarkis-Onofre R, Schuch HS, Nör JE, Demarco FF. A scoping review of root canal revascularization: relevant aspects for clinical success and tissue formation [Internet]. Vol. 50, International Endodontic Journal. Blackwell Publishing Ltd; 2017 [cited 2020 Aug 2]. p. 860-74. Available from: https://pubmed.ncbi.nlm.nih.gov/27770435/.
3. Naumann M, Koelpin M, Beuer F, Meyer-Lueckel H. 10-year survival evaluation for glass-fiber-supported postendodontic restoration: a prospective observational clinical study. J Endod [Internet]. 2012 Apr [cited 2018 Mar 5];38(4):432-5. Available from: http://linkinghub.elsevier.com/retrieve/pii/S009923991200057X.
4. Faria-e-Silva AL, Menezes M de S, Silva FP, Reis GR dos, Moraes RR de. Intra-radicular dentin treatments and retention of fiber posts with self-adhesive resin cements. Braz Oral Res [Internet]. [cited 2018 Mar 5];27(1):14-9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23306622.
5. Cecchin D, Almeida JFA, Gomes BPFA, Zaia AA, Ferraz CCR. Deproteinization technique stabilizes the adhesion of the fiberglass post relined with resin composite to root canal. J Biomed Mater Res B Appl Biomater [Internet]. 2012 Feb [cited 2018 Mar 5];100(2):577-83. Available from: http://doi.wiley.com/10.1002/jbm.b.31946.
6. Lisboa DS, Santos SV dos, Griza S, Rodrigues JL, Faria-e-Silva AL. Dentin deproteinization effect on bond strength of self-adhesive resin cements. Braz Oral Res [Internet]. [cited 2018 Mar 5];27(1):73-5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23306629.
7. Barbosa De Souza F, Sinclér Delfino C, Lacalle Turbino M, Braz R. Deproteinized dentin: a favorable substrate to self-bonding resin cements? J Biomed Mater Res B Appl Biomater [Internet]. 2011 Aug [cited 2018 Mar 5];98(2):387-94. Available from: http://doi.wiley.com/10.1002/jbm.b.31863.
8. Gerth HUV, Dammaschke T, Züchner H, Schäfer E. Chemical analysis and bonding reaction of RelyX Unicem and Bifix composites-A comparative study. Dent Mater [Internet]. 2006 Oct [cited 2020 Jul 29];22(10):934-41. Available from: https://pubmed.ncbi.nlm.nih.gov/16364427/.
9. Al-Assaf K, Chakmakchi M, Palaghias G, Karanika-Kouma A, Eliades G. Interfacial characteristics of adhesive luting resins and composites with dentine. Dent Mater [Internet]. 2007 Jul [cited 2018 Mar 13];23(7):829-39. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0109564106001837.
10. Monticelli F, Osorio R, Mazzitelli C, Ferrari M, Toledano M. Limited decalcification/diffusion of self-adhesive cements into dentin. J Dent Res [Internet]. 2008 Oct [cited 2020 Jul 29];87(10):974-9. Available from: https://pubmed.ncbi.nlm.nih.gov/18809754/.
11. Barcellos DC, Batista GR, Da Silva MA, Rangel PM, Torres CRG, Fava M. Evaluation of bond strength of self-adhesive cements to dentin with or without application of adhesive systems. J Adhes Dent [Internet]. 2011 [cited 2020 Jul 29];13(3):261-5. Available from: https://pubmed.ncbi.nlm.nih.gov/21734960/.
12. Skupien JA die, Sarkis-Onofre R, Cenci MS érgi, Moraes RR att. de, Pereira-Cenci T. A systematic review of factors associated with the retention of glass fiber posts [Internet]. Vol. 29, Brazilian oral research. Braz Oral Res; 2015 [cited 2020 Jul 31]. Available from: https://pubmed.ncbi.nlm.nih.gov/26083089/.
13. Pisani-Proença J, Erhardt MCG, Amaral R, Valandro LF, Bottino MA, Del Castillo-Salmerón R. Influence of different surface conditioning protocols on microtensile bond strength of self-adhesive resin cements to dentin. J Prosthet Dent [Internet]. 2011 Apr [cited 2018 Mar 5];105(4):227-35. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0022391311600371.
14. Santos MJMC, Bapoo H, Rizkalla AS, Santos GC. Effect of dentin-cleaning techniques on the shear bond strength of self-adhesive resin luting cement to dentin. Oper Dent [Internet]. 2011 Oct [cited 2018 Mar 5];36(5):512-20. Available from: http://www.jopdentonline.org/doi/10.2341/10-392-L.
15. Gu XH, Mao CY, Liang C, Wang HM, Kern M. Does endodontic post space irrigation affect smear layer removal and bonding effectiveness? Eur J Oral Sci [Internet]. 2009 Oct [cited 2020 Jul 29];117(5):597-603. Available from: https://pubmed.ncbi.nlm.nih.gov/19758258/.
16. Morris MD, Lee KW, Agee KA, Bouillaguet S, Pashley DH. Effects of sodium hypochlorite and RC-prep on bond strengths of resin cement to endodontic surfaces. J Endod [Internet]. 2001 Dec [cited 2018 Mar 28];27(12):753-7. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0099239905610345.

17. Sung EC, Tai ET, Chen T, Caputo AA. Effect of irrigation solutions on dentin bonding agents and restorative shear bond strength. J Prosthet Dent [Internet]. 2002 [cited 2020 Jul 28];87(6):628-32. Available from: https://pubmed.ncbi.nlm.nih.gov/12131885/.
18. Sarkis-Onofre R, Skupien JA, Cenci MS, Moraes RR, Pereira-Cenci T. The role of resin cement on bond strength of glass-fiber posts luted into root canals: a systematic review and meta-analysis of in vitro studies. Oper Dent [Internet]. 2014 Jan [cited 2018 Mar 6];39(1):E31-44. Available from: http://www.jopdentonline.org/doi/10.2341/13-070-LIT.
19. Abo T, Asmussen E, Uno S, Tagami J. Short- and long-term in vitro study of the bonding of eight commercial adhesives to normal and deproteinized dentin. Acta Odontol Scand [Internet]. 2006 Aug 2 [cited 2018 Mar 5];64(4):237-43. Available from: http://www.tandfonline.com/doi/full/10.1080/00016350600633177.
20. Seballos VG, Barreto MS, da Rosa RA, Machado E, Valandro LF, Kaizer OB. Effect of post-space irrigation with NaOCL and CaOCL at different concentrations on the bond strength of posts cemented with a self-adhesive resin cement. Braz Dent J [Internet]. 2018 Sep 1 [cited 2020 Jul 31];29(5):446-51. Available from: https://pubmed.ncbi.nlm.nih.gov/30517442/.
21. Daleprane B, Pereira CNB, Bueno AC, Ferreira RC, Moreira AN, Magalhães CS. Bond strength of fiber posts to the root canal: Effects of anatomic root levels and resin cements. J Prosthet Dent [Internet]. 2016 Sep 1 [cited 2020 Jul 31];116(3):416-24. Available from: https://pubmed.ncbi.nlm.nih.gov/27086107/.
22. Pedreira APRDV, D'Alpino PHP, Pereira PNR, Chaves SB, Wang L, Hilgert L, et al. Effects of the application techniques of self-adhesive resin cements on the interfacial integrity and bond strength of fiber posts to dentin. J Appl Oral Sci [Internet]. 2016 Sep 1 [cited 2020 Jul 31];24(5):437-46. Available from: https://pubmed.ncbi.nlm.nih.gov/27812613/.
23. Barreto MS, Rosa RA, Seballos VG, Machado E, Valandro LF, Kaizer OB, et al. Effect of intracanal irrigants on bond strength of fiber posts cemented with a self-Adhesive resin cement. Oper Dent [Internet]. 2016 Nov 1 [cited 2020 Jul 31];41(6):e159-67. Available from: https://pubmed.ncbi.nlm.nih.gov/27603176/.
24. Grischke J, Müller-Heine A, Hülsmann M. The effect of four different irrigation systems in the removal of a root canal sealer. Clin Oral Investig [Internet]. 2014 Sep 1 [cited 2020 Jul 28];18(7):1845-51. Available from: https://pubmed.ncbi.nlm.nih.gov/24317958/.
25. Prati C, Zamparini F, Spinelli A, Pelliccioni GA, Pirani C, Gandolfi MG. Secondary Root Canal Treatment with Reciproc Blue and K-File: Radiographic and ESEM-EDX Analysis of Dentin and Root Canal Filling Remnants. J Clin Med [Internet]. 2020 Jun 18 [cited 2020 Jul 28];9(6):1902. Available from: /pmc/articles/PMC7355850/?report=abstract.
26. Saleh IM, Ruyter IE, Haapasalo MP, Ørstavik D. Adhesion of endodontic sealers: Scanning electron microscopy and energy dispersive spectroscopy. J Endod [Internet]. 2003 [cited 2020 July 28];29(9):595-601. Available from: https://pubmed.ncbi.nlm.nih.gov/14503835/.
27. Kenshima S, Reis A, Uceda-Gomez N, Tancredo L de LF, Filho LER, Nogueira FN, et al. Effect of smear layer thickness and pH of self-etching adhesive systems on the bond strength and gap formation to dentin. J Adhes Dent [Internet]. 2005 [cited 2018 Mar 13];7(2):117-26. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16052760.
28. Bitter K, Maletic A, Neumann K, Breschi L, Sterzenbach G, Taschner M. Adhesive durability inside the root canal using self-adhesive resin cements from luting fiber posts. Oper Dent [Internet]. 2017 Nov 1 [cited 2020 Aug 2];42(6):E167-76. Available from: https://pubmed.ncbi.nlm.nih.gov/28976849/.
29. Han L, Okamoto A, Fukushima M, Okiji T. Evaluation of physical properties and surface degradation of self-adhesive resin cements. Dent Mater J [Internet]. 2007 Nov [cited 2018 Mar 15];26(6):906-14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18203498.
30. Gandolfi MG, Taddei P, Pondrelli A, Zamparini F, Prati C, Spagnuolo G. Demineralization, collagen modification and remineralization degree of human dentin after EDTA and citric acid treatments. Materials (Basel) [Internet]. 2019 Dec 21 [cited 2020 Jul 28];12(1). Available from: https://pubmed.ncbi.nlm.nih.gov/30577625/.
31. Leitune VCB, Collares FM, Werner Samuel SM. Influence of chlorhexidine application at longitudinal push-out bond strength of fiber posts. Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology [Internet]. 2010 Nov 6 [cited 2020 Jul 31];110(5):e77-81. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1079210410002994.
32. Siqueira FSF, Cardenas AFM, Gomes GM, Chibinski AC, Gomes OMM, Bandeca MC, et al. Three-Year effects of deproteinization on the in vitro durability of resin/dentin-eroded interfaces. Oper Dent. 2018 Jan 1;43(1):60-70.
33. Alshaikh KH, Hamama HHH, Mahmoud SH. Effect of smear layer deproteinization on bonding of self-etch adhesives to dentin: a systematic review and meta-analysis. Restor Dent Endod [Internet]. 2018 [cited 2020 Aug 2];43(2). Available from: https://pubmed.ncbi.nlm.nih.gov/29765895/.
34. Augusto MG, Torres CRG, Pucci CR, Schlueter N, Borges AB. Bond stability of a universal adhesive system to eroded/abraded dentin after deproteinization. Oper Dent [Internet]. 2018 May 1 [cited 2020 Aug 2];43(3):291-300. Available from: https://pubmed.ncbi.nlm.nih.gov/29676982/.
35. Torres CRG, Barcellos DC, Batista GR, Pucci CR, Antunes MJS, De La Cruz DB, et al. Five-year clinical performance of the dentine Deproteinization Technique in non-carious cervical lesions. J Dent [Internet]. 2014 [cited 2020 Aug 2];42(7):816-23. Available from: https://pubmed.ncbi.nlm.nih.gov/24739486/.
36. Perdigão J, Gomes G, Augusto V. The effect of dowel space on the bond strengths of fiber posts. J Prosthodont [Internet]. 2007 May [cited 2018 Mar 13];16(3):154-64. Available from: http://doi.wiley.com/10.1111/j.1532-849X.2006.00166.
37. Osorio R, Ceballos L, Tay F, Cabrerizo-Vilchez MA, Toledano M. Effect of sodium hypochlorite on den-

