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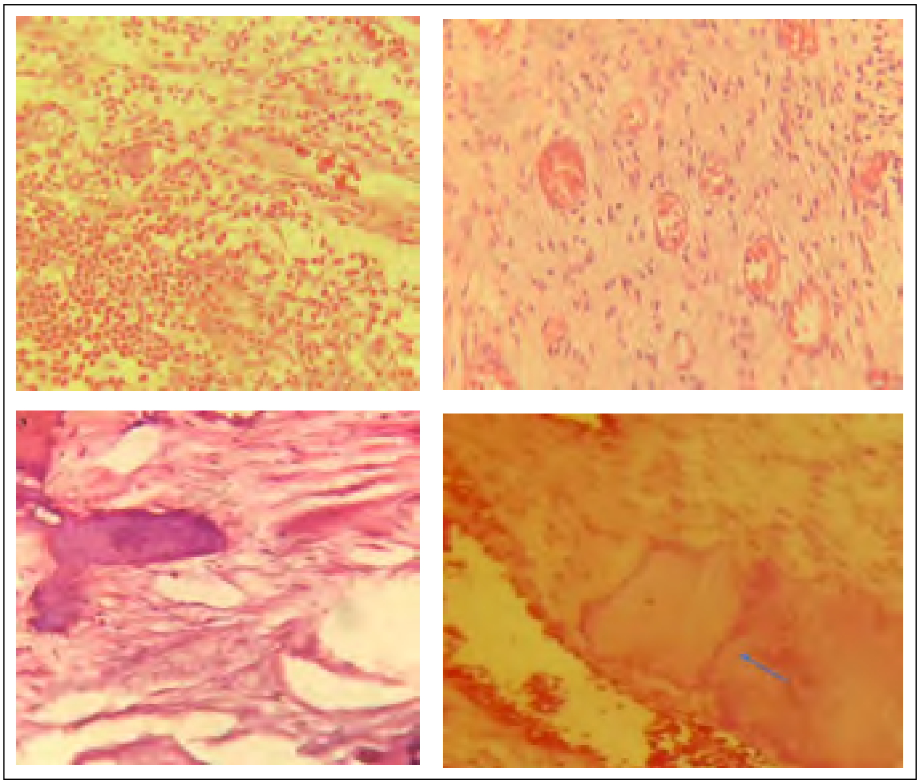

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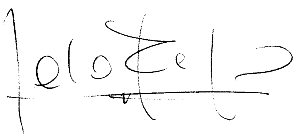

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After a full year of worldwide pandemic, the start of 2021 marks the desire for a strong rebirth and need of new perspectives.
One of the main discussed topic in endodontics is represented by the chance to "regenerate" the pulp tissue of necrotic tooth. The maintain of tooth vitality is of paramount importance to guarantee the long-term tooth survival. In this light, vital pulp therapy has gaining more and more interest, not only in the treatment of permanent immature teeth but also in mature ones. The possibility to preserve over time the pulp vitality of permanent teeth presenting inflammatory response represents a new challenge in endodontic field that, however, is not yet supported by strong scientific evidence. Calcium-silicate based cements had provided successful results when applied to preserve tooth vitality and are promising involved in total or partial pulp regeneration. Thanks to their biocompatibility and sealing ability, bioceramic cements are effectively used as perforation repair materials, endodontic sealers, root-end fillings (apical plug) and pulp dressing materials in vital pulp therapy. Nowadays, available evidence recommends MTA and calcium hydroxide as the gold standard materials for vital pulp therapy, even though other available calcium-silicate based cements provided comparable results.
In the present issue are collected several scientific articles that assess calcium-silicate based cements, particularly MTA, in distinct area of endodontics. Specifically, the improvement of physical-chemical properties and effectiveness of sealing ability in terms of bacterial leakage were evaluated. In addition, an interesting comparison of MTA and platelet-rich fibrin applied in direct pulp capping was conducted, providing preliminary but very interesting results. Therefore, the future trends will be represented by the improvement of traditional endodontic treatment, namely removal of inflamed or necrotic pulp tissue, and the introduction of alternative approaches of pulp tissue repair/regeneration using newly developed biomaterials and innovative technologies. Naturally, the evolution of latest materials should strictly respect the biological principles that represent the basis of tissue response.

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CASE REPORT

Multidisciplinary management of an external cervical resorption and cemental tear

ABSTRACT

Aim: *The case report presents the management of two different pathologies, external cervical resorption (ECR) and cemental tear, in two different central incisors, owing to the same predisposing factor i.e. trauma from occlusion.*
Summary: *A 53-year-old man was referred to the University Dental Clinic complaining of a pink spot that he noticed on his right maxillary central incisor. After thorough clinical and radiographic examination, including (FOV) cone-beam computed tomography (CBCT), revealed ECR classified as class 3Bp in tooth 11 and a probable cemental tear on tooth 21. A root canal treatment was performed on tooth 21 using a bioceramic sealer (Bioroot™ RCS). Subsequently, a modified papilla preservation flap was raised over the ECR lesion of tooth 11, followed by a complete rubber dam isolation, and blocking the root canal with a single gutta-percha cone. The defect was then restored with a resin modified glass ionomer cement (Geristore®). A simplified papilla preservation technique was then extended to treat the cemental tear on tooth 21, after which the root canal treatment for tooth 11 was completed. A 10-months follow-up examination showed a successful outcome with clinically stable gingival margins and no further evidence of ECR recurrence.*
Key learning points
- *ECR and cemental tear can occur together owing to the same predisposing factor.*
- *CBCT proves to be an indispensable tool in the detection and extent of ECR and cemental tears.*
- *The case report also confirms the easy handling, favorable physical and biological properties of Geristore® cement to restore ECR cavities.*

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Introduction

External cervical resorption (ECR) is a pathology that is often misdiagnosed as internal resorption or subgingival caries leading to tooth loss or incorrect treatment (1, 2). Heithersay classified ECR lesions from class 1 to class 4 based on two-dimensional (2D) radiography, according to the extent of the lesion (3). He also demonstrated that after a 3-year follow-up, the success rate of a class 1 lesion was 100% and a mere 12.5% for the class 4 category (3, 4). Patel et al. (2018) recently categorized this type of lesion using three-dimensional (3D) cone beam computed tomography (CBCT), according to the height, circumferential spread and proximity of the lesion to the root canal (5). This classification allows clinicians to perceive the extent of the lesion and plan an appropriate treatment (6).

The main predisposing factors for ECR are orthodontics, previous traumatic injury, poor oral health, and malocclusion (7). Some predisposing factors of ECR are also shared by cemental tears, such as previous traumatic injury, malocclusion, and even occlusal overloading. Cemental tear mostly affects the maxillary and mandibular incisors, is common in men aged over 60 years, and is only detected radiographically in 56.3% of the cases, although its detectability using CBCT is still questionable (8). In most cases its apicocoronal location makes accessibility difficult non-surgically and is a factor that usually inclines clinicians to choose a surgical approach to remove cemental tears.

Figure 1
A pre-operative clinical picture showing patients general oral health and a pink spot over the cervical third of tooth 11.

The occurrence of external cervical resorption and cemental tear together is a rare entity and according to the authors limited knowledge has not been reported yet in the literature. This case report describes the successful multidisciplinary management of two maxillary central incisors, one that presented with an external cervical resorption and the other with cemental tear, both most probable due to occlusal trauma.

Case Report

A 53-year-old male was referred to the Department of Endodontics (University dental clinic at the International University of Catalunya) complaining of a pink spot in his maxillary front tooth. Intraoral examination revealed probing depths of 7 mm with profuse bleeding on probing (BOP) on the buccal aspect of tooth 11 (Figure 1). Tooth 21 had probing depths of 7 mm on the buccal aspect with a gingival recession of 4 mm, resulting in a clinical attachment loss of 11 mm. Both teeth did not respond to neither cold nor electric vitality tests and had a Miller grade 1 mobility (9). Tooth 11 was negative to percussion/palpation while tooth 21 was positive to vertical percussion and palpation. Radiographic evaluation revealed a significant radiolucent area in the cervical portion of tooth 11 while tooth 21 had periapical radiolucency (Figure 2). A CBCT scan, taken with a limited field of view (FOV) of 5 x 8 cm (Planmeca Oy, Helsinki, Finland) at 0.8 mA and 85 kV, revealed an external cervical resorption with tooth 11. As the lesion was extending into the middle third of the root, with a circumferential spread between >90 to <180 degrees and seemed to have probable pulpal involvement, it was classified as 3Bp, according to Patel et al. (2018) (5). Tooth 21 also showed a radiopaque chip separated along the root that was presumed to be a cemental tear (Figure 2). Based on the radiographic and clinical examination, tooth 11 was diagnosed as a necrotic tooth with asymptomatic apical periodontitis with an external cervical resorption (classification 3Bp), and tooth 21 as necrotic pulp with symp-

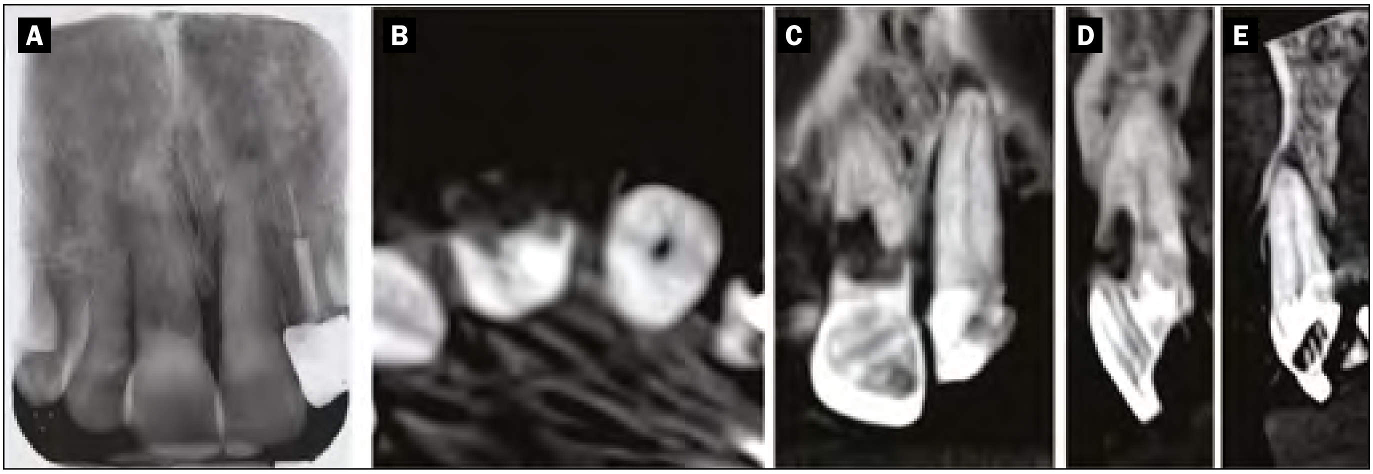

Figure 2
(A) Pre-operative periapical radiograph showing a radiolucent cervical lesion with tooth 11 and apical lesion with tooth 21.
(B) Axial CBCT cross-section showing the circumferential extent of the resorption with tooth 11.
(C) Sagittal CBCT cross-section showing the apico-coronal extent of the resorption with tooth 11 and apical radiolucency with tooth 21.
(D, E) Coronal CBCT cross-section showing the proximity of the resorption with the pulp space with tooth 11 and a cemental tear on the middle third of the root with 21.

tomatic apical periodontitis with recurrent periodontal abscess due to a probable cemental tear. From a periodontal point of view the diagnosis was periodontitis stage III grade B, given the attachment loss of ≥5 mm and no teeth lost; and since the percentage of bone loss to age was between 0.25 and 1.0. The patient also presented gingival recessions classified as RT2 and RT3 according to Cairo et al. 2011 (10); as well as generalized secondary occlusal trauma due to the loss of periodontal support.
The treatment plan was explained and an informed consent was obtained from the patient.
The treatment plan consisted of an initial phase I periodontal therapy (hygienic phase) with simultaneous occlusal adjustment. This was followed by an endodontic treatment of tooth 21 which was performed using absolute rubber dam isolation (Hu Friedy, Chicago, IL). After the access opening, a 10-k file (Mani, Inc, Shioya, Japan) with an electronic apex locator (Root ZX mini, J Morita Corp, Tokyo, Japan) was used to achieve patency and determine the working length. A radiograph was performed using a 15 k-file to confirm the working length and the canal was instrumented with Reciproc 25 (VDW GmbH, Munich Germany) using intermittent irrigation with 4.25% sodium hypochlorite (NaOCl) and 17% EDTA solution. Apical enlargement was performed using a 35.04 Profile instrument (Dentsply Maillefer, Ballaigues, Switzerland).
Final irrigation was performed with 96% alcohol and the root canal was dried and filled using a single master gutta-percha cone calibrated at 35.04 (Autofit, Sybron Endo, Kerr, USA) and a bioceramic sealer (Bioroot™ RCS, Septodont, France) according to manufacturer instructions. The tooth was immediately restored using a nano hybrid composite (Filtek™ Z250 XT, 3M, ESPE).
Due to the extent of the ECR lesion (Figure 2) on tooth 11 and the position of the cemental tear on tooth 21, an external and internal treatment approach was planned such that within a single intervention both lesions could be treated simultaneously. This plan also included orthodontic treatment to align and correct the malocclusion. A modified papilla preservation technique (Cortellini et al. 1995) (11) was performed over tooth 12 and 11 to expose the resorptive lesion. The granulation

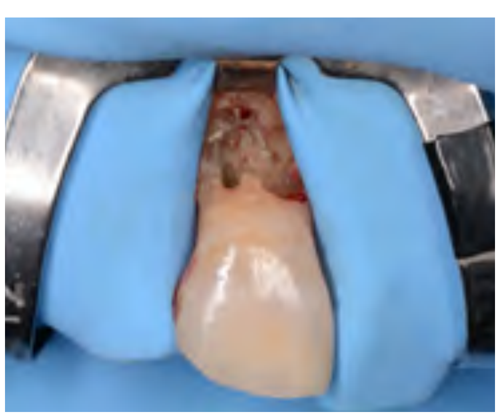

Figure 3
An absolute isolation of the resorptive cavity after excavation of the resorptive tissues.

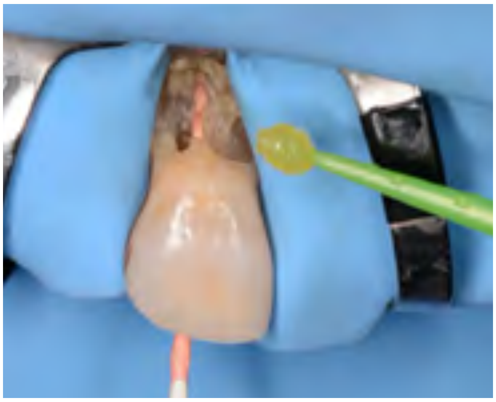

Figure 4
A gutta-percha cone used to block the root canal and the application of the bonding agent to receive the restorative material.

tissue was removed using small excavators and absolute rubber dam isolation was performed (Figure 3, see supplementary video). An access cavity was made and reciprocating instrumentation using Reciproc 25 up to the middle third was performed to temporarily block the canal using a gutta-percha cone as an aid in endodontic treatment following surgery (Figure 4). A cotton pellet impregnated in 90% trichloroacetic acid was applied to the cavity intermittently for 5 minutes. Thereafter, the cavity was irrigated with saline and etched with 35% phosphoric acid for 15s. A dual cure adhesive (ExciTE® F DSC, Ivoclar Vivadent, Ellwangen, Germany) was rubbed over the cavity for 10 seconds and then air-dried for 2 s. The cavity was then sealed with a dual-cure cement (Geristore, DEN-MAT Corporation, Santa Maria, CA), applied directly with its mixing tip. It was then light-cured for 40 s and polished using Perio-set (Intensiv SA) and Soflex discs (3M ESPE, St Paul, MN, USA). As already planned, the flap was then extended to the tooth 21 using also the simplified papilla preservation technique between tooth 21 and 22. Deep scaling and root planning were performed with ultrasonic and manual scalers, and cemental tears were carefully removed (Figure 5 and 6, see supplementary video).

After a thorough cleaning, the flap was repositioned and sutured. After 7 days the sutures were removed and root canal treatment was completed for tooth 11, which was then filled using warm vertical condensation (Elements Free, SybronEndo; Orange, CA). At the 11-day post-surgery control visit, the patient was completely asymptomatic. The patient refused orthodontic treatment, opting instead for a more aggressive prosthetic treatment. A 10-month follow-up radiograph showed that both the teeth were healing (Figure 7) and were clinically asymptomatic (Figure 8). A flow chart demonstrated the summary of the treatmnets performed (Figure 9).

Discussion

The effectiveness of CBCT for planning and treating ECR has been shown previously (12). One study demonstrated the use of CBCT-generated digital models of ECR to help evaluate the tooth structure

Figure 5
An intra-operative picture demonstrating complete removal of cemental tears from the root surface of tooth 21.

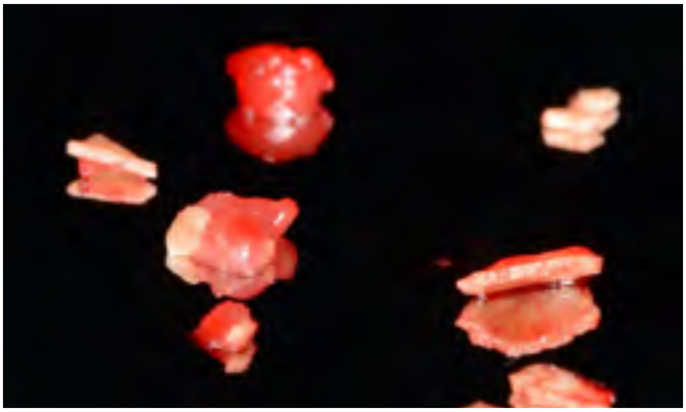

Figure 6
An image of the cemental tears removed from the root surface of tooth 21.

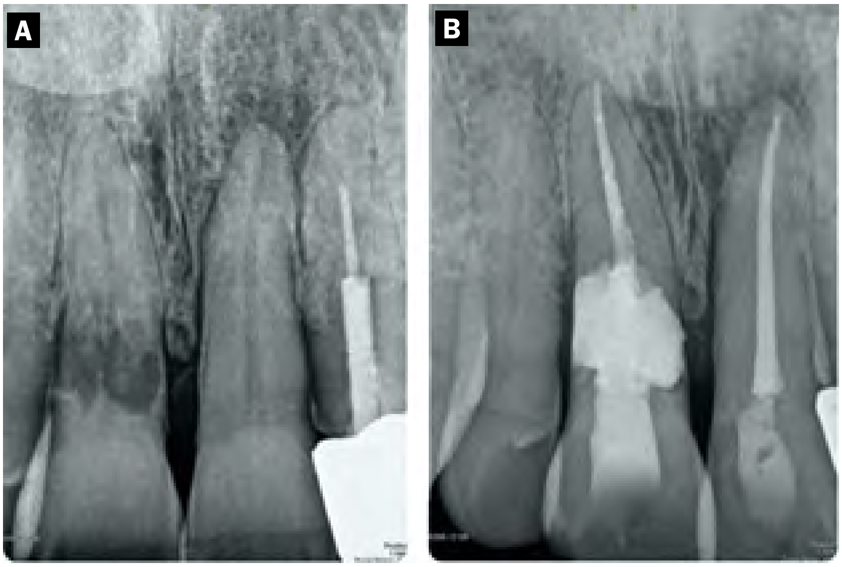

Figure 7
A comparison of **(A)** pre-operative and **(B)** 10-months post-operative periapical radiograph images demonstrating stability with no evidence of resorption recurrence.

Figure 8
A 10-month follow-up clinical picture demonstrating soft tissue stability with no evidence of resorption recurrence.

and plan a more conservative treatment (13). The recent 3D classification by Patel et al. (5) overcomes the limitations of the previous 2D classification by Heithersay (4) while also allowing clinicians to plan an effective and more conservative treatment. While radiographically cemental tears are only detected half of the time (8), in this case report it was only detected through CBCT. Although, a small chip on the apical third along the root surface of tooth 21 was observable on the CBCT scan, during the surgical intervention it became clear that more incomplete separated chips were present. This might indicate the low sensitivity of CBCT towards cemental tear in this case, but is still a better alternative to periapical radiography.

Heithersay recommended glass-ionomer cement as the material of choice for the restoration of ECR defects, although in this case Geristore®, a resin-modified glass ionomer cement was used. This material, compared to mineral trioxide aggregate (MTA) and glass-ionomer cement (GIC), has demonstrated to have enhanced biological behavior in human periodontal ligament cells and superior biocompatibility (14). Its fluoride-releasing properties and low shrinkage make this material a more reliable choice (15, 16).

The mechanism of cemental tear is unknown, but it has been linked with some predisposing factors such as >60 years of age, occlusal trauma, occlusal overloading, thicker or higher fragility of cementum, or also previous periodontal treatment. It largely affects the middle and apical third of the root and is related to recurrent periodontal abscesses (8). Hsueh-Jen Lin et. al. (8) had a success rate of 51.5% in 33 teeth treated for cemental tears. Almost half of the teeth (57.7%) were treated surgically, as was performed in the present case report. Lin et al. 2011 (8) considered cemental tear to be one of the reasons for the exposure of dentin that initiates ECR, however in this case it was not evident with tooth 11. External cervical resorption and cemental tear maybe difficult to diagnose clinically, although in this case report CBCT proved to be very helpful in detecting both the pathologies (6, 17). Some predisposing factors like occlusal trauma, occlusal overloading and even previous periodontal treatment are common for both the pathologies, which might be the reason of development of ECR in one and cemental tear in the other tooth. Moreover, neither the coexistence of ECR and cemental tear due to the same predisposing factor nor any protocol to treat the same has been published in the literature. A multidisciplinary approach should be considered and the elimination of the predisposing factor is as necessary for the complete resolution of similar lesions.

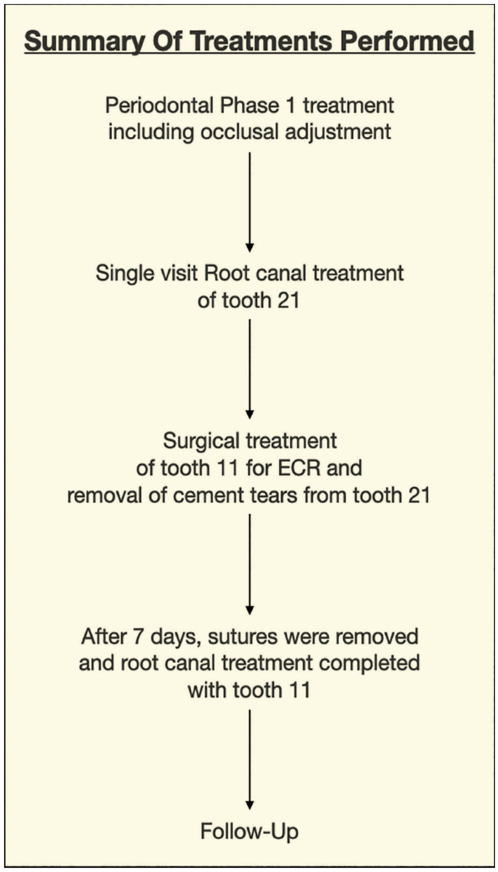

Figure 9
A flowchart demonstrating the summary of treatments performed.

Conclusions

The simultaneous occurrence of two different pathologies, ECR and cemental tear, in this case report had developed due to the same predisposing factor of trauma from occlusion. CBCT is an essential tool to diagnose cemental tear, although it cannot be relied on completely. An extensive evaluation of the restorability and the treatment outcome with the help of appropriate tools and knowledge should always be considered before treating such complex pathologies.

Clinical Relevance

Although occurrence of ECR and cemental tear is rare, a multidisciplinary approach with the correct use of CBCT is inevitable in such cases.

Conflict of Interest

The authors deny any conflicts of interest.

Acknowledgement

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CASE REPORT

Mandibular first molar root canal retreatment with the presence of a missed middle mesial root canal: a report of two cases

ABSTRACT

Aim: *The purpose of the present case report is to document the root canal retreatment of two mandibular first molars with a previously unnoticed MMC.*
Summary: *Mandibular molars may present a third root canal in the mesial root, defined as middle mesial root canal. This challenging anatomical variation should not be disregarded by the clinicians in order to minimize the possibility of an endodontic failure. This paper reports two cases of non-surgical endodontic retreatment of the mandibular first molar in which this complexity of the root canal system was missed in the first treatment, which may help to explain the treatment failure resulting in a periapical diagnosis of asymptomatic and symptomatic apical periodontitis in the first and second case, respectively.*
Key learning points
• The use of magnification, with the dental operating microscope, and of ultrasonic tips was of the utmost importance in locating this additional root canal orifice.
• Both cases were able to achieve a successful outcome only after a correct chemical-mechanical disinfection and three-dimensional obturation of all identifiable anatomy.

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Introduction

In order to achieve the best root canal treatment prognosis, the root canal system space should be properly scouted, debrided, disinfected and obturated so the occurrence, or continuity, of periapical periodontitis can be minimized (1, 2, 3, 4).
Due to its small prevalence and small diameter, root canals such as maxillary molars third mesiobuccal (MB3) (5) or mandibular molars middle mesial canal (MMC) (3the incidence of MMC was 18.6% (48 out of 258 molars), may go unnoticed, which may compromise the root canal treatment.
The presence of an MMC in the mandibular molars was first reported by Vertucci et al. (6) and Barker et al. (7), both in 1974. Pomeranz et al. (8), has classified the MMC into three categories. According to the author, the MMC may be presented as: "fin", when an instrument can pass freely between the mesiobuccal (MB) or mesiolingual (ML) canal and the MMC; "confluent", when the MMC starts as an independent pulp chamber orifice and joins apically with the MB or ML root canal; and "independent" when the MMC starts as a separate pulp chamber orifice and ends in an independent apical foramen. Moreover, and according to the same author, a broad single mesial canal in which three master cones could be cemented to the apex simultaneously can also be described as an independent MMC (8).
According to a clinical study from Weinberg et al. (4), an MMC prevalence of 13.7% may be expected, whereas the proportion of an ambiguous isthmus may reach the 52.9%, both assessed clinically and on cone-beam computed tomography (CBCT) imaging. However, and among the studies that have reported it, the MMC prevalence may range from 0.3% (9) to 46.2% (10) depending on the study criteria. Furthermore, Azim et al. (10) concluded that regardless of the reports of a higher prevalence of intercanal communications in mandibular molars mesial root (up to 83.0%), success in locating and accessing an MMC ranges from 1.0% to 25.0%, and failure to locate it may jeopardize the therapy.
The purpose of the present case report is to document, following the PRICE guidelines (11), the root canal retreatment of two mandibular first molars with a previously unnoticed MMC.

Case report

Case #1
A 47-year-old female patient was referred to the Endodontics Postgraduation clinic at University of Lisbon School of Dentist-

Figure 1
Pre-operative radiograph of tooth 46.

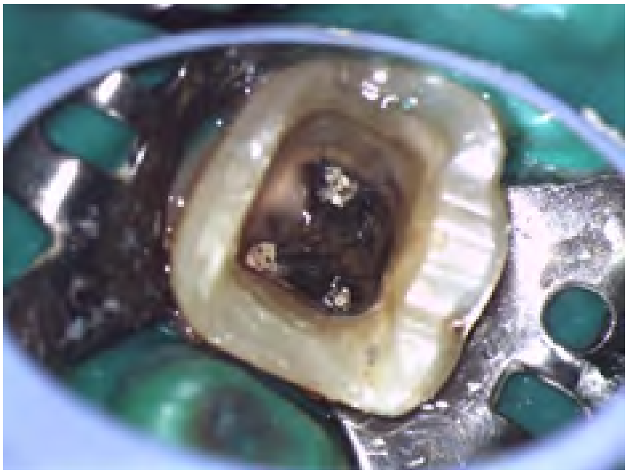

Figure 2
After endodontic access, two mesial obturated root canals were identified.

Figure 3
Working length radiography after identifying the MMC.

Figure 4
Pulp chamber view of the root canals instrumented.

Figure 5
Pulp chamber view of the root canals obturated.

Figure 6
Final disto-angulated radiograph of tooth 46.

Figure 7
12-month follow-up radiograph of tooth 46.

ry (FMDUL), in order to perform a non-surgical endodontic retreatment of tooth 46 (mandibular right first molar). Clinically a large restoration filling was present, and probing was within normal depths in all surfaces except distolingual, where a localized 7 mm pocket was observed. The tooth was asymptomatic and no pain to percussion was noted. Upon radiographic examination, a previous root canal treatment was observed, which was associated with a periapical lesion (Figure 1). According to the patient, the endodontic treatment had been performed a few years ago. A diagnosis of previous endodontic treatment and asymptomatic apical periodontitis was made. The root canal retreatment was suggested and accepted.

The treatment was performed under a Leica M320 dental operating microscope (M320, Leica Microsystems, Wetzlar, Germany), in multiple visits without using any intracanal medication in between visits. After proper anesthesia and rubber dam isolation, the access cavity was established (Figure 2) with round burs and improved with Start-X ultrasonic tips (Start-X, Dentsply Maillefer, Baillagues, Switzerland). The ultrasonics exploration of the isthmus connecting both mesiobuccal and mesiolingual canals, as previously documented (12), revealed an MMC. In addition two distal root canals were also observed. The root canals were negotiated with 0.10 and 0.15 stainless-steel K-files (Ready Steel, Dentsply Maillefer, Baillagues, Switzerland), and the working length determination was performed with a Root ZX electronic apex locator (Root ZX, Morita, Komuro, Japan) and confirmed radiographically (Figure 3). All root canals were shaped with R25 and R40 Reciproc files (Reciproc, VDW, Munich, Germany) according to the manufacturer's instructions (Figure 4). Copious irrigation with 5.25% sodium hypochlorite (Denta Flux, J. Ripoll SL, Murcia, Spain) using a 5 ml syringe and a 27G notched needle (Canal-Pro Slotted-End Tips, Coltene, Lezzenes, France) was performed throughout all the root canal treatment. In the last appointment, the root canals were dried with Zipperer paper points (Zipperer, VDW, Munich, Germany) and filled with gutta-percha and epoxy resin based sealer (AH Plus, Dentsply DeTrey, Konstanz, Germany) with a continuous wave of obturation technique (B&L, Biotech, Seoul, Republic of Korea) (Figures 5 and 6). The canals were sealed with a flowable composite resin (Supraflow, R&S CFPM, Tremblay-en-France France) and the crown was subsequently restored with a direct composite resin. At 12 months follow-up, the patient was completely asymptomatic and periapical radiography showed resolution of the periapical lesion (Figure 7).

Case #2

A 37-year-old female was observed in an endodontic appointment reporting permanent pain on tooth 36 (mandibular left first molar). Clinically it was possible to observe a large crown filling with no visible sec-

Figure 8
Pre-operative radiograph of tooth 36.

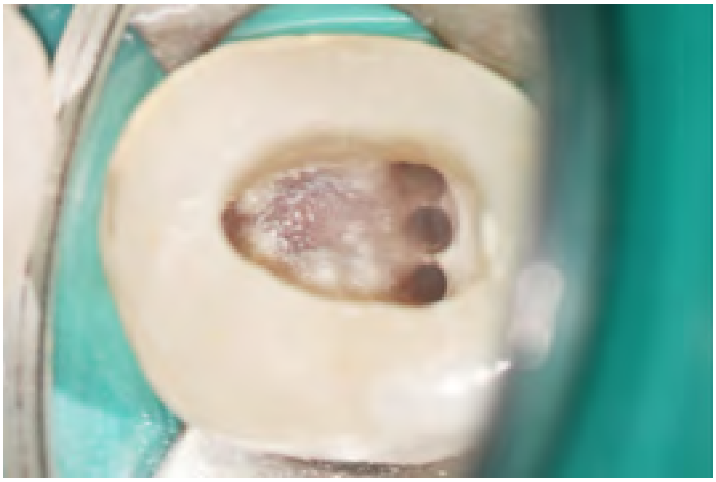

Figure 9
Pulp chamber view of the root canals instrumented.

ondary decay or fractures. The mobility and periodontal probing were within normal limits and the tooth was tender to percussion. Tooth 35 presented normal response to the cold sensitivity test (Endo cold spray, Henry Schein, Langen, Germany). The radiographic examination revealed a previous root canal treatment with an associated periapical lesion (Figure 8). A diagnosis of previous endodontic treatment and symptomatic apical periodontitis was made. The root canal retreatment was proposed and accepted by the patient.

The treatment was performed in two appointments. The tooth was anaesthetized with a buccal infiltration using 4% articaine with 1:200.000 epinephrine (Artinibsa, Inibsa, Barcelona, Spain), and a proper rubber dam isolation was achieved. After initial occlusal reduction, the proper access cavity was achieved using a round diamond and endo-z high speed burs. The main root canals (mesiobuccal, mesiolingual and distal) were identified and the previous gutta-percha filling removed with the help of chloroform solvent. The root canals were negotiated with 0.10 stainless steel K-files (Ready Steel, Dentsply Maillefer, Baillagues, Switzerland), and

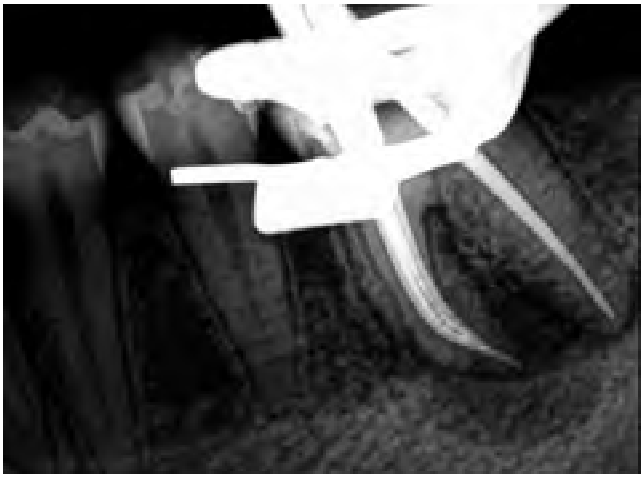

Figure 10
Master cone fit radiograph of tooth 36.

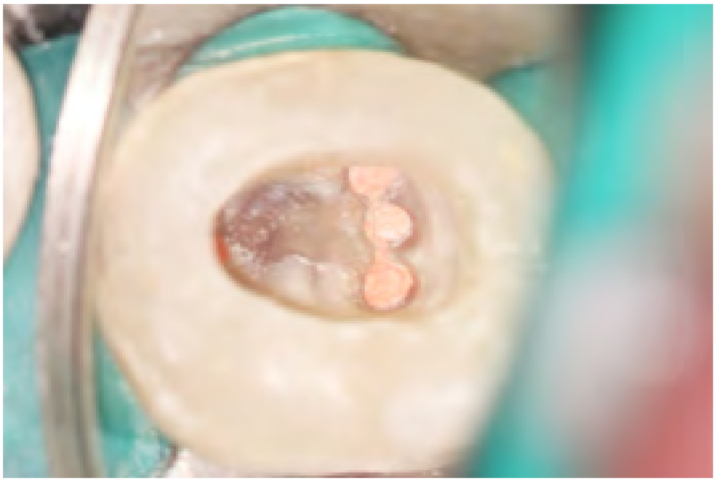

Figure 11
Pulp chamber view of the root canals obturated.

Figure 12
Final mesial-angulated radiograph of tooth 36.

Figure 13
32-month follow-up radiograph of tooth 36.

the working length determined by both radiograph and electronic apex locator (Root ZX, Morita, Komuro Japan). The identified root canals were shaped using the ProTaper Universal NiTi rotary system (ProTaper Universal, Dentsply Maillefer, Baillagues, Switzerland), according the recommendations of the manufacturer, up to F2 file in the mesial canals and F3 in the distal. Under the magnification of a dental operating microscope (Opmi Pico, Carl Zeiss, Jena, Germany) a groove in between mesial canals was explored using #2 ProUltra ultrasonic tip (ProUltra, Dentspy Maillefer, Baillagues, Switzerland), as recommended (12), and an MMC identified (Figure 9). This canal was shaped as previously mentioned for the others up to an F2 instrument. A continuous irrigation of 5.25% sodium hypochlorite was performed during the procedure. The root canals were dried and a calcium hydroxide paste (Ultracal, Ultradent, South Jordan, USA) was used as medication in between appointments. In the second visit the tooth was asymptomatic. A final irrigation protocol was performed with 17% EDTA and 5.25% sodium hypochlorite. The root canals were dried with paper points (ProTaper Universal, Dentsply Maillefer, Baillagues, Switzerland) and the final root canal filling was performed with gutta-percha and an epoxy resin based sealer (AH Plus, Dentsply De-Trey, Konstanz, Germany) using the continuous wave of obturation technique accomplished with a System B unit (System B, Sybron Endo, West Collins, USA) and with an Obtura II unit (Obtura II, Obtura Spartan, Chicago, USA) (Figures 10, 11 and 12). The crown was provisionally restored with Cavit (Cavit W, 3M ESPE, Seefeld, Germany). The tooth was sent to proper crown rehabilitation in the Oral Rehabilitation appointment. A radiographic 32 months follow up showed periapical healing and the patient remained asymptomatic (Figure 13).

Discussion

Although several factors have already been shown to be associated with higher prevalence of periapical lesion (13, 14), missed anatomy is one of the main causes for root canal treatment failure 15). Cantatore et al. (1) stated that missed canals resulted in high percentage of apical periodontitis and failed endodontically treated teeth. Moreover, the prevalence of post treatment disease was more commonly found in multirooted molars where the chance of missing an extra canal was higher (1, 16). Traditionally, mandibular molars are described as presenting two roots with two root canals on the mesial root and one or two canals in the distal one (17). However, the mesial root internal anatomy can be highly variable and complex. Anatomic features such as branching, accessory or dividing and rejoining main root canals, isthmuses and fins, at different levels, can be found on this particular root. Therefore locating an existing MMC, in order to decrease the microbial load in it, is of the outmost importance when aiming to maximize the root canal treatment success (2, 3, 18, 19). Regarding retreatment cases of mandibular molars a pooled success rate of 85.0% has been reported by Ng et al. (20), who also stated that the presence of pre-operative periapical lesion, apical extension of root canal filling and quality of coronal restoration, were significant prognostic factors that would, in fact, compromise the retreatment outcomes (20).

Methods of studying the tooth internal anatomy include the clearing technique (6, 8), plastic resin injection (21), histology (22), radiography (10, 17, 23), scanning electron microscopy (23), CBCT imaging (15, 22, 24), micro-computed tomographic (micro-CT) (16, 23, 25), micro-computed tomographic (micro-CT) imaging (2, 3, 26) or simply clinical findings (10, 17, 27). Each one of them presenting their own MMC proportions. When using magnification, such as loupes or microscope, the MMC percentages vary from 12.0% (8) to 46.2% (10). The results from populations assessment by using CBCT range from 0.3% in Korea (n=1952) (9) to 27.0% in Brazil (n=44) (28), although the sample size used in the latter was quite low. A large sample size screening on a Portuguese subpopulation confirms the lower MMC prevalence of 5.5% (n=450) (25). Regarding the micro-CT investigations, Versiani et al. (3) reported an MMC proportion of 18.6% (48 out of 258 mandibular first molars). A condition which was significantly higher in the Brazilians (22.1%) when compared to Turkish (14.8%) samples. Keles & Keskin (2), in a Turkey study, found 32 MMC in 106 mandibular first molars (30.2%) with 8 cases showing two MMC simultaneously. Such differences between methodologies might have origin on different sensitivity of the methods, the nature of the sample or different concepts of morphology (3, 10, 17).

Clinically, adequate coronal access and the use of the dental operating microscope are both crucial to overcome this challenge (28). Azim et al. (10) suggested troughing in the mesial root pulp chamber floor level, by 2-mm depth using a 1-mm-diameter round Munce bur head as a depth guide under a dental operating microscope visualization. However, this may result in iatrogenic mishaps while attempting to locate extra root canal orifices. The use of ultrasonic tips to clean the cervical isthmus, as performed on both presented cases, enables the identification of the majority of MMC (28), allows a more precise troughing and avoids iatrogenic procedural errors such as perforations or unnecessary weakening of the dentinal walls (3). On the other hand, and according to Keles & Keskin (19), 77.4% of the MMC orifices were located at the cemento-enamel junction level between the mesiobuccal and mesiolingual canal orifices, which means that troughing is not always mandatory in order to locate these extra canals.

The instrumentation of MMC with endodontic files is able to provide access and space for irrigating solutions into, otherwise inaccessible, isthmuses, reducing the bacterial load which can be suspected to be present in the initial radiographs of both presented cases (Figure 1 and 8) which presented apical radiolucencies. Choosing the right instrument to clean and shape the root canal space is as important as any other step, and in the present case report both reciprocation and rotary systems were use. It is known that the presence of the tissue remnants may affect the quality of canal filling and became a substrate for bacterial growth in unprepared areas (29), therefore several studies have been made comparing reciprocation and rotary instruments in regards to volume changes, increased surface area, and also the remaining unprepared areas (29). A recent systematic review on micro-CT studies concluded that both kinematics leave unprepared areas and produce changes in volume and surface area, and while reciprocating systems had higher volume and surface areas, the rotary ones left less unprepared surface areas (29). Having this into consideration both kinematics are apparently valid.

In the first case, the MMC was independent at the orifice of the pulp chamber floor and confluent to the mesiobuccal canal at its apical third, such as presented by Weinberg et al. (4) in 85.7% of their assessed sample. As for the second case, all the three canals started independently and joined in a single one at the apical third, which in the available literature was also documented by Azim et al. (10) and Akbarzadeh et al. (18) in 78.3% and 76.0% of their cases, respectively. The finding by Versiani et al. (3) that in the case of specimens with independent root canal orifices, MMC opening was found at the same mean distance between the mesiobucal and mesiolingual orifices, can be applied in our cases too.

Conclusions

Complex morphologies such as MMC may interfere with treatment prognosis. Nonetheless, as long as an effective chemo-mechanical disinfection and a three-dimensional obturation are achieved, the endodontic treatment outcome may turn out predictable and favorable.
This case reports highlights the importance of magnification and illumination when less common anatomic configurations are encountered. The clinician needs to be aware of the existence of this anatomy in order to achieve the best possible outcome.

Clinical Relevance

Mandibular molars may present a third root canal in the mesial root, defined as middle mesial root canal. The use of magnification, with the dental operating microscope, and of ultrasonic tips was of the utmost importance in locating this additional root canal orifice.

Conflict of Interest

None.

Acknowledgements

None.

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ORIGINAL ARTICLE

Effectiveness of a new electronic apex locator in two modalities in detecting the working length: an *ex vivo* study

ABSTRACT

Aim: *This ex vivo study aims to compare the accuracy of two electronic apex locators, Wirele-X (Forum Engineering Technologies Ltd., Israel), tested in two modalities, alone and connected to the display screen and Dentaport ZX (J. Morita, Tokyo, Japan) in determining the working length.*

Methodology: *15 single-rooted teeth were selected for this study. The actual microscopic working length was measured using a size 10 K-file that was advanced until the tip of the file could be visualized just within the apical foramen under a stereomicroscope. Then each tooth was placed into the alginate to simulate the clinical conditions. Wirele-X and Dentaport ZX were used according manufacturer's instructions. Three measurements were performed for each tooth and each apex locator and modality and differences between the electronic and actual working lengths were calculated. Positive values indicated measurements that extruded beyond the apical foramen, while negative values indicated measurements that were short of the apical foramen. Means and standard deviation were calculated and the statistical analysis was performed using One-way ANOVA and Tukey tests (P<0.05).*

Results: *The difference between electronic and actual working length was 0.05±0.34 mm for Wirele-X alone, 0.003±0.37 mm for Wirele-X connected to the display screen and 0.08±0.35 mm for Dentaport ZX. No statistical differences were found among Wirele-X alone, Wirele-X connected to the display screen and Dentaport ZX (p>0.05).*

Conclusions: *Wirele-X in both modalities and Dentaport ZX showed a high accuracy in determining working length and were accurate to within ±0.5 mm, without any statistical differences among them.*

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Introduction

The outcome of root canal treatment depends on shaping, cleaning and disinfection, removing the microorganisms from the entire root canal space, followed by an homogenous root canal filling (1).
The ideal end-point of a root canal therapy have been debated by many authors (2, 3). The cemento-dentinal junction is credited to be the ideal limit of a root canal therapy (4, 5), but this position is usually variable and cannot be clinically detected (3, 6). Clinically, the success rate of a root canal therapy increase when the shaping, cleaning and filling are located within 2 mm from the radiographic apex, in the region of the apical constriction (7, 8). However, the apical constriction, usually the narrowest part of the root canal, is also not easily identified (6).
Different methods have been used to establish the working length: radiography, tactile sensation, the anatomical average length of teeth and moisture of a paper point (5). The measuring of working length using radiographs has been used for many years and it had the limit of providing a two-dimensional image of a three-dimensional complex structure. ElAyouty et al. showed that the use of radiographs alone in working length determination led to an overstrumentation in 33% of molars and 56% of premolars (9). The introduction of electronic apex locators (EALs) has enabled, in addiction to appropriate radiographs, to determine a more predictable and accurate working length (10,11) and led to a reduction of the patient x-ray radiation exposure (12). In the last decades, different generations of EALs have been developed. The first generation measured the electrical resistance while the last generations measure alternating current impendance using one or more frequencies (13). Several studies have been conducted on different EALs to evaluate their accuracy in different conditions (14-17). Dentaport ZX (J. Morita, Tokyo, Japan) is a third generation EALs based on dual frequencies (8 and 0.4 kHz) and it is considered the gold standard EAL to which any new device should be compared. In fact, several studies have clearly demonstrated *ex vivo* (18) and *in vivo* (19) its precision. Among these studies, as an example, Puri et al. (2013) showed that Dentaport ZX had an accuracy in 93.3% of the samples and found a difference of the electronic measurement with the actual working length of 0.05±0.25 mm (20).
Wirele-X (Forum Engineering Technologies Ltd., Israel) is a new wireless EAL that can be used alone or in association with a 7" high-resolution touch display screen. The measurements are performed utilizing alternating current signals at two frequencies (500 Hz and 8 kHz) and are transmitted from the EAL to the display unit using Bluetooth technology. The manufacturer claims that the frequencies are alternated and not mixed, thus canceling the need for signal filtering and eliminating the noise caused by non-ideal filters. The signal measuring method utilized in Wirele-X has been patented (US Patent No. 6,425,875). To calculate file tip position, the RMS (Root Mean Square) level of the signal is used and not signal amplitude or phase. The RMS value, representing the energy level of the signal, is much more immune to various kinds of electromagnetic noises then other parameters of the measured signal.
To our knowledge, scientific data on this new EAL are still not available in the literature. Thus, the aim of this *ex vivo* study was to compare the accuracy of two EALs, Wirele-X in two modalities, alone and connected to the display screen and Dentaport ZX in determining working length in extracted teeth.

Materials and Methods

15 freshly extracted single-rooted teeth were selected for this study. Teeth were collected, debrided and disinfected in 5.25% sodium hypochlorite (NaOCl) for 2 hours and then stored in normal saline (0.9% NaCl) until used.
Two digital radiographs were taken in a bucco-lingual and mesio-distal direction to exclude samples with two canals, open

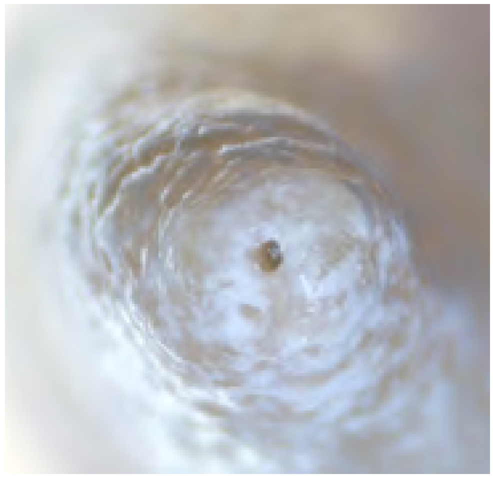


Figure 1
The determination of the actual working length (AWL) under stereomicroscope at 20X magnification. The file is visible through the major apical foramen.

apices, amalgam or composite fillings and previous root canal treatments. An access cavity was performed and size 10 and 15 K-files were inserted until the apex to confirm patency. When a size 20 K-file reached the apex, the tooth was excluded and replaced.
The actual microscopic working length (AWL) was measured using a size 10 K-file that was advanced until the tip of the file could be visualized just within the apical foramen under a stereomicroscope Zeiss Axiophot (Carl Zeiss Jena Gmbh, Zeiss Group, Jena, Germany) connected with a digital camera (Moticam Pro SMP) at a 20X microscopic magnification (Figure 1). Double stoppers were positioned for all measurements taken to decrease the possibility of stopper movement during all measurements.
Then each tooth was placed in a container filled with alginate that was obtained mixing the alginate powder with physiological solution to replicate the electric conductivity and simulate the oral environment. The teeth were inserted leaving 5 mm of the coronal root surface exposed (21). The lip clip was placed into the alginate and the wire of the EAL was connected to the file (Figure 2).
Wirele-X and Dentaport ZX were used according to the manufacturer's instructions. When the Wirele-X was used alone without the Bluetooth connection with the display unit, the size 10 K-file was inserted gently until the last red bar appeared on the device and then retracted until the apical position was reached (orange bar at the mark "0.0") (Figure 3). When the Wirele-X was used connected via Bluetooth with its display unit, the size 10 K-file was gently advanced until the red "blood drop" icon appeared on the screen and warning sound designated that the file has passed the Apex and then withdrawn to the red bar at the mark "0" and reading "APEX" (Figure 4). For Dentaport ZX the size 10 K-file was inserted until the apex reading was reached at the first red bar and then withdrawn to the last green flashing bar on display (Figure 5). Measurements were considered as valid if the reading remained stable for at least 5 seconds. Each measurement was repeated three times for each tooth and each EAL and modality and all working lengths were measured on the file using a digital caliper.
Differences between the electronic working length (EWL) and the AWL were calculated. Positive values indicated measurements that extruded beyond the apical foramen, while negative values indicated measurements that were short in the apical foramen. Means and standard deviation were calculated for each group and the statistical analysis was performed using One-way ANOVA and Tukey tests with a significant difference set at $P<0.05$.

Figure 2
Image showing the experimental set-up.

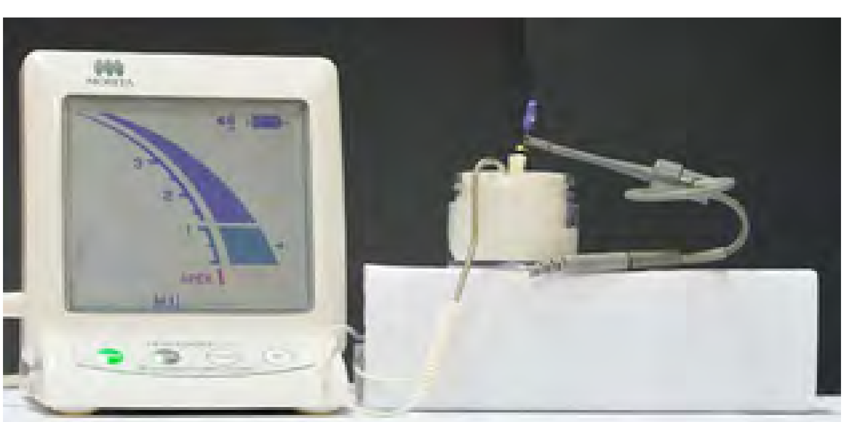
Results

Considering the margin of accuracy ±1 mm, all the EALs showed an accuracy of 100%. When considering the margin of accuracy ±0.5 mm, Wirele-X alone, Wire-

Figure 3
The orange bar at the mark "0.0" of Wirele-X device that appears when the file reached the apical position.

Figure 4
The display unit connected via Bluetooth to Wirele-X device with the screen showing the red bar at the mark "0".

Figure 5
Dentaport ZX showing the last green flashing bar on display.



le-X/Display and Dentaport ZX showed an accuracy of 88.8 %, 82.2% and 86.6% respectively. The main difference between EWL and AWL was 0.05±0.34 mm for Wirele-X, 0.003±0.37 mm for Wirele-X/Display and 0.08±0.35 mm for Dentaport ZX (Figure 6). No statistical differences were found among Wirele-X alone, Wirele-X/Display and Dentaport ZX (p>0.05).

Discussion

The goal of this study was to evaluate *ex vivo* the accuracy of the new Wirele-X EAL in two modalities, alone and connected to the display screen and compare it to the Dentaport ZX. Many authors have evaluated the accuracy of EALs considering the apical constriction (22) or the major foramen (23), which seems to be more reproducible (24).

Several materials have been proposed by many authors to simulate periodontal ligament to test *in vitro* EALs: gelatin (25), agar-agar (26), saline (27), flower sponge soaked in saline (28) or alginate (21). Alginate as a substitute for periodontal ligament was investigated by Lipski *et al.* who showed a 100% rate of correct measurement (29). On the contrary, gelatin, agar-agar, saline and flower sponge soaked in saline showed a rate of 96.7%, 76.7%, 73.4% and 63.4% respectively (28). For this reason, alginate was used in the present study to ensure the best medium possible for testing the EALs *ex vivo.*

In the present study, single-rooted teeth with narrow root canals were selected to standardise the samples and a size 10 K-file was used to obtain all the AWLs and EWLs. In fact, Ebrahim et al. reported that, when the diameter of a root canal increased, the electronic measurement with a small K-file become shorter (30).

The accuracy of the majority of the latest generations of EALs is not affected by irrigants within the root canal (31). Çınar et al. compared *in vivo* the accuracy of Propex Pixi, Mini Root ZX, Raypex 5 in determining working length in presence of blood-pulp tissue or sodium hypochlorite using micro-computed tomography. There were no differences among working lengths measured in different conditions. In a systematic review and meta-analysis Tsesis et al. similarly stated that the presence of vital or necrotic pulp has not effect on the precision of EALs (32). In the present experiment, conducted in normal condi-

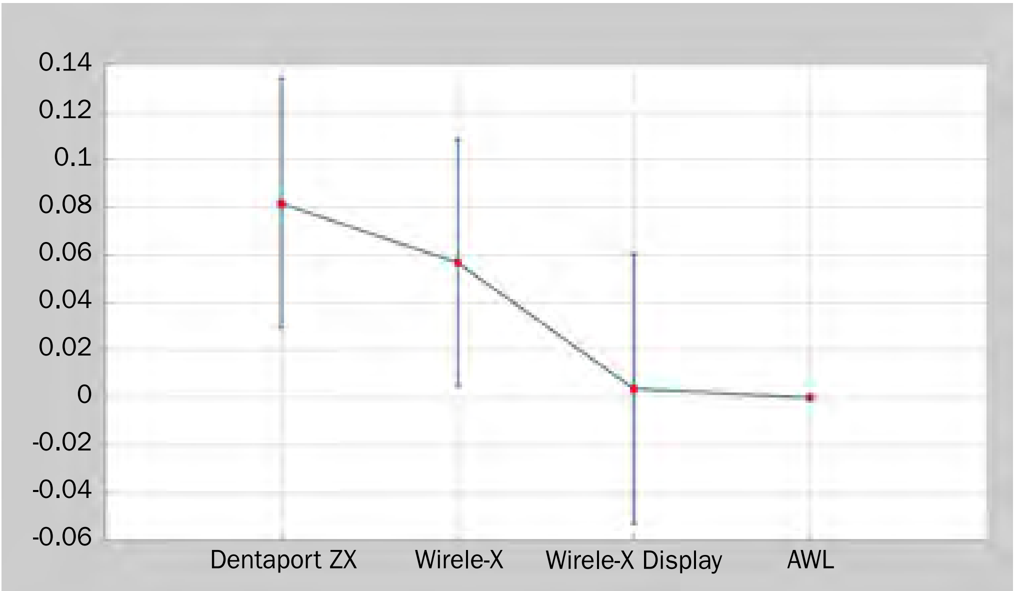


Figure 6
Distribution of positive and negative values of electronic working length (EWL), standard deviation and the actual working length (AWL).

tions, the EWL measurements have been very accurate for both Wirele-X, alone or connected to the display screen, and Dentaport ZX.

Regarding the accuracy of Dentaport Zx, the results of the presence study are generally consistent with previous *in vivo* and *in vitro* investigations. Saatchi et al. *in vivo* found that Dentaport ZX showed an accuracy within ±0.5 mm of 93.8% and 93.3% in presence, or not, of apical periodontitis (33). Piasecki et al. showed *in vivo* that the apical foramen was accurately located by Root ZX II within ±0.5 mm in 83% of the teeth with apical periodontitis and in 100% of vital teeth (34). Comparing working length determination *in vivo* and *in vitro*, Duran-Sindreu et al. reported that Root ZX was accurate 74% of the time to ±0.5 mm *in vitro* and 78.3% of the time to ±0.5 mm *in vivo* (35). Connert et al., using Micro-CT to calculate the distance between the K-file and the minor and the major foramen, found an accuracy of Dentaport ZX of 99% and 100% in detecting major foramen, within a tolerance of ±0.5 mm or ±1 mm respectively (14). Stöber et al., under *in vivo* clinical conditions, measured a mean distance from the AWL to the file tip of 0.146 ± 0.43 mm and an accuracy of 72% within ±0.5 mm and 100% of the time within ±1 mm (36). Pascon et al. reported, within a tolerance of ±0.5 mm or ±1 mm, an accuracy of Dentaport ZX of 39% or 90% respectively (18). The results obtained in the present study are in agreement with most of the mentioned studies. The different percentages obtained in all these studies for the accuracy of Dentaport XZ could be explained by the method used to establish the actual working length (AWL).