tin bonding with a polyalkenoic acid-containing adhesive system. J Biomed Mater Res [Internet]. 2002 May [cited 2018 Mar 28];60(2):316-24. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11857439.
38. Sauro S, Mannocci F, Tay FR, Pashley DH, Cook R, Carpenter GH, et al. Deproteinization Effects of NaOCl on Acid-etched Dentin in Clinically-relevant vs Prolonged Periods of Application. A Confocal and Environmental Scanning Electron Microscopy Study. Oper Dent [Internet]. 2009 Mar [cited 2018 Mar 5];34(2):166-73. Available from: http://www.jopdentonline.org/doi/10.2341/08-56.
39. Rocha AW, de Andrade CD, Leitune VCB, Collares FM, Samuel SMW, Grecca FS, et al. Influence of Endodontic Irrigants on Resin Sealer Bond Strength to Radicular Dentin. Bull Tokyo Dent Coll [Internet]. 2012 Jan 1 [cited 2020 Jul 30];53(1):1-7. Available from: http://jlc.jst.go.jp/JST.JSTAGE/tdcpublication/53.1?lang=en&from=CrossRef&type=abstract.
40. Souza SFC, Bombana AC, Francci C, Gonçalves F, Castellan C, Braga RR. Polymerization stress, flow and dentine bond strength of two resin-based root canal sealers. Int Endod J [Internet]. 2009 Oct 1 [cited 2020 Jul 30];42(10):867-73. Available from: http://doi.wiley.com/10.1111/j.1365- 2591.2009.01581.
41. Gomes GM, Gomes OMM, Reis A, Gomes JC, Loguercio AD, Calixto AL. Regional bond strengths to root canal dentin of fiber posts luted with three cementation systems. Braz Dent J [Internet]. 2011 [cited 2018 Mar 13];22(6):460-7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22189640.
42. Giachetti L, Scaminaci Russo D, Baldini M, Bertini F, Steier L, Ferrari M. Push-out strength of translucent fibre posts cemented using a dual-curing technique or a light-curing self-adhering material. Int Endod J [Internet]. 2012 Mar [cited 2018 Mar 13];45(3):249-56. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22007679.
43. Brichko J, Burrow MF, Parashos P. Design Variability of the Push-out Bond Test in Endodontic Research: A Systematic Review [Internet]. Vol. 44, Journal of Endodontics. Elsevier Inc.; 2018 [cited 2020 Aug 2]. p. 1237-45. Available from: https://pubmed.ncbi.nlm.nih.gov/29935874/.
44. Bazzo J, Pedriali MBP, Guiraldo R, Berger S, Moura S, de Carvalho R. Push-out bond strength of different translucent fiber posts cemented with self-adhesive resin cement. J Conserv Dent [Internet]. 2016 Nov 1 [cited 2020 Aug 2];19(6):583. Available from: http://www.jcd.org.in/text.asp?2016/19/6/583/194036.

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ORIGINAL ARTICLE

The effect of calcium hydroxide on the apical microleakage of canals filled with bioceramic and resin sealers

ABSTRACT

Aim: *To evaluate the impact of calcium hydroxide (Ca[OH]2) on the apical microleakage of canals filled with the bioceramic Total Fill BC (TF BC) and the resin AH 26 sealers.*
Methodology: *A total of 104 human single-canal maxillary teeth were prepared with rotary files. The teeth were randomly divided into two main test groups (n=48) and two control groups, one positive and one negative (n=4). In one test group, $Ca(OH)_2$ was used; in the other test group, no dressing was placed within the canal. Samples in each test group - with or without $Ca(OH)_2$ dressing - were randomly divided into two subgroups. In one subgroup, AH 26 sealer was used and in the other one, TF BC sealer was used. The specimens with $Ca(OH)_2$ were kept at 37 °C in 100% humidity. A dye penetration technique was used to evaluate the leakage. The data were analyzed by Kolmogorov-Smirnov, Kruskal-Wallis, Mann-Whitney U, and Fisher exact tests.*
Results: *The mean microleakage in the subgroups filled with TF BC sealer, with or without $Ca(OH)_2$, was significantly greater than that in the subgroups filled with AH 26 sealer. In addition, the microleakage of the TF BC specimens with $Ca(OH)_2$ was significantly higher than the microleakage of TF BC specimens without the dressing (P<0.001).*
Conclusions: *The findings of this study showed that use of $Ca(OH)_2$ as an intracanal dressing negatively affected the sealing potential of TF BC.*

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Introduction

Bacteria and their products are the main causes of pulpal necrosis and periapical diseases (1). Although chemical and mechanical canal cleaning techniques have been developed over time, none of the current techniques is able to completely clean the root canal system (2). As a solution, studies have suggested the use of an effective intracanal dressing to reduce the number of bacteria in the root canal system (3).

Calcium hydroxide, $Ca(OH)_2$, has commonly been used as an intracanal antimicrobial dressing (4). However, some studies on the removal of intracanal $Ca(OH)_2$ by chemical and mechanical methods have shown that no method is effective at completely removing the dressing (5-7). Incomplete removal of $Ca(OH)_2$ may interfere with the sealing, bonding, and penetration of endodontic sealers, thus negatively affecting the performance of the sealer and possibly impacting the long-term prognosis of root canal treatment (8, 9).

A root canal filling is aimed to prevent reinfection of the canal by providing an adequate seal against bacteria and their toxins (10). Canal filling is done with two materials: a solid core and a sealer (11). Gutta percha is the most commonly accepted solid core root canal filling material to fill the anatomical variations that cannot be cleaned mechanically or chemically (12-14). Various types of sealers, based on zinc oxide, $Ca(OH)_2$, glass ionomer, epoxy resin, silicon, and methacrylate, have been introduced to dentists. In recent years, bioceramic sealers have been introduced to the market. These sealers, according to the manufacturers' claims, are insoluble, radiopaque, aluminum-free, and need water to harden. This type of sealer is hydrophilic and has self-sealing properties owing to its bonding during hardening (15).

The present research was performed to assess the effect of $Ca(OH)_2$ on the microleakage of root canals filled with a new bioceramic sealer, Total Fill BC (TF BC) (FKG Dentaire), and a conventional sealer, AH 26 (Dentsply Sirona).

Materials and Methods

This empirical study was performed on 104 human maxillary single-canal teeth. All the teeth had only one straight canal with no root curvature. The roots were investigated for fractures or cracks by a stereomicroscope (Motic, Xiamen, China) with 40× magnification. Special care was given to ensure the absence of apical and external root resorptions or canal calcification.

To homogenize the specimens, all dental crowns were cut by a diamond disc and a laboratory handpiece so that the length of the remaining root was 15±1 mm. To determine the working length, a No. 20 K-file (Mani, Tochigi, Japan) was placed in the canal until its tip reached the tip of root apex. Then, a rubber stop was placed tangential to the coronal surface of the root, 1 mm was reduced from this length, and the resultant length was recorded as the working length. This length was confirmed with radiographs.

The preparation of each canal was carried out with Mtwo rotary files (VDW, Munich, Germany) up to file No. 40, according to the manufacturer's instructions. The canals were washed with normal saline during preparation.

The teeth were randomly classified into two major test groups (A and B) with 48 specimens in each and four control groups (two positive and two negative) with two specimens in each. A thick $Ca(OH)_2$ paste was prepared with distilled water (3:1 weight ratio of powder to liquid). A No. 30 Lentulo (Medin, Nové Město na Moravě, Czech Republic) and a low-speed handpiece was used to place the $Ca(OH)_2$ paste within the canals of group A and two specimens of each of the positive and negative control groups. No $Ca(OH)_2$ dressing was placed within the canals of group B. Specimens of group B and the four control teeth without $Ca(OH)_2$ were immediately proceeded to the next steps of final rinse and obturation. While specimens of group A and the four control teeth with $Ca(OH)_2$ needed an additional step of 7-day-incubation. The dentinal part of each specimen in group A and the four control teeth with $Ca(OH)_2$ was temporarily restored to the depth of 3 mm with Cavit (3M ESPE, MN, USA). Then, the specimens were placed first in a closed container in holes created in a saline-soaked sponge and then in an incubator (Memmert, Schwabach, Germany) at 37 °C and 100% humidity. After seven days, the specimens were removed from the incubator, and the $Ca(OH)_2$ paste in the canals was extracted by application of master apical file up to the working length. The canals were then washed with 5 mL each of 5% sodium hypochlorite and 17% ethylenediaminetetraacetic acid solution (Ariadent, Tehran, Iran). This final washing process was also repeated for the specimens in the group B and control specimens without $Ca(OH)_2$ in order to remove the smear layer. A radiograph was obtained from samples with $Ca(OH)_2$ to ensure that the paste has been completely removed from the root canal. Finally, the canals of all the specimens were washed with 5 mL of distilled water and dried with paper points.

The two test groups of A and B - with or without $Ca(OH)_2$ pretreatment, respectively - were divided equally into two subgroups. The canals of 24 specimens pretreated with $Ca(OH)_2$ and 24 specimens without $Ca(OH)_2$, along with two canals in the negative control group - one with and one without $Ca(OH)_2$ - were filled with gutta percha (Gapadent, Tianjin, China) and TF BC sealer by lateral condensation technique. The canals of 24 specimens pretreated with $Ca(OH)_2$ and 24 specimens without $Ca(OH)_2$, along with two canals in the negative control group - one with and one without $Ca(OH)_2$ - were filled with gutta percha and AH 26 sealer using the lateral condensation method. The four specimens of the positive control group - two with and two without $Ca(OH)_2$ - were filled with gutta percha after preparation.

The roots of all specimens except the negative control group were covered with three layers of nail polish up to the apical 2 mm of the root canal. In the negative control group, all root surfaces were covered with nail polish completely. An interval of 24 hours was allowed between the layers of nail polish in order for them to dry completely.

The specimens were then immersed in India ink solution (Pelikan, Hannover, Germany). The height of ink in each container was the same for all specimens to ensure that the hydrostatic pressure of the liquid was equal. To this end, the specimens were immersed in a cryogenic container (Isolab, Bavaria, Germany). After 48 hours, the specimens were removed from the ink and washed with water for 10 minutes.

Afterward, the nail polish was removed from the specimens with acetone. For decalcification, the specimens were put in 10% acid nitric solution (Merck & Co, NJ, USA). The roots were cross-sectioned from the longitudinal direction with a No. 15 scalpel (Aesculap, Tuttlingen, Germany).

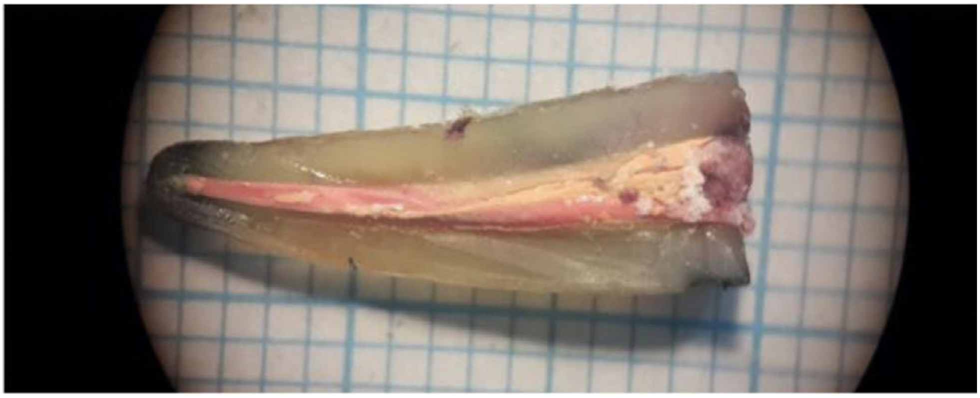

Figure 1.
Demonstration of a sample under microscope.

To determine the penetrability of the dye, prepared sections of specimens were analyzed under stereomicroscope (Figure 1). The maximum level of dye penetration for each specimen was observed under the stereomicroscope with 40× magnification and recorded based on the microscopic scale. All the specimens were investigated by two observers, and the mean of the values obtained for each specimen was considered to be the microleakage of that specimen.
The results were analyzed by SPSS (version 16, IBM Corporation, IL, USA) software using Kolmogorov-Smirnov, Kruskal-Wallis, Mann-Whitney U, and Fisher exact tests. The significance level was set at $P<0.05$.