Wirele-X was tested for the first time in the present study as no previous scientific literature has been published on this EAL, which has obtained comparable results with Dentaport ZX in both modalities tested ($p>0.05$). Wirele-X/Display showed the best results concerning the difference between EWL and AWL, demonstrating that the connection via Bluetooth has not affected the accuracy of this EAL. Possible advantages in the use of Wirele-X EAL can be the notably small size of the EAL unit and the possibility to attach it to the dental dam for a more comfortable and ergonomic use. The Wirele-X shows the movement of the file inside the canal from the beginning of the measurements to the end, providing uninterrupted feedback. Proprietary software algorithms are used for calculations of file tip position and file movement in different parts of root canal. Clearly distinguished graphical readings in the apex region accompanied by audio signals enable better control over the file advance. In case of over-instrumentation a red "blood drop" icon and warning sound designate that the file has passed the Apex. Numerical values changing from +0.1 to +0.5 indicate relative depth of over-instrumentation, a useful feature for patency testing. If the file tip penetrates deeper, the "OVER" reading appears.

Conclusions

Under the limitations of this *ex vivo* study, Wirele-X, alone and connected via Bluetooth with its display, and Dentaport ZX showed high accuracy in detecting the working length and were accurate to within ± 0.5 mm, without any statistical differences among them.

Clinical Relevance

All apex locators tested in this study can be recommended for clinical practice.

Conflict of Interest

The authors declare that there is no conflict of interest.

Acknowledgements

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ORIGINAL ARTICLE

Effects of mineral trioxide aggregate and platelet-rich fibrin on histological results of direct pulp capping in dogs

ABSTRACT

Aim: This study aimed to compare the effects of mineral trioxide aggregate (MTA) and platelet-rich fibrin (PRF) on histological results of direct pulp capping (DPC) in dogs.
Methodology: In this animal study, 36 class V cavities were prepared in the incisors of adult healthy mixed-breed dogs. The teeth were then randomly divided into three groups. No material was placed in the control teeth. MTA and PRF were placed on the exposure site in groups 2 and 3, respectively. After two months, the teeth were extracted under general anesthesia and were histological analyzed regarding inflammation, calcified bridge formation and necrosis. Data were analyzed using non-parametric Kruskal-Wallis test. Pairwise comparisons were made using the Mann-Whitney U test.
Results: There are no statistically significant differences in terms of pulp inflammation, dentinal bridge formation and necrosis among the treatment groups capping with MTA and PRF. (P Value>0.05), however, PRF and MTA were the same in all parameters, these groups were both significantly superior to the control group.
Conclusions: Within the limitations of this study, PRF can be used for DPC as an alternative to MTA.

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Introduction

The viability of dentin-pulp complex is imperative for tooth vitality and is therefore a priority in clinical control strategies. Vital pulp therapy is performed aiming to preserve the vitality of the residual healthy pulp tissue for structural and functional regeneration of dentin-pulp complex (1). Vital pulp therapy is commonly used for carious teeth or those with traumatic injuries (2). Vital permanent teeth with no sign of irreversible pulpitis or apical periodontitis may be good candidates for vital pulp therapy (3) since they have the potential for regeneration and healing of the remaining radicular tissue, and the materials used for this purpose, have to be biocompatible (4).
Vital pulp therapy requires biomaterials to cover the exposed pulp tissue as a protective barrier in direct pulp capping or pulpotomy (2, 4).
Novel biological treatments are required to resolve the pulpal inflammation and induce dentinogenesis by the pulp tissue in order to increase the success rate of vital pulp therapy.
The pulp capping agents should have bio-interactivity (release of biological ions) and also be able to produce apatite crystals (2, 5). The pulp capping agents should provide a suitable environment for regeneration of dentin-pulp complex. Moreover, they have to be non-toxic and possess antibacterial activity to induce the differentiation of odontoblast-like cells (2, 6).
Calcium hydroxide and its derivatives have been the gold standard for preservation of pulp vitality in pulp capping treatments since the 1920. However, high solubility and early loss are among the drawbacks of calcium hydroxide (1). A previous study evaluated different formulations of calcium hydroxide and indicated the formation of dentinal bridge in 50% to 87% of the cases (7). Also, teeth pulp capped with calcium hydroxide have shown its limited efficacy for pulp tissue healing and regeneration. Thus, research is ongoing to find biocompatible materials inducing pulp tissue regeneration in clinical studies. Despite the numerous applications of calcium hydroxide, it has shortcomings such as creation of tunnel-like defects in the induced dentinal bridge, poor adhesion to dentin and absence of permanent seal.
Calcium-silicate based cements, the most important of which being mineral trioxide aggregate (MTA), are promising alternatives to calcium hydroxide for this purpose since they have shown favorable properties in animal models (8).
Following the hydration of MTA in presence of blood and other biological fluids, calcium hydroxide is formed. Moreover, MTA biologically induces the pulp cells (2, 8). MTA provides a long-term seal and is biocompatible. Freshly mixed MTA is relatively cytotoxic due to its high pH; however, it has applications in vital pulp therapy, and increased durability of teeth pulp capped with MTA has been reported (9).
Recently, platelet concentrates are increasingly used to enhance wound healing and cause soft and hard tissue regeneration after different surgical procedures. Blood clots after surgical procedures initiate the process of repair and regeneration of the hard and soft tissues. Use of platelet concentrate is one strategy to enhance natural wound healing mechanisms. A natural blood clot mainly includes red blood cells, around 5% platelets and less than 1% white blood cells. In fact, platelets not only participate in forming clot but also release important growth factors that initiate and support wound healing (10). Assessment of the mechanisms of actions of growth factors and their extraction from platelets led to the increasing use of platelet-rich plasma (PRP) in different fields of oral surgery.
Platelet-rich fibrin (PRF) is obtained by eliminating the middle layer of a centrifuged blood sample. PRF was first described by Choukran et al. (2006) and is known as the second-generation platelet concentrate. Also, PRF has numerous advantages over PRP including easy preparation and absence of blood manipulation, which indicates the obligation for it to be autologous (11).
PRF clot forms a strong natural fibrin

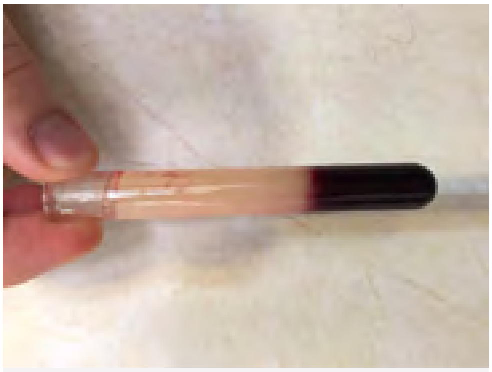

Figure 1
White buffy coat above the clot (PRF).

Figure 2
Cavity preparation using an inverted bur and pulpal exposure.

Figure 3
Restoring the cavities with Zonalin.

matrix, which includes all the platelets and growth factors of the blood sample and has a complex structure, which can best serve as a matrix for regeneration and healing. It possesses some favorable mechanical properties that other platelet concentrates do not have. PRF can serve as a biomaterial with a natural fibrin matrix to enhance micro-vascularization and guide cell migration towards the wound site. Thus, PRF has been recommended as a pulp capping agent for formation of reparative dentin or as a biomaterial for pulp regeneration (12).
This study aimed to assess the effects of application of MTA and PRF on the success rate of DPC in incisor teeth of dogs.

Materials and Methods

This animal study was conducted in line with the guidelines for the care and use of laboratory animals. In order to prepare PRF, 10 mL of blood was collected from the jugular vein of dogs. Healthy, mixed-breed adult dogs weighing 18±3 kg were chosen for this study. The dogs were refrained from eating for 8 hours prior to the surgical procedure. The blood was collected in tubes without anticoagulant agents such as EDTA. After 1 minute, the tube containing the blood was centrifuged for 10,000 cycles for 12 minutes. The white buffy coat (Figure 1) above the clot, which was PRF, was collected. General anesthesia was then induced using 0.01 mg/kg acepromazine as premedication. A combination of ketamine and diazepam with 8.5 mg/kg and 0.2 mg/kg dosage, respectively was administered intravenously for anesthesia induction. Anesthesia was continued by inhalation of isoflurane in oxygen following intubation.
Oral cavity was rinsed with 1.2% chlorhexidine, and infiltration anesthesia was administered using lidocaine plus epinephrine. Cervical cavities were prepared in nine maxillary and mandibular incisor teeth using an inverted conical bur. As soon as the pink shadow of the pulp tissue was observed through a thin layer of dentin, the teeth were isolated with cotton rolls and the pulp chamber was exposed using a #2 dental explorer. The cavities were rinsed with saline. Cervical cavities were prepared in a total of 36 incisor teeth (Figure 2). The teeth were then randomly divided into three groups. In the control group, no pulp capping agent was placed

Table 1

Degree of inflammation in the control, MTA and PRF groups

inflammation Degree Group	0×	1××	2×××	3××××	Total
Control	0	2 (16.07%)	5 (41.07%)	5 (41.07%)	12 (100%)
MTA	7 (58.03%)	4 (33.03%)	1 (8.03%)	0	12 (100%)
PRF	3 (25.00%)	9 (75.00%)	0	0	12 (100%)
Total	10 (27.08%)	15 (41.07%)	6 (16.07%)	5 (13.09%)	36 (100%)

× *Absence of inflammatory cells*
×× *Mild inflammation*
××× *Moderate inflammation*
×××× *Severe inflammation*

on the exposure site. In groups 2 and 3, MTA and PRF were applied on the exposure site, respectively. Then entire cavity in all groups, was restored with Zonalin (Zinc oxide eugenol, Purton, Wiltshin, Sweden) (Figure 3). The teeth were evaluated in terms of discoloration and inflammation on a weekly basis.
After 2 months, the teeth in all three groups were extracted under general anesthesia (13). The teeth were fixed in 10% buffered formalin, decalcified and subjected to histological analysis. Tissue sections were embedded in paraffin blocks and stained with hematoxylin and eosin (14). The stained slides were observed under a light microscope at x10 and x40 magnifications. One pathologist observed the slides and scored them according to the degree of inflammation, formation of hard tissue and dentinal bridge and occurrence of necrosis. The pathologist was blinded to the group allocation of specimens and type of pulp capping agent used. Inflammation was scored as follow.
0: Absence of inflammatory cells
1: Small number of neutrophils and mononuclear inflammatory cells
2: Moderate infiltration of inflammatory cells, neutrophils and leukocytes
3: Severe infiltration of inflammatory cells, neutrophils and leukocytes such that they occupied more than two/thirds of the pulp chamber.
Hard tissue and dentinal bridge formation in the samples was scored as follow.
0: No formation of dentinal bridge
1: Slight deposition of hard tissue beneath and at the margins of the pulp capping agent
2: Moderate deposition of hard tissue beneath and at the margins of the pulp capping agent
Presence of denatured and autolyzed proteins in the pulp tissue indicated the presence of necrosis.

Statistical analysis
Data were analyzed using SPSS version 21. The frequency and percentage of scores for degree of inflammation, formation of dentinal bridge and presence/absence of necrosis in the three groups were calculated and reported. The non-parametric Kruskal-Wallis test was used to compare the three groups in terms of degree of inflammation, dentinal bridge formation and presence/absence of necrosis. Pairwise comparisons were carried out using non-parametric Mann Whitney U test. Considering the significance of the topic, the mean and standard deviation of degree of inflammation and dentinal bridge formation scores were separately calculated

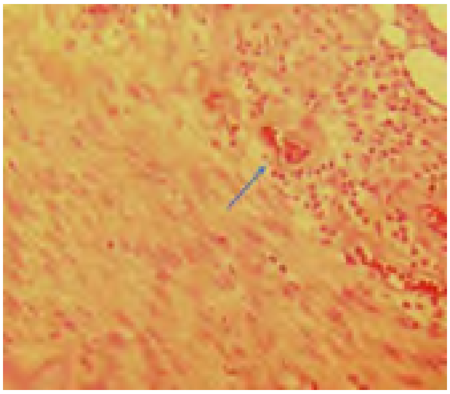

Figure 4
Moderate inflammation in the PRF group.

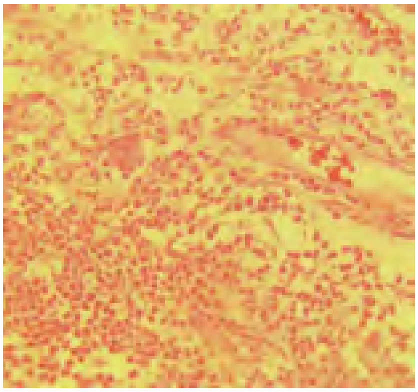

Figure 5
Severe inflammation and pulp necrosis in the control group (x40 magnification).

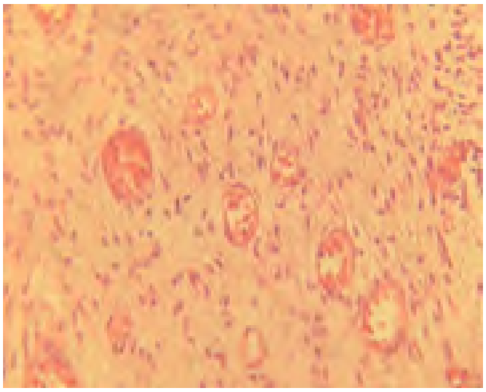

Figure 6
Angiogenesis and fibroplasia in the PRF group (x40 magnification).

in the control and PRF groups and were compared using the Mann Whitney U test. Level of significance was set at 0.05.

Results

Inflammation
Three groups were significantly different in terms of degree of inflammation. Significant differences were noted between the MTA and control (P<0.0001) and also between the PRF and control (P<0.002) groups in terms of degree of inflammation but the difference between the MTA and PRF groups (P=1.0) was not significant in this regard.
Table 1 shows the degree of inflammation in the three groups. Figures 4 to 6 indicate different degrees of inflammation and angiogenesis in the PRF and control groups.

Dentinal bridge formation
The results showed that the MTA and PRF groups had equal performance with regard to hard tissue and dentinal bridge formation (P=1) when used as pulp capping agent and their efficacy was significantly higher than that of the control group (P=0.0001). Table 2 shows the degree of hard tissue

Table 2

Hard tissue and dentinal bridge formation in the control, MTA and PRF groups

Dentinal bridge formation Group	0×	1××	2×××	Total
Control	12 (100%)	0	0	12 (100%)
MTA	0	4 (33.03%)	8 (66.03%)	12 (100%)
PRF	0	4 (33.03%)	8 (66.07%)	12 (100%)
Total	12 (33.03%)	8 (22.02%)	16 (44.04%)	36 (100%)

× *No dentinal bridge formation*
×× *Slight deposition of hard tissue*
××× *Moderate deposition of hard tissue*

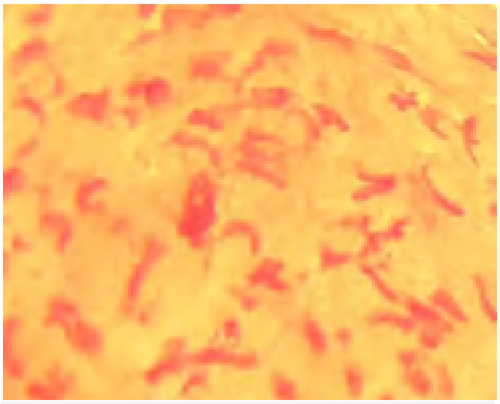

Figure 7
No dentinal bridge formation in pulp in the control group (x10 magnification).

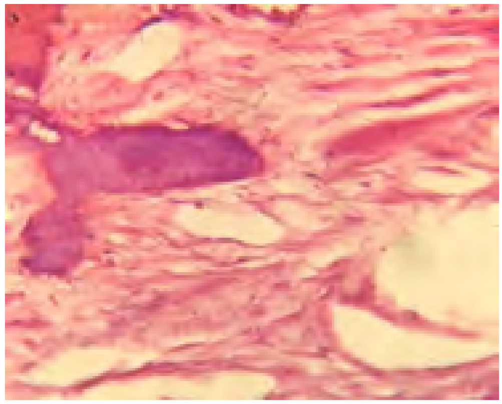

Figure 8
Islands of deposited hard tissue (initiation of dentinal bridge formation) in the MTA group (x40 magnification).

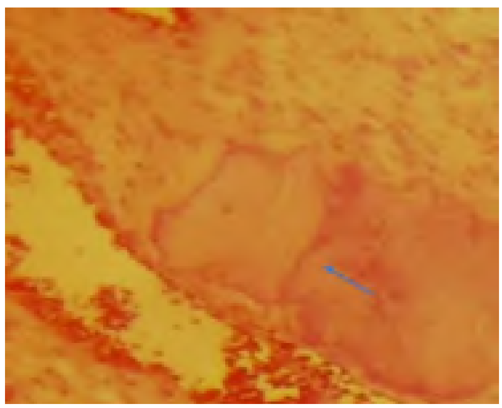

Figure 9
Thick dentinal bridge formation in the PRF group (x40 magnification).

and dentinal bridge formation in the three groups. Figures 7 to 9 show hard tissue and dentinal bridge formation in different groups.

Necrosis
MTA and PRF had equal efficacy (P=1) in prevention of necrosis when applied as pulp capping agents and their efficacy was significantly higher than the control group (P=0.0001). Table 3 shows presence/absence of necrosis in the three groups. Figure 10 shows necrosis in the control group.

Table 3
Presence/absence of necrosis in the control, MTA and PRF groups

Necrosis Group	absence×	presence××	Total
Control	0	12 (100%)	12 (100%)
MTA	12 (100%)	0	12 (100%)
PRF	10 (83.03%)	2 (16.07%)	12 (100%)
Total	22 (63.01%)	14 (38.09%)	36 (100%)

× Absence of necrosis
xx Presence of denatured and autolyzed proteins in the pulp tissue

Discussion

In the recent years, attempts have been made to find a material to directly cap the exposed vital pulp and enhance regeneration (1). In this study, different histological parameters (dentinal bridge formation, degree of inflammation and presence of necrosis) were compared to assess the efficacy of MTA and PRF for pulp capping in dogs.
In this study, the incisor teeth of dogs were selected because the anatomy of dog teeth has some similarities to that of human teeth. Thus, they are commonly used in experimental models, yielding favorable results (15).
The current results showed that inflammation occurred in all groups; however, degree of inflammation in the control group was higher than that in the MTA and PRF groups. The MTA and PRF groups showed almost equal degrees of pulpal inflammation. Also, dentinal bridge formation was the same in both MTA and PRF groups and significantly higher than that of the control group.
The ability of MTA in dentinal bridge formation is related to its excellent sealing ability and polymerization. Thus, when applied as a pulp capping agent, it is not disseminated into the adjacent tissues and decreases subsequent microleakage (16).
MTA releases hydroxyl and calcium ions when in contact with water and tissue fluids and can induce proliferation of pulp fibroblasts. Takita et al. (2006) evaluated the effects of MTA and calcium hydroxide on human dental pulp stem cells in vitro and showed that MTA, compared with the control group, induced cell proliferation within 12 days. However, calcium hydroxide did not show such results (17). The optimal properties of MTA are responsible for its favorable results when used as a DPC agent. These properties include insolubility, minimal toxicity, excellent marginal

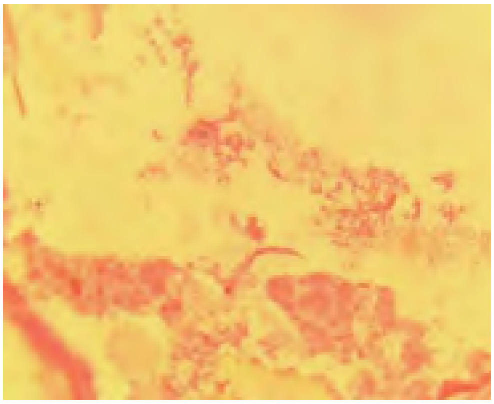

Figure 10
Complete pulp necrosis in the control group (x40 magnification).

adaptation, high setting pH after 3-4 hours and induction of cytokine production by human osteoblasts (16). Shi et al. (2016) evaluated the pulpal reactions to DPC with MTA and another biomaterial in dog teeth and showed that the majority of teeth in both groups demonstrated evidence of calcified bridge formation and did not have pulpal inflammation (18). These observations are in agreement with our results regarding MTA.
Li et al. (2015) in a meta-analysis introduced MTA as a suitable alternative to calcium hydroxide for DPC considering insignificant inflammatory reactions, formation of dentinal bridge and high success rate (19).
Considering all the above and the current results, MTA seems to bring about optimal outcomes when used for DPC. Comparison of PRF and MTA in our study had adequate methodological reliability and considering the similar histological findings in the PRF and MTA groups, it may be concluded that PRF can be used for DPC.
PRF is a non-homogenous biomaterial, on which cells are cultured. It contains plasma, cytokines, leukocytes and different proteins trapped in a very dense fibrin membrane. The fibrin matrix has significant effects on differentiation of osteoblasts (20). Also, growth factors/PRP fibrin matrix may be responsible for dual actions of osteoblasts. Huang et al. (2010) evaluated the biological effects of PRF on human dental pulp cells based on histological observations and showed that PRF stimulates the proliferation and differentiation of human dental pulp cells by up-regulation and expression of alkaline phosphatase (21).
Moreover, PRF can cause controlled release of growth factors over time such that the level of tumor growth factor B1 and platelet-derived growth factor-AB increases to day 14 and decreases afterwards. PRF contains a dense fibrin matrix along with leukocytes, cytokines, glycoproteins and growth factors. Leukocytes in PRF are mainly responsible for the release of growth factors and anti-inflammatory activity (22). This explains the presence of moderate inflammation following DPC with PRF.
Pathak et al. (2014) confirmed the clinical and radiographic success of pulpotomy with PRF in immature human permanent molars in a case report (23). This finding was in agreement with our results. Yang et al. (2013) evaluated the effects of PRF on proliferation and chemotaxis of autogenous dental pulp cells and evaluated the results following its application as a DPC agent in vital pulp therapy. They reported that PRF was biocompatible with human dental pulp cells and use of adequate concentration of PRF exudate enhanced the proliferation and migration of pulp cells. These parameters play a role in pulp healing in vital pulp therapy (24).
In a case report, Lee et al. (2013) reported that pulpotomy with PRF is a biocompatible treatment and PRF in direct contact with the pulp tissue can enhance root development due to its growth factor content (25).
Hiremath et al. (2012) showed that PRF serves as a physiological structure and supports root development. In total, pulpotomy with PRF can replace pulpotomy with MTA or other biomaterials in permanent molars with pulpitis (26).
The current results revealed that dentinal bridge did not form in any of the control samples due to high degree of inflammation. Moreover, PRF and MTA showed similar degrees of dentinal bridge formation. Evidence shows that growth factors present in PRF are released within 1 to 4 weeks after the application of PRF.
The regenerative properties of PRF are evident in its application as a DPC agent.

Also, the expected time for pulp regeneration is the first 3 weeks following application, which was also reported in our study (27).
Thus, observation of reparative dentin in pulp tissue is a sign of pulpal regeneration, which was reported in the MTA and PRF groups after DPC in our study. Huang et al. (2010) demonstrated that PRF can induce proliferation of human dental pulp cells, increase the expression of osteoprotegerin proteins and increase the alkaline phosphatase activity. Thus, in presence of a small number of vital pulp cells, odontoblast-like cells are produced and dentin-pulp complex is formed (28). On the other hand, Wang et al. (2010) showed that cells present in dental pulp may be stem cells and play a role in regeneration of dental pulp (29). Similar results were reported in our study.
Dentinal bridge formation is imperative for pulp vitality. Reparative dentin is formed in the pulpal remnants. Thus, in absence of bleeding, PRF provides growth factors and a potential network for pulp regeneration (27). The present study showed mild to moderate inflammation and slight to moderate deposition of hard tissue beneath and at the margins of the pulp capping agent in incisor teeth of dogs following DPC with PRF and MTA.
Dentinal bridge formation at the interface of pulp tissue-DPC agent is a debated topic because presence of dentinal bridge does not necessarily indicate a healthy pulp status and does not protect the pulp tissue against bacterial penetration. However, it may be a sign of recovery or reaction to stimulation. Dentinal bridge was not formed in any of the control samples in our study. On the other hand, dentinal bridge formation has been noted in exposed pulps without applying a biomaterial (30).
The extracellular matrix on the surface of wound is comprised of healing connective tissue, which is physiologically formed in exposed pulp tissues following deposition of osteodentin or fibrodentin. In this region, odontoblast-like cells produce reparative dentin and eventually show evidence of normal pulp function (31).
Since dental pulp has adequate viable tissue, it seems that DPC treatments can be successful in asymptomatic pulp exposures. A clinical study stated that teeth with asymptomatic pulp exposures remain vital for averagely 12 years following DPC treatment (32).
The efficacy of PRF for enhancement of wound healing has been evaluated and confirmed in different tissues. Growth factors are highly important in signaling, formation and regeneration of dentin-pulp complex. On the other hand, recapitulation of these procedures may lead to pulpal regeneration. Also, micron-scale angiogenesis in PRF fibrin network causes cell migration (26).
As expected, the exposed and uncapped pulp tissue led to necrosis in the control group. Necrosis was also noted in the MTA and PRF groups in lower rate. This can be due to organization of inflammation and pulpal infection following inflammation. If pulp tissue inflammation overcomes the existing infection, subsequent regeneration would occur. Since infection was limited in the MTA and PRF groups, the inflammation overcame it and regeneration occurred in the pulp tissue.
In the present study, PRF was prepared using blood collected from dogs and applied at the site of pulpal exposure. The process of PRF preparation is simple and fast and it does not require activation by bovine thrombin as does the PRP. On the other hand, PRF is an autologous material and risk of transmission of infection and other diseases is insignificant compared with the use of other allografts, xenografts and biomaterials used for DPC (26, 33).
Further in vitro and clinical studies are required to accurately determine the mechanism of action of PRF in pulp tissue regeneration. In vitro studies are highly effective to determine the biological effects of PRF; however, generalization of their results is limited since they cannot well simulate the clinical setting. Future studies are required to assess the effects of PRF and MTA on expression of differentiating odontoblastic markers and dentin matrix proteins.

Conclusions

Comparison of the effects of treatment with MTA and PRF when used as DPC agents in incisor teeth of dogs based on histopathological observations revealed that MTA and PRF both had equal performance with regard to degree of inflammation, dentinal bridge formation and necrosis when used as DPC agents and were superior to the control group.
Considering the optimally equal results of application of PRF and MTA as DPC agents, and easy preparation of PRF compared with PRP, it seems that PRF can bring about optimal results when used for DPC. However, long-term clinical studies are required on this topic.

Clinical Relevance

Knowledge about tissue responses to different pulp capping materials is essential for improving the outcome of direct pulp cap (DPC) treatment. Clinical success of DPC results in more longevity of tooth.

Conflict of Interest

The authors declare no conflict of interest.

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Ethical considerations

The study protocol was in line with the Declaration of Helsinki regarding the care and use of laboratory animals. Animal's rights were followed and no unnecessary harm was done to animals.

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ORIGINAL ARTICLE

Comparison of apically extruded debris during canal shaping with single-file systems

ABSTRACT

Aim: *To assess the amount of apically extruded debris during root canal shaping using various nickel-titanium single-file systems.*
Methodology: *Seventy-two extracted single-rooted human mandibular incisors were assigned randomly to six groups (n=12). The canals were instrumented with the following nickel-titanium single-file systems 25 diameter at the tip: WaveOne (Dentsply Sirona Endodontics), WaveOne Gold (Dentsply Sirona Endodontics), Reciproc (VDW, Munich, Germany), Reciproc Blue (VDW, Munich, Germany), OneShape (Micro-Mega, Besançon Cedex, France) and Hyflex EDM (Coltene/Whaledent AG, Switzerland). Apically extruded debris during root canal shaping were collected into pre-weighed Eppendorf tubes stored in an incubator at 70 °C for five days. The weight of the dry extruded debris was established by subtracting the pre-instrumentation and post-instrumentation weight of the Eppendorf tubes for each group. Data were analyzed using one-way analysis of variance (ANOVA) and Tukey's post hoc tests.*
Results: *All the tested files were associated with apical extrusion of debris. There was no significant difference between examined files regarding the amount of debris extruded during canal shaping (P>0.05).*
Conclusions: *Single-file tested systems produced debris extrusion, and the amount of debris was independent of the used instrumentation technique.*

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Introduction

One of the most fundamental aspects of root canal treatment is the chemo-mechanical preparation that includes mechanical cleansing with instruments and the use of irrigant solutions. However, dentinal chips, pulpal fragments, necrotic debris, and microorganisms may be accidentally pushed out from the root canal into periapical tissues during canal preparation. Extrusion of these elements into periradicular space may cause undesired consequences such as induction of inflammation, postoperative pain, and delayed periapical healing (1, 2). Both manual and rotary Nickel-Titanium (NiTi) preparation sequences are demonstrated to be inevitably associated with extrusion of debris (3).

In the last years, instrument sequence simplification has been proposed through single-file systems as canal preparation may be reliable and faster than that obtainable with conventional multi-file sequences (4, 5).