Results

In the four negative control group specimens, no color penetration was observed. In the four specimens of the positive control group, color penetration was seen all along the canal.
Microleakage was found in 44 AH 26 specimens (91/7%) and 48 TF BC specimens (100.0%). The Fisher exact test revealed that the number of canals exhibiting microleakage did not significantly correlate with the type of sealer ($P=0.117$).
The minimum microleakage was found in the AH 26 sealer specimens without $Ca(OH)_2$ paste and the maximum microleakage was reported in the TF BC sealer specimens with $Ca(OH)_2$ paste (Table 1).
The results of the Kolmogorov-Smirnov test showed that the microleakage did not follow a normal distribution pattern ($P=0.038$); therefore, nonparametric tests were used for data analysis. Microleakage in the canals filled with AH 26 and TF BC sealers was reported to be significantly different ($P<0.001$; Mann-Whitney U test). Moreover, the Kruskal-Wallis test showed that the microleakage in the canals with and without Ca(OH)2 and filled with AH 26 and TF BC sealers was significantly different ($P<0.001$). According to the results of Mann-Whitney U test, all binary comparisons of these four groups were significantly different ($P<0.05$).

Discussion

Removal of microorganisms and prevention of reinfection are the major objectives of endodontic treatment, both of which can be achieved by disinfecting and filling the canal space completely. In addition to the cleaning, correct shaping, and sealing of both the root canal and access cavity, intracanal dressings have also been suggested. Although application of $Ca(OH)_2$ has been proposed, currently there is no method available to completely remove it from the canal walls, and the remaining $Ca(OH)_2$ can affect the apical seal of the filled canals. Hence, this study was an attempt to evaluate the effect of $Ca(OH)_2$ on apical microleakage in canals obturated with gutta percha and sealed with AH 26 or TF BC.
The results showed that microleakage was greater in canals filled with TF BC than in canals filled with AH 26. AH 26 is a resin-based sealer that sets quickly. While this sealer has a tendency to exhibit shrinkage and therefore may separate more

Table 1
Mean (SD) microleakage of specimens in the present study

Sealer	Intracanal dressing	Mean (SD) mm
AH 26	$Ca(OH)_2$ paste	3.79 (2.70)
AH 26	None	1.20 (1.64)
TF BC	$Ca(OH)_2$ paste	8.15 (2.51)
TF BC	None	6.10 (2.71)

Abbreviations: $Ca(OH)_2$=calcium hydroxide; TF BC=Total Fill BC.

easily from the canal walls, AH 26 has beneficial factors, such as better penetration into canal irregularities and its ability to creep, which enable it to bond to dentin better than other sealers (16).
TF BC is a calcium silicate-based sealer that has recently been introduced to the market. As mentioned previously, it is a bioceramic sealer that is hydrophilic and expands during setting. This chemical expansion and micromechanical bonding causes the sealer to bond to the canal wall (17, 18). In this type of sealer, hydroxyapatite crystals are formed between the dentin and sealer to the extent that separating these crystals from the dentin walls and dentinal tubules may be challenging (19). It can be hypothesized that the better penetration of AH 26 into a canal compared to TF BC may be the reason for the better sealing potential. The hydroxyapatite crystals formed at the interface of dentin and sealer (20) may result in increased leakage. In addition, since the setting of a bioceramic sealer requires the presence of water (21), drying the canal prior to obturation removes the humidity required for the setting of the sealer, which in turn can explain the increased microleakage.
Kim et al analyzed the impact of $Ca(OH)_2$ and epoxy resin on endodontic retreatment (4). Their findings indicated that the penetration of AH 26 sealer was greater than that of bioceramic sealer, which is in line with the results of the present study. Further, Jafari et al concluded that sealing ability of AH 26 sealers are significantly higher than a bioceramic sealer used in their study (Apatite sealer) (22). However, Mohammadian et al incorporated scanning electron microscopy in order to evaluate the dentin-sealer interface in extracted human single rooted teeth treated using different sealers (23). Their results showed no significant difference in the mean dentin-sealer gap in the coronal, middle, and apical area between AH plus and BC sealer. The inconsistency between findings of the two studies can be attributed to different methods for evaluation of sealing ability.
Moreover, the results of the present study revealed that the amount of microleakage in the canals sealed with TF BC and AH 26 increased in the presence of $Ca(OH)_2$. Due to its alkaline pH, $Ca(OH)_2$ is extensively used for disinfecting root canals. However, it cannot be removed completely from the root canal, which prevents the penetration of sealer into the dentinal tubules of the canal, thereby reducing the adhesion of the sealer to the canal wall. This can cause microleakage in the long term (24).
$Ca(OH)_2$ dressing has shown to have higher microleakage compared to other medications when used with Biodentine plug and gutta percha filling (25). The results of the curent study showed that the number of canals with microleakage with and without $Ca(OH)_2$ was not significantly different.
The present study has some limitations. Micro computed tomography is a better alternative for dye penetration technique. However, dye penetration technique is still used for this purpose. Further studies on new sealers must be performed in order to evaluate the effects of $Ca(OH)_2$ on apical seal.

Conclusions

The findings of this present study showed that $Ca(OH)_2$ as an intracanal dressing affected the sealing ability of the bioceramic sealer TF BC.

Clinical Relevance

Remnants of calcium hydroxide in root canals compromise the sealing ability of TF BC and AH26 sealers.

Conflict of Interest

The authors have no financial, economic, commercial, or professional interests related to topics presented in this article.

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References

1. Jhajharia K, Parolia A, Shetty KV, Mehta LK. Biofilm in endodontics: a review. J Int Soc Prev Community Dent. 2015;5:1.
2. Plotino G, Ahmed HMA, Grande NM, Cohen S, Bukiet F. Current assessment of reciprocation in endodontic preparation: a comprehensive review—part II: properties and effectiveness. J Endod. 2015;41:1939-50.
3. Jacobs JC, Troxel A, Ehrlich Y, Spolnik K, Bringas JS, Gregory RL, et al. Antibacterial effects of antimicrobials used in regenerative endodontics against biofilm bacteria obtained from mature and immature teeth with necrotic pulps. Journal of endodontics. 2017;43:575-9.
4. Kim D, Kim E. Antimicrobial effect of calcium hydroxide as an intracanal medicament in root canal treatment: a literature review-Part II. in vivo studies. Restor Dent Endod. 2015;40:97-103.
5. Topçuoğlu H, Düzgün S, Ceyhanlı K, Aktı A, Pala K, Kesim B. Efficacy of different irrigation techniques in the removal of calcium hydroxide from a simulated internal root resorption cavity. Int Endod J. 2015;48:309-16.
6. Ma J, Shen Y, Al-Ashaw A, Khaleel H, Yang Y, Wang Z, et al. Micro-computed tomography evaluation of the removal of calcium hydroxide medicament from C-shaped root canals of mandibular second molars. Int Endod J. 2015;48:333-41.
7. Zorzin J, Wießner J, Wießner T, Lohbauer U, Petschelt A, Ebert J. Removal of radioactively marked calcium hydroxide from the root canal: influence of volume of irrigation and activation. J Endod. 2016;42:637-40.
8. Ghabraei S, Bolhari B, Yaghoobnejad F, Meraji N. Effect of intra-canal calcium hydroxide remnants on the push-out bond strength of two endodontic sealers. Iran Endod J. 2017;12:168.
9. Uzunoglu-Özyürek E, Erdoğan Ö, Türker SA. Effect of calcium hydroxide dressing on the dentinal tubule penetration of 2 different root canal sealers: a confocal laser scanning microscopic study. J Endod. 2018;44:1018-23.
10. Al-Haddad A, Kasim NHA, Ab Aziz ZAC. Interfacial adaptation and thickness of bioceramic-based root canal sealers. Dent Mater J. 2015;34:516-21.
11. Celikten B, Uzuntas CF, Orhan AI, Tufenkci P, Misirli M, Demiralp KO, et al. Micro-CT assessment of the sealing ability of three root canal filling techniques. J Oral Sci. 2015;57:361-6.
12. Camilleri J. Sealers and warm gutta-percha obturation techniques. J Endod. 2015;41:72-8.
13. Lee D-K, Kim SV, Limansubroto AN, Yen A, Soundia A, Wang C-Y, et al. Nanodiamond–gutta percha composite biomaterials for root canal therapy. ACS Nano. 2015;9:11490-501.
14. Fernández R, Restrepo J, Aristizábal D, Álvarez L. Evaluation of the filling ability of artificial lateral canals using calcium silicate-based and epoxy resin-based endodontic sealers and two gutta-percha filling techniques. Int Endod J. 2016;49:365-73.
15. Jitaru S, Hodisan I, Timis L, Lucian A, Bud M. The use of bioceramics in endodontics-literature review. Clujul Med. 2016;89:470.
16. Tuncer AK. Effect of QMix 2in1 on sealer penetration into the dentinal tubules. J Endod. 2015;41:257-60.
17. Tanomaru-Filho M, Torres FFE, Chávez-Andrade GM, de Almeida M, Navarro LG, Steier L, et al. Physicochemical properties and volumetric change of silicone/bioactive glass and calcium silicate–based endodontic sealers. J Endod. 2017;43:2097-101.
18. Yap WY, Ab Aziz ZAC, Azami NH, Al-Haddad AY, Khan AA. An in vitro comparison of bond strength of different sealers/obturation systems to root dentin using the push-out test at 2 weeks and 3 months after obturation. Med Princ Pract. 2017;26:464-9.
19. Donnermeyer D, Dornseifer P, Schäfer E, Dammaschke T. The push-out bond strength of calcium silicate-based endodontic sealers. Head Face Med. 2018;14:1-7.
20. Kim H, Kim E, Lee S-J, Shin S-J. Comparisons of the Retreatment Efficacy of Calcium Silicate and Epoxy Resin–based Sealers and Residual Sealer in Dentinal Tubules. Journal of Endodontics. 2015;41:2025-30.
21. Loushine BA, Bryan TE, Looney SW, Gillen BM, Loushine RJ, Weller RN, et al. Setting properties and cytotoxicity evaluation of a premixed bioceramic root canal sealer. Journal of endodontics. 2011;37:673-7.
22. Jafari F, Sobhani E, Samadi-Kafil H, Pirzadeh A, Jafari S. In vitro evaluation of the sealing ability of three newly developed root canal sealers: A bacterial microleakage study. J Clin Exp Dent. 2016;8:e561-e5.
23. Mohammadian F, Farahanimastary F, Dibaji F, Kharazifard MJ. Scanning electron microscopic evaluation of the sealer-dentine interface of three sealers. Iran Endod J. 2017;12:38.
24. Dadresanfar B, Abbas FM, Bashbaghi H, Miri SS, Ghorbani F. Intra-canal calcium hydroxide removal by two rotary systems: A comparative study. J Conserv Dent. 2015;18:257.
25. Srivastava AA, Srivastava H, Prasad AB, Raisingani D, Soni D. Effect of Calcium Hydroxide, Chlorhexidine Digluconate and Camphorated Monochlorophenol on the Sealing Ability of Biodentine Apical Plug. J Clin Diagn Res. 2016;10:ZC43-ZC6.

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ORIGINAL ARTICLE

The impact of kinematics, single-file technique and preparation time on the apical extrusion of debris

ABSTRACT

Aim: *To compare canal preparation time and apical extrusion of debris during instrumentation with the ProTaper Next (PTN), HyFlex CM (HCM), HyFlex EDM (HEDM), WaveOne Gold (WOG), and Reciproc Blue (RCB) systems.*
Methodology: *Seventy-five roots of extracted mandibular first molars, with curved mesiobuccal canals (10–20°) and independent foramina, were distributed across 5 experimental groups (n=15 each) according to the instrumentation system used. Roots were secured in Eppendorf tubes, the canals were irrigated with double-distilled water, and the instrumentation time was recorded. After instrumentation, the roots were removed from the Eppendorf tubes and the amount of extruded debris was calculated by subtracting the initial weight from the final weight. The assumption of normality was rejected by the Shapiro–Wilk test, followed by the Kruskal–Wallis test with a post-hoc Dunn's test. A simple linear regression analysis was run to test for correlation between amount of extruded debris and time required for instrumentation.*
Results: *The PTN and HCM systems were associated with significantly (p<0.05) greater amounts of extruded debris and longer instrumentation time (PTN and HCM>HEDM, WOG, RCB). There was no significant difference between the PTN and HCM groups (p>0.05), nor between the HEDM, WOG, and RCB groups (p>0.05). Simple linear regression demonstrated a positive correlation (r=0.74, p<0.05) between the amount of debris extruded and instrumentation time.*
Conclusions: *The RCB, WOG, and HEDM systems were associated with less debris extrusion and shorter instrumentation time when compared to the PTN and HCM systems.*

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Introduction

During root canal preparation, apical extrusion of debris, irrigants, and/or bacteria can occur, potentially leading to such complications as post-operative pain, flare-ups, or even treatment failure (1, 2). Apical extrusion has been reported as the main cause of pain after completion of endodontic treatment (3). Which factors increase the amount of extruded debris remains controversial; studies have demonstrated associations with the type of file motion, type of irrigant, working length, cross-section, tip, taper, flexibility, heat treatment, and number of files used (4-7).
Advances in metallurgy and kinematics have reduced the number of instruments needed instrument a root canal system (8). However, the literature clearly shows that all systems in use, however advanced, lead to apical extrusion of debris. It is thus important to investigate the amount of debris extruded and the factors associated with this reduction or increase (9-11). Instrumentation time is another important factor, as several systems (whether reciprocating or rotary) are based on a "single-file" philosophy for canal preparation and previous work has shown that a shorter file activity time within the canal system can generate less debris extrusion (12, 13).
Within this context, the objective of this study was to compare canal preparation time and apical extrusion of debris during instrumentation with the ProTaper Next (PTN), HyFlex CM (HCM), HyFlex EDM (HEDM), WaveOne Gold (WOG), and Reciproc Blue (RCB) systems. The null hypothesis is that there would be no difference in the amount of extruded debris or the time required to perform instrumentation across these different systems.

Materials and Methods

Specimen selection and preparation
Once approval from the local Research Ethics Committee had been obtained (opinion no. 2,379,268), 75 mandibular first molars which had been extracted for various reasons were included in the present study. Teeth with fully formed roots showing independent foramina, curvature angles of 10-20° (14), absence of calcifications, resorption, or previous endodontic treatment, and with an initial apical canal diameter corresponding to that of a #15 K-file (Maillefer Corp, Ballaigues, Switzerland) were selected and disinfected by soaking in 0.5% chloramine-T trihydrate solution for one week.
Sample size calculation was performed in G*Power 3.1.9.4 software, The amount of debris extruded was considered the primary outcome of interest. Based on prior work by Uslu et al. (15), to detect a difference of 0.0024 between groups, with a standard error of 0.0025, statistical power of 0.80, and an alpha level of 0.05, a sample size of 15 specimens per group would be required.
All tooth crowns were sectioned at the cementoenamel junction with a diamond disc (Horico Dental Hpf; Ringleb, Berlin, Germany) coupled to a slow-speed handpiece powered by a micromotor, under constant refrigeration, to generate specimens 13 mm in length, as confirmed by a digital caliper (500 series, DIN 862; Mitutoyo, São Paulo, SP, Brazil). The initial diameter of the mesiobuccal canal was established by advancement of a #15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) until it fit snugly within the canal and its tip was just visible in the apical foramen under an operating microscope at 12.5x magnification (Stemi 508; Carl Zeiss, Jena, Germany). The same procedure was used to determine the working lengths of the specimens. Canals that did not meet this criterion were excluded from the study and replaced with new specimens. Two #10 K-files were introduced into each mesial root canal, in a clockwise/counterclockwise motion with slight apical pressure, to confirm the presence of independent foramina under 8x magnification (Stemi 508; Carl Zeiss, Jena, Germany).

Group allocation
Specimens were randomly allocated (www.random.org) across 5 groups (n=15 each) depending on the system used for instrumentation of the mesiobuccal canals.

The mesiolingual canals were not exposed to any type of instrumentation or irrigation at any point in the experiment.

Instrumentation of sample groups

PTN group: an X1 (17.04) PTN file (Dentsply Maillefer, Ballaigues, Switzerland) was used in rotary motion (300 rpm, 2 N·cm). Three in-and-out movements (pecks), with a stroke amplitude of 3 mm, were performed in each third of the canal (cervical, middle, and apical) until the WL was reached (1 mm short of the apical foramen). The exact same sequence was then followed with an X2 (25.06) instrument.

HCM group: the 25.08 instrument (500 rpm; 2.5 N·cm) of the Hyflex CM – Controlled Memory System (Coltène, Altstätten, Switzerland) was used to prepare the cervical third. The canal was then instrumented in sequence with the 20.04, 25.04, 20.06, and 25.06 instruments, using the same speed and torque settings, the same type and amplitude of motion, and the same WL determined for the PTN group.

HEDM group: the OneFile instrument (25/~, variable taper) of the Hyflex EDM rotary system (Coltène, Altstätten, Switzerland) was used, again employing the same speed and torque (500 rpm, 2.5 N·cm), type and amplitude of motion, and WL used for the X1 instrument of the PTN group.

RCB group: an R25 (25.08) RCB file (VDW, Ballaigues, Switzerland) was used in reciprocating motion. Three in-and-out movements (pecks) with a stroke amplitude of 3 mm were performed in each third of the canal (cervical, middle, and apical) until the WL was reached (1 mm short of the apical foramen).

WOG group The primary instrument of the WOG system (25.07) was used in a manner similar to that described for the RCB group.

Instrumentation of the respective experimental groups was performed with the aid of an X-Smart Plus motor (Dentsply Maillefer, Ballaigues, Switzerland), adjusted for each system, always by the same operator. Regardless of system, each file was used only once, for the preparation of only one canal, and later discarded.

Throughout instrumentation, the specimens were irrigated with 3 mL of double-distilled water per root third, through a 30G NaviTip needle (Ultradent Products Inc, South Jordan, UT). In all groups, after each cycle of instrumentation and irrigation, foramen patency was controlled with a #10 K-file advanced 1 mm beyond the foramen. At the end of the instrumentation, a final irrigation with 1 mL of the same irrigant used throughout was performed, never exceeding the total amount of irrigant standardized for all specimens (10 mL). Canals were evacuated with the aid of capillary tips (Ultradent, South Jordan, UT) and further dried with the paper points provided with the respective systems.

Fabrication of devices for collection and weighing of extruded debris

This study followed the methodological parameters proposed by Myers and Montgomery (16) and modified by other authors (7, 17) to quantify the amount of debris extruded through the apical foramen after instrumentation. The initial weight of each Eppendorf tube (Eppendorf do Brasil, São Paulo, SP, Brazil) was determined by weighing three consecutive times on a precision balance (Ohaus Corporation, Parsippany, NJ, USA) with a resolution of 10^{-5} g. The tip of a #2 hand plugger (SS White Artigos Dentários Ltda, Rio de Janeiro, Brazil) was heated and used to puncture a hole in the stopper of each Eppendorf tube. The root was pushed through this hole and a rubber dam (Madeitex, São Paulo, SP, Brazil) was placed for isolation, simulating a clinical procedure. To equalize inner and outer air pressure levels, a 27G short disposable anesthetic needle (Unoject DFL Ltda, Rio de Janeiro, RJ, Brazil) was inserted through the rubber dam and stopper. Each Eppendorf/root assembly was then placed into an opaque vial to prevent the operator from having any visual contact with the inside of the tubes. Instrumentation was then performed, and any apically extruded debris was thus collected inside the Eppendorf tube.

To collect any residual debris still adherent to the outer root surface, 1 mL of double-distilled water in a 10 mL hypodermic syringe (BD Plastipak, Curitiba, Brazil) was used to rinse the root; any debris thus removed was caught in the Eppendorf tube. In order to allow complete evaporation of water from the Eppendorf tubes and subsequent weighing of the extruded debris, the tubes incubated for 5 consecutive days at a constant temperature of 70 °C (Model EL-14, Odontobrás, São Paulo, Brazil). In all experimental groups, each Eppendorf tube was weighed in triplicate after instrumentation, using the same procedure described above. The mean of the three weights was recorded as the final value. The weight of the extruded debris in grams was obtained by subtracting the mean final weight from the mean initial weight of each Eppendorf tube.

Assessment of overall instrumentation time
The entire instrumentation sequence was timed (Seiko, Japan). The timer was started only when the instrument was activated and introduced into the channel and stopped whenever the instrument was removed, thus yielding the actual instrumentation time.

Statistical analysis
Data on debris weight and instrumentation time were entered into BioEstat 5.0 for analysis. The Shapiro-Wilk test rejected the assumption of normality for both outcomes of interest (amount of extruded debris and instrumentation time).
Descriptive analyses were performed. The nonparametric Kruskal-Wallis test (with Dunn's post-hoc test) was used, at a significance level of 5%.
A simple linear regression analysis was run in Minitab (version 16) to test for correlation between amount of extruded debris and time required for instrumentation.

Results

Figure 1 represents the amount of debris extruded and the actual instrumentation time (in seconds) for all groups. The systems associated with the greatest amount of extrusion were PTN and HCM, with both yielding significantly more debris ($p<0.05$) than the HEDM, WOG, and RCB systems (Figure 1A). There was no significant difference between the PTN and HCM groups ($p>0.05$), nor between the HEDM, WOG, and RCB groups ($p>0.05$).
Regarding instrumentation time, the HCM and PTN systems were associated with significantly longer time ($p<0.05$) than the HEDM, RCB, or WOG systems (Figure 1B). Again, there was no significant difference between the HCM and PTN groups ($p>0.05$), nor between the HEDM, WOG, and RCB groups ($p>0.05$).
Simple linear regression analysis demonstrated a positive correlation ($r=0.746$, $p<0.05$) between the amount of debris extruded and instrumentation time (Figure 2).

Discussion

The null hypothesis was rejected, as there were significant differences both in the amount of extruded debris and in the time required for instrumentation across the systems compared herein.
The inclusion criteria of this study are factors that ordinarily have an influence on the amount of debris extruded. The experiment was performed on mesial roots of mandibular molars because curved roots are usually associated with a greater amount of debris extrusion when compared to straight roots, mainly due to the challenging preparation of the former (18). Teeth with a single root canal are widely used in such research because both instrumentation and debris collection are easier; however, this practice can skew the results, because the canals of these teeth are very wide. Conversely, molars have narrow, curved root canals, generating more debris extrusion and thus making the results of the study closer to the experience of everyday clinical practice (19). It is important to note that all root lengths were standardized at 13 mm, avoiding any influence of canal length on instrumentation time or amount of debris extruded. Controversy remains as to whether variation in working length can lead to changes in the amount of debris extruded. However, as assessing this was not within the scope of the pres-

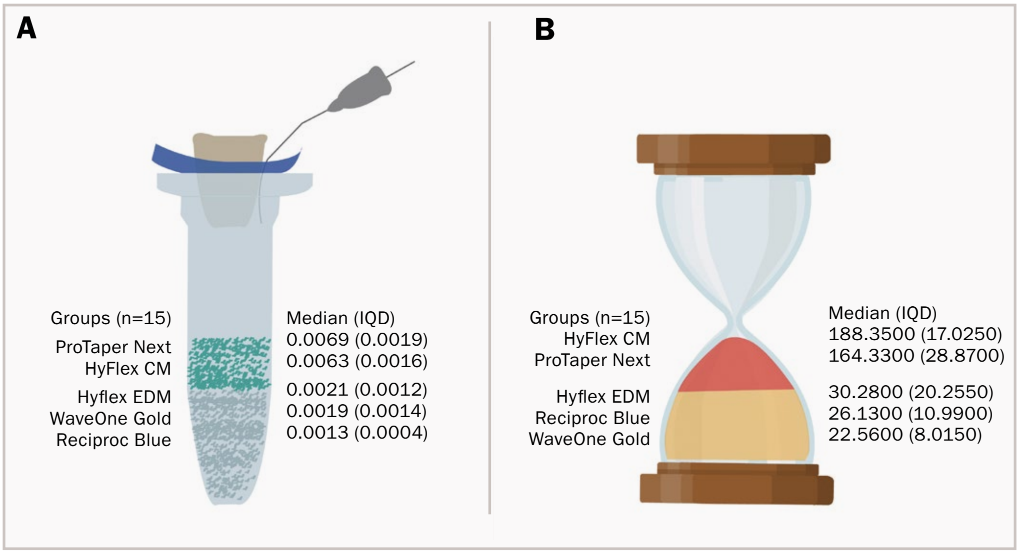

Figure 1. Amount of debris extruded during instrumentation with each system **(1A)**. Time (in seconds) required to perform instrumentation with each system **(1B)**. Different colors denote statistically significant differences (p<0.05). IQR, interquartile range n=15 per group.

ent study, the working length was established at 1 mm short of the apex for all groups (8, 20, 21).

Double-distilled water was chosen as the irrigant because any extravasation that occurs during instrumentation will evaporate completely while the Eppendorf cubes are incubated, without leaving any residue that might interfere with weighing, as sodium hypochlorite or chlorhexidine would (22).