Improved mechanical properties of single-file NiTi systems achieved through various thermomechanical treatments aimed to optimize the microstructure of the alloys (6) and different kinematics such as reciprocating motion (7) have been proposed as an alternative to the conventional continuous rotation for single-file NiTi systems.

OneShape (MicroMega, Besançon, France) was launched on the dental market in 2011 as the first single-file shaping system conceived for a continuous rotation movement. Made of a conventional austenite 55-NiTi alloy, the design consists of a size 25/.06 file with a passive tip and three different cross-section zones; the first with a 3-cutting-edge design. The second changes from 3 to 2 cutting edges, and the last (coronal) is provided with two cutting edges (8).

In 2011, Dentsply Tulsa Dental (Tulsa, OK, USA) developed the first NiTi endodontic instrument with a molecular structure altered by heat treatment. There are now Gold and Blue heat-treated NiTi systems used in a reciprocating motion (WaveOne Gold, Dentsply Sirona Endodontics; Reciproc Blue, VDW). They are considered as the improved version of the precursors WaveOne and Reciproc, respectively. WaveOne Gold files are manufactured using gold heat treatment performed by heating and then slowly cooling the file after production (9). The reciprocating motion of WaveOne was maintained, but their geometry was altered. The cross-section of the WaveOne Gold was modified to a parallelogram, having two cutting edges and the off-center design. As Reciproc file, Reciproc Blue has an S-shaped cross-section, two cutting edges, and a noncutting tip. However, Reciproc Blue files are manufactured by altering the molecular structure through a new heat treatment that creates a blue-colored titanium oxide layer to increase the cyclic fatigue resistance (9).

Hyflex EDM (Coltene/Whaledent) is manufactured through an innovative patented treatment that is the electro-discharge machining (EDM) process. HyFlex EDM OneFile 25/.~ has a constant 8% taper in the apical 4 mm; the taper decreases to 4% toward the coronal region. Throughout the entire working part of the file, the horizontal cross-section changes from quadratic in the apical region to trapezoidal in the middle region, and almost triangular in the coronal region (10).

This *ex vivo* research aimed to compare the amount of apically extruded debris after the preparation of straight root canals in extracted human teeth using four reciprocating single-file systems (Reciproc, Reciproc Blue, WaveOne, WaveOne Gold) compared with two rotary single-file systems (OneShape, Hyflex EDM).

Materials and Methods

Sample size calculation

A previous study (5) was used to identify an effect size of 0.50 required to calculate the total sample size for this study. a-type error=0.05 and power b=0.80 were also input. A total of 72 samples were indicated as the minimum to observe differences between the systems (F test family, ANOVA, G*Power for Mac).

Selection of teeth

The Research Ethics Committee approved this study's protocol of the Faculty of Dentistry, Ain Shams University, Egypt. Seventy-two mandibular incisors were selected from a collection of teeth extracted for reasons unrelated to this study. Specimens were stored in 4 °C distilled water until use. Soft tissue and calculus were removed mechanically from the root surfaces with a periodontal scaler. Teeth were radiographed form the facial and proximal aspects. The exclusion criteria were a tooth having more than one root canal and apical foramen, previous root canal treatment, internal/external resorption, immature root apices, caries/cracks/fractures on the root surface, root canal curvature more than 10 degrees, and/or teeth in which the apical minor constriction was gauged larger than a size 20 hand file.

Preparation of teeth

An access cavity was prepared in each tooth using a high-speed handpiece and a round bur under water cooling. Canal patency was achieved using a size of 10 K-file. The tooth length (TL) was determined by introducing a size 15 K–file into the canal until the tip of the file was visible from the apex. The working length (WL) was determined by subtraction of 1 mm from TL. The incisal edges were slightly flattened to obtain comparable working lengths 21±1mm. A mechanical glide path was established for all groups using the R Pilot file (12.5 /.04) (VDW, Munich, Germany) before shaping with the respective single-file system.

The experimental model described by Myers & Montgomery (11) was used. The stoppers were separated from Eppendorf tubes, and their initial weight was determined using an analytical balance with an accuracy of 10^{-4} g. Each tube was weighed five times, and the mean value was calculated. Each tooth was inserted up to the cementoenamel junction (CEJ), and a 27-gauge needle was placed alongside the stopper to balance the air pressure inside and outside. Then, each stopper with the tooth and the needle was attached to its Eppendorf tube, and the tubes were fitted into the vials.

The samples were assigned randomly to six experimental groups (n=12) as follows.

Group 1: prepared with WaveOne (size 25/08 taper) reciprocating instruments.

Group 2: prepared with WaveOne Gold (size 25/.07 taper) reciprocating instruments.

Group 3: prepared with Reciproc (size 25/.08 taper) reciprocating instruments.

Group 4: prepared with Reciproc Blue (size 25/.08 taper) reciprocating instruments.

Group 5: prepared with One Shape (size 25/.06 taper) rotating instruments.

Group 6: prepared with Hyflex EDM (size 25/.08 taper) rotating instruments.

All instruments were used in a slow in-and-out pecking motion with an amplitude of about 3 mm. The instruments' flutes were cleaned after three in-and-out movements (pecks) by insertion into a sponge. Each root canal was irrigated with a total volume of 8 mL of distilled water for 4 min divided into four phases (2 mL /1 min each) as follows: before instrumentation, after reaching one-third WL, after reaching two-thirds WL, and after reaching full WL using a 30-gauge needle (NaviTip; Ultradent, South Jordan, UT, USA). Apical patency was maintained using a size 10 K-file. Once the 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. To avoid inter-operator variability, a single experienced operator (SS) performed all preparations under 2.5x magnification and LED illumination (Heine, Herrsching, Germany).

Evaluation of apically extruded debris

After instrumentation, stopper, needle, and tooth were separated from the Eppendorf tube, and the debris adhering to the root surface was collected by washing the root with 1 mL distilled water while in the tube. The tubes were stored in an incubator at 70 °C for five days to evaporate the distilled water, and the weight calculation was performed by a second examiner (TM) who was blinded to the group assignment. The Eppendorf tubes, including the extruded debris, were weighed again in the same way to obtain the final weights of the tubes. Each of the tubes was weighed five times, and the mean value was calculated. The amount of the extruded debris was calculated by subtracting the initial weight from the final weight.

Statistical analysis

Data assumed normal distribution. Hence it was analyzed by parametric tests using a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. The level of significance was set at $P<0.05$. All statistical analyses were performed with SPSS version 16.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Apical extrusion of debris was observed in all the tested groups. The mean values and the standard deviation data of each experimental group are shown in Table 1. Statistical analysis of the results showed the differences between the single-file systems used for root canal shaping were non-significant ($P>0.05$).

Discussion

Concern has been raised regarding the extrusion of debris using different instrumentation systems and how it impacts the patient's postoperative comfort level and treatment outcome. It has been suggested that techniques that minimize apically extruded debris should be sought (12, 13). Therefore, this *ex vivo* study was performed to quantify the amount of extruded debris associated with root canal shaping using six different single-file systems. Up to our knowledge, they were not compared all together before. Their manufacturers claim that most of these instruments have improved clinical performance following their modified design features and the proprietary thermomechanical treatment (14).

All methodologies for evaluating the apical extrusion of debris are based on the quantitative measurement of debris, liquid, or bacteria. The generally accepted method of Myers & Montgomery (11) was used to collect apically extruded debris. Some limitations in this experimental model could affect the results, such as the absence of apical backpressure, lack of control of dentine microhardness, sensitivity of the analytical balance, and hydration of samples due to humidity (15). Also, the implications of a vital or necrotic pulp and the presence of a lesion of endodontic origin in the apical extrusion of debris remain not clear (16). In the present study, straight single-rooted teeth were used to eliminate possible complications, such as WL loss or nonstandard preparation and irrigation in the curved root canals (17). The incisal edges were also slightly flattened to obtain comparable working lengths for the specimens. The results of the current investigation revealed that all the single-file systems caused apical extrusion of debris during canal shaping. This is consistent with other apical extrusion studies (14, 18-20) and reinforces that this shaping sequela is unavoidable.

Apical extrusion of debris is the consequence of the interplay amongst several variables, including the shaping technique, movement kinematics, and instrument design. The results of our study imply that adhering to a strict shaping protocol is the most critical variable. A

Table 1

The mean and standard deviation (SD) values for the amount of apically extruded debris in each study group expressed in milligrams

File type	Amount of debris (mean ± SD)
Wave One	.0002 ± .00007[a]
Wave One Gold	.0001 ± .00003[a]
Reciproc	.0002 ± .00011[a]
Reciproc Blue	.0002 ± .00009[a]
One Shape	.0002 ± .00010[a]
Hyflex EDM	.0001 ± .00003[a]
P-value	.217

Similar superscript letters in the same column indicate a non-significant difference among groups.

standardized mechanical glide path was established in our study before shaping to minimize the incidence of procedural errors and reduce the amount of apically extruded debris (21). The crown down technique was used with all file systems, as suggested by manufacturer recommendations.

Moreover, the apical diameter of all teeth was standardized to a size 25 after instrumentation. In all canals, a standard volume of distilled water was used as an irrigation solution to avoid any possible crystallization of sodium hypochlorite that could alter the weight of dentine debris and compromise the results (13).

The impact of movement kinematics on the apical extrusion of debris is controversial. Bürklein & Schäfer (5) stated that reciprocal movement might enhance debris transportation towards the apex and that continuous rotation may improve the coronal transportation of dentine chips and debris by acting as a screw conveyor. A similar finding was reported by Topcuoglu et al. (22), who found that Reciproc produces more debris extrusion than continuous rotation files as OneShape single rotary files.

Contradicting findings were reported (17) on reciprocating WaveOne files that extruded significantly less debris than the ProTaper Next rotary system because of the reciprocating action act as a type of mechanized balanced forced pressure-less technique (7, 23). Other researches (24) reported that WaveOne and Reciproc systems extruded fewer bacteria than the multifile rotary system BioRaCe; that Reciproc and WO extruded significantly less debris compared to ProTaper with no differences between them (16), and that Reciproc instruments extruded less debris when used in reciprocating motion than in continuous rotation (25). The present study reported a similar amount regarding apical extrusion of debris irrespective of the selected rotary or reciprocating instrumentation system, corroborating previous findings (26).

With the limits of this study that did not evaluate different NiTi alloys as an independent variable, present research did not find any statistically significant differences concerning different heat-treated wires. Gold thermal treatment of the WaveOne Gold and electro-discharge machining process of Hyflex EDM instruments were associated with less debris extrusion (14, 27) during treatment procedures; however, these differences were not significant. Recently (28) it was reported that Reciproc Blue extruded significantly less debris than M-Wire Reciproc during retreatment procedures. Instrument flexibility and alloy microhardness (10, 28-30) were altered by proprietary thermal treatments, which might explain the reduced amount of debris extrusion herein found. All the evaluated single-file systems (except OneShape) are characterized by different regressive taper values from the tip to the shank. Although the instrument taper of the tested files was slightly different, this aspect did not result in significant differences between systems. The cross-section design of the files is different, with a non-identical number of cutting-edge contacts against the canal wall and different symmetrical or off-centered section. Further investigations are needed to confirm the present findings.

Conclusions

Based on the results of this study, single-file tested systems produced debris extrusion; the amount of debris was independent of the kinematics or file design.

Clinical Relevance

All the single-file systems caused apical extrusion of debris during canal shaping. This reinforces the concept that shaping sequela is unavoidable.

Conflict of Interest

Authors deny any conflict of interest related to this research.

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The present research was self-founded.

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ORIGINAL ARTICLE

Residual effect of sodium hypochlorite on pulp chamber dentin adhesion

ABSTRACT

Aim: *This study has evaluated the residual effect of NaOCl on resin-pulp chamber dentin bond strength after 7 and 14 days, using a three-step etch-and-rinse adhesive system.*
Methodology: *Forty pulp chamber dentin from bovine incisors crowns were randomly allocated to one of the following groups: G1, immersion in 0.9% saline solution for 30 minutes (control); G2, immersion in 5.25% NaOCl for 30 minutes; G3, immersion in 5.25% NaOCl for 30 minutes and stored for 7 days; and G4, immersion in 5.25% NaOCl for 30 minutes and stored for 14 days. After restoration, the dentin/resin interface was tested by microtensile bond strength, and failure mode was analyzed by Scanning Electron Microscopy. Data were analyzed by ANOVA followed by Tukey.*
Results: *G1 had higher bond strength than the rest of the groups. There were no statistically significant differences among G2, G3, and G4 (p>0.05).*
Conclusions: *The adverse effect of NaOCl on bond strength persisted even after 14 days following exposure.*

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Introduction

A major goal of endodontic therapy is to eliminate microorganisms from the root canal system to create an environment that is most favorable for healing (1). This is accomplished as a result of mechanical cleaning and shaping along with irrigation using antibacterial agents (2). In order to keep the root canals free of contamination after endodontic treatment, a final restoration of the involved tooth should be performed with a careful technique (3). The infiltration of oral microorganisms in the root canal filling is favored by the lack of a proper coronary restoration, which impairs the prognosis of endodontic treatments (4). Likewise, restoration is important to preserve the remaining dental structure, avoiding fractures that could lead to tooth loss (5). Moreover, the restoration of endodontically treated teeth is critical for clinical success achievement (6-10).

One of the reasons for loss of adhesion of adhesive restorative materials and consequently an improper restoration may be the chemical substance used during the endodontic treatment irrigation (11-13). Sodium hypochlorite (NaOCl) is a widely used chemical irrigant for the biomechanical preparation of root canals due to its antimicrobial properties and capacity to dissolve organic matter (14). However, NaOCl can affect the structure of the dentin surface and degrade collagen fibers (12, 15). Sodium hypochlorite liberates chlorine in its cleavage (Cl_2) and oxidizing compounds; Cl_2 generates dissolution of collagen fibers and the oxidizing compounds interfere with the closing of the polymeric chain of the resin during the set time, causing incomplete polymerization and reduced adhesion to dentin. Once the bond of adhesive systems is due to the infiltration of resin monomers in the collagen layer, this degradation of the organic matrix will be a detrimental factor (16-19).

NaOCl is one of the most used substances for irrigation and decontamination during root canal treatment. Composite resin restorations have been used more and more worldwide. However, residual sodium hypochlorite in porosities of mineralized dentin may give rise to uncured resin polymerization, and thus undermine bond strength (20). The latter may explain a reduction in adhesive bond strength after its use. The aim of this study was to evaluate the residual effect of NaOCl on resin-pulp chamber dentin bond strength after 7 and 14 days, using a three-step etch-and-rinse adhesive system. There is a hypothesis that waiting 7 or 14 days to restore the teeth would not interfere in the reestablishment the bond strength between composite resin and pulp chamber dentin.

Materials and Methods

Forty bovine incisors were stored in 0.5% thymol and used within 2 months of extraction. Methods were performed by a single operator. The teeth were cut to expose the pulp chamber dentine of the middle third of the buccal part of the crown (3). Five mm was cut horizontally from the incisal portion of the crown with a double-sided diamond disc (KG Sorensen, Barueri, SP, Brazil) under refrigeration. Next, the middle third of the crown was removed at a height of approximately 8 mm corresponding to the double-sided diamond disc radius (KG Sorensen, Barueri, SP, Brazil), which then drilled into the middle third of the incisal border aligned to the long axis of the tooth, removing the buccal surface of the crown fragment. Once the samples were acquired, the pulp tissue was cautiously taken out with a spoon excavator. After, the intracoronary dentin was polished for 30 seconds with wet 180- and 600-grit silicon carbide abrasive paper under running water (Struers, Abramin, Copenhagen, Denmark) to plane and create a standardized smear layer. Forty rectangular dentin pieces were acquired.

Specimens were randomly allocated into four groups, with ten specimens in each group, according to the chemical irrigants used and time of storage, as follows: G1, immersion in 0.9% saline solution for 30 minutes (control); G2, immersion in 5.25% NaOCl for 30 minutes; G3, immersion in 5.25% NaOCl for 30 minutes and stored

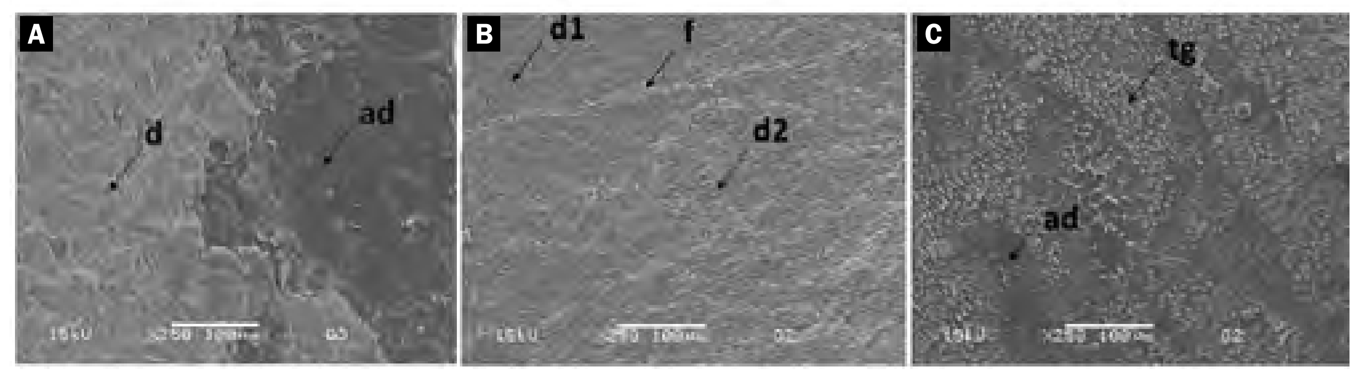

Figure 1
Scanning electron microscopic images of the different fracture patterns taken at 15 kV under x250 magnification. In **A**, a mixed fracture is noticed; it exhibits adhesive area (ad) and dentin (d). In **B**, it is shown the characteristic of two aspects of cohesive fracture in dentin (d1, d2), and the fracture line (f). And in **C**, both tags (tg) and fractured tags can be seen inside the adhesive matrix (ad), illustrating the adhesive failure.

for 7 days; and G4, immersion in 5.25% NaOCl for 30 minutes and stored for 14 days. During immersion, the samples were kept shaking, and the chemical substance was renewed every 10 minutes. Sample storage was done at 37 °C in a 100% humidified environment. After 14 days, 7 days, or immediately after immersion, according to each group, the adhesive system followed by composite resin (Scotchbond Multi Purpose and Filtek Z250, 3M ESPE, St Paul, MN, USA) were applied in the pulp chamber of all samples at the same day to avoid variants. Before the bonding procedures, all specimens were dried with absorbent papers and etched with 37% phosphoric acid for 15 seconds, rinsed for the same time, and dried with cotton balls. A total etching adhesive system was applied to the surface of the pulp chamber dentin according to the manufacturer's instructions. Three layers with 1 mm of a resin composite were incrementally added to the bonded dentin (21), and each one was light-cured for 20 seconds by using a halogen light-curing unit operating at 1200 mW/cm^2 (Optilight Max; Gnatus, Ribeirão Preto, SP, Brazil). After the composite filling of dentin, the blocks were stored in distilled water at 37 °C for 24 hours.

After 24 hours, specimens were removed from the water, dried, and fixed to an acrylic plate to allow the creation of serial cross-sections using a diamond saw operating at 300 rpm (Isomet; Buehler, Lake Bluff, IL). Twenty-five rectangular sticks (1±0.1 mm^2) were obtained from each group from the middle portion of the crown portion to ensure the existence of a linear resin/dentin interface. The sticks were individually attached to a testing apparatus [ie, the Geraldeli's device (22)], with a cyanoacrylate adhesive (Superbonder Gel, Loctite Adesivos, Itapevi, SP, Brazil), and the dentin/resin interface was submitted to microtensile bond test in a universal testing machine (EZ Test, Shimadzu Co., Kyoto, Japan) at a crosshead speed of 1 mm/min until failure with 500N load cell adjustment.

The sticks cross sections were measured using a caliper (Vonder Digital Electronic Caliper, Curitiba, PR, Brazil) for calculating the bond area. The microtensile bond strength was determined and analyzed applying the analysis of variance and the Tukey test for post-hoc comparisons (α=0.05) using the BioEstat 2.0 program (CNPq 2000, Brasília, DF, Brazil). The failure modes were examined under a scanning electron microscopy (Figure 1) and classified into one of four types: adhesive (interfacial failure), adhesive/cohesive (mixed), cohesive in resin, and cohesive in dentin. The specimens were sputter-coated with gold in a Denton Vacuum Desk II Sputtering device (Denton Vacuum, Cherry Hill, NJ, USA) and observed by scanning electron microscopy (JSM/5600 LV - JEOL Ltd., Tokyo, Japan) operating at 15 kV.

Results

The microtensile bond strength means and standard deviations are presented in Table 1. Statistical analysis of the data revealed significant differences between pairs of means (P<0.05). The control group had the

highest bond strength compared to the other groups, although no statistically significant difference was observed between irrigation with 0.9% saline solution or 5.25% NaOCl in the immediately restored group (P>0.05). Moreover, no statistically significant differences were observed in irrigation among groups with 5.25% NaOCl (P>0.05).
Table 1 shows the failure modes observed in each group. All groups studied presented two or more failure modes and the predominating failure is the adhesive. The control group had a higher percentage of fractures in cohesive in resin than others, while the group irrigated with NaOCl and stored for 14 days was the only group with failure in dentin.

Discussion

The bond of therapeutic materials to dentin is a critical factor as it would avoid microleakage, favoring the results of the endodontic treatment (6). There are different restoration materials, such as amalgam, glass ionomer, and composite resin. Amalgam has been less used over time, as it has non-aesthetic characteristics, in addition to containing mercury in its composition. Composite resin has been the material of choice to restore endodontically treated teeth because of its hardness in comparison to glass ionomer, avoiding fracture of the teeth (23).
The use of chemical substances during endodontic treatment may interfere with the adaptation of the restorative material. A well-known interaction is the use of sodium hypochlorite with adhesive restorations (24). Sodium hypochlorite can decrease the bond strength of the adhesive materials to the dentin because it liberates oxidizing compounds and further degradation of the collagen fibers (20). However, it was unknown until the present study if, with time, this bond strength could be restored.
In this *in vitro* study, the coronal part of bovine teeth was used as a replacement for human teeth (25). The methodology was adapted (12) in order to analyze the bond strength of adhesive materials to the intracoronary dentin, where it is affected by chemical substances used during endodontic treatment. This is a unique study aimed to evaluate the bond strength of composite resin to the pulp chamber dentin after the use of sodium hypochlorite immediately, 7, and 14 days. We failed to reject the null hypothesis, which held that waiting 7 or 14 days to restore the teeth would not interfere to re-establish the bond strength between composite resin and pulp chamber dentin.
The results have shown there was no statistically significant difference between G1 and G2. We suggest there was not sufficient time to degrade the collagen fibers. However, G1 has had more cohesive in resin failures than the other groups, suggesting the control group as the one with the strongest bond strength.
There was also no statistically significant

Table 1

Bond strength mean and standard deviations (in MPa) and failure mode (%) according to the experimental groups

Group	Mean (Standard Deviation)	Failure Mode			
		Adhesive	Mixed	Cohesive in resin	Cohesive in dentin
Control	62.42 (37.74)[a]	44.1	14.7	41.2	0
0 days	48.10 (32.49)[a,b]	76.5	17.6	5.9	0
7 days	35.17 (26.66)[b]	54.3	37.1	8.6	0
14 days	35.57 (25.37)[b]	71.0	18.4	8.0	2.6

Same superscript letters in the column are not statistically significant (P>0.05).

difference among G2, G3, and G4 and it reveals the decreased bond strength generated by the sodium hypochlorite in accordance with the literature (24). Sodium hypochlorite can alter the mechanical features of the dentin, such as diminishing the flexural strength, elastic modulus, and hardness (26). Indeed, sodium hypochlorite is an oxidizing chemical substance that causes solid restraint of the interfacial polymerization of adhesive restorative materials (27). In this context, the reactive residual-free radicals from the sodium hypochlorite in the dentin compete with the propagating vinyl-free radicals from the resin light activation system, and consequently, a premature chain termination is formed along with an unfinished polymerization (18, 28).

We suggest that oxygen is not the only factor of this adverse effect on dentin since this effect would be restored after 14 days. Another study used 35% hydrogen peroxide bleached teeth, another oxidant agent, and was restored immediately and, also, after a week; they did not find a difference between the groups (29), which is in accordance with our study. Indeed, it could be that degradation of the collagen fibers also contributes to decreased bond strength, which because of the chlorine content, it breaks the bonds of the collagen fibers (15). The adverse effect of sodium hypochlorite continued to affect the dentin with time; this is believed by the type of fracture found in G4, cohesive in dentin, showing a weakness in the dentin structure. Hence, G1 and G2 have not shown a significant difference.

EDTA (ethylenediaminetetraacetic acid) is a chelating agent used in the field of Endodontics (30). It is able to demineralize the dentin and to rearrange the collagen structure after its use (31). However, after some time of the use of the EDTA, remineralization can occur (31). When EDTA is used after NaOCl, it is able to improve the bond strength (18, 32). EDTA might increases bond strength due to its anti-oxidant power via redox reaction, allowing for free radicals polymerization without premature chains or failures (18).

Alteration of the adhesive system to the sodium hypochlorite could prompt in higher bond strength. However, in this research, the adverse effect of this irrigating solution persisted even after 14 days. It should be due to the slow liberation of the oxidizing agents released by the sodium hypochlorite, persisting in a high amount at the dentinal structure. The degradation that occurred in the dentin is not re-established by the time. The literature demonstrates that sodium thiosulfate is able to restore the dentin bond strength after the use of sodium hypochlorite, allowing the restoration of the tooth immediately after the irrigation protocol (24). Researches seeking to restore collagen fibers or a deep investigation in the antioxidant agents are needed since it would be more favorable to restore the tooth immediately after endodontic treatment.

Conclusions

Within the limitations of this study, the adverse effect of NaOCl on bond strength persisted even after 14 days of its exposure.

Clinical Relevance

The restoration of endodontically treated teeth is critical for clinical achievement. This study showed that restoring the teeth immediately after NaOCl irrigation resulted in not significantly different bond strength than irrigating with saline. However, restoring the teeth 7 or 14 days after NaOCl irrigation resulted in significantly reduced bond strength.

Conflict of Interest

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

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ORIGINAL ARTICLE

Influence of sodium hypochlorite and chlorexidine on the dynamic cyclic fatigue resistance of XP-Endo Finisher instruments

ABSTRACT

Aim: *This study evaluated the cyclic fatigue resistance of XP-Endo Finisher (XPF), associated with two different irrigation solutions: sodium hypochlorite 6% (NaOCl) or chlorhexidine digluconate gel 2% (CHX) in a dynamic model. The null hypothesis tested was that there was no statistically significant difference.*

Methodology: *30 new files were divided into three groups (n=10) based on the irrigation solution used. Group 1: XPF using CHX as an irrigation solution; Group 2: XPF using NaOCl as an irrigation solution; Group 3 (control group): XPF using lubricating oil (LO). The artificial canal was manufactured measuring 1.5 mm wide, 20 mm long and 3.5 mm deep with a straight cervical segment measuring 14.29 mm. A curved apical segment 4.71 mm with 3 mm radius and 90° of curvature and an ending with straight apical portion measuring 1 mm long. Resistance to cyclic fatigue was determined by recording time to fracture, and the file surface was examined under scanning electron microscopy.*

Results: *Data were tabulated and subjected to statistical analysis of variance. Student-Newman-Keuls test for multiple comparisons, with a significance level of 5%. The average time took for file fracture was 306.7 seconds for 2% chlorhexidine and 67,6 for 6% sodium hypochlorite. 2% CHX was significantly different from the NaOCl and LO groups (P<.001).*

Conclusions: *XPF presented the best results of fracture time and number of cycles to fracture when used along with 2% chlorhexidine in canals with severe apical curvature, and there was no statistical difference on fragment size.*

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Introduction

The improvement of new thermal treatment of conventional NiTi alloy, corroborate with better execution of chemical mechanical preparation stages of the root canal, as well as its completion, improving fracture resistance. Thus, this evolution provided a higher index of success, improving the technique speed and rate of file fracture within the root canal (1). However, although advances have been achieved with the development of technological innovations in endodontics, nickel-titanium instruments fracture still occur and can be caused in two ways: torsional or bending fracture (2). XP-Endo Finisher (3) is a new instrument presented as a final step in the disinfection of the root canal system and finishing protocol. It is an instrument made of MaxWire alloy, triangular cross-section, which offers flexibility, cyclic fatigue resistance, and ability to penetrate the canals easily and quickly. With ISO #25 diameter and initial taper of .00, the XP-Endo Finisher expands when inserted into the root canal, at body temperature, respecting the original anatomy and effectively clearing irregular areas due to its greater flexibility (3). Several studies have been carried out in the search for irrigants that have better properties, including antimicrobial activity, low periapical toxicity, solubility and the ability to dissolve organic tissues (4). Of all the substances currently used, sodium hypochlorite (NaOCl) seems to be ideal because it covers more requirements for endodontic irrigator than any other known solution. Pitome et al. (5) evaluated concentrations of NaOCl as an irrigator in endodontics and showed that one of the characteristics of this substance is its ability to dissolve organic tissues, although this has been questioned. The antibacterial efficacy and tissue dissolution ability of NaOCl are related to concentration (6).
Chlorhexidine is a cationic compound, with excellent antibacterial properties, just like 5.25% NaOCl, and has a better residual effect because it shows substantivity (up to twenty-four hours), although it cannot dissolve the pulp tissue (7). The use of chlorhexidine in contact with vital tissues reveals biocompatibility. As an endodontic irrigant, it is used in concentrations of 2% and also presents excellent clinical performance and rheological action. Its lubricating action promotes a more significant intensification of the canal cleaning since it acts to reduce the friction of the file with the canal interface, reducing the fracture rate (8).
After immersion in chlorhexidine, no data were reported on the fatigue behavior of XPF instruments. Therefore, the aim of this study was to evaluate cyclic fatigue resistance of XPF-file in simulated canals with two different types of auxiliary chemical substances. The null hypothesis tested was that there was no statistically significant difference between NaOCl and CHX gel 2% in the dynamic cyclic fatigue resistance.