The study followed the method proposed by Myers and Montgomery (16) for collection and weighing of debris extruded through the apical foramen, a widely cited protocol that is extensively represented in the existing literature (6, 22-24). However, we did modify the Myers and Montgomery method on the basis of recent work (7, 17, 18). In addition, the Eppendorf tubes were placed into opaque vials with a diameter similar to that of the tube itself, thus ensuring that only the root canal access opening was visible during instrumentation. This not only ensured blinding of the operator but also made the conditions of the experiment closer to those of clinical

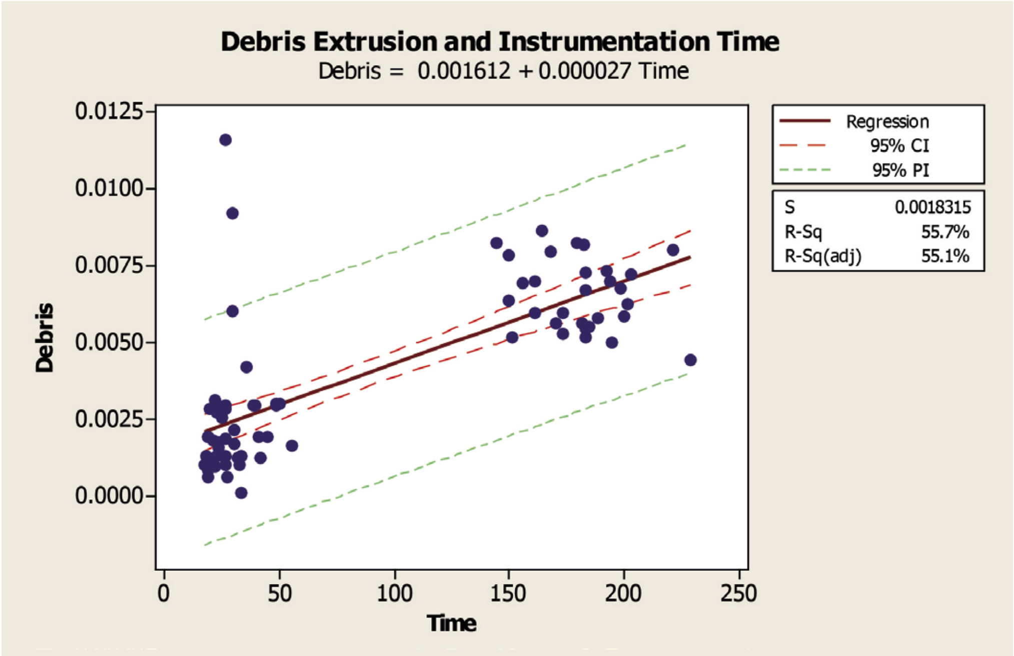

Figure 2. Association between amount of debris extruded and the required instrumentation time. Statistically significant on linear regression analysis (p<0.05).

practice and prevented handling of the Eppendorf tube by the operator, which might have influenced the final weight of the specimens.
The systems associated with the most debris extrusion were those that required longer instrumentation times, indicating a possible correlation - as demonstrated by simple linear regression - that the longer the instrumentation time, the greater the amount of debris extruded. This corroborates the previous findings of Karatas et al. (12). Likewise, Dincer et al. (5) demonstrated that the PTN system extruded more debris and required longer instrumentation times compared to the WOG system, as did Ehsani et al. (25), who observed greater extrusion of debris with those systems in which instrumentation took longer. The authors believe this might be explained because using a greater number of files naturally requires a longer instrumentation time, which means more time spent cutting dentin and, consequently, greater formation of debris, which may eventually be extruded through the apex. Thus, when working with this type of system, the use of irrigation protocols capable of removing debris from the canal and isthmus is paramount (26).
Reciprocating instruments extruded less debris when compared to rotary systems with multiple files, as in other studies (17, 19, 27). This may explain the finding of a recent systematic review by Martins et al. (3) that reciprocating systems are associated with less postoperative pain when compared to rotary systems. However, when the reciprocating systems included in this study were compared to the HEDM system (which, although rotary, is also a single-file system), the amount of extruded debris was found to be similar.
Gummadi et al. (28) observed greater debris extrusion with the WaveOne system when compared to the One Shape single-file rotary system; however, they analyzed the first generation of this reciprocating system, while the present study used the later WaveOne Gold iteration.
The findings of this experiment demonstrate that instrumentation kinematics play a relevant role the amount of debris extruded, but that even rotary systems which employ a single file to simplify preparation reduce the risk of debris extrusion compared to multiple-file systems. This can be explained by the fact that using a greater number of instruments can generate a greater amount of debris. (17, 29)
Our findings contradict those of previous studies (15, 30, 31) that reported greater extrusion of reciprocating instruments, probably due to differences in instrumentation protocol. When reciprocating preparation is done by thirds, alternating with glide path maneuvers - which have been proven to lead to less debris extrusion (10) - and combined with abundant irrigation, these instruments are probably associated with a similar or even reduced amount of extrusion compared to rotary systems.
Despite being mentioned by other authors such as Frota et al. (20) and Amaral et al. (1) as a possible interfering factor in the extrusion of debris, instrument taper was not relevant in the present study. In our experiment, #25 rotary instruments but with smaller tapers generated more debris than reciprocating instruments with the same diameter but a relatively larger taper, a finding also reported by Dincer et al. (5).
The systems evaluated in this study all have different cross-sections, but again, this was not a determining factor in the amount of debris extrusion observed. The literature on the matter is controversial (25, 30, 32). The RCB instrument, which has an S-shaped section with two sharp cutting edges, was not associated with greater debris extrusion than the WOG system, which has a parallelogram-shaped cross-section with alternating points of contact. Although the initial file of the HEDM rotary system has a unique variable cross-section design along the active cutting shaft, this was not associated with any difference in the amount of debris extruded as compared to other single-file systems.
Another important point is the difference in heat treatment of the tested instruments. Among the rotary systems analyzed, PTN uses an M-Wire alloy, while the HCM system employs a memory NiTi wire and would thus theoretically be capable of greater canal-centering ability, with less

deviation, thus allowing more conservative preparations (33). Nevertheless, this potential advantage was not associated with any difference in the amount of debris extruded through the apical foramen of curved canals between the two systems. The HEDM rotary instrument, which is manufactured using the electric discharge machining (EDM) heat treatment, was associated with a smaller amount of debris extrusion compared to the other rotary systems evaluated; however, this result may be attributable more to the fact that the system allows preparation of the entire canal with one instrument (single-file endodontics) (34).
The reciprocating systems all achieved similar results in terms of debris extrusion, regardless of their unique heat treatments and cross-section designs. The greater flexibility of these systems is probably associated with greater canal-centering ability, but has no bearing on the amount of debris extruded through the apical foramen.

Conclusions

Within the limitations of this study, we conclude that debris extrusion occurred with all systems. The RCB, WOG, and HEDM systems were associated with less debris extrusion and shorter instrumentation time when compared to the PTN and HCM systems.

Clinical Relevance

Apical extrusion has been reported as the main cause of pain after endodontic treatment.

Conflict of Interest

The authors deny any conflict of interest.

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References

1. *Amaral AP, Limongi PBOC, Fontana CE, et al. Debris Apically Extruded by Two Reciprocating Systems: A Comparative Quantitative Study. Eur J Dent 2019;13:625-8.*
2. *Tavares SG, Fontana CE, Martin AS De, et al. In Vivo Evaluation of Painful Symptomatology after Endodontic Treatment Performed Using Two Different Irrigation Needle Insertion Depths. Eur J Dent 2020;14:274-280.*
3. *Martins CM, Batista VE, Souza AC, et al. Reciprocating kinematics leads to lower incidences of postoperative pain than rotary kinematics after endodontic treatment: A systematic review and meta-analysis of randomized controlled trial. J Conserv Dent 2019;22:320-331.*
4. *Silva EJNL, Teixeira JM, Kudsi N, et al. Influence of apical preparation size and working length on debris extrusion. Braz Dent J 2016;27:28-31.*
5. *Dincer A, Guneser M, Arslan D. Apical extrusion of debris during root canal preparation using a novel nickel-titanium file system: WaveOne gold. J Conserv Dent 2017;20:322-5.*
6. *Sen OG, Bilgin B, Kocak S, et al. Evaluation of Apically Extruded Debris Using Continuous Rotation, Reciprocation, or Adaptive Motion. Braz Dent J 2018;29:245-8.*
7. *Boijink D, Costa DD, Hoppe CB, et al. Apically Extruded Debris in Curved Root Canals Using the WaveOne Gold Reciprocating and Twisted File Adaptive Systems. J Endod 2018;44:1289-1292.*
8. *Mendonça de Moura JD, Bueno CE da S, Fontana CE, Pelegrine RA. Extrusion of Debris from Curved Root Canals Instrumented up to Different Working Lengths Using Different Reciprocating Systems. J Endod 2019;45:930-4.*
9. *Pedrinha VF, Brandão JM da S, Pessoa OF, Rodrigues P de A. Influence of File Motion on Shaping, Apical Debris Extrusion and Dentinal Defects: A Critical Review. Open Dent J 2018;12:189-201.*
10. *Plotino G, Nagendrababu V, Bukiet F, et al. Influence of Negotiation, Glide Path, and Preflaring Procedures on Root Canal Shaping - Terminology, Basic Concepts, and a Systematic Review. J Endod 2020;46:707-729.*
11. *Ahn SY, Kim HC, Kim E. Kinematic effects of nickel-titanium instruments with reciprocating or continuous rotation motion: A systematic review of in vitro studies. J Endod 2016;42:1009-1017.*
12. *Karataş E, Ersoy İ, Gündüz HA, et al. Influence of Instruments Used in Root Canal Preparation on Amount of Apically Extruded Debris. Artif Organs 2016;40:774-7.*
13. *Li H, Zhang C, Li Q, et al. Comparison of cleaning efficiency and deformation characteristics of Twisted File and ProTaper rotary instruments. Eur J Dent 2014;8:191-6.*
14. *Schneider SW. A comparison of canal preparations in straight and curved root canals. Oral Surg Oral Med Oral Pathol 1971;32:271-5.*
15. *Uslu G, Özyürek T, Yılmaz K, et al. Apically Extruded Debris during Root Canal Instrumentation with Reciproc Blue, HyFlex EDM, and XP-endo Shaper Nickel-titanium Files. J Endod 2018;44:856-9.*
16. *Myers GL, Montgomery S. A comparison of weights of debris extruded apically by conventional filing*

and Canal Master techniques. J Endod 1991; 17:275-9.
17. Silva EJ, Carapia MF, Lopes RM, et al. Comparison of apically extruded debris after large apical preparations by full-sequence rotary and single-file reciprocating systems. Int Endod J 2016;49:700-5.
18. Karataslioglu E, Arslan H, Er G, Avci E. Influence of canal curvature on the amount of apically extruded debris determined by using three-dimensional determination method. Aust Endod J 2019;45:216-224.
19. De-Deus G, Neves A, Silva EJ, et al. Apically extruded dentin debris by reciprocating single-file and multi-file rotary system. Clin Oral Investig 2015;19:357-361.
20. Frota MMA, Bernardes RA, Vivan RR, et al. Debris extrusion and foraminal deformation produced by reciprocating instruments made of thermally treated NiTi wires. J Appl Oral Sci 2018;26:1-8.
21. Silva EJ, Teixeira JM, Kudsi N, et al. Influence of Apical Preparation Size and Working Length on Debris Extrusion. In: Braz Dent J 2016;27:28-31.
22. Silveira C, Pimpão MV, Fernandes LA, et al. Influence of different irrigation solutions and instrumentation techniques on the amount of apically extruded debris. Eur Endod J 2019;4:122-6.
23. Caviedes-Bucheli J, Castellanos F, Vasquez N, et al. The influence of two reciprocating single-file and two rotary-file systems on the apical extrusion of debris and its biological relationship with symptomatic apical periodontitis. A systematic review and meta-analysis. Int Endod J 2016;49:255-270.
24. Doğanay Yıldız E, Arslan H. The effect of blue thermal treatment on endodontic instruments and apical debris extrusion during retreatment procedures. Int Endod J 2019;52:1629-1634.
25. Ehsani M, Farhang R, Harandi A, et al. Comparison of Apical Extrusion of Debris by Using Single-File, Full-Sequence Rotary and Reciprocating Systems. J Dent 2016;13:394-9.
26. Duque JA, Duarte MAH, Canali LCF, et al. Comparative Effectiveness of New Mechanical Irrigant Agitating Devices for Debris Removal from the Canal and Isthmus of Mesial Roots of Mandibular Molars. J Endod 2017;43:326-331.
27. Lu Y, Chen M, Qiao F, Wu L. Comparison of apical and coronal extrusions using reciprocating and rotary instrumentation systems. BMC Oral Health 2015;15:92.
28. Gummadi A, Panchajanya S, Ashwathnarayana S, et al. Apical extrusion of debris following the use of single-file rotary/reciprocating systems, combined with syringe or ultrasonically-facilitated canal irrigation. J Conserv Dent 2019;22:351-5.
29. Ehsani M, Farhang R, Harandi A, et al. Comparison of Apical Extrusion of Debris by Using Single-File, Full-Sequence Rotary and Reciprocating Systems. J Dent 2016;13:394-9.
30. Labbaf H, Moghadam KN, Shahab S, et al. An in vitro comparison of apically extruded debris using reciproc, protaper universal, neolix and hyflex in curved canals. Iran Endod J 2017;12:307-311.
31. Delvarani A, Mohammadzadeh Akhlaghi N, Aminirad R, et al. In vitro Comparison of Apical Debris Extrusion Using Rotary and Reciprocating Systems in Severely Curved Root Canals. Iran Endod J 2017;12:34-7.
32. Vivekanandhan P, Subbiya A, Mitthra S, Karthick A. Comparison of apical debris extrusion of two rotary systems and one reciprocating system. J Conserv Dent 2016;19:245-9.
33. Razcha C, Zacharopoulos A, Anestis D, et al. Micro-Computed Tomographic Evaluation of Canal Transportation and Centering Ability of 4 Heat-Treated Nickel-Titanium Systems. J Endod 2020;46:675-681.
34. Pedullà E, Lo Savio F, Boninelli S, et al. Torsional and Cyclic Fatigue Resistance of a New Nickel-Titanium Instrument Manufactured by Electrical Discharge Machining. J Endod 2016;42:156-9.

Lettera DEL PRESIDENTE

Si sta per concludere un anno che difficilmente verrà dimenticato.
La memoria di eventi così traumatici non potrà essere cancellata, purtroppo li ricorderemo a livello globale.
Il Consiglio Direttivo ha lavorato attivamente, con tenacia, ha regnato un clima rilassato e costruttivo, ogni proposta è stata tenuta in considerazione e valutata con spirito di collaborazione: tutti uniti in un momento così destabilizzante.
Ed è questo aspetto che voglio ricordare della mia Presidenza: un clima di collaborazione, scevro da ineleganti individualismi, il cui intendimento comune è stata la determinazione di attraversare le difficoltà nel modo migliore possibile per il bene della SIE.
Voglio ora tirare le fila di un anno di lavoro intenso e ringraziare i membri del nostro Consiglio per questo *annus horribilis,* che speriamo non si ripeta.
Di solito, alla fine di ogni relazione si ringrazia la nostra unica, indispensabile, insostituibile, ineguagliabile ma anche decisa, gentile, simpatica e bella Gaia, senza la quale saremmo fortemente penalizzati… e questa volta voglio ringraziarla per prima: grazie Gaia!
Roberto Fornara, che ha preso sulle proprie spalle il coordinamento dei lavori in un momento davvero critico per la nostra Società. Mettendo da parte la carica di Presidente Eletto si è fatto promotore di scelte che mai avremmo pensato di dover prendere ma che, con il passare del tempo, si sono dimostrate vincenti per la nostra Società.
Cristian Coraini, il nostro Tesoriere: non avremmo potuto ben valutare e concretizzare le nostre decisioni se non fossero state accompagnate dalle sue stime economiche diligentemente stilate con dovizia di particolari.
Andrea Polesel, che con la sua passione è stato in grado di scegliere argomenti attraenti per sollecitare il richiamo e l'interesse da parte dei soci e dei non soci ai quali erano rivolti i Webinar, in fase di lockdown; non credo possa essere considerato un lavoro facile, ma lui ha centrato l'obiettivo vista la numerosa ed entusiastica partecipazione.
Denise Pontoriero, che da buona combattente ha saputo ben gestire la "comunicazione" con decisione e gentilezza. Le donne, infatti, anche in tempi attuali, devono dimostrare sempre più degli uomini per essere apprezzate e per questo bisogna avere coraggio di tirare fuori il carattere.
Massimo Gagliani e Claudio Pisacane, nuovi membri del Consiglio Direttivo, che sono stati fonte di utili consigli e proposte.
Grazie per il loro sostegno a entrambi i Revisori dei Conti Katia Greco e Franco Ongaro, amici da sempre.
Da parte mia voglio ringraziare tutti con un abbraccio forte e pare che, pur non avendo scelto, non solo non mi sono accontentata ma, certamente, sono stata accompagnata e sostenuta.

Ancora Grazie e Arrivederci all'Endodontic Week!

Il Presidente SIE
Dott.ssa Maria Teresa Sberna

In memoriam
del Prof. Gianfranco Borsotti

Lo scorso febbraio è mancato il Prof. Gianfranco Borsotti, Socio Fondatore della Società Italiana di Endodonzia, Presidente della nostra Associazione all'inizio degli anni Ottanta.
Già Libero Docente presso l'Università degli Studi di Milano, il Prof. Borsotti fu custode, in un modo signorile e raffinato, di quella che potremmo definire la prima Endodonzia nostrana; cultore dello scrupolo professionale che la disciplina imponeva in un'epoca ove la qualità della stessa appariva approssimativa.
In quell'epoca si disegnavano i contorni della materia che aveva vissuto periodi fulgidi nell'Europa pre-guerra, cancellati dalla cultura americana post-bellica; Gianfranco Borsotti aveva saputo connettere, con il tratto signorile, a volte quasi austero, le competenze del nostro continente con le novità tecniche di oltreoceano.
Nei giorni più spumeggianti della moderna Endodonzia, pur con il garbo che i suoi modi gli imponevano, era sempre presente nelle discussioni per ribadire, qualora si dimenticasse, il predominio della biologia sulla tecnologia e per enfatizzare il ruolo che le conoscenze mediche avevano nel determinare le scelte terapeutiche troppo spesso motivate da questo o quell'artificio meccanico.
Salutiamo nel Prof. Gianfranco Borsotti una figura, insignita a buon diritto del titolo di Socio Onorario, cardine nella nostra Società: quel che si dice un puntuto signore d'altri tempi, che aveva fatto del rigore, anche verso sé stesso, una ragione di vita.
Un esempio che non dovremmo dimenticare.

Il Presidente SIE
Dott.ssa Maria Teresa Sberna

Il Segretario SIE
Prof. Massimo Gagliani

STRUTTURA SOCIETARIA

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Zaccheo Dott. Fabrizio
Zerbinati Dott. Massimo
Zilocchi Dott. Franco

SOCI AGGREGATI
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Cuppini Dott.ssa Elisa
Fordellone Dott. Francesco
Giovinazzo Dott. Luca
Milani Dott. Stefano

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Revisore dei Conti
Greco Dott.ssa Katia
Revisore dei Conti
Ongaro Dott. Franco

SOCI SCOMPARSI
Ricordiamo con affetto e gratitudine i Soci scomparsi:

Attanasio Dott. Salvatore
Socio Attivo
Borsotti Prof. Gianfranco
Socio Onorario
Castagnola Prof. Luigi
Socio Onorario
De Fazio Prof. Pietro
Socio Attivo
Dolci Prof. Giovanni
Socio Onorario
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Socio Onorario
Spina Dott. Vincenzo
Socio Onorario
Zerosi Prof. Carlo
Socio Onorario

COME DIVENTARE SOCIO ATTIVO/AGGREGATO

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

SOCIO AGGREGATO

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

DOCUMENTAZIONE NECESSARIA PER DIVENTARE SOCIO AGGREGATO

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

SOCIO ATTIVO

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

DOCUMENTAZIONE NECESSARIA PER DIVENTARE SOCIO ATTIVO

Qualsiasi Socio Ordinario, con i requisiti necessari, può presentare la documentazione per ottenere la qualifica di Socio Attivo. Il Socio Aggregato che volesse presentare la documentazione scientifica e clinica a integrazione di quella clinica già approvata dalla CAS per lo status di socio Aggregato, potrà farlo già dall'anno successivo all'ottenimento della sua qualifica.
Un meccanismo a punti è stato introdotto per valutare il candidato a Socio Attivo. Un minimo di 200 punti è richiesto per divenire Socio Attivo.
Nella domanda non potranno essere presentati casi la cui somma superi i 240 punti per la qualifica di Socio Attivo.
La documentazione scientifica potrà essere presentata, a completamento della documentazione clinica, solo per la domanda per divenire Socio Attivo e non potrà superare i 100 punti.
La documentazione clinica dovrà presentare un minimo di sei casi, di cui almeno 4 di molari pluriradicolati con delle precise tipologie: tra questi casi almeno uno deve essere un ritrattamento con lesione visibile nella radiografia preoperatoria e dei restanti tre almeno due devono avere una lesione visibile nella radiografia preoperatoria.
La documentazione clinica non deve presentare più di un caso di Endodonzia Chirurgica Retrograda con immagini e non più di uno senza immagini.
La documentazione scientifica non potrà presentare più di due articoli come coautore.

MODALITÀ DI DOCUMENTAZIONE DEI CASI CLINICI

Criteri e modalità per la valutazione dei casi clinici idonei ad accedere alle qualifiche di Socio Aggregato e di Socio Attivo sono espressi nell'apposita sezione del Regolamento della Società Italiana di Endodonzia (SIE) all'indirizzo web: www.endodonzia.it.

CRITERI DI VALUTAZIONE

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

ADEMPIMENTI DEL CANDIDATO

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

PRESENTAZIONE DEI CASI ALLA COMMISSIONE

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

LA COMMISSIONE ACCETTAZIONE SOCI

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

MEMBRI DELLA COMMISSIONE ACCETTAZIONE SOCI BIENNIO 2019-2020

Past President della Società
Dott. **Enrico Cassai**
Dott. **Marco Colla**
Dott. **Mario Mancini**
Dott. **Pier Luigi Schirosa**

A cura del Dr. Davide Fabio Castro, Coordinatore di macroarea nord-ovest

Le nuove frontiere cliniche nell'Endodonzia moderna

Resoconto della giornata endodontica di macroarea nord-ovest

Viareggio, 26 settembre 2020 - Hotel Sina Astor

I relatori

In data 26 settembre 2020 si è svolta la Giornata Endodontica SIE di macroarea nord-ovest presso l'Hotel Sina Astor di Viareggio.
L'affluenza è stata molto soddisfacente. L'evento si è aperto alle 9,15 con un saluto da parte del Coordinatore di macroarea nord-ovest alle autorità presenti e un ringraziamento a tutte le società patrocinanti. I Relatori invitati hanno portato contenuti di altissima valenza scientifica.
Il dr. Castellucci ha trattato l'Endodonzia chirurgica mini-invasiva. Hanno fatto seguito il prof. Cantatore e la dr.ssa Greco che hanno presentato la loro esperienza clinica sui nuovi materiali bioceramici. Il prof. Pasqualini ha parlato dell'utilizzo della Cone Beam nei ritrattamenti mentre il dr. Alovisi delle tecniche di rigenerazione pulpare. Dopo il pranzo, condiviso dalle due tavole cliniche di Simit e Sweden&Martina, il dr. Polesel ha esposto i protocolli nei traumi dentali seguito poi dal dr. Zerbinati che ha trattato i riassorbimenti interni ed esterni. L'ultimo Relatore, dr. Papaleoni, ha concluso con la riabilitazione conservativa post-endodontica con indici di silicone. Una tavola rotonda finale ha offerto numerosi spunti di confronto clinico tra i partecipanti, chiudendo i lavori alle 17,45.

La platea

Le tavole cliniche

A cura del Dr. Luigi Scagnoli, Coordinatore di macroarea Centro

Decision Making in Endodonzia

Resoconto della giornata endodontica di macrearea Centro

Pesaro, 3 ottobre 2020 - Uappala Hotel Cruiser

Pesaro 3 ottobre 2020, la SIE c'è!
In mezzo a mille difficoltà, rinvii, rischi di cancellazione dell'ultim'ora, paura di non avere un pubblico adeguato, gli sponsor preoccupati da questa alternanza di sì e di no, siamo riusciti a tener fede all'impegno di svolgere la giornata della macroarea SIE del centro Italia.
L'evento ha avuto successo. Più di 80 partecipanti in una sala da 250 posti ridotti a circa 90 per le note restrizioni anti-Covid, una nutrita rappresentanza di sponsor e lo svolgimento di due tavole cliniche organizzate dalle aziende presenti. La sala, con una meravigliosa vista sul mare, ha fatto da cornice ai relatori che si sono susseguiti sul palco.
Tutto si è svolto seguendo alla lettera le indicazioni del Ministero, grazie all'organizzazione dell'hotel che ci ha ospitato e alla costante sorveglianza della nostra Gaia che si è adoperata in modo encomiabile affinchè tutto avvenisse nel pieno rispetto delle regole.
Il titolo della manifestazione, Decision Making in Endodonzia, è stato ampiamente rispettato dalle relazioni che hanno spaziato in quasi tutti i campi della disciplina endodontica, dal piano di trattamento nei casi multidisciplinari (Roberto Mancini e Giorgio Ban), strategie di sagomatura (Vito Antonio Malagnino), la CBCT (Emanuele Ambu), strategie in pedodonzia (Maurizio Bossù e Gianni Di Giorgio), rigenerativa e cementi bioceramici (Manuele Mancini), come valutare la guarigione delle lesioni endodontiche (Mario Mancini), il restauro post-endodontico (Marco Forestali), rendendo vivace, interessante e varia la giornata.
Ringrazio tutti i relatori che sono intervenuti, in special modo, e non me ne vogliano coloro che hanno avuto la fortuna di abitare nelle vicinanze di Pesaro, chi si è "sobbarcato" lunghi viaggi di andata e ritorno nella stessa giornata, per essere presente, non curante delle difficoltà e dei disagi da affrontare.
Questa partecipazione è stata un segnale importante da parte sia dei relatori che dei soci e degli studenti intervenuti, facendo così capire quanto la Società sia viva e vogliosa di riprendere una normale attività culturale e mettendo in evidenza l'attaccamento che noi tutti nutriamo per la SIE.