Materials and Methods

In this experiment, thirty new endodontic NiTi files XP-Endo finisher, with 0.25 mm tip diameter and 25mm length were divided into 3 groups (n=10) according to the auxiliary chemical substance used.
Group 1: XPF instruments using 2% chlorhexidine gel as an irrigation solution;
Group 2: XPF using 6% sodium hypochlorite as an irrigation solution;
Group 3 (control group): XPF instruments using lubricating oil (WD-40, Milton Keynes, UK).
For resistance to cyclic fatigue a stainless steel plate, with one artificial groove measuring 1.5 mm wide, 20 mm long and 3.5 mm deep with a straight cervical segment measuring 14.29 mm, with an arc length of curvature of 4.71 mm, with a radius of curvature of 3 mm, and ending in a linear apical segment of 1 mm simulating root canals was used according to Figure 1.
The purpose of these measurements is for the file to rotate freely in the same position in the canal. An acrylic plate was attached to the stainless-steel plate so that the separate fragment would not be lost and to contain the solution within the artificial canal.

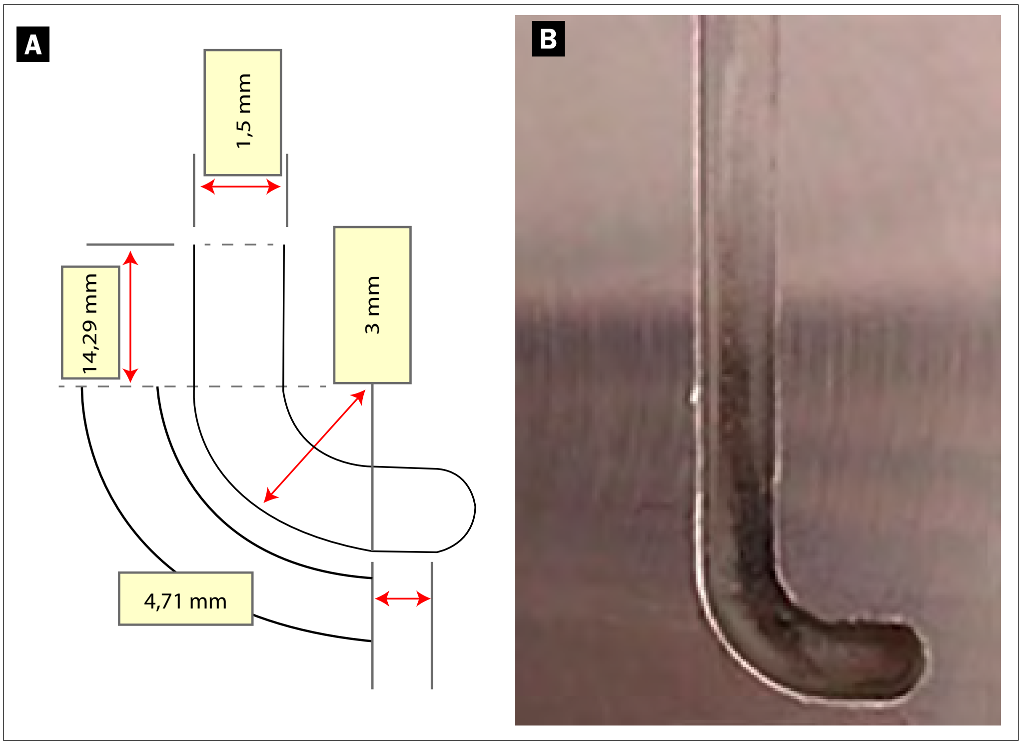

Figure 1
Artificial canal:
A) Schematic drawing;
B) Simulated stainless steel root canal.
Source: Private Clinic in Rio Branco Laboratory, AC, place of tests.

Fatigue resistance test
The files were placed within the simulated canals and triggered by VDW reciprocating motor (VDW GmbH Munich, Germany) following the manufacturer's recommendations of programming, where the speed is 900 RPM and torque 1 Ncm. The oil that was used as a control and the different chemical substances were used in the canals.
The files were inserted in the simulated canals until the length of 20 mm, using a stop to register this length. All files were dynamically driven until a visual fracture occurred. The irrigant was taken to the artificial canal with Navi tip plastic needles, with a 21 mm long flexible tip penetrating 1 mm into the cervical region of the simulated canal with the needle tip without touching the moving file, maintaining the canal filled with the solution. All file rotation time within the simulated canal was recorded with iPhone 6s mobile device, (Apple Cupertino, California, USA) from the beginning of the rotational test until its fracture, to enable the timing of the fracture. The analysis was performed in movie maker program of Microsoft Corporation (Redmond, Washington, USA). The mathematical formula calculated the number of cycles for the fracture (NCF) is

NCF=Time to fracture (seconds)
x Rotation speed (RPM)/60

Evaluation of the fracture pattern under scanning electron microscopy
After collecting the samples, all fragments were cleaned in an ultrasonic vat and dried in an incubator at 37 °C for 24 hours, being separated in glass tubes containing the group identification and instrument number. Subsequently, they were analyzed under scanning electron microscopy. The fractured files were positioned on their long horizontal axis to verify if there was any plastic deformation; after the file was placed in a vertical position to observe the area of the fractured segment to verify if there was any fracture pattern between the endodontic files of each group (Figure 2).

Evaluation of the length of the file fragments
The fragments were measured with digital caliper 150 mm, with an accuracy of ±0.03

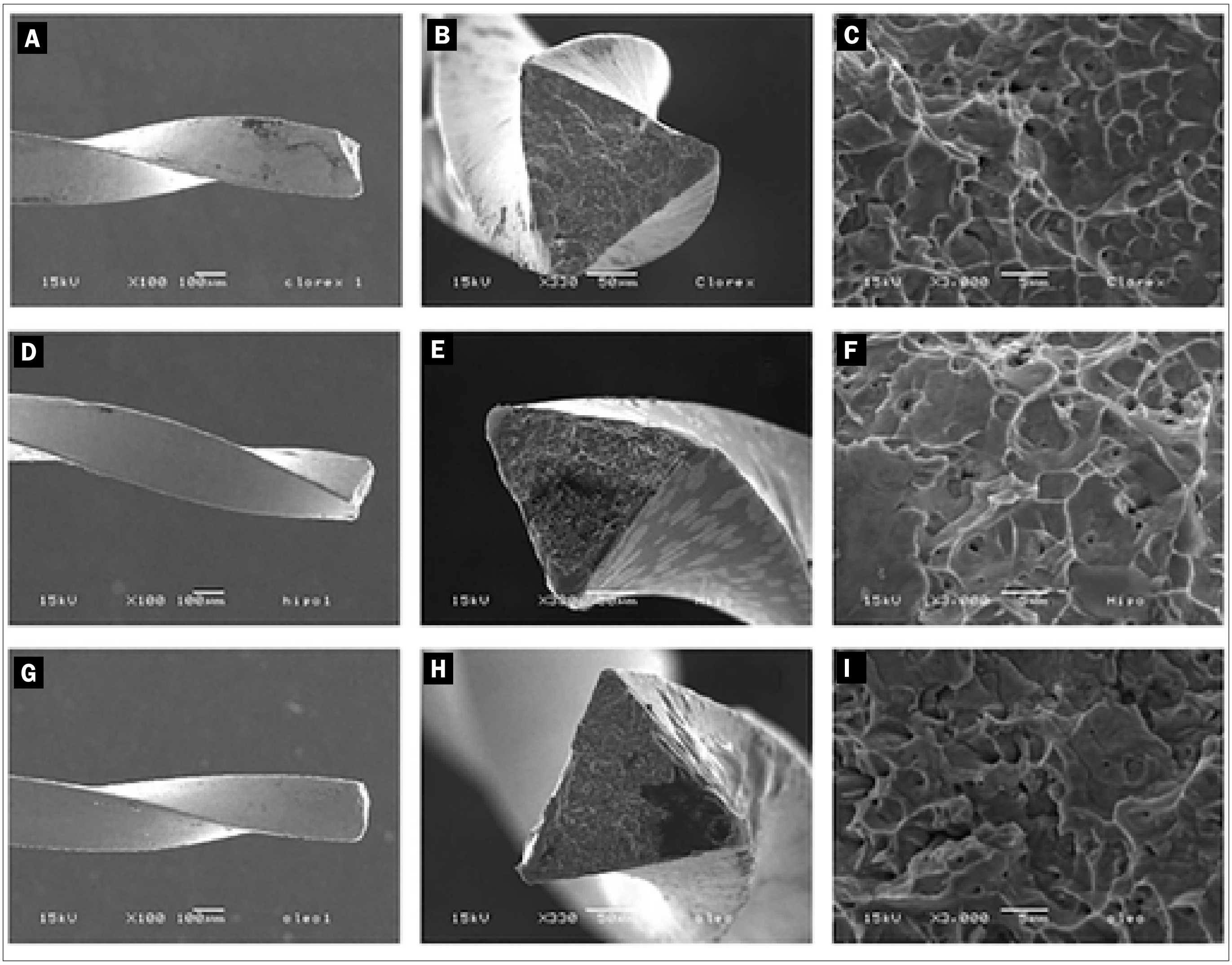

Figure 2
SEM images showing the cross-section of the fractured instrument. **(A, B, C)** CHX, **(D, E, F)** NaOCl, **(G, H, I)** LO. **(A, D, G)** Absence of plastic deformation in the helical shaft of all instruments. **(B, E, H)** ductile morphologic characteristics on fractured surfaces. **(C, F, I)** Magnified area of **(B, E, H)**.

mm/0.001. Fracture length patterns of the files of each group and the location of the maximum point in both artificial canals of the test were analyzed.

Statistical analysis

After collecting the fracture times of the files in each of the three groups, the data were tabulated and submitted to statistical analysis undergoing variance test and Student-Newman-Keuls test for multiple comparisons, with a significance level of 5%

The Kolmogorov Smirnov normality test (P<0.05) confirmed the normality of the data, thus using parametric tests to compare the means. The ANOVA test, followed by the Post-Hoc, Tukey, was used to compare the time means, cycle number until file fracture according to the type of irrigation substance (k groups=3).

Results

The mean and standard deviations of the variables studied, such as time and number of cycles to fracture (NCF) for each group, are shown in Table 1.

In the chlorhexidine group, the time ranged from 3.06 to 8.39 min and NCF from 2,790 to 7,785. With sodium hypochlorite group, the time ranged from 0.25 to 3.22 and NCF from 375 to 3,030. For the oil group, the time ranged from 0.23 to 1.45 and the NCF from 345 to 1,575.

The time and number of cycles until frac-

Table 1

Mean and standard deviation (SD) of time (minutes) to fracture and NCF for each type of irrigation substance

Irrigating solution	Time Mean (SD)	NCF Mean (SD)
CHX	4.99 (1.62)[a]	4558.5 (1495.33)[a]
NaOCl	0.92 (0.91)[b]	1014.00 (795.34)[b]
Oil	0.67 (0.41)[b]	832.50 (354.42)[b]
P-value	<.001	<.001

Note: Vertical, for columns, different lowercase letters indicate statistical difference, ANOVA test, with Post-hoc Tukey's test, P<0.05.

Table 2

Mean and standard deviation (SD) of file fragments fracture size for each type of irrigation substance

	Fragments (mm) Mean (SD)
CHX	3.90 (0.188)
NaOCl	4.05 (0.236)[a]
LO	3.82 (0.187)
P valor	<.05

Note: Vertical, for columns, lowercase letters indicate statistical difference, ANOVA test, with Post-hoc Tukey's test, P<0.05.

ture (NCF) for chlorhexidine were statistically different from the other groups (P<.001). There was no significant difference between hypochlorite and oil in time (P=0.876), and also in NCF (P=0.913).
There were no significant differences among the groups in terms of the mean fracture length (4.5 mm) regardless of conditions (P>.05) (Table 2).

Discussion

This study was designed to compare cyclic fatigue resistance of the XPF instrument using two different substances (CHX or NaOCl) as irrigants. Lubricating oil was used as a control because it is a well-established substance that has been used in other experiments (9). The obtained fatigue tests results were evaluated according to the type of substance used. CHX was more effective in improving the instruments resistance to cyclic fatigue, so, the null hypothesis was rejected. Current findings might be explained by the corrosive effect of NaOCl on endodontic NiTi instruments (10), that may negatively affect their physical properties (11). Although NaOCl is the irrigation solution of choice for clinicians, CHX emerges as a new perspective in Endodontics, and when it is used in gel form facilitates root canal instrumentation (7).
Dynamic evaluation of a NiTi rotary files during root canal instrumentation is often recommended and could be performed evaluating the instrument shaping ability (12). The dynamic test was conducted in a standard metal canal to ensure the standardization of the experiment (13), such as the entire length of the canal, the length of the curvature ratios and the length of the arc (14). In the dynamic test, the instrument moves axially along within the canal, which allows stresses to be distributed along the instrument shaft, extending the fatigue life of the file (15). The fatigue life of the XPF was significantly higher when the CHX was used as opposed to NaOCl. Although comparing to static models, the dynamic model enhances fracture resistance (1), and could reproduce a clinical up and down motion (9) none of which influenced fracture morphology (15).
Files breaking in the root canal can influence treatment prognosis and is a challenge faced by clinicians, so reducing the risk of instrument fracture is critical to clinical success (16). The different pH of NaOCl could influence metal corrosion (17) and can explain the better results for the CHX. The instruments were exposed to the solutions at body temperature throughout the experiment to ensure better clinical condition, although this condition reduces fracture resistance (18) NaOCl is the irrigating substance most used for root canal irrigation in endodontics due to its properties. Despite this, the substance may cause micro cratering by removing nickel from the instrument surface (19), decreasing the resistance to cyclic fatigue. CHX

is a substance that has also been used in canal treatments, up to this moment no study has evaluated the effect of immersion in these substances in the cyclic fatigue resistance of XPF.
NaOCl, when compared with water, negatively affect the fatigue resistance of NiTi instruments (20), especially at higher concentrations. On the contrary, Cheung et al. (21) found that the immersion of instruments in NaOCl before cyclic fatigue testing for 3 to 5 minutes did not affect the cyclic fatigue of NiTi endodontic instrument. However, they do not reflect the real clinical condition of root canal preparation in that it is performed in the presence of the irrigants in the root canal.
Fatigue failure can be caused initially by cracking on the surface of the instrument, and this may be due to concentration of chloride ions in corrosion under a titanium gap (22), influencing fatigue resistance.
The radius and the angle of canal curvature are known to have a significant role in cyclic fatigue failure, so in this study, a severely curved at the apex artificial canal was used. The mean lengths of fractured segments were recorded to evaluate the correct positioning of the tested files inside the canal curvature. There was no significant difference between the groups regardless of the lengths of the broken pieces (≈3,92 mm). This matches with the location of the file at the maximum curvature (4.0 mm from the tip). The point of maximum stress was similar in each condition, suggesting the standardization of the experiment (23).
Two mechanisms are involved in the fracture of NiTi rotary files: cyclic fatigue and torsional failure. The morphological characteristics of the fracture surface can be characterized by a smooth, shiny, or ductile surface, with presenting hemispheric dimples (24, 25).
The use of different lubricants during testing may be responsible for inconsistent results (26). Our result may be due by the fact that CHX acts as an anti-corrosive agent, which can increase the cyclic fatigue resistance. Moreover, it has superior lubrication quality, reducing friction of the instrument with the surface of the artificial canal (27). Additionally, CHX did not cause surface damage to the NiTi alloy, in contrast, NaOCl and EDTA endodontic irrigants caused alterations in the surface of instruments (28).
In conclusion, results showed that XPF had a long fatigue life when CHX was used as an irrigant. The enhanced resistance may be associated with lubricant and non-corrosive properties of CHX gel. Although CHX increases cyclic resistance, it does not comply with all the requirements of an endodontic irrigant (no dissolving action). Further studies are required to evaluate other irrigation solutions and their influence on the cyclic fatigue behavior of XPF instruments.

Clinical Relevance

The present study has shown that XP-Endo Finisher exhibited higher dynamic cyclic fatigue resistance when used in association with 2% chlorhexidine gel as an irrigation solution in a severe curved simulated canal.

Conflict of Interest

The authors deny any conflicts of interest.

Acknowledgements

None.

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ORIGINAL ARTICLE

Impact of different flow rate on apically extruded debris and irrigants

ABSTRACT

Aim: *Root canal instrumentation might lead to extrusion of debris and irrigants, leading to postoperative pain and compromised outcomes. Several factors might impact extrusion during root canal instrumentation, including the flow rate in which the irrigant solution is applied inside the root canal. The aim of this study was to assess the extrusion of irrigants and debris with different flow rates of 0.9% saline solution.*
Materials and Methods: *Thirty mandibular premolars presenting single roots and straight root canals were used in this study. The roots were standardized in 17 mm and inserted in Eppendorf tubes. A 1.5% agar gel was placed inside the tube that involved the roots, while the coronal part of the roots was kept visible. The combination of tube and agar gel was weighted. Then, the specimens were randomly distributed to 3 different groups (n=10) according to the flow rate of the irrigation: Control Group (CG) 5 mL/min, High Flow rate Group (HG) 10 mL/min, and Ultra-High flow rate (UG) 60 mL/min. The canals were instrumented with a Reciproc Blue instrument size 25.08 up to the length of the root, following the manufacturer guidelines. After the instrumentation, the roots were removed and the tube and agar were weighted again. The difference between the final weight and the initial weight represented the total amount extruded beyond the apex. For statistical analysis, the ANOVA test (post-hoc Bonferroni) was used at P>0.05.*
Results: *The mean weight of the extruded debris and irrigants for CG, HG, and UG was respectively 0.0079 ± 0.0087, 0.0110 ± 0.0093, and 0.0083 ± 0.0091. There were no statistically significant differences among the groups.*
Conclusions: *Within the limitations of this study, it can be concluded that the flow rate of irrigants does not impact the extrusion of debris and irrigants.*

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Introduction

Root canal therapy involves the removal of vital or necrotic pulp tissue, bacteria, and its by-products. Root canal instrumentation using different instruments and irrigants is the most common method for the removal of such contents. While irrigation is of major importance in the reduction of bacterial load and removal of pulp tissue coronally, there is a risk of extrusion of debris and/or irrigants into periapical area.

Sodium hypochlorite has been largely applied for root canal irrigation for several years (1). The main benefits of NaOCl are the disinfection and tissue dissolution inside the root canal. However, NaOCl might impose severe damage in case of extrusion beyond the apex; for many years, there have been reports of accidents due to NaOCl extrusion (2-4). Therefore, different irrigants have been proposed for replacement of or in combination with NaOCL (5). Chlorhexidine (CHX) has been demonstrated to be comparable to NaOCl in regards to root disinfection. The use of 2% CHX gel proved more efficient than 5% NaOCl in a previous study (6). However, CHX gel alone is not able to properly remove the contents from the root canal system. Therefore, it is used in combination with saline solution or distilled water, with adequate flow rate is necessary for a better cleaning of the root canal system (7).

Several factors might contribute to the extrusion of irrigants: volume used, type and position of the needle, and flow rate of the irrigation (8-10). While different studies have assessed the impact of such variables on the extrusion of debris, the effects of different flow rates have scarcely been assessed in recent literature (8). Therefore, the aim of this study is to assess the extrusion of debris and irrigants with different flow rates of saline solution.

Materials and Methods

This study was reviewed and approved by the Ethical Committee of the Faculdade de Odontologia São Leopoldo Mandic (# 2.973.441). This *ex vivo* study comprised 30 first or second mandibular premolars extracted for reasons not related to this study. Only single rooted teeth presenting a single canal as well as fully formed apices were included. The teeth were radiographed in both bucco-lingual and mesio-distal direction in order to select only teeth presenting curvatures smaller than 5°, following Schneider´s method (11). The teeth with cracks or fractures, caries, calcification, resorption, or any sign of previous root canal therapy were excluded from the experiment.

Sample Size Calculation

The sample size calculation was based on a previous study by Lu et al. (12) that compared three different systems in regards to apically extruded debris. For achieving an alpha-type error of 0.5 and study power of 95% were input to test using a G*Power 3.0 for Mac statistical package. The results indicated a total of 10 teeth per group in order to observe differences in apically extruded debris and irrigants.

Preparation of the Specimens

After the selection, the teeth were decoronated with a diamond bur and the length of the roots was standardized in 17 mm. The content of the canals was gently removed with a size 10 k-file under 5% NaOCl irrigation. Then, a size 15 k-file was inserted up to the major foramen (MF) in order to assure the selection of specimens with MFs of 0.15 mm in diameter.

The roots were covered with a teflon tape, allowing only 1 mm of the apical third to be exposed. The lids of the Eppendorf tubes (Eppendorf do Brasil, São Paulo, SP, Brazil) were perforated with a diamond bur, the roots were inserted in these lids, and the margins were sealed with cyanoacrylate glue and a layer of gingival dam. Each set of root and lid was weighted 3 times on a scale with 10^{-4} precision (Nowak, São José do Rio Preto, Brasil), and the mean weight was registered in a spreadsheet. Then, 1.5% agar gel (Kasvi, São José dos Pinhais-PR, Brasil) was placed inside the Eppendorf tubes, and each set of root and lid was inserted in the flasks in order to have the roots completely involved in the agar gel. After 24 hours of setting, the whole set was weighted 3 times and the mean weight was calculated. The initial considered weight of the tooth-free flask with agar was the difference between the 2 means.

Root Canal Preparation
The root canals were prepared using a Reciproc Blue (VDW, Munich, Germany) reciprocating instrument (25.08) in the VDW Silver motor (VDW) in the "Reciproc ALL" setting. The instrument moved to the WL in an in-and-out fashion of three movements with an amplitude of 3-4 mm. After each set of movements, the instrument was removed and cleaned with an alcohol-soaked gauze. The canal was again irrigated and the apical patency checked with a size 10 k-file. These movements were repeated until the instrument reached the WL that was previously determined (at the MF), assuring the instrumentation of the whole length of the root.

Irrigation Protocols
The specimens were randomly assigned to three different groups, according to the irrigation protocol. The canals were irrigated with 0.9% saline solution at different flow rates, controlled by a peristaltic pump (LPD 101-3, MS Tecnopon, Piracicaba, Brazil). In the CG, the irrigation was done at 5 mL/min, in the HG at 10 mL/min, and in the UG at 60 mL/min. The same volume (8 mL) of saline solution was used for each specimen. The gauge 31 endo-eze (Ultradent, South Jordan, UT) side-vented needle, placed as close as possible to the WL, was used in all samples. All procedures were carried out by a single operator.

Post-Instrumentation Analysis
After the root canal instrumentation, the lids with the roots were removed and the set of Eppendorf tubes with the agar gel was weighted three times. Then, the mean weight was calculated. The final weight that included both irrigants and debris extruded was the difference between this final weight and the previously calculated weight of the whole content. All weighting measures were conducted by a operator blinded to the group of the specimen.

Statistical Analysis
The data was submitted to the Kolmogorov-Smirnov test that assumed the normality of the results. Therefore, the ANOVA, post-hoc Bonferroni, was used for statistical analysis for differences among the groups. The Statistical Package for Social Sciences (SPSS 20.0 for Windows) was chosen and the $P<0.05$ was considered for statistical differences.

Results

The mean weight of the extruded debris and irrigants for CG, HG, and UG was respectively 0.0079 ± 0.0087, 0.0110 ± 0.0093, and 0.0083 ± 0.0091. There were no statistically significant differences among the groups $P>0.05$.

Table 1
Mean and standard deviation of the amount of extruded debris in each group

Group	n	Mean (g)	SD
Control	10	0.0110	0.0093
High Flow Rate	10	0.0083	0.0091
Ultra-High Flow Rate	10	0.0079	0.0087
P value		.719	

Discussion

The traditional methodology for assessment of apically extruded debris is based on the collection of extruded material in an empty flask (13). Then, the flask is dried, the irrigant evaporates, and the remaining debris can be properly weighted. The major drawback in this methodology is that the amount of extruded irrigants cannot be assessed. The methodology herein adopted uses a 1.5% agar gel for collecting the apically extruded material (12). The advantage of this methodology is the possibility of collecting not only debris, but also irrigants pushed beyond the apex. Moreover, the agar gel promotes a better

simulation of the counter pressure promoted by the periapical tissues (5, 12). The characteristics of the gel also prevent the observation of the extruded debris, diminishing the risk of bias.
Different technologies were proposed and demonstrated to diminish the risk of apically extruded debris, including negative apical pressure (NAP) irrigation, and lasers (14). Contrary to suggested, there are studies and meta-analysis that shows the lasers have the potential for extrusion of fluid from the apex (5), and there has been no evidence yet if NAP prevents the extrusion of debris (15). It was also observed that all the irrigant activation devices, including sonics and ultrasonics, extruded debris and irrigants from the apex (16). Due to all of these contradictory results, in this study investigating the effect of different flow rates on debris and irrigant extrusion, any irrigant activation devices were not used and only the side-vented needle was used to deliver the solution into the canal.
The WL adopted in the present study allows preparation in the whole extension of the canal, rather than only up to the apical constriction. By doing so, a larger preparation of the apical third is achieved, aiming the reduction of the bacterial load (17, 18). The use of 2% CHX gel in association with 0.9% saline was proposed to avoid the harmful effects of extruded NaOCl. However, a gel does not possess the proper surface tension to effectively flush the entire root canal system; therefore, saline solution is used for the removal of the entire contents of the root canal. In this study, the needle was positioned at the WL, aiming to ensure a better disinfection (19); the increase in flow rate theoretically could improve the cleaning effects. One might infer that the combination of foraminal enlargement and active irrigation leads to painful postoperative results; however, the effects on postoperative pain are controversial (20, 21). A previous study showed fewer irrigant extrusions when a side-vented needle was used (5); the results of the present study suggest that preparation at the MF associate with high flow rate irrigation does not increase apical extrusion. Boutsioukis et al. (22) demonstrated that a higher flow rate of 0.26 mL/s (15.6 mL/min) increased the amount of extruded irrigants when compared to 0.14 mL/s (8.4 mL/min) in both open-vented and side-vented needles. A recent study also demonstrated that a flow rate of 6 mL/min is related to higher amounts of irrigant extrusion than 3 mL/min (23). In that study, both open- and side-vented needles were used. In both aforementioned studies, only the extrusion of irrigants was assessed, while in the present study the combination of irrigants and debris extrusion could be evaluated. In disagreement with those findings, the present study showed no difference in apical extrusion when 5 mL/min, 10 mL/min, or 60 mL/min were used. It seems clear that the extrusion of irrigants increases when a higher flow rate is applied. The findings of the present study suggest that the total combination of irrigants and debris extruded seems to be unaltered when a high flow rate (60 mL/min) is used with side-vented needles.
In the present study, the instrumentation was performed at the MF, promoting the disruption of the constriction. While this maneuver presents controversial results, our findings need further investigation (20, 21). Further studies should investigate the impact of high flow rate with different needle types in addition to the effects of the variation in flow rate in the cleaning effects.

Conclusions

Within the limitations of the present study, it can be concluded that a higher irrigant flow rate did not impact apical extrusion of debris and irrigants.

Clinical Relevance

Extrusion of debris and irrigants might increase inflammation and promote postoperative pain, especially in necrotic teeth. The different flow rates applied during irrigation might impact the extrusion, therefore resulting in increased postoperative pain. This study aimed to evaluate the impact of different flow rates on the extrusion of irrigants and debris.

Conflict of Interest

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

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None.

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ORIGINAL ARTICLE

Micro-computed tomography evaluation of filling material removal by three reciprocating systems with different thermal treatments

ABSTRACT

Aim: This study compared the efficacy of three reciprocating systems with different heat treatments in removing obturator material from lower molar mesial canals.
Methodology: The mesial canals of 18 lower molars were instrumented with ProTaper Next 25/0.06 and filled with gutta-percha and AHPlus. The teeth were randomly divided into three groups (n=6) according to the system used to remove the filling material: Reciproc (REC R25), Reciproc Blue (RECB R25) and Wave One Primary (WO). The desobturation was considered complete when the working length was reached, and remnants of obturator material were no longer found in the instrument and also in the canal walls, observed through the dental operating microscope.
Results: The results were analyzed using Biostat 4.0 software. Shapiro-Wilk and ANOVA (Tukey) tests with a significance level of 5% were applied. The volume of obturator material decreased in the cervical and middle thirds after the use of REC, RECB and WO systems (P<.01), but there was no significant difference between the three systems (P>.05). In the apical third, there was no significant reduction in the remaining obturator material independent of the operating system (P>.05).
Conclusions: REC, RECB and WO systems showed similarity in the removal of obturator material and were inefficient specifically in the apical third, showing the limitation of these systems in removing obturation material from curved canals.

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Introduction

Although endodontic treatment has high success rates (1, 2), failures can occur, requiring reintervention. The percentage of success and survival of root canal treatment over 2-10 years ranged between 86% and 93% (3). Conventional retreatment is the first option for the removal of the filling material (4).
Some rotary Ni-Ti systems, such as Mtwo Retreatment (VDW, Munich, Germany) and ProTaper Retreatment (Dentsply Maillefer, Ballaigues, Switzerland) have been developed for the retreatment of root canals. Although Reciproc (WDW, Munich, Germany), Reciproc Blue (Munich, Germany) and WaveOne (Dentsply Maillefer, Ballaigues, Switzerland) reciprocating instruments have not been designed for root canal retreatment, studies have demonstrated their efficacy for the removal of the filling material (5-7).
The REC and RECB reciprocating systems are S-shaped cross-sections, the first with M-Wire alloy and the second with blue-Wire alloy. The WO system has a triangular cross-section and an M-Wire alloy. Researches performed on rotary and/or reciprocating instruments for retreatment has shown that these are not effective for the removal of gutta-percha from the canal system, especially in the apical region (8-10). Therefore, it is important to study whether blue heat-treated systems can effectively remove all the obturator materials from the root canals.
This study aimed to evaluate the volume of filling material remaining in mesial root canals of human lower molars after the use of Reciproc (REC), Reciproc Blue (RECB), and WaveOne (WO) reciprocating systems. The null hypothesis was that the type of instruments, with different thermal treatments of the alloy, would not interfere with the remaining volume of the obturator material in the mesial canals of lower molars.

Materials and Methods

The study protocol was approved by the Institutional Research Ethics Committee (register No. 2.332.674) because it is a study involving biological material/humans. Eighteen first and second molars were selected and maintained in 0.1% thymol solution. The sample calculation was performed using the ANOVA test that indicated a total of 6 samples per group as the ideal size required to notice significant differences among the groups. The sample had the alpha type error of 0.05 and a test power of 80%.
Teeth with mesial roots with two canals and independent foramina, complete rhizogenesis, and angle of curvature between 20° and 40° were included in the study and measured with Image J program (National Institutes of Health, Maryland, USA) according to the method of Schneider (11).
Teeth were excluded with a previous endodontic treatment, pulp calcifications, resorptions, root fractures, and canals whose patency was not obtained after the access surgery. The teeth were washed in running water, and the root surface was cleaned by ultrasound and kept hydrated in saline until the beginning of the experiment. The size of the teeth was standardized at 18 mm by cutting the occlusal surfaces using a diamond disk (FKG Dentaire, La Chaux-de-Fonds, Switzerland).
The working length (WL) was defined by placing a size 15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) inside the canal until its tip was visible at the apical foramen, subtracting 1 mm. The patency was maintained with the use of a size 10 K-file (Dentsply Maillefer), passing 1 mm beyond the WL. All treatments were performed using the dental operative microscope (Alliance, São Carlos, São Paulo) with the magnification of 8x. Each instrument was used in four canals (12) and then discarded. Irrigation was performed with 20 mL of 2.5% sodium hypochlorite (NaOCl). The smear layer was removed at the end of instrumentation with 5mL of 17% ethylenediaminetetraacetic acid (EDTA) for 3 minutes.
The canals were instrumented by a single qualified specialist in endodontics with ProTaper Next (Dentsply Maillefer) X3 files (30/.07) in the cervical and middle thirds

and X2 files (25/.06) in the apical third, with the aid of an electric motor with torque control, 300 RPM and 2 N (X-Smart, Dentsply/Maillefer).

The teeth were dried with absorbent paper and sealed by the single cone technique (X2, Dentsply Maillefer) and AHPlus sealer (Dentsply Maillefer). The sealer was placed in the cone and inserted into the canal with a single movement. The excess cone was cut with Touch'n Heat thermo-compactor (SybronEndo, Orange, CA), and then vertical cold condensation was performed. Crowns were sealed with temporary Coltosol cement (Coltene, Whaledent, Cuyahoga Falls, OH) and stored at 100% humidity at 37° C for 30 days (13). The teeth were radiographed mesiodistally and buccolingually to evaluate the quality of the obturation, which was confirmed by the micro-CT examinations. All of the root canal fillings were adequate without presence of underfilling or overfilling. The teeth were divided into 3 groups (n=6): REC R25, RECB R25, and WO Primary.

Removal of obturator material

The removal of the filling material was accomplished using a endodontic motor (VDW Silver; VDW GmbH, Munich, Germany). Reciproc and Reciproc Blue instruments with the Reciproc ALL program and the WaveOne instruments with the Wave One ALL program. Each instrument was used only once and discarded. The instruments were used with in-and-out movements against the walls of the canals until the WL was reached. The foraminal patency of the canals was maintained with a size 15 K-file to remove the complete obturator material. The filling removal procedure was considered complete when the WL was reached, and the remnants of the obturator material were no longer found on the instrument and the canal walls were seen through the dental operative microscope (16x). Irrigation was performed with 5 mL of 2.5% NaOCl between each file, after three in-and-out movements with a total volume of 20 mL. The final irrigation protocol was performed with 5 mL of 2.5% NaOCl, followed by 5 mL of EDTA, using a 31 G NaviTip (Ultradent, South Jordan, UT, USA).

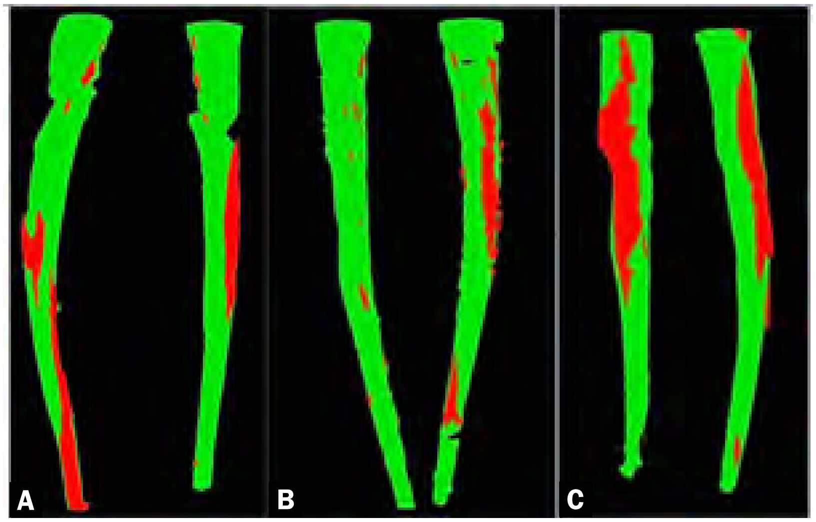

Figure 1
Superimposed pre- and post retreatment micro-CT images of a representative sample of each group. Green corresponds to the initial root canal filling and red to the filling remaining after using the reciprocating instruments. REC **(A)** three-dimensional model of a tooth after filling and retreatment procedure with Reciproc; RECB **(B)** three-dimensional model of a tooth after filling and retreatment procedure with Reciproc Blue; and WO **(C)** three-dimensional model of a tooth after filling and retreatment procedure with Wave One.

Micro-CT Scans

Preoperative and postoperative microtomographic examinations were performed for the non-destructive evaluation of the volume of obturator material remaining in the entire canals and also in the apical, middle, and cervical thirds. For this step, a SkyScan 1173 X-ray Microtomography (Bruker microCT; Bruker, Aartselaar, Belgium) was used.

The specimens were scanned with a power of 90 KV and 278 μA, rotated 360° with 0.5° of rotation step, producing an image with voxel size to be defined (from 6 to 20μm). The filter used was 0.1 mm copper. The images were analyzed with the program CTAn v.1.15 (Bruker microCT), and the volumes of obturator material (mm 3) after the obturation and the filling removal procedures were calculated. Then, the 3D models were created and visualized through the program CTVol v.2.3 software (Bruker microCT).

Statistical analysis

The results were analyzed using the Biostat 4.0 Program. The Shapiro-Wilk normality test was applied. The sample presented normal behavior. The ANOVA (Tukey) test was applied with a significance level of 5%.

Results

None of the complications had occurred during instrumentation nor than retreatment procedure. The volume of obturator material decreased in the cervical and middle thirds after the use of WO, REC and RECB systems (P<.01), but there was no significant difference (Figure 1) between the three systems (P>.05). In the apical third (Table 1) there was no significant reduction in the remaining obturator material independent of the operating system (P>.05).

Discussion

Removal of all the obturator material from the root canal system in cases of retreatment is essential for effective action of irrigation solutions and instruments, as the remaining gutta-percha and sealer can shelter microorganisms, which will lead to failure (14-17). This study aimed to evaluate, by micro-CT, the amount of filling material remaining in curved canals after the use of three reciprocating instruments. The null hypothesis was accepted as there were no differences between the groups in the volume of obturator material removed. It is a consensus in the literature that no technique can completely remove the obturator material from the root canal system (18-20).

The reciprocating instruments REC, RECB, and WO were developed to perform the instrumentation of the root canals, presenting similar shaping ability (21). Although the reciprocating instruments used in this study were not designed for retreatment, these have already been tested for this purpose in other studies (7, 22) with controversial results. Notably, procedural errors may occur in curved root canals (20); however, no instrument presented deformation or fracture in this study. The reciprocating systems are as efficient as the rotary in retreatment, regardless of the morphology of the canals (14).
Several methodologies, such as cleavage (5, 24), radiographs (25), and diaphanization (26), were already used to evaluate the amount of remaining obturator material; however, in this study, micro-CT was used as a 3D method, non-destructive and accurate (27).
In this study, there were no significant differences between the systems in the quantity of initial and final remaining obturator material in the apical third of the studied groups. This can be justified by the fact that the instruments used in the removal of root canal obturator material have the same tip (0.25) as the last instrument used during the preparation of the root canals. Also, moderately curved root canals

Table 1

Arithmetic means, standard deviations, and statistical analysis of ANOVA (Tukey) of the total volume of filling material and the remaining amount in the apical, middle, and cervical thirds after using Reciproc (REC), Reciproc Blue (RECB) and WaveOne (WO)

	Filling Material Total	Filling Material Apical	Filling Material Middle	Filling Material Cervical
WO-I	7.43 (3.09)^{A}	1.14 (0.79)^{A}	4.00 (1.24)^{A}	2.29 (1.06)^{A}
WO-F	2.15 (2.15)^{B}	0.70 (0.68)^{A}	0.58 (0.63)^{B}	0.87 (0.84)^{B}
REC-I	7.47 (1.47)^{A}	1.03 (0.39)^{A}	4.31 (0.82)^{A}	2.13 (0.26)^{A}
REC-F	1.48 (1.22)^{B}	0.41 (0.26)^{A}	0.61 (0.61)^{B}	0.46 (0.35)^{B}
RECB-I	5.95 (0.85)^{A}	0.80 (0.25)^{A}	3.35 (0.37)^{A}	1.80 (0.23)^{A}
RECB-F	0.87 (0.81)^{B}	0.24 (0.21)^{A}	0.37 (0.37)^{B}	0.26 (0.23)^{B}
(P-value)	<.01	>.05	<.01	<.01

I: initial, F: final, different uppercase letters in the vertical direction: statistically significant differences.

were used in this study. The use of more tapered instruments than the last instrument used in the preparation of the canals would be ideal for promoting more significant apical enlargement and consequently higher removal of the gutta-percha (28, 29). Furthermore, Rodig et al. (20) used the R25 file followed by R40 file (40/.06) in WL, and this may have contributed to the higher percentage of gutta-percha removal. Although Bernardes et al. (30) have shown that reciprocating systems with larger tapers are more efficient than rotary ones, the Twist File Adaptive (TFA) and PUR provided the most significant removal of filling material then REC (31). Ozyurek et al. (32) have shown that ProTaper Next and PUR rotational systems were superior to REC and TFA. Other studies (9, 14, 19, 20), found that rotary and reciprocating instruments are effective in removing the filling material, regardless of canal morphology. Comparing REC and RECB, De Deus et al. 2019 (7) found no differences; however, Bago et al. (22) have shown that REC was superior than RECB in the removal of the gutta-percha from the root canal system. RECB and K-manual files were able to remove great part of Thermafil and AH Plus fillings (33).

The single cone technique was used in this study as one of the most commonly used techniques. Higher volume of remaining sealing material was found in the canals filled by the Continuous Condensation Wave Technique than in those in which the Lateral Condensation Technique (29).

Although Cavenago et al. (34) reported that the use of xylene improved the removal of gutta-percha remnants, the solvent was not used in this study because of cytotoxicity (35) and the increase in retreatment time, without much efficacy. It can also increase the adhesion of the gutta-percha to the walls of the canals (29). Another study (6) reported the agitation of xylene with ultrasonic tips after the use of reciprocating instruments, which slightly increased the removal of the remaining obturator material, but it was not statistically significant. Complementary techniques to improve the removal of the remaining filling material, such as XP-Endo Finisher (36), have been studied with satisfactory results. Although the Passive Ultrasonic Irrigation has not been sufficient for the removal of gutta-percha in maxillary molars (19), it was useful when used in mandibular oval incisors after the use of REC 50 (30).

Conclusions

No protocol could effectively remove all the gutta-percha and sealer from the root canal system. There was no significant reduction of the remaining obturator material in the apical third in the three systems studied, which shows that these systems were equivalent but not efficient for the removal of the gutta-percha in curved canals in mandibular molars. More studies are needed to evaluate the best instrument for gutta-percha removal during root canal retreatment, as well as the need for additional techniques to increase root canal cleansing (36, 37).

Clinical Relevance

The present study aims to help clinicians understand the ability of different reciprocating instruments in the removing of sealer material from curved canals.

Conflict of Interest

Nothing to declare.

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ORIGINAL ARTICLE

Bacterial leakage assessment of bioceramic sealers

ABSTRACT

Aim: *To assess the bacterial leakage of two bioceramic sealers and compare them to an epoxy resin-based sealer.*
Methodology: *A sample of 94 one-rooted premolars was obtained and separated into three experimental groups of 30 teeth; two samples were used for negative controls and two for positive controls. The experimental groups were obturated using the hydraulic compaction technique: Group 1 AH Plus® sealer, Group 2 Bioroot® RCS and Group 3 TotalFill® BC Sealer. The teeth were mounted on bacterial leakage devices and incubated at 37 °C for 30 days in a bilin esculin agar culture with* Enterococcus Faecalis.
Results: *Leakage occurred in 27 roots (30%) of the entire sample. Group 1 (AH® Plus) showed the highest percentage of leaked samples (40%) and also took the least average days to leak (16,9) compared to Bioroot® (19,7) and TotalFill® (19,5), but the differences found were not significant (p>0,05).*
Conclusions: *None of the sealers analyzed in this study produced an effective apical seal in which no bacterial leakage occurred. A third of the total sample presented leakage, but the differences between groups were not significant.*

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Introduction

One of the main objectives of root canal treatment is the three-dimensional stable seal of the root canal system to avoid fluid, bacteria, and bacterial byproducts filtration (1, 2). This obturation is usually obtained through a gutta-percha core and an endodontic sealer that improves wall adaptation and fills irregularities and spaces inside the core (1). There is no endodontic sealer that allows a fluid-tight seal of the root canal system, and that also presents the ideal qualities of insolubility, biotolerability, fluidity, and physicochemical stability (3). Epoxy resin sealers are widely used in clinical endodontics (4), and they are considered the gold standard to compare new sealers (5), mainly because they present favorable qualities such as low solubility, adequate radiopacity (6) and dimensional stability (7). However, they have the disadvantage of producing a high initial inflammatory response (8).

Since biocompatibility and sealing ability of traditional sealers should be improved (3), bioceramic cements were developed few year ago to overcome these drawbacks. These sealers present several advantages over epoxy resin sealers, including bioactivity and biotolerability. Bioactivity refers to the stimulation of mineralization through the deposit of apatite crystals over the dentinal walls, generating a chemical bond between dentin and obturation material (9); and good biotolerability refers to the fact that, when compared to others sealers, they induce less citotoxicity (10, 22). They are hydrophilic, which allows setting in the presence of humidity, and they also present a significant rise of pH during the first few weeks after the initial setting (6, 9, 11).

TotalFill® BC Sealer (FKG Dentaire, SA, La-Chaux-de-Fonds, France) is amongst the most frequently used bioceramic sealers. It is a premixed calcium silicate-based sealer, composed of zirconium oxide, calcium silicate, monobasic calcium phosphate ($CaH_4P_2O_8$), calcium hydroxide, tantalum peroxide (9) and thickening agents. Its reported properties are adequate radiopacity, good fluidity because it is nanoparticulated, and a high pH of 11-12 for several days after its initial setting (9), giving the sealer an antibacterial effect. However, it has been reported that it presents higher solubility, porosity and water absorption than other sealers, possibly because of its longer setting time and hydrophilia (9); this factor may affect its capacity to produce a fluid-tight seal (12).

Bioroot® RCS (Septodont, Saint-Maur-des-Fosses, France) is a calcium silicate-based endodontic sealer presented commercially as a powder-liquid material. The powder comprises of tricalcium silicate, zirconium oxide as an opacifier, and excipients; the aqueous solution is composed of calcium chloride and excipients (6, 7). It is a hydrophilic sealer that presents a pH rise of up to 11-12 during the first 14 days after the initial setting (6). It has an antibacterial property (13) given by a sustained pH of 10 for up to six months (11). Moreover, it has good biotolerability (8, 14, 15) adequate radiopacity and excellent capacity to release calcium ions and apatite formation, but has higher solubility than other sealers such as AH Plus® and MTA Fillapex® (6). This high solubility has also been observed in TotalFill® BC Sealer (9); it is proposed for both cements that the sealing capacity could be unaffected by this solubility, because the apposition of apatite could mitigate it (11).

A treated root canal must be adequately sealed to prevent microleakage, because this could lead to the development of an apical pathology (16), mainly because bacteria, cell wall components and soluble byproducts of bacterial metabolism can easily penetrate inside the root canal (5). One of the methods used to evaluate the effectiveness of materials and obturation techniques is to verify the leakage of fluid, bacteria, or chemical substances through the interphase between the radicular wall and the obturation material or through the spaces inside the material itself (5).

This study aimed to evaluate the sealing ability through the bacterial leakage of two bioceramic sealers: TotalFill® BC Sealer and Bioroot® RCS and compare them to an epoxy resin-based endodontic sealer: AH Plus® (Dentsply-Sirona, Ballaigues, Switzerland).

Materials and Methods

An experimental ex vivo study was conducted on extracted human one-rooted lower premolars obtained on the public dental services of Curicó province, Chile, with the previous approval of the ethics committee of the San Sebastian University (resolution Nº 2019-53). The teeth were cleaned of organic residue with a Scaler (NSK Varios 570, Japan), stored in saline solution, and a periapical x-ray was taken. Inclusion criteria consisted of roots with small curvatures (according to the Schneider Method), the presence of only one canal, no intracanal calcifications, complete radicular formation, and absence of resorptions.
The obtained sample consisted of 94 teeth, which were then decrowned 1 mm coronal to the cemento-enamel junction. Working length was obtained through introducing a K10 File until it was visualized through the apical foramen, and 1 mm was subtracted of this length. Canal shaping was performed with WaveOne® Primary files (Dentsply-Sirona, Ballaigues, Switzerland) and frequent irrigation and aspiration, of 5% Sodium Hypochlorite.
The final irrigation protocol was performed with 17% EDTA for 60 seconds, followed by 5% Sodium Hypochlorite and Saline Solution. Canals were dried with paper points and then distributed randomly in three different experimental groups of 30 samples each, to be obturated with the Synchronized Hydraulic Condensation Technique with WaveOne® Primary gutta-percha cones (Dentsply-Sirona, Ballaigues, Switzerland). Group 1: AH Plus® sealer (Dentsply-Sirona, Ballaigues, Switzerland); Group 2: Bioroot® RCS® (Septodont, Saint-Maur-des-Fosses, France); Group 3: Total-Fill® BC Sealer® (FKG Dentaire SA, La Chaux-de-Fonds, Switzerland). After root canal obturation, each root's coronal end was sealed with a glass ionomer self-curing cement (Chemfill® Superior, Dentsply, Germany) and exteriorly sealed with methacrylate varnish leaving the most apical two millimeters uncovered. Two roots were sealed entirely with glass ionomer cement after obturation, and were used as negative controls. One root was not obturated and was covered only with methacrylate varnish, and used as positive control.
According to the bacterial leakage model, the roots were mounted on a device built specifically for this study (Figure 1). The device consisted of two chambers connected by the endodontically treated tooth (17) (De Deus, 2007): the superior chamber was an Eppendorf tube (Biologix Research Company, USA) cut to a capacity of 1.5 ml of volume with a hermetic seal lid, and the inferior chamber was a 5ml glass bottle with a plastic lid. The union between the tube and the tooth was sealed with industrial silicone (Silicon Seal, Lanco, Orlando, Florida, USA). After that, the Eppendorf tubes were fixed to the glass chambers with cyanoacrylate incorporating a 30G Luer type needle, which allowed both the entry of fresh broth and the liberation of gases (Figure 1).
The devices were left to settle for 24 hours and then were sterilized with Ethylene

Figure 1
Bacterial leakage device composed of two chambers connected by the endodontically treated tooth.

Oxide; they were posteriorly opened in a sterile environment obtained through the use of three burners, and Bile Esculin Agar broth (Becton Dickinson and Co. Sparks, USA) was loaded into the superior chamber.
The inferior chamber was loaded with an *Enterococcus Faecalis* suspension in bile esculin agar broth, adjusted to 0,5 McFarland (1,5x108 CFU/ml) (Probac do Brasil, Sao Paulo, Brasil) leaving the root apex submerged in the contaminated broth. Then all the samples were incubated for four weeks at 37 °C in a Binder culture incubator (Tuttlingen, Germany), with frequent introduction of fresh nutritional broth. Bacterial growth was determined through the appearance of turbidity in the superior chamber. The number of days in which turbidity occurred was registered according to the group, and the data was analyzed with IBM SPSS 24.0 statistics program. A sample was taken of each broth that presented turbidity and again cultured in bile esculin in 6.5% NaCLO agar to confirm the bacterial strain.
A Shapiro-Wilk test was applied determining that data distribution was non-normal ($p<0,05$), proceeding then to the application of Kruskal-Wallis non-parametric test to analyze independent samples.

Results

Out of the 90 experimental samples observed, 27 presented bacterial leakage, which corresponds to 30%. Table 1 shows leakage results per group, including the average day in which turbidity occurred. Group 1 (AH Plus®) showed the highest bacterial leakage with 12 samples (40%), Group 3 (TotalFill® BC Sealer) presented eight samples with leakage (27%), and Group 2 (BioRoot® RCS) presented seven leaked samples (23%). The group that took the least amount of days to leak was Group 1 (AH Plus®) with 16,9 average days, followed by Group 3 (TotalFill® BC Sealer) with 19,5 days average and Group 2 (Bioroot® RCS) with 19,7 days. No turbidity was observed on the negative controls, and on the positive controls, turbidity was observed on the third day for both samples. The differences within the samples were not statistically significant ($p>0.05$).

Discussion

Endodontic sealers should ideally produce a long-term fluid and bacteria-tight seal of the root canal system, but this has not been achieved in the past with the available sealers. One of the objectives of obturation is the entombment of remaining bacteria, which cannot occur if there is apical filtration. To overcome these limitations, new materials and obturation systems are continuously developed.
In the present study, leakage was observed in all groups with no statistical differences between them; 30% of all samples presented bacterial leakage, which in general terms means that none of the sealers produced an effective seal. These results agreed with Yanpiset et al. were leakage was observed in 20 to 45% of all studied groups (18).
The group that presented the highest amount of leaked samples was the AH Plus® group (Group 1). It also showed the shortest median time to leak, without significant differences compared to the other groups. These results matched with what was observed by Viapiana et al. who evaluated the sealing ability of BioRoot® RCS and AH Plus® through fluid transportation and microsphere leakage, and obtained similar results in both groups (4). Zhang and Yanpiset et al. compared Bioceramic Sealers to AH Plus® sealer, and they also did not found any significant differences between groups (1, 18).
Contrary to the results obtained in this study, Pawar et al. observed that the bioceramic cement' sealing ability was better than AH Plus® sealer (19). But the method they used to measure leakage was Blue Methylene dye, which has a low molecular weight, and has a different penetration pattern (5). El Sayed et al. found that leakage was significantly less in a Bioceramic sealer (Endosequence BC Sealer, Brasseler, USA, Savannah, GA) when combined with a hydrophilic gutta-percha C-point (Brasseler, USA, Savannah, GA). However, when they used bioceramic sealers with conven-

Table 1
Bacterial leakage results per group and average day in which turbidity occurred with standard deviation

	Bacterial Leakage		Average days to leak
	YES	NO	
Group 1 (AH Plus)	12 (40%)	18	16,9 d (9,5 SD)
Group 2 (Bioroot)	7 (23%)	23	19,7 d (9,5 SD)
Group 3 (Totalfill)	8 (27%)	22	19,5 d (7,3 SD)

tional gutta-percha, they registered no significant difference between them and epoxy resin sealers (20). Muedra et al. (21) found higher sealer penetration for Endosequence BC sealer when compared to Bioroot and AHPlus sealer, although the difference was not significant. They attributed the difference between the syringe sealer and the powder/liquid sealer, possibly to particle size, presentation and fluidity.
Leakage occurred on average at 16 to 19 days for all groups, which is earlier than what was observed in other studies in which leakage occurred mainly after the fifth week of observation (5) and after nine weeks for 30% of the resin sealer samples (17). This early leakage may have occurred because the synchronized hydraulic technique uses just one tapered gutta-percha cone and a considerable amount of sealer, which could compromise the obturation if and when some parts of the sealer contracts or dissolves.
The mentioned studies used lateral compaction technique for their samples (5, 17), which might require further evaluation, because lateral compaction would be a more favorable technique for bioceramic sealers. A study conducted by Yanpiset et al. used a similar obturation protocol, but filtration occurred on days 42 to 52 on average (18). The difference with the outcomes reported in the present study could be due to the use of distobuccal roots instead of one-rooted premolars, which have a more irregular transversal shape.
There are different methods to measure leakage, such as dye penetration and diffusion, bacterial and endotoxins leakage, fluid, glucose, caffeine, and radioisotope filtration (2). The methods that use bacterial or bacterial byproduct penetration are considered more clinically and biologically relevant than the dye penetration method (16). *Enterococcus Faecalis* could be even more appropriate because it is one of the microorganisms related to apical periodontitis in previously treated teeth (13).

Conclusions

Under the conditions of this study, it is possible to conclude that none of the materials used in this study offer a 100% effective seal. All groups presented leakage, without significant differences between them, but with slightly better behavior for bioceramic sealers.

Clinical Relevance

The use of Bioceramic Sealers is widespread because of their biocompatibility. This study shows that their sealing ability is similar to resin sealer AH Plus, which is the gold standard to measure endodontic sealers. This result gives more confidence to use these relatively new sealers.

Conflict of Interest

The authors declare no conflicts of interest.

Acknowledgements

None.

Ethics approval

The ethics committee of the San Sebastian University (resolution Nº 2019-53).

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ORIGINAL ARTICLE

Association between psychological factors and pain perception in patients with symptomatic irreversible pulpitis during endodontic treatment

ABSTRACT

Aim: *To investigate the association of pain perception of inferior alveolar nerve (IAN) block injection and access cavity preparation with psychological factors in patients undergoing endodontic treatment.*
Methodology: *In this observational study, out of 208 patients who had lower molar with symptomatic irreversible pulpitis, 165 patients completed the study. Psychological factors comprising anxiety, depression and personality traits were evaluated by Hospital Anxiety and Depression scale and short form of NEO Five-Factor Inventory. Procedural pain comprising needle insertion and anesthesia solution deposition during IAN block injection as well as access cavity preparation was rated based on the Heft-Parker visual analog scale. Binary logistic regression was used to determine odd ratio (OR) with 95% confidence interval (CI).*
Results: *The mean (standard deviation) age of patients was 34.63 (12.42) in which, females comprised 72.7% (n=120). Considering the psychological factors, the anxious and depressed individuals constituted 38.8 % and 32.7% of the participants, respectively. By adjusting the socio-demographic factors, depression score during needle injection and anesthetic solution deposition were the risk factors for higher levels of pain (OR=1.12; 95% CI=1.03_1.29 and OR=1.13; 95% CI=1.05_1.32 respectively). Among the personality traits, just neuroticism at needle insertion and anesthetic solution deposition associated with higher levels of pain (OR=1.11; 95% CI=1.02_1.28 and OR=1.09; 95% CI=1.01_1.20 respectively).*
Conclusions: *Coupling with the effect of physiological aspects (depression and neuroticism) on the perception of pain at the needle insertion and anesthetic solution during IAN block injection, a multidisciplinary effort both by dentists and by psychologists might improve dental services for some patients.*

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Introduction

Perceptions of pain related to dental treatment may be variated not only by procedural issues, but also by many environmental and psychological factors (1-4). Gender, age, education, anxiety, fear, depression (2, 3) as well as former experiences, expectancy level of control (1) are some of the factors which may affect the perceptions of pain in dental setting.

Dental-related anxiety, which is likely to affect 10 % of people, is one of the chiefs impediments for dental treatment and can adversely affect the patient-practitioner interaction (5). Likewise, depression, a psychiatric condition that harms behavioral patterns, satisfaction and temper over the time, is related to a wide-range of dental diseases (6) and may be associated with dental pain (3, 7). However, the role of anxiety and depression on the pain perception has not been comprehensively studied in the field of Endodontology.

Although the effect of personality traits, behavioral patterns affecting the way of thinking about oneself and the surroundings, on the dental belief (8) and dental anxiety (9, 10) have been evaluated, the role of personality characters in pain perception still remains a mystery in the field of dentistry.

Owing to lack of evidence regarding association among psychological factors, particularly personality characters with perception of pain in the field of Endodontology, the present study aimed to investigate the association of anxiety, depression and personality traits with pain perception during inferior alveolar nerve (IAN) block injection and access cavity preparation for patients with symptomatic irreversible pulpitis undergoing endodontic treatment.

Materials and Methods

This observational study was performed from January, 2016, to January, 2017. 208 patients attending the Department of Endodontic at Dentistry School, Isfahan University of Medical Sciences, who had at least one mandibular molar tooth with symptomatic irreversible pulpitis entered the study. Inclusion criteria included participants being able to complete the questionnaires, no sign of periodontal disease or apical radiolucency (except periodontal ligament widening), no history of allergic reaction and systemic diseases that contraindicate lidocaine injections, no medication history that may alter pain perception or interact with lidocaine. Exclusion criteria included confronting a necrotic pulp after access cavity preparation and no sign of successful anesthesia after 15 minutes.

The study was performed after ethical and scientific approval of the Regional Bioethics Committee of Isfahan University of Medical Sciences (IR.MUI.REC.1395.3.375). This research was done in complete agreement with the World Medical Association Declaration of Helsinki. Information about this study was given to patients in waiting room and they were asked to sign the informed consent. In order to maintain the privacy of the patients and confidentiality of the research, questionnaires were given to them in sealed packs.

All eligible patients with the history of pain were tested with a Frisco spray (ad-Arztbedarf GmbH, French, Deutschland) and Gentle Pulse electrical pulp tester (Parkell Inc., Farmington, NY), to confirm symptomatic irreversible pulpitis diagnosis.

Using the standard IAN block injection (11), a cartridge of 2% lidocaine hydrochloride with 1:80,000 epinephrine (Darou Pakhsh, Tehran, Iran) with 27-gauge 1¼-inch needle (Septoject, Septodont, Saint-Maur-des-fosses cedex, France) attached to an aspirating syringe (Aspirating Dental Injection Syringe, Novocol Ontario, Canada) was administrated for each patient. All IAN blocks were injected at the same manner and location by an endodontist (A.Kh). 0.2 mL of anesthetic solution was deposited during the advancement of needle toward the bone at 1cm higher than occlusal plan. After contacting needle to the bone, it was drawn back for 1 mm and after negative aspiration, the rest anesthetic solution was deposited in 60 seconds. The second IAN block was immediately

Table 1

Socio-demographic characteristics, psychological variables and level of pain of study population (n=165)

Variables	Mean (SD)	Frequency (%)
Age	34.63 (12.42)	
Educational year	11.72 (4.21)	
Gender		
Female		120 (72.7)
Male		45 (27.3)
Marital status		
Married		111 (67.3)
Widow		1 (0.6)
Divorced		7 (4.2)
Single		44 (26.7)
Type of tooth		
First molar		93 (56.4)
Second molar		49 (29.7)
Third molar		23 (13.9)
Depression score	6.18 (3.21)	
Depressed individual		54 (32.7)
Anxiety score	6.83 (3.40)	
Anxious individuals		64 (38.8)
NEO Five Factor Inventory		
Neuroticism	22.15 (3.44)	
Extraversion	28.56 (5.77)	
Openness	25.19 (6.76)	
Agreeableness	29.94 (5.49)	
Conscientiousness	31.37 (6.51)	
Level of pain at T1		
Low		102 (61.8)
High		63 (38.2)
Level of pain at T2		
Low		96 (58.2)
High		69 (41.8)
Level of pain at T3		
Low		113 (68.5)
High		47 (28.5)

Abbreviations: T1, time of needle insertion; T2, time of anesthetic solution deposition; T3, time of access cavity preparation.
Pain according to Heft-Parker visual analog scale.

injected again using second cartridge in the same manner.

If the lip numbness was not obtained within 15 minutes after the injection, the block would be considered missed and patients dismissed and reappointed. In cases with profound lip numbness achievement, the tooth would be tested again with cold spray to confirm pulp anesthesia. Then access cavity would be prepared (12).

Socio-demographic factors, depression and anxiety, personality traits, and pain perception of participants were obtained by questionnaires. Socio-demographic factors including age, gender, educational years and marital status (widowed, divorced, married or unmarried) were recorded. The Hospital Anxiety and Depression scale comprising two subscales, each of which included seven items which were ranked according to a four-point rate was used for measuring the participants' anxiety and depression. Each subscale had a range from 0 to 21 and scores equal or higher than 11 were considered as clinically anxious and depressed (13, 14). To study personality traits, the short form of NEO Five-Factor Inventory scale which is made of 60 questions (12 items for each personality) was used and each question was scored from 1 for agreeing strongly to 5 for disagreeing strongly (15). If a score of each personality trait was higher than the median, the patient would be associated with that trait. The five personalities studied in this questionnaire were extraversion; neuroticism; agreeableness; openness to experience; conscientiousness.

Before the procedure, the patients were instructed to rate pain at three phases of procedure; needle insertion, anesthetic solution deposition as well as access cavity preparation according to the 170-mm Heft-Parker visual analogue scale (HPS) (16). This scale was rated 0 to 54-mm and 55 to 170-mm corresponding with low pain and high pain respectively (17). The needle insertion and anesthetic solution deposition pain were recorded immediately after injection. In addition, after the access cavity was prepared, the pain of entering the dentin was recorded.

For describing continuous variables, mean with standard deviation (SD) was used. Comparison between groups' means was performed by t-test. Association between pain perception and psychological factors was analyzed with the use of a binary logistic regression test. Corresponding confidence interval (CI) of 95% was used for reporting odd ratio (OR). The dependent variable was the level of pain (low/high) and the independent variables were socio-demographic factors including sex,

Table 2
Comparison of mean (SD) of psychological variables between levels of pain perception (low or high) at different time of intervention

	Level of pain at T1			Level of pain at T2			Level of pain at T3		
	Low	High	P-value^2	Low	High	P-value^2	Low	High	P-value^2
Depression score^1	5.98(2.19)	6.88(2.26)	0.04	5.74(2.29)	6.78(2.80)	0.04	6.20(3.29)	6.26(3.22)	0.94
Anxiety score^1	6.75(4.71)	6.91(3.92)	0.82	6.66(4.01)	6.94(4.68)	0.69	6.19(4.56)	7.16(4.44)	0.74
NEO Five Factor Inventory^1									
Neuroticism	21.05(2.04)	23.74(3.17)	0.04	21.21(3.30)	23.46(3.69)	0.03	22.37(4.55)	22.64(3.43)	0.78
Extraversion	28.59(5.56)	28.57(6.14)	0.98	28.71(5.76)	28.33(5.81)	0.71	28.78(5.83)	27.37(5.34)	0.21
Openness	25.22(6.07)	25.15(5.28)	0.93	25.88(4.72)	26.62(4.80)	0.37	25.52(5.01)	24.51(4.76)	0.26
Agreeableness	30.36(6.83)	29.80(5.70	0.64	29.90(5.80)	29.90(5.04)	0.91	30.45(5.51)	29.56(5.35)	0.28
Conscientiousness	32.36(6.89)	30.78(6.83)	0.15	31.68(6.61)	30.93(6.40)	0.49	31.42(6.82)	30.79(5.66)	0.59

Abbreviations and notes: SD, standard deviation; T1, time of needle insertion; T2, time of anesthetic solution deposition; T3, time of access cavity preparation. ^{1}Mean (SD), ^{2}t-test.

Pain according to Heft-Parker visual analog scale.

age, educational and marital status as well as depression (yes/no), anxiety (yes/no) and personality traits (yes/no). Analysis was performed using SPSS, version 21 (IBM Corp, Armonk, NY) and P<0.05 was considered as the level of significance.

Results

A total of 208 individuals were eligible to be included in this study. The profound lip numbness was not achieved in 17 patients, 2 teeth were diagnosed as partially necrotic pulp after access preparation, and 24 patients failed to fill the questionnaires completely (participants who failed to answer more than 10% of questions). Finally, the mean (SD) age of 165 patients who completed the study was 34.63 (12.42) years ranging from 19 to 70 years. Females comprised 72.7% (n=120) of the sample. The distributions of anxious and depressed individuals were 38.8% and 32.7%, respectively. Based on HPS, 41.8%, 38.2%, and 28.5% of patients reported high levels of pain for anesthetic solution deposition, needle insertion, and access cavity preparation respectively (Table 1).
Higher levels of pain at needle insertion and anesthetic solution deposition phase were associated with depression score and neuroticism traits (P<0.05, t-test). There was no association between higher pain of access cavity preparation with any psychological factors (P≥0.05, t-test, Table 2).
After adjusting the socio-demographic factors, binary logistic regression model demonstrated that by increasing a unit of depression score, the odd of pain perception at high level would be 1.12 and 1.13 times greater during needle injection and anesthetic solution deposition respectively (OR=1.12; 95% CI=1.03_1.29 and OR=1.13; 95% CI=1.05_1.32 respectively). Moreover, neuroticism during needle insertion (lower median VS. upper median: OR=1.11; 95% CI=1.02_1.28) and during anesthetic solution deposition (lower median VS. upper median: OR=1.09; 95% CI=1.01_1.20) were the risk factors associated with feeling higher levels of pain (Table 3).

Discussion

The etiology, persistence and perception of pain, which is common in dental environment, is a multifaceted agenda (18). Beside the neurobiological aspects of pain perception, many psychological factors comprising attention, feeling either positive or negative, social interaction and

Table 3

Estimated odds ratio from binary logistic regression model predicts the probability of level of pain (low or high) at T1, T2 and T3 as a dependent variable of psychological variables in 165 patients undergoing endodontic treatment

	T1	T2	T3
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Depression score	1.12 (1.03_1.29)	1.13 (1.05_1.32)	1.01 (0.93_1.11)
Anxiety score	1.01 (0.98_1.10)	1.02 (0.91_1.07)	1.01 (0.92_1.10)
NEO Five Factor Inventory			
Neuroticism			
Lower median	Ref	Ref	Ref
Upper median	1.11 (1.02_1.28)	1.09 (1.01_1.20)	1.02 (0.94,1.09)
Extraversion			
Lower median	Ref	Ref	Ref
Upper median	1.00 (0.94_1.06)	0.99 (0.93_1.07)	0.93 (0.85_1.00)
Openness			
Lower median	Ref	Ref	Ref
Upper median	1.00 (0.91_1.10)	1.07 (0.97_1.18)	0.99 (0.89_1.13)
Agreeableness			
Lower median	Ref	Ref	Ref
Upper median	1.01 (0.94_1.08)	1.01 (0.94_1.08)	1.00 (0.93_1.08)
Conscientiousness			
Lower median	Ref	Ref	Ref
Upper median	0.98 (0.95_1.09)	1.00 (0.94_1.06)	1.00 (0.94_1.07)

Abbreviations: CI, Confidence interval; OR, Odd ratio; T1, time of needle insertion; T2, time of anesthetic solution deposition; T3, time of access cavity preparation.
The outcome variable: level of pain at T1, T2 and T3 (low=0 and high=1). Adjusted based on age, gender and type of tooth.

seeing the other agony may modify it in dental setting (19).
Notwithstanding controversies with respect to the role of psychological factors of patient in pain perception in dental environment, the present study demonstrated that depression and neuroticism trait were risk factors for feeling higher levels of pain at either needle insertion or solution deposition. The consequence of depression on the feeling of pain in dentistry has not been thoroughly understood yet. Although pain perception after dental implant insertion was not modified by the level of depression of patients (20), the post-operative oral sugary pain was found to be influenced by depression and distress (3).
Moreover, the Korea National Health and Nutrition Examination Survey revealed that depressed individuals indicated higher dental related pain, particularly allied to pulpits (7).
Similarly, the present study also indicated the depression score influenced the pain at two stages of injection.
These contrary findings related to the association between depression and pain perception may be attributed to different methodologies and time of measuring pain or as well as diverse dental procedures. Notably, although some models were introduced to explain the co-occurrence of pain and depression, the direct relation remained uncertain (7).
In general, contrary findings observed about the impact of anxiety on pain perception in dental setting mostly concluded the anxiety may alter the dental-related pain (1, 2, 18, 20-23). Notably, in the field

of Endodontology, a moderate association between pain level and anxiety were found (24) the main sources of which were cognitive conditioning and parental pathways (25). The present study also indicated that the anxiety was not a risk factor for pain perception at any phase, which was different from previous studies due to the different dental procedures, population, scale and time of pain rating (2, 21). Considering the high rate of dental anxiety in dental setting, endodontists should know how to recognize and manage anxious patients. Both subjective and objective check would benefit a dentist to recognize an anxious patient and consequently apply psychology intervention or medication administration or multifaceted approaches (26).
It is worthy to mention that even the use of anxiety control protocols alone may not always result in endodontic pain avoidance. The present study discovered that just neuroticism characteristic is associated with both needle insertion and solution deposition pain during the IAN block injection. Feinmann et al. (27) determined, beside anxiety, neuroticism was a risk factor for feeling higher levels of post-operative pain in patients undergoing minor dental surgery. On the other hand, Abu Alhaija et al. (28) reported personality traits did not affect patients' way of thinking about orthodontic therapy and pain. A critical review also releveled the modest association of neuroticism with pain, predominantly with regard to alteration of chronic pain (29), which may be attributed to the fact that neuroticism is correlated with the trait of harm avoidance (30). Additionally, personality parameters may act as moderators for the dental beliefs, fear and anxiety in dental setting (8), hence may causes pain perception indirectly. Above all, the neuroticism traits may be related to the pain perception in dental setting, more studies are recommended.
The use of a self-administered questionnaire for assessing pain without examining any biomarkers, not evaluating other socio-demographic factors as well as merely investigated the procedurals pain can be considered as limitations of the present study.

Conclusions

Depression and neuroticism might be related to higher levels of pain perception at needle insertion and solution deposition during IAN block injection. Therefore, bearing in mind the physiological aspects of pain during endodontic treatment, promoting awareness of endodontists about the identification and managing of physiological factors related to pain as well as informing them about the appropriate time to refer their patients suffering from high pain to psychologists is encouraged to improve dental services.

Clinical Relevance

Depression and neuroticism were associated with a higher level of pain perception during the inferior alveolar nerve block administration.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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ORIGINAL ARTICLE

Shaping ability of WaveOne Gold reciprocating file with and without glidepath in artificial S-shaped canals

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ABSTRACT

Aim: *Most authors have recognized that coronal pre-flaring has several advantages and it provides accordance with the objectives of endodontic treatment. Together with glidepath, it constitutes the most appropriate work sequence for the success of canal shaping. The purpose of this study was to compare the influence of glidepath on canal transportation and centring ability, using the WaveOne Gold with and without glidepath.*
Methodology: *40 standard clear resin "S" shaped Endo Training Blocks* (Dentsply, Maillefer, Switzerland) *divided into two groups of 20 blocks were used in this study. The first group was prepared using WaveOne Gold Glider and WaveOne Gold while the second group was prepared with WaveOne Gold only. After the preparation, the amount of canal transportation, centring ratio, angle and radius of curvature, were measured on superimposed photos taken with an optical microscope. The two groups were statistically compared with analysis of variance, Kolmogorov-Smirnov and Student significant difference test. The level of significance was set at P<0.05.*
Results: *Less transportation and better centring ability occurred when using glidepath before root canal preparation (P<.0001). In these two groups there was a significant variation in the angles and radius of curvature compared to the initial values and this variation was found to be reduced when using glidepath.*
Conclusions: *Canal modifications seem to be significantly reduced when previous glide path was performed before using the WaveOne Gold. The use of glidepath reduced significantly the root canal transportation, minimized the curvatures straightening after the preparation and revealed a better centring ability.*

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Introduction

The objectives of root canal treatment are the elimination of irritants from the canal system, multidimensional obturation in order to block the entry and the exit doors of bacteria and to promote bacterial entombing after canal disinfection, prevention of the recontamination of the canal systems and preservation of the initial canal trajectory (1). Canal transportation is a common error to all canal preparation techniques. It is directly related to canal curvature as well as variations in the centre of gravity (2). According to the AAE (American Association of Endodontics), canal transportation is the concept according to which the instruments tend to regain their initial shape at the level of the external curvature of the apical third, and this by eliminating the canal wall (3). Centring ability preserves the initial canal trajectory and reduces the risk of canal transportation, thus minimizing errors such as ledges, straightening of curvatures and apical enlargement (4).

According to West 2010 (5), canal preparation consists of three stages: the initial canal negotiation, therefore scouting and pre-flaring which is always necessary when the canal is not wide enough, the creation of glidepath and finally canal shaping. The glidepath is a smooth canal tunnel from the orifice of the canal to the foraminal constriction. The glidepath must be discovered if it is already present in the canal anatomy or it must be prepared if it is not. The original canal anatomy can influence the glide path creation (5). Coronal pre-flaring and glidepath minimize errors during canal preparation, since they prevent taper lock, broken instruments and aberrations.

The introduction of nickel-titanium (NiTi) instruments' super elastic alloy which provides great flexibility for shaping the root canal, improved the effectiveness of endodontic treatment, thus reducing preparation time and iatrogenic errors (6). These instruments are associated with lower canal transportation than manual stainless steel files because of their ability to maintain the original curvature of the canal and because of their memory shape (7). However, the resistance of these instruments when it comes to fracturing, is lower and remains disadvantageous compared to manual files (8). The reciprocating movement could reduce the continuous fatigue caused by the rotation of the NiTi instruments, relatively to the continuous motion. The WaveOne Gold which is a unique instrumentation system, introduced by Dentsply Maillefer, is composed of four single-use files: Small (ISO 20 tip and 7% cone) for fine root canals; primary (ISO 25 tip and 7% tapered) for most of the canals; and large (ISO 35 and 6% cone-ISO 45 and 5% cone) for large canals. The files are made of Gold-Wire NiTi alloy. The WaveOne Gold glider (ISO 15 tip and 2 to 6% tapered), introduced by Dentsply Maillefer also, is a single instrument that works in reciprocating motion and is made of gold-Wire, designed in order to be used before the WaveOne Gold system.

The aim of the study was to compare the shaping ability of WaveOne Gold and its impact on the modification of the canal curvature and axis in artificial resin blocks, with and without glidepath.

Materials and Methods

The protocol was approved by the Ethics Committee of Saint Joseph University on March 20.

Forty ISO 15,0.02 taper S-shaped Endo Training Blocks (Dentsply, Maillefer. Ballaigues, Switzerland) were used. Green ink (Pelikan, Hannover, Germany) was injected with a syringe into each of the blocks. Holders were made with putty (ZETAPLUS, Zhermax) and ice cube tray for each of the blocks (Figure 1) in order to organize the blocks during work. Each block was photographed with an optical microscope (Olympus, E330-ADU1 2X, Japan) at 1.25x/0.04/FN 26.5 magnification preoperatively and postoperatively while maintaining the same photography conditions in order to superimpose the photos during the analysis (Figure 2). An opaque slide was chosen to improve the brightness of the photo and therefore increase the accu-

Figure 1
Preparation of the blocks in holders made with putty and ice cube tray.

Figure 2
Olympus optical microscope at 1.25x/0.04/FN 26.5 magnification.

racy of the analysis. Distilled water was used to remove the ink and the blocks were randomly divided into two groups (n=20).
Group A: Catheterization with K file #10 (Dentsply, Sirona) and determination of the working length (WL) and mechanical glidepath using WaveOne Gold Glider (Dentsply, Sirona, Ballaigues, Swizerland). The system consists of a single file with a parallelogram shaped cross-section, 1 size glider 15, and variable taper 2% to 6, used in reciprocating motion with WaveOne Gold settings. The glider was used at the WL. Each canal was shaped with the WaveOne Gold Primary (Dentsply, Sirona, Ballaigues, Swizerland) according to the sequence of pecking motion with parietal support and vertical back and forth to allow the progression of the instrument apically with a light pressure, use of the instrument about three times in average in this group, cleaning of the instrument with a compress soaked in sodium hypochlorite (NaOCl, 2.5%) after each movement, irrigation using half a syringe 3cc of distilled water and permeabilization using K file #10, brushing motion for the elimination of coronary interference and finally, arrival of the instrument at the WL.
Group B: Catheterization with K file #10 (Dentsply, Sirona) and determination of the working length, no glidepath performed and final shaping with the WaveOne Gold Primary (Dentsply, Sirona) using the same sequence as group A. For both groups, the motor used was the E-Connect PRO Endo Motor (Eighteeth medical) in mode: Fwd 30° and Rev 150° according to the manufacturer's recommendations. All procedure was done by the same operator.

Measurements
Pre and post-operative images were overlaid using CS4 extended Adobe Photoshop Program software (San Jose, CA, USA) (Figure 3). The width and angle measurements were made using AutoCAD software (Autodesk Inc, San Rafael, California). Twelve defined measurement points were traced over the entire length of each block and perpendicular to the axis of the root canal.
These levels were designed according to the method described by Madureira et al. (9) (Figure 4): level 1 at the beginning of the root canal and level 3 at the end of the coronal zone before the first curvature, level 2 in the middle between level 1 and level 3 while level 12 is at the apex. In addition, seven more equidistant levels were drawn between levels 3 and 12 and numbered 4, 5, 6, 7, 8, 9 and 10. Level 11 was halfway between 10 and 12. Finally, the images were evaluated in three areas: coronal area (CZ) of level 1-3, area of first curvature of level 4-7 (FCZ) and area of second curvature or apical curvature (AZ) of level 8-12 (Figure 5).

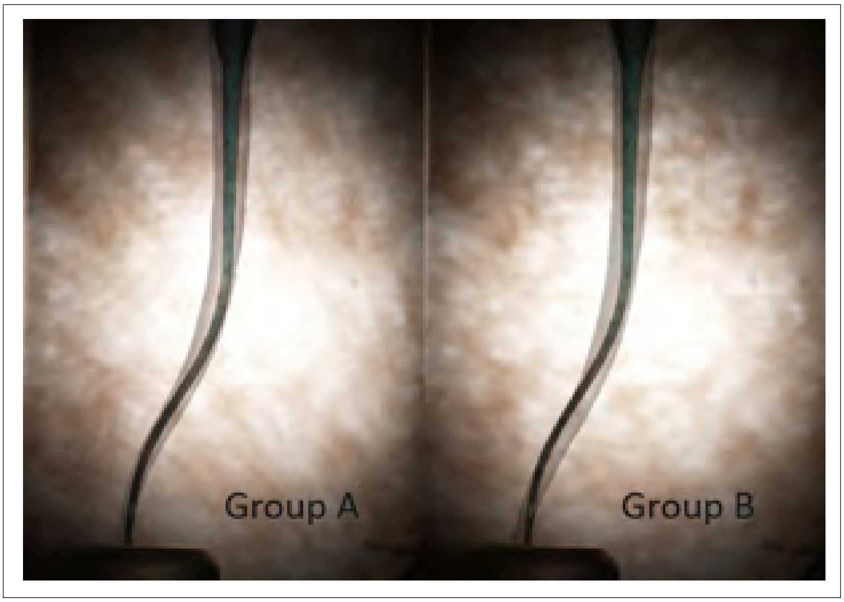
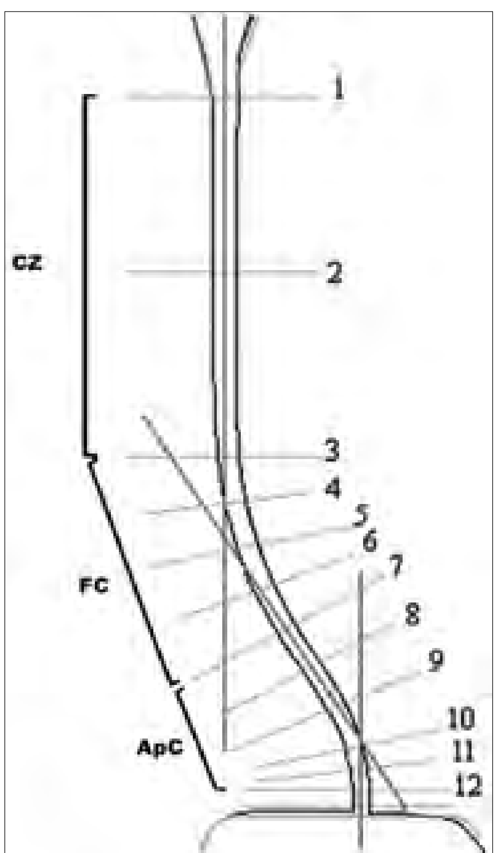


Figure 3
Pre- and post-operative images of resin blocks with (Group A) and without glidepath (Group B).

The comparison between the two groups in pre and post-operative was obtained according to the following factors: the quantification of the canal transport, the quantity of resin eliminated, the centring ratio, the variation of the angles of curvature and the variation of the radius of curvature. The canal was divided into two sides: the first defined as the external aspect of the apical curvature and the internal aspect of the coronal curvature while the second side defined by the opposite (10). To facilitate the measurements and their analysis, the first side was designated by the letter R and the second by the letter L indicating the right and left sides (Figure 6).

The widths of resin removed was calculated at each of the 12 levels divided according to the technique of Madureira described above. The amount of canal transportation is the difference between the widths of resin removed from both sides of the canal while resin removal was calculated by summing the widths of resin removed from both sides of the canal and the centring ratio was calculated by dividing the narrowest width of resin removal by the widest at each sides (11).

For the measurement of the angles of curvature a line A along the axis of the coro-



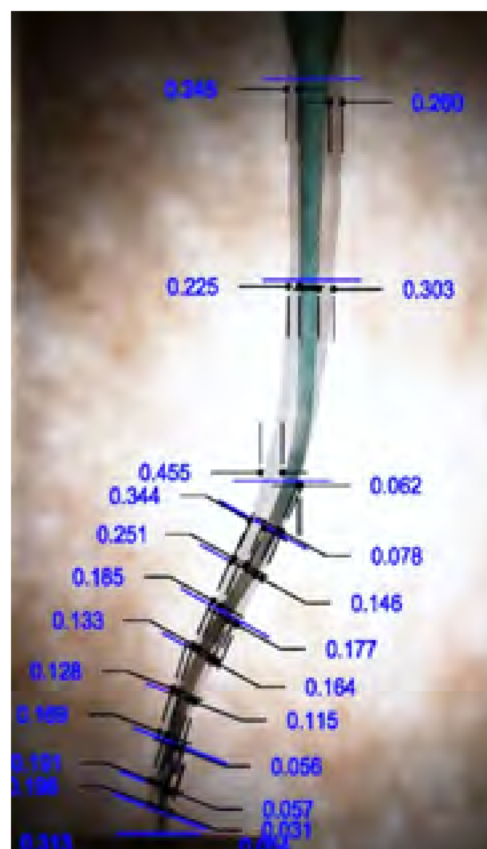


Figure 4
Madureira's technique to divide the entire canal into 12 sections perpendicular to the axix of the root canal.

Figure 5
Measurements of the widths of resin removed from both sides of the canal.

Figure 6
Right and left sides of the canal.