Apertura dei lavori.

Purtroppo quest'anno non si svolgerà il Congresso Nazionale in presenza e questi "piccoli" eventi assumono un valore ancora più importante in un momento così difficile, come anche la giornata della macroarea del nord-ovest svolta a Viareggio sotto la mirabile organizzazione dell'amico Davide Castro anch'essa seguita da un alto numero di partecipanti.
Un grazie ad Alessandra, Lucio e Stefano per il supporto datomi nell'organizzazione, che si sono preoccupati e occupati di tutte quelle piccole grandi cose di contorno che alla fine fanno la differenza.
Nonostante le paure e le ansie dei giorni che hanno preceduto l'ultimo l'evento del mio mandato posso dire con assoluta serenità che non poteva andare meglio di così. Rivolgo il mio ultimo ringraziamento al Consiglio Direttivo della SIE per avermi, e averci, concesso, questa possibilità.

A sinistra: la platea. In centro: un sentito grazie a chi ha organizzato e reso possibile questo evento. A destra: Relatore e Presidente di sessione.

MTWO, L'EVOLUZIONE DELLA TECNICA SIMULTANEA: LA NUOVA SEQUENZA

La sezione a *S italica* è stata "ripetuta" oramai da innumerevoli aziende, così come la sequenza dallo strumento piccolo verso i più grandi.
Come sempre accade, gli originali restano, comunque, gli *originali*: mai completamente replicabili. Gli *originali* si chiamano Mtwo, sono nati 20 anni fa e sono semplicemente *non migliorabili*.
Negli ultimi anni diverse aziende hanno provato a proporre delle misure per canali piccoli, affiancando semplicisticamente alle conicità 06 le conicità 04.
Sebbene riguardi un numero di canali non elevato, ci siamo posti il problema di una preparazione più "conservativa", diciamo "minimamente invasiva", per quei canali dove si voglia preservare (ulteriormente) lo spessore parietale.
Ne è nata una sequenza nuova, ancora una volta originale, breve, con 3 strumenti dalle misure inedite e non casuali: 10/.035, 17.5/.0475, 25/.05.
Questa sequenza è particolarmente indicata nei canali mesiali di molari inferiori di piccole dimensioni. In questi casi la parete distale dei canali mesiali è quella il cui spessore può essere critico. Il pericolo non è (utilizzando la sequenza standard) quello di assottigliare fino a perforare: questa è una evenienza mai descritta a proposito degli Mtwo; il pericolo è quello di lasciare degli spessori sottili che rendano il dente vulnerabile agli stress masticatori, e quindi passibile di frattura.
In realtà non è chiara la consistenza di questo rischio; riteniamo che un certo grado di preparazione nei canali sia indispensabile e che preparazioni troppo piccole possano condurre ad una insufficiente detersione e in alcuni casi a difficoltà nella gestione dell' otturazione.
A motivo di ciò ci siamo posti il problema di quale potesse essere la misura giusta per completare la preparazione in questi canali; dopo vari studi di analisi degli spessori tagliati e varie osservazioni cliniche, siamo arrivati alla conclusione che la taglia giusta fosse il 25 di conicità .05.
Lo strumento iniziale di questa nuova sequenza è un 10 di conicità .035, leggermente più piccolo dell'ormai tradizionale 10/.04, quindi più appropriato in canali di ridotte dimensioni. Lo strumento intermedio è quello risultato adatto a lavorare dopo il 10/.035 e, nello stesso tempo, in grado di lasciare il canale con delle misure abbordabili per il 25/.05.
Nessuno vieta di integrare alcuni passaggi con alcune misure della sequenza standard, ma questo potrà raramente rendersi utile, se non in casi molto difficili, non solo per dimensioni ma anche per lunghezza e traiettoria.

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Per maggiori informazioni: **www.sweden-martina.com**

La soluzione... il nuovo sistema di matrici circonferenziali Palodent 360 di Dentsply Sirona.

Le matrici Palodent 360 sono la miglior scelta per un restauro di II classe quando non è possibile utilizzare una matrice sezionale e per le ricostruzioni dei monconi. Uniscono tutti i vantaggi delle matrici circonferenziali Automatrix (best seller!) con quelli delle matrici sezionali Palodent V3, permettendo al contempo una semplicità assoluta di applicazione senza la necessità di retainer e di conseguenza anche una maggior visibilità e garantendo una forma della ricostruzione più anatomica.

L'assortimento del sistema di matrici circonferenziale Palodent 360 è composto da tre diverse misure:

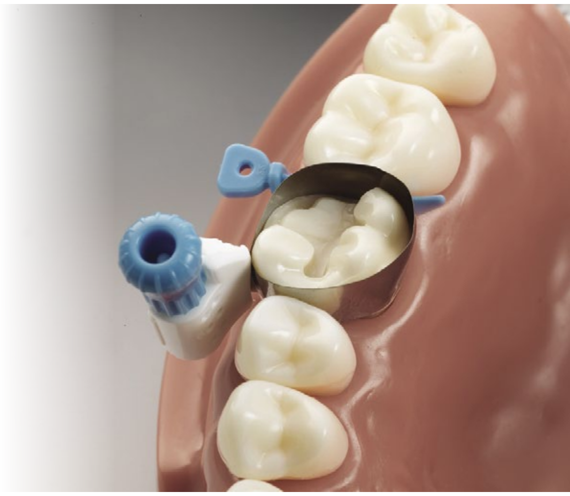
- 4,5mm
- 5,5mm (misura intermedia per la maggior parte dei casi)
- 6,5mm (dotata anche di estensione gengivale)

La matrice è composta da un corpo sul quale è posizionata una rotellina che permette di stringere la banda sagomata attorno al dente.
La banda sagomata che avvolge il dente ha uno spessore di 30µm (0,03mm).

Con due semplici gesti (posiziona e stringi) è possibile ora coprire tutte le necessità per le I e le II Classi e oltre.
Il posizionamento è facile e viene garantita una perfetta adattabilità.
Mezzo giro con le vostre dita è tutto quello che vi serve per ottenere punti di contatto precisi e un accurato profilo anatomico, senza essere ostacolati da scomodi applicatori e retainer.
Questo significa che potrete avere delle operazioni di rifinitura ridotte al minimo e ottenere dei risultati ineccepibili in brevissimo tempo.

Palodent 360, per restauri migliori, più veloci e più facili di quanto avreste mai potuto immaginare.

Nuove PALODENT 360...il successo arriva a tutto tondo.



Restauri efficienti

- Punti di contatto precisi
- Accurato profilo anatomico
- Ridotti tempi di finitura

Senza applicatori e retainer

- Posizionamento versatile
- Accesso facilitato e grande visibilità

PRENDI PARTE AI NUVI MODERN WORKSHOP, COLLEGATI CON IL NOSTRO ESPERTO VIVI UNA NUOVA ESPERIENZA OVUNQUE TI TROVI

Morita: Modern treatment & Modern Workshop

By Riccardo Tonini

TR ZX II l'unico, l'inimitabile

Da anni ormai **Il TR ZX2** è l'unico sistema del suo genere che integra la localizzazione Apicale e la preparazione del canale radicolare in un unico manipolo: è stato progettato per la massima sicurezza ed è dotato delle ormai consolidate funzioni **OTR** ed **OGP** .

L'OTR Garantisce sicurezza in qualsiasi tipo di canale, passando automaticamente dalla rotazione continua, quando il canale radicolare non presenta stress eccessivi, ad una di "reciprocazione" quando il canale diventa più ostico, cosi facendo, lo strumento endodontico utilizzato diminuirà la fatica ciclica e di conseguenza un eventuale frattura. Inoltre questo sistema aiuta a conservare la morfologia originale dei canali radicolari evitando false strade e facilita la rimozione dei detriti. Tutti questi fattori si combinano per aumentare la sicurezza abbreviando il tempo del trattamento.

L'OGP viene definita la nuova frontiera dello scouting Meccanico, fornisce una preparazione rapida e automatizzata del glide-path, che è la prima fase di preparazione effettiva, il funzionamento è molto semplice ed intuitivo. Impostato a 90 gradi di svincolo e 100 rpm, simulerà una tecnica manuale a carica d'orologio, come se fossero le vostre dita a compiere il movimento. Il vantaggio sarà però quello di poterlo utilizzare con i comuni strumenti Endodontic in Ni-TI che consentono maggiore flessibilità e sicurezza risparmiando inoltre la fatica ed il tempo della tecnica manuale. Inoltre grazie al display sempre posizionato davanti ai vostri occhi, avrete in tempo reale la posizione del vostro strumento.

La sua **piccola testina e il peso ridotto** (140 g), lo rendono maneggevole. La sua batteria a litio gli consentono un autonomia di oltre 11 ore.

MODERN WORKSHOP LIVE

Mandaci una E.mail all'indirizzo mc3com@hotmail.it, prenota il tuo Modern Workshop Live, potrai decidere se ricevere il materiale che verrà utilizzato compreso il nuovissimo **TRZX II** che potrai acquistare inseguito oppure renderlo senza impegno.

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L'applicazione alla strumentazione rotante della nuova tecnologia **FireWire™** rende gli strumenti più flessibili e più resistenti alla fatica ciclica.
Consente altresì un approccio minimamente invasivo sulla dentina, in virtù di un minore ritorno elastico; ciò permette agli strumenti di rispettare meglio l'anatomia originale e seguire il tragitto canalare in modo più semplice, preciso ed efficace.
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La parola ai clinici che usano con grande soddisfazione i file EdgeEndo

"Ho recentemente paragonato i file **EdgeEndo NiTi** alle sistematiche da me utilizzate. Sono stato piacevolmente sorpreso dalla loro flessibilità, durevolezza e resistenza alla frattura. Vantaggio più importante per me è stato

poterli utilizzare senza apportare variazioni alla mia tecnica di preparazione, ottenendo risultati altrettanto validi con un significativo risparmio economico. EdgeEndo è entrato a far parte della quotidianità dei miei trattamenti clinici".
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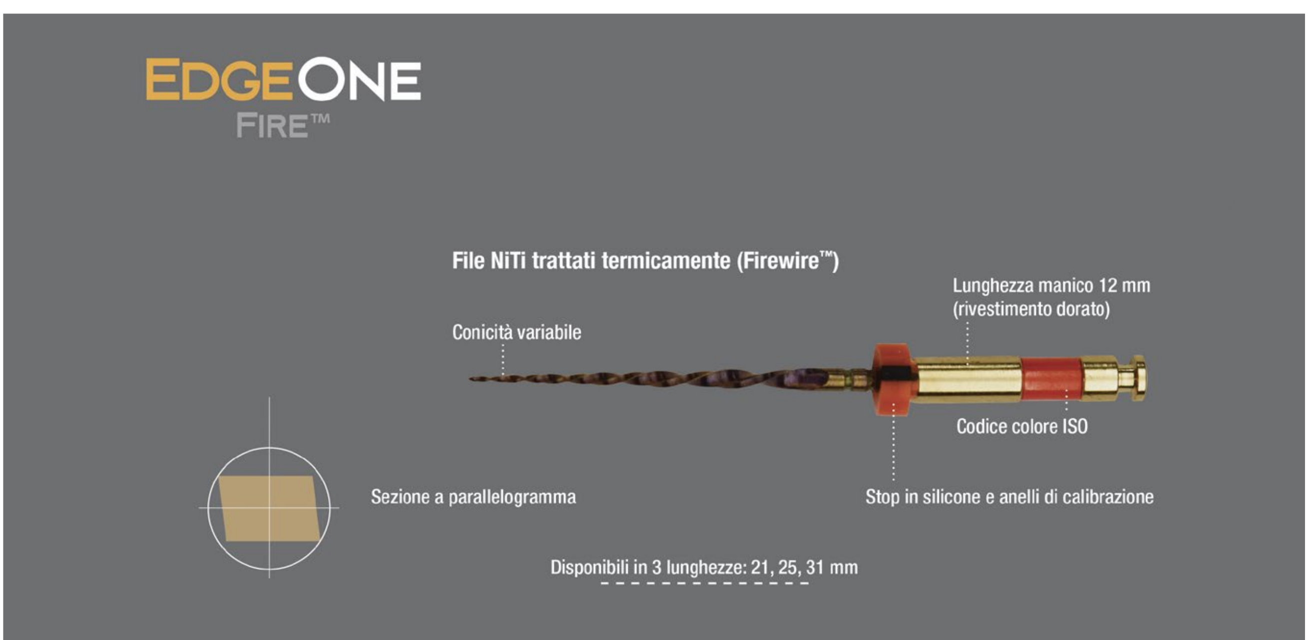