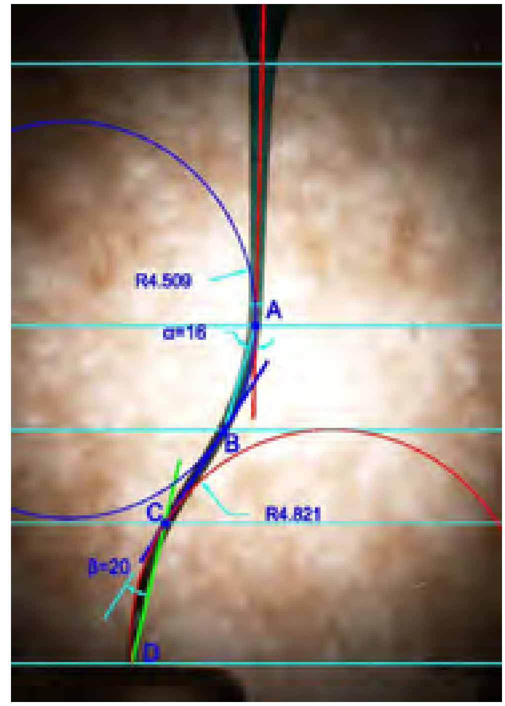


Figure 7
Measurements of the angles and radius of curvatures.

nary part of the canal was drawn passing through the canal entrance and by point A marking the end of the straight coronal part and the beginning of the first curvature. A point B at the start of the deviation of the first curvature and thus forming the first point of inflection. Point C at the beginning of the second curvature and therefore forming the second point of inflection, Point D at the apex.
The inflection points were the exact locations of the change of the direction of the canal. Lines joining A to B, B to C and C to D were also drawn. The angle α of the first curvature represented the intersection of line A with the line AB with vertex the point A. The angle β of the second curvature the intersection of lines BC and CD with the vertex the point C. The measurement of these angles was carried out pre-operatively by obtaining: α is 16˚ and β is 20˚. Post-operative measurements were performed on each of the canals of the two study groups (Figure 7).
For the measurement of the radius of curvature the hypothetical circle of centre O of the first curvature represents the circle passing through the points A and B and whose radius is: Radius 1=AB/2sinα. Whereas the hypothetical circle of centre O' of the second curvature is the circle passing through points C and D and whose radius is: Radius 2=CD/2sinβ.
It should be noted that the more the amount of the initial angle decreases and the more the radius of curvature increases, the more the curvature is straightened. The variation of the distances between points A and B, C and D plays an essential role in the variation of the radius of curvature and it must be taken into consideration (Figure 7).

Statistical analyses
The IBM SPSS Statistics (version 25.0) was used for statistical analyses. The level of significance was set at p≤0.05. Repeated-measure analyses of variance with one between-subjects factor (with or without glidepath) and one within-subjects factor (coronal, first and second curvature zones) were used to compare the canal transportation, resin removal and centring ratio between groups; they were followed by univariates analyses and Bonferroni multiple comparisons tests.
Student t-tests were also used to compare the mean angle and radius of curvature variations between groups (with and without glidepath). One Sample t-tests were

used to compare the mean angles and radius variations with the theoretical values which indicate the absence of a significant variation.

Results

Canal transportation
The mean canal transportation was significantly smaller when using glidepath ($p<0.001$). Without glidepath, the mean canal transportation was significantly smaller at the coronal zone ($p=0.002$), and the difference was not significant between the first and the second curvature zone ($p=0.243$). However, with glidepath, the canal transportation was not significantly different within levels ($p=0.504$) (Table 1).

Centring Ratio
The mean centring ratio was significantly greater with glidepath at the apical level ($p<0.001$). The centring ratio was significantly smaller on the apical zone, intermediate on the middle zone and elevated on the coronal zone for specimen shaped with ($p<0.001$) or without glidepath ($p<0.001$) (Table 1).

Resin removal
The mean resin removal was significantly greater on specimen shaped without glidepath ($p<0.001$). Moreover, the amount removed was smaller on the apical zone and greater on the coronal or middle zone ($p<0.001$) (Table 1).

Angles and radius curvature variations
The variation of alpha and beta angles, the variation of distances and radius curvature were significantly greater in group shaped without glidepath ($p<0.001$). All these measurements were different significantly compared to the reference values ($p<0.001$) (Table 2).

Discussion

A technique advocated by Pr. G. Yared used a single instrument without the glidepath only by using a K-file 08 for the canal scouting (12). Therefore, this tech-

Table 1
Canal transportation, centring ratio and resin removal of the groups

	Canal transportation (mm)			
	Coronal zone	**First curvature**	**Second curvature**	**p**
With glidepath	0.1374±0.0302	0.1362±0.0257	0.1236±0.0277	0.504
Without glidepath	0.1725±0.0430[a]	0.2343±0.0743[b]	0.2077±0.0505[b]	0.002
p	0.005	<0.001	<0.001	
	Centring Ratio			
	Coronal zone	**First curvature**	**Second curvature**	**p**
With glidepath	0.6147±0.0817[c]	0.4952±0.0752[b]	0.4007±0.0748[a]	<0.001
Without glidepath	0.6276±0.0681[c]	0.4343±0.1200[b]	0.2994±0.0717[a]	<0.001
p	0.591	0.062	<0.001	
	Resin Removal (mm)			
	Coronal zone	**First curvature**	**Second curvature**	**p**
With glidepath	0.535±0.0508[b]	0.503±0.0250[b]	0.435±0.0617[a]	<0.001
Without glidepath	0.669±0.0504[b]	0.713±0.0679[b]	0.575±0.0962[a]	<0.001
p	<0.001	<0.001	<0.001	

a, b, c: different letters indicate the presence of a significant difference according to Bonferroni multiple comparisons.

Table 2

Angle curvature (degree), radius curvature (mm) and distance (mm) in different groups

Reference values	With glidepath	Without glidepath	p
Angle alpha=16 degrees	14.725±0.2918 -1.275 degrees	13.965±0.3573 -2.035 degrees	<0.001
Angle Beta=20 degrees	13.535±0.4771 -6.465 degrees	11.990±0.4327 -8.01 degrees	<0.001
Distance AB=2.486 mm	3.764±0.2096 +1.278 mm	4.424±0.4813 +1.938 mm	<0.001
Distance CD=3.298 mm	3.38± 0.1208 + 0.086 mm	3.583±0.2757 0.285 mm	0.005
Radius 1=4.509 mm	7.403±0.3678 +2.894 mm	9.175±1.0569 +4.666 mm	<0.001
Radius 2=4.821 mm	7.241±0.4094 +2.42 mm	8.627±0.6231 +3.806 mm	<0.001

nique encourages shaping without the preliminary creation of a manual or rotary glidepath, an idea subject to several controversies (13).
However, studies have shown that when the tip of an instrument faces a part of the canal that is smaller than its diameter, it locks up and quickly reaches a high torque. If the latter reaches a very high level, the instrument will cause structural deformations (14). Therefore, according to a study by Berutti and Pasqualini in 2004, it is necessary and crucial to carry out a pre-flaring in order to reduce the friction forces and the damage on the instruments (15). Berutti et al also demonstrated in 2009 that the use of Pathfiles to secure the path of the shaping instruments is an essential condition (14). Peter et al in 2003 (16) and Patino et al. in 2005 (17) demonstrated that the canal must be widened with a K 15 lime at least before any use of rotary instruments, regardless of what technique they used.
The main goal of this experimental research was to demonstrate the impact of glidepath on the shaping ability of WaveOne Gold and compliance with the initial canal trajectory. An instrument specially designed with the properties as the WaveOne Gold was used: the WaveOne Gold Glider. This instrument works in reciprocating motion and is made of gold-Wire, it is designed by the same company specially to assist the WaveOne Gold system. In fact, it has been shown that an ideal endodontic treatment begins with a coronal pre-flaring, followed by the realization of a glidepath, preferably rotary and in reciprocating motion, to finally move on to shaping (6,18). In our study, we ensured these conditions in order to be able to compare as accurately as possible the two groups A and B.
We have chosen to use simulated curved S-shaped canals to compare the shaping ability of this system based on the technique of superimposing the contours of the pre- and post-operative root canals, which allowed a direct visual comparison of the changes. However, the blocks were photographed using an optical microscope in order to make sure of the precision of its overlay, unlike several other studies

which have used digital photography, including the studies of Yilmaz et al. (11), Keskin et al. (18), Nazarimoghadam et al. (19).

For other authors, the evaluation criteria is mainly based on the quantification of canal transport, canal centring, the incidence of aberrations and the amount of resin removed (11, 18) who proved that glidepath promotes the maintenance of the initial canal trajectory, minimizes the modifications of the canal curvature as well as the occurrence of iatrogenic errors. However, the modification of canal trajectory is defined by the involuntary deviation of the path of the initial canal which generates the modification canal curvature, the decrease in angle of curvature, leading to the straightening of the canal base. In order to increase the precision of comparison, the straightening of the curvature must be added as an evaluation parameter. In this perspective, we have studied the variation of the angle of curvature as well as the radius of curvature, in addition to other criteria subjects present in several studies.

Our results confirm the initial problem and demonstrate that glidepath is an essential step which increases the success of canal preparation and promotes the maintenance of the initial canal trajectory. Indeed, there was a significant difference between the two groups of studies showing that glidepath reduced canal transportation and resin removal and increased the centring ratio especially at the apical third (p value<0.001). A considerable variation of the radius and the angle of curvature is found in the two groups of study. Glidepath reduced the amount of variation. In a study by Yilmaz (11) in which the shaping ability of WaveOne was evaluated with or without the use of Pathfiles, the results showed that no significant difference concerning canal transportation and resin removal between the two groups. Centring ratio has been shown to be better with the glidepath. Burklein et al. (20), in 2014, demonstrated that glidepath had no influence on transportation or canal centring. This difference may be explained by the experience of the operator which differs between studies, or by the use of a reciprocating glidepath instrument shown to be better compared to the continuous glidepath (18). Coelho et al. (21) and Vorster et al. (22) showed, as well, that glidepath did not reduce canal transportation. This difference can be explained by the use of extracted teeth instead of resin blocks.

Berutti et al. (23) studied the influence of glidepath on canal curvature and the modification of the axis using the WaveOne. They found a significantly greater alteration in canal curvature in the absence of glidepath, and the results of the present study were in agreement with these results. However, it should be noted that straightening was observed even with glidepath, and this was in agreement with the results of Berutti et al. (24) who reported a consequent reduction in the working length of curved root canals. A second check of the working length is recommended after the pre-flaring and before the preparation of the apical part in particular with the WaveOne system. Lim et al. (25) reported that the WaveOne and Reciproc systems remain more centred after the glidepath at the apical third, while Nazarimoghadam et al. (19) showed a significant reduction in canal transportation in the apical third in the presence of glidepath. Their results support the results of this study which showed significantly higher values for canal centring with glidepath.

However, in this study, resin simulators were used and it is well proved that resin differs from dentin in terms of hardness or surface texture (26), as it can be softened due to the heat of friction of mechanical instruments (23). The extracted teeth, on the other hand, reflect better the clinical conditions since two root canals are never identical to each other, they cannot be standardized anatomically, and they can include different variables which can influence the results of the study (27). For this reason, artificial resin canals were used in the present study in order to be able to standardize the sample and get accurate results. Further research on extracted teeth and using CBCT (Cone Beam Computerized Tomography) or Micro-CT (Microcomputed tomography) should be considered in order to increase the precision of the study parameters and reproduce the specific factors of teeth including the variation of the canal anatomy and the dentin properties.

Conclusions

Under the conditions of our study and within its limitations, the canal preparations with the WaveOne Gold enabled better compliance with the canal trajectory in the apical, middle and coronary areas of the double curvature canals with the use of the reciprocating file, WaveOne Gold glider. The use of the mechanical reciprocating glidepath seems to increase the shaping ability of WaveOne Gold by reducing modifications in canal curvature and trajectory. This concept, studied by many authors, remains subject to several controversies influenced by factors that should probably be better explored.

Clinical Relevance

Highlights of the study: Glidepath reduces canal transportation and resin removal and increases centring ratio while reduces canal straightening. Angles of curvatures have higher values in the presence of glidepath and radius of curvature are smaller.

Conflict of interest

The authors deny any conflict of interest.

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ORIGINAL ARTICLE

Antimicrobial activity of tt-farnesol associated with an endodontic sealer against *Enterococcus faecalis*

ABSTRACT

Aim: *This study aimed to evaluate* in vitro *the antibacterial activity of trans-trans farnesol (tt-farnesol) associated with Sealapex sealer against* Enterococcus faecalis.

Methodology: *Initially, the minimum bactericidal concentration (MBC) of tt-farnesol was determined by microdilution technique. The sealer was associated with 350 µg/g tt-farnesol (GS+0.35f); 1,750 µg/g tt-farnesol (GS+0.175f); or only Sealapex (GS). For antimicrobial activity test,* E faecalis *suspension was added in tubes containing the sealer samples and incubated for 24, 48, 72, 96, 120 and 144 h. After each time point, two blinded and calibrated evaluators performed the CFU count. Data were analyzed statistically by one-way ANOVA and Tukey* post-hoc *tests (significance level P<0.05).*

Results: *It was observed difference in the CFU count between G_S, GS+0.35f and GS+0.175 after 48 and 72 h, and between GS for the other groups in 96, 120 and 144 h (P<0.05). The CFU count was lower in GS+0.35f than in GS+0.175f after 48 and 72 h (P<0.05). In GS+0.35f, there was a decrease in CFU count after 48 h and in GS+0.175f after 72 h (P<0.01).*

Conclusions: *The association of tt-farnesol with Sealapex decreased* E faecalis *growth* in vitro *after 48 h of incubation. The MBC of 0.35 and 0.175 mg/mL of tt-farnesol reduced the CFU count after 48 and 72 h, respectively.*

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Introduction

The presence of microorganisms is one of the main conditions for the maintenance of periapical pathologies, after endodontic treatment (1). Therefore, the effectiveness of disinfection and preparation of root canals are crucial, to avoid secondary endodontic infections (1, 2). In addition to the chemical and mechanical preparation, the use of an intracanal dressing and an adequate filling of the root canal are necessary to eliminate pathogenic microorganisms and achieve more success (2).
The root canal filling is the final stage of endodontic treatment and requires a three-dimensional filling for the success of the treatment (3). It was performed using a material, usually of thermoplastic origin, in combination with an endodontic sealer (3). The filling materials should promote a great sealing of the pulp cavity, avoiding recontamination or proliferation of microorganisms that eventually survive the endodontic preparation (3, 4). Besides, it is interesting that endodontic sealers have an antibacterial effect against microorganisms that remain in isthmuses, dentinal tubules and apical deltas (3, 5). There are endodontic sealers of different bases, such as zinc oxide-eugenols, calcium hydroxide, epoxy resins and bioceramics (6-8).
Enterococcus faecalis is a Gram+ bacteria commonly found in cases of secondary endodontic infections (9-12), with a prevalence of 24% to 80% (10, 11), corresponding to 9-99.8% of the total bacterial count (12). It can invade and survive inside dentinal tubules, where it forms a resistant biofilm, which is difficult to eliminate during the root canal preparation (13, 14). The persistence of this bacteria in hostile environments and its resistance to endodontic dressings that raise the pH of the dentin (such as calcium hydroxide) can contribute to damage the periapical tissues, which can be considered a possible cause of post-treatment apical periodontitis (14-16). Also, *E. faecalis* produces several virulence factors, such as surface adhesins and gelatinase, which contribute to bacterial adhesion, colonization, biofilm formation and tissue damage (17-19). Therefore, the elimination of this resistant microorganism is essential to prevent root canal reinfection.
Propolis has been used in Dentistry since the 1990s and consists of an association of wax, oils and bioactive compounds known as bioflavonoid or terpenoid, such as trans-trans farnesol (tt-farnesol), which has antimicrobial activity (20-22). The association between different therapies and bioactive compounds of propolis has been studied. One example is the tt-farnesol that has been associated with fluoride, dental adhesives and glass ionomer cement in anti-caries therapy, and recently used in primary teeth endodontic therapy (21, 23, 24). Combination therapy negatively influenced the virulence of *Streptococcus mutans* biofilms, being effective in controlling the growth of cariogenic bacteria (25-29). However, few studies have attempted to evaluate tt-farnesol action against *E faecalis*. Therefore, this study aimed to evaluate the antibacterial activity of tt-farnesol associated with an endodontic sealer against *E faecalis*.

Material and Methods

Minimum bactericidal concentration determination
Initially, the minimum bactericidal concentration (MBC) of tt-farnesol ($C_{15}H_{26}O$ 96% Aldrich Chemistry INC; St. Louis, USA) was determined through the microdilution technique (30). The diluent was made of 20% 92.8° alcohol (Alcool Santa Cruz; São Paulo, Brazil), 0.75% dimethyl sulfoxide (DMSO, Rio de Janeiro, Brazil) and 79.25% distilled water, totaling 100 mL.
For dilution, 10 ml of diluent was added to tt-farnesol (28 mg), which resulted in a final concentration of 2.800 µg/mL. The weight of tt-farnesol solution was determined using an analytical balance. Then, tt-farnesol was diluted successively, obtaining final concentrations of 1.4, 0.7, 0.35, 0.175, 0.0875, 0.043, 0.021, 0.010 and 0.0054 mg/mL.

For the inoculum preparation, 100 µL of *E faecalis* (ATCC 10542) was transferred to a tube containing 5 mL of brain heart infusion (BHI) broth (DIFCO, São Paulo, Brazil) and incubated at 37 ºC in 5% CO_2 for 18 h. An aliquot was grown on BHI agar plates and incubated at 37 ºC in 5% CO_2 for 24 h, to obtain isolated colonies and confirm the uniform growth of *E faecalis*. The purity of the inoculum was confirmed by optical microscopy (x1000). The BHI agar plates provided five isolated colonies of *E faecalis* that were replicated, in a tube containing 5 mL of BHI, which was further incubated at 37 ºC in 5% CO_2 for 18 h.
After bacterial growth, the test tubes (n=18) were filled with 100 µL of the diluted solutions. Subsequently, it was filled with the inoculum and standardized using a spectrophotometer (330 model Metrolab, Buenos Aires, Argentina) with a wavelength of 625 nm and absorbance of 0.09. The cultures were adjusted to 0.5 Mc Farland standard (an approximation of $1.5x10^8$ colony forming units - CFU/mL).

Table 1
CFU count (mean) in different concentrations of tt-farnesol and controls in the incubation times

Solution concentration	E faecalis (CFUx10^6)
tt-farnesol (mg/mL)	
1.4	0
0.7	0
0.35	0
0.175	9
0.0875	50
0.043	Uncountable
0.021	Uncountable
0.010	Uncountable
0.0054	Uncountable
Positive control	Uncountable
Negative control	0
Buffer solution control	0
Culture medium control	0

To test the antimicrobial activity of tt-farnesol, the wells of a 96 ELISA tray were filled with 100 µL of BHI. The wells (n=7) received 100 µL of each diluted solution and added with the inoculum. Then it was incubated at 37 ºC in 5% CO_2 for 24 h. Positive control (BHI + inoculum), negative control (BHI + inoculum + 0.12% chlorhexidine), buffer solution control (DMSO 100 µL + BHI 100 µL) and culture medium control (BHI) were also performed in the wells (n=7).
An aliquot from each well was grown on BHI agar plates. Two blindly calibrated evaluators (*Kappa* test=1.0) performed the CFU count and the mean values are shown in Table 1. The solution was considered effective when 99.9% growth of the inoculum was inhibited. The concentrations of 0.35 and 0.175 mg/mL of tt-farnesol were those chosen, as they were the borderline results between inhibiting or not bacterial growth.

Sealer preparation
The sealer used is this study was Sealapex (Kerr, Washington, EUA). This sealer is an endodontic sealer calcium hydroxide-based and its mechanism of action is obtained through ionic dissociation of Ca^{2+} and OH^- ions (31). The addition of calcium hydroxide in root canal sealers improves physico-chemical properties, mainly due to a decrease in the flow rate of the sealer (31). The sealer and tt-farnesol were weighed with a precision scale (Ohaus Corporation Pine Brook, New Jersey, USA). The sealer was prepared by mixing 0.025 g of Sealapex paste and 0.025 g of Sealapex catalyst associated with different proportions of tt-farnesol: only Sealapex (GS); Sealapex + 350 µg/g tt-farnesol (GS+0.35f); Sealapex + 1750 µg/g tt-farnesol (GS+0.175f). The sealer was mixed following the manufacturer's instructions. Then, it was dispensed in 2 mm-diameter and 6 mm-long polyethilene tubes (n=108) with a Centrix syringe (DFL, Rio de Janeiro, Brazil), to avoid bubbles inside the sealer. The end of the tubes was sealed with plastic tape to prevent the material from overflowing. Once the initial hardening time was reached, the polyethilene tubes were cut with a

scalpel blade (15C model Swann-Morton, Sheffield, United Kingdom) and the sample size was confirmed with the help of a calliper.
Each group was placed in a previously identified 3 mL Eppendorf tubes and incubated at 37 °C for 7 days. Thus, the setting could be attained and, as a result, by-products could be released during the setting reaction.

Inoculum preparation
E faecalis culture was prepared in Mueller-Hinton broth (BD, New Jersey, USA) and adjusted to a concentration of 10^6 CFU/mL simulating body fluid (SBF). The adjustment was performed in a spectrophotometer. The absorbance was measured at 660 nm.

Antimicrobial activity test of sealer
The bacterial suspension was then added, in a 1:10 ratio (weight/volume), into the Eppendorf tubes containing the samples. Therefore, at the initial time, all tubes had the same concentration of bacteria. In specific times, 100 µL of each group were removed and twofold serial dilution (decimal dilution) were prepared in saline solution, to obtain a concentration of 10^6 CFU/mL. Subsequently, the aliquots were immediately seeded in BHI agar (Isofar Indústrias Comércio de Produtos Químicos, Rio de Janeiro, Brazil) and incubated at 35°C for 24, 48, 72, 96, 120 and 144 h. After each time, they were removed and two blindly calibrated evaluators (*Kappa* test=1.0) performed the CFU count by group (n=10), under an x25 magnification with the aid of stereomicroscopic analysis. The mean values obtained were considered for each group at different times.
All manipulations were carried out in aseptic conditions and a laminar flow cabinet, to minimize the risks of contamination.

Statistical Analysis
Statistical difference between the groups was tested by the Analysis of Variance (one-way ANOVA). Tukey HSD multiple comparison post-hoc test was used to complement the analysis. The level of significance was set at 5%. The analysis was performed with the aid of SPSS 20 Software for Windows (IBM SPSS Statistics, Chicago, Il, USA).

Results

The mean of CFU count by the group at incubation times are shown in Table 2. It was observed a statistically significant difference in the CFU count between the groups after 48 h (one-way ANOVA, *P*0.01). The post-hoc test revealed differences between GS, GS+0.35f and GS+0.175 after 48 and 72 h, and between GS and all other groups in 96, 120 and 144 h of incubation (Tukey HSD, $P<0.05$). The CFU count was lower in GS+0.35f than in GS+0.175f after 48 and 72 h (Tukey HSD, $P<0.05$). In GS+0,35f, there was a decrease in CFU count after 48 h, in GS+0.175f after 72 h and in GS only after 120 h of incubation (one-way ANOVA $P<0.01$, Tukey HSD $P<0.05$).

Discussion

Secondary endodontic infection occurs due to the presence of microorganisms that resist to chemical and mechanical preparation of root canal (1, 2). *E faecalis* is a microorganism associated with the appearance or maintenance of periapical pathologies after endodontic treatment (9-12). The use of irrigation solution and intracanal dressing contributes to the root canal disinfection, but it is not always able to eliminate *E faecalis* (16). The use of an endodontic sealer with antibacterial activity can favour the elimination of microorganism that can survive in isthmuses, dentinal tubules and apical deltas, prolonging this effect even after root canal filling and preventing the recontamination (13, 14).
An ideal endodontic sealer must have antimicrobial activity, good sealing ability, be highly penetrating, have good fluidity and be able to stimulate the repair of periapical tissues (3-5). In this study, Sealapex was the endodontic sealer chosen for presenting good sealing capacity and flow (32, 33). Also, it is calcium hydroxide-based, which is favorable for repairing the periapical tissues (32-34). However, previous

Table 2

CFU count by groups in the incubation times

Incubation time	CFU count – mean (±SD)			
	GS	**GS+0.35f**	**GS+0.175f**	***P***
0 h	$1.36x10^{7} (\pm 3.2x10^{6})^{Aa}$	$1.38x10^{7} (\pm 1.6x10^{6})^{Aa}$	$1.7x10^{7} (\pm 4x10^{6})^{Aa}$	0.069
24 h	$5.4x10^{7} (\pm 8x10^{6})^{Aa}$	$2.8x10^{7} (\pm 1.6x10^{7})^{Aa}$	$5.2x10^{7} (\pm 1.4x10^{7})^{Aa}$	0.055
48 h	$4x10^{7} (\pm 1x10^{7})^{Aa}$	$1.7x10^{7} (\pm 4x10^{6})^{Bb}$	$8x10^{6} (\pm 3.7x10^{6})^{Ca}$	<0.01
72 h	$4.3x10^{7} (\pm 1.8x10^{7})^{Aa}$	$7x10^{5} (\pm 4x10^{5})^{Bb}$	$3.2x10^{6} (\pm 1.4x10^{6})^{Cb}$	<0.01
96 h	$2.6x10^{6} (\pm 1.2x10^{6})^{Aa}$	$1.96x10^{4} (\pm 1.5x10^{4})^{Bb}$	$0 (\pm 0)^{Bb}$	<0.01
120 h	$3.5x10^{5} (\pm 1x10^{5})^{Aa}$	$0 (\pm 0)^{Bb}$	$0 (\pm 0)^{Bb}$	<0.01
144 h	$4.3x10^{5} (\pm 1.4x10^{5})^{Aa}$	$0 (\pm 0)^{Bb}$	$0 (\pm 0)^{Bb}$	<0.01
P	<0.01	<0.01	<0.01	

SD Standard Deviation.
Same lower letter indicate statistically similarity between groups in lines and same capital letter represents statistically similarity between groups in columns (Tukey's test, P>0.05).

studies have shown that this endodontic sealer was not able to eliminate *E faecalis*, because its antibacterial activity occurs through ionic dissociation of calcium hydroxide, which increases the pH but is unable to reach deeper into the dentinal tubules, where microorganisms may be located (35-37). Therefore, to improve Sealapex antibacterial activity, this study proposed an association with the bioactive compound known for tt-farnesol.

Tt-farnesol is a terpenoid responsible for the antibacterial activity of propolis. Its use in Dentistry is safe, because of its pharmacological characteristics such as low cytotoxicity and genotoxicity, which allows it to be used as an active or adjuvant medication (38-40). Rezende et al. (24) evaluated two pastes containing propolis extract associated with calcium hydroxide for root canal filling of primary molars. The agar-well diffusion technique showed that the association between propolis and calcium hydroxide promoted greater inhibition zones of bacterial growth, being effective in the control of dental infections *in vitro*. These findings corroborate with this study, which showed that the association of the Sealapex sealer with tt-farnesol, a component of propolis, promoted inhibition of *E faecalis* growth in vitro, which could contribute to prolonging the disinfection of the root canals after filling. Studies show that tt-farnesol was effective in dental caries control and inhibition of *S mutans* growth (21, 23, 25, 37). Thus, the application Sealapex sealer associated with tt-farnesol in endodontic therapy has great importance, mainly because it is an original combination. A methodological advantage of this study was the use of simulated body fluid (SBF) as the medium for sealer and bacterial inoculum interaction (25, 26). SBF is a medium that favors the dissolution of the sealer and has similarity to the human body fluid, which resembles the real conditions of the root canal. It is different from studies that use only the agar culture medium (25, 26).

In this study, there was a decrease in CFU count in $GS+0.175_{f}$ after 72 h and GS+0.35f after 48 h of incubation. In the GS group, which has no association with tt-farnesol, CFU count was higher after 48 h, compared to the other groups. The association of tt-farnesol with Sealapex sealer seemed to inhibit bacterial growth. This result can be explained by the study by Schäfer and Zandbiglari (32) which demonstrated that Sealapex sealer presents low solubility in

the first hours, but increase its solubility over time, reaching 10% after 28 days.
In this study, CFU count decreased in G_S only after 120 h of incubation. This can be explained by the ability of *E faecalis* to remain viable, even after raising the medium pH (14-16). The association between Sealapex and tt-farnesol seems to improve the action of the sealer against *E faecalis*. The action mechanism of tt-farnesol (as well as other terpenoids) is in its ability to act on the bacterial membrane, promoting the lysis of the microorganism (22, 26). Koo et al. (26) demonstrated that tt-farnesol promoted a visible rupture of the membrane of *Streptococcus* in phase-contrast microscopy.
The anti-inflammatory activity of propolis was associated with its ability to remove free radicals through its bioactive compounds, which favors tissue regeneration and repair, stimulating the formation of hard tissue (22). Based on the results of the present study and considering tt-farnesol as an anti-inflammatory agent that favors histological repair, it seems essential to develop further research to understand the mechanism of action of this substance, particularly to provide fundamentals for combinatorial therapy. This would provide this therapy with stronger biological support, thereby allowing it to be clinically tested in the future.

Conclusions

From this study, we can conclude that the association of tt-farnesol with Sealapex