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It is a peer-reviewed journal, only available in electonic format and publishes original scientific articles, reviews, clinical articles and case reports in the field of Endodontology. Scientific contributions dealing with health, injuries to and diseases of the pulp and periradicular region, and their relationship with systemic well-being and health. Original scientific martticles are published in the areas of biomedical science, applied materials science, bioengineering, epidemiology and social science relevant to endodontic disease and its management, and to the restoration of root-treated teeth. In addition, review articles, reports of clinical cases, book reviews, summaries and abstracts of scientific meetings and news items are accepted. Please read the instructions below carefully for details on the submission of manuscripts, the journal's requirements and standards as well as information concerning the procedure after a manuscript has been accepted for publication in Giornale Italiano di Endodonzia. Giornale Italiano di Endodonzia is indexed in Scopus, Science Direct, Embase and published online by Ariesdue, Milan, Italy and hosted by PAGEPress, Pavia, Italy. All articles are available on www.giornaleitalianoendodonzia.it. The Journal is issued twice a year, in June and November.

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Ethical guidelines

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

Authorship and Acknowledgements

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

Manuscript preparation

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

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

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

Manuscript Types Accepted

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

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

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

Clinical Articles are suited to describe significant improvements in clinical practice such as the report of a novel technique, a breakthrough in technology or practical approaches to recognised clinical challenges. They should conform to the highest scientific and clinical practice standards.

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

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

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The **official language** of the publication is **English**. It is preferred that manuscript is professionally edited. All services are paid for and arranged by the author and use of one of these services does not guarantee acceptance or preference for publication.

Authors should pay special attention to the **presentation** of their research findings or clinical reports so that they may be communicated clearly.

Technical **jargon** should be avoided as much as possible and clearly explained where its use is unavoidable. **Abbreviations** should also be kept to a minimum, particularly those that are not standard. Giornale Italiano di Endodonzia adheres to the conventions outlined in *Units, Symbols and Abbreviations: A Guide for Medical and Scientific Editors and Authors.* If abbreviations are used in the text, authors are required to write full name+abbreviation in brackets [e.g. Multiple Myeloma (MM)] the first time they are used, then only abbreviations can be written (apart from titles; in this case authors have to write always the full name).

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The **background** and **hypotheses** underlying the study, as well as its main conclusions, should be clearly explained.
Titles and abstracts especially should be written in language that will be readily intelligible to any scientist.

Structure
All manuscripts submitted to Giornale Italiano di Endodonzia should include Title Page, Abstract, Main Text, References, Clinical Relevance, Conflict of Interest and Acknowledgements, Tables, Figures and Figure Legends as appropriate.
Title Page should bear:
I. Title, which should be concise as well as descriptive (no more than 150 letters and spaces);
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Abstracts should be no more than 250 words giving details of what was done.
Abstract for Original Scientific Articles should be no more than 250 words giving details of what was done using the following structure:
- **Aim:** give a clear statement of the main aim of the study and the main hypothesis tested, if any.
- **Methodology:** describe the methods adopted including, as appropriate, the design of the study, the setting, entry requirements for subjects, use of materials, outcome measures and statistical tests.
- **Results:** give the main results of the study, including the outcome of any statistical analysis.
- **Conclusions:** state the primary conclusions of the study and their implications. Suggest areas for further research, if appropriate.

Abstract for Review Articles should be non-structured, no more than 250 words giving details of what was done including the literature search strategy.
Abstract for Mini Review Articles should be non-structured of no more than 250 words, including a clear research question, details of the literature search strategy and clear conclusions.
Abstract for Case Reports and **Case Series** should be no more than 250 words using the following structure:
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- **Summary:** describe the methods adopted including, as appropriate, the design of the study, the setting, entry requirements for subjects, use of materials, outcome measures and analysis if any.
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- **Methodology:** describe the methods adopted.
- **Results:** give the main results of the study.
- **Conclusions:** state the primary conclusions of the study.

THE STRUCTURE

Main text for Original Scientific Articles
should include Introduction, Materials and Methods, Results, Discussion and Conclusion.
Introduction: should be focused, outlining the historical or logical origins of the study and gaps in knowledge. Exhaustive literature reviews are not appropriate. It should close with the explicit statement of the specific aims of the investigation, or hypothesis to be tested.

Material and Methods must contain sufficient detail such that, in combination with the references cited, all clinical trials and experiments reported can be fully reproduced.
(I) Clinical Trials: should be reported using the CONSORT guidelines available at www.consort-statement.org A CONSORT checklist and flow diagram (as a Figure) should also be included in the submission material.
(II) Experimental Subjects: experimentation involving **human subjects** will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki (version 2008) and the additional requirements, if any, of the country where the research has been carried out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. A statement regarding the fact that the study has been independently reviewed and approved by an ethical board should also be included. Editors reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used. When **experimental animals** are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations. All studies using human or animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study, if applicable. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

(III) Suppliers of materials should be named and their location (Company, town/city, state, country) included.

Results should present the observations with minimal reference to earlier literature or to possible interpretations. Data should not be duplicated in Tables and Figures.

Discussion may usefully start with a brief summary of the major findings, but repetition of parts of the abstract or of the results section should be avoided. The Discussion section should progress with a review of the methodology before discussing the results in light of previous work in the field. The Discussion should end with a brief conclusion and a comment on the potential clinical relevance of the findings. Statements and interpretation of the data should be appropriately supported by original references.
Conclusions should contain a summary of the findings.

Main Text of Review Articles
should be divided into Introduction, Review and Conclusions.

The **Introduction** section should be focused to place the subject matter in context and to justify the need for the review. The **Review** section should be divided into logical subsections in order to improve readability and enhance understanding. Search strategies must be described and the use of state-of-the-art evidence-based systematic approaches is expected. The use of tabulated and illustrative material is encouraged. The **Conclusion** section should reach clear conclusions and/or recommendations on the basis of the evidence presented.

Main Text of Mini Review Articles
should be divided into Introduction, Review and Conclusions; please note that the **Conclusions section** should present clear statements/recommendations and suggestions for further work. The manuscript, including references and figure legends, should not normally exceed 4,000 words.

Main Text of Case Reports and Clinical Articles
should be divided into Introduction, Report, Discussion and Conclusion. They should be well illustrated with clinical images, radiographs, diagrams and, where appropriate, supporting tables and graphs. However, all illustrations must be of the highest quality.

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Manuscript that do not conform to the general aims and scope of the Journal will be returned immediately without review. All other manuscripts will be reviewed by experts in the field (generally two referees). Giornale Italiano di Endodonzia aims to forward referees´ comments and to inform the corresponding author of the result of the review process. Manuscripts will be considered for fast-track publication under special circumstances after consultation with the Editor. Giornale Italiano di Endodonz-

ia uses **double blinded review** which menas that the names of the reviewers will thus not be disclosed to the author submitting a paper and the name(s) of the author(s) will not be disclosed to the reviewers. To allow double blinded review, please submit your main manuscript and title page as separate files.

Acknowledgements. Giornale Italiano di Endodonzia requires that all sources of institutional, private and corporate financial support for the work within the manuscript must be fully acknowledged, and any potential conflicts of interest noted. Grant or contribution numbers may be acknowledged, and principal grant holders should be listed. Acknowledgments should be brief and should not include thanks to anonymous referees and editors. Under this section please specify contributors to the article other than the authors accredited. Please also include specifications of the source of funding for the study.

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References should be prepared according to the **Vancouver style**. References must be numbered consecutively in the order in which they are first cited in the text (not alphabetical order), and they must be identified in the text by Arabic numerals in brackets [example (34)]. References to personal communications and unpublished data should be incorporated in the text and not placed under the numbered references [Example: (Wright 2011, unpublished data) or (Wright 2011, personal communication)]. Where available, URLs for the references should be provided directly within the MS-Word document.

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III. journal titles mentioned in the References list should be abbreviated according to the following websites:
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Examples of correct forms of reference follow.
Standard journal article
(1) Somma F, Cammarota G, Plotino G, Grande NM, Pameijer CH. The effectiveness of manual and mechanical instrumentation for the retreatment of three different root canal filling materials. J Endod 2008;34:466-9.

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

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

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

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

Published proceedings paper
DuPont B Bone marrow transplantation in severe combined immunodeficiency with an unrelated MLC compatible donor. In: White HJ, Smith R, eds. Proceedings of the Third Annual Meeting of the International Society for Experimental Rematology; (1974), pp. 44-46. Houston, TX, USA: International Society for Experimental Hematology.

Agency publication
Ranofsky AL Surgical Operations in Short-Stay Hospitals: United States-1975 (1978). DHEW publication no. (PHS) 78-1785 (Vital and Health Statistics; Series 13; no. 34.) Hyattsville, MD, USA: National Centre for Health Statistics.8

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

URLs
Full reference details must be given along with the URL, i.e. authorship, year, title of document/report and URL. If this information is not available, the reference should be removed and only the web address cited in the text.

Tables, Figures and Figure Legends

Tables should be submitted as word format, numbered and cited in the text of the manuscript. Units of measurements must be included in the column title or in the figure legend or caption. Figure files accepted: TIF, EPS, JPEG.

- color (saved as CMYK): minimum 300 dpi;
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- one column width (8.0 cm) or 1.5 column widths (13.0 cm) or 2 columns widths (17.0 cm).

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Authorship

All persons designated as authors should qualify for authorship according to the ICMJE criteria. Each author should have participated sufficiently in the work to take public responsibility for the content. Authorship credit should only be based on substantial contributions to

i) conception and design, or analysis and interpretation of data;
ii) drafting the article or revising it critically for important intellectual content;
iii) final approval of the version to be published.

These three conditions must all be met. Participation solely in the acquisition of funding or the collection of data does not justify authorship. General supervision of the research group is not sufficient for authorship. Any part of an article critical to its main conclusions must be the responsibility of at least one author. Authors should provide a brief description of their individual contributions.

Obligation to Register Clinical Trials
http://www.icmje.org/#clin_trials
The ICMJE believes that it is important to foster a comprehensive, publicly available database of clinical trials.
The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or concurrent comparison or control groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Medical interventions include drugs, surgical procedures,

devices, behavioral treatments, process-of-care changes, etc.
Our journals require, as a condition of consideration for publication, registration in a public trials registry.
The journal considers a trial for publication only if it has been registered before the enrollment of the first patient.
The journal does not advocate one particular registry, but requires authors to register their trial in a registry that meets several criteria. The registry must be accessible to the public at no charge. It must be open to all prospective registrants and managed by a non-profit organization.
There must be a mechanism to ensure the validity of the registration data, and the registry should be electronically searchable. An acceptable registry must include a minimum of data elements.
For example http://www.clinicaltrials.gov, sponsored by the United States National Library of Medicine, meets these requirements.

Protection of Human Subjects and Animals in Research
When reporting experiments on human subjects, authors should indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2013 (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects). If doubt exists whether the research was conducted in accordance with the Helsinki Declaration, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study. When reporting experiments on animals, authors should indicate whether institutional and national standards for the care and use of laboratory animals were followed. Further guidance on animal research ethics is available from the World Medical Association and from the International Association of Veterinary Editors' Consensus Author Guidelines on Animal Ethics and Welfare.
When reporting experiments on ecosystems involving non-native species, Authors are bound to ensure compliance with the institutional and national guide for the preservation of native biodiversity.

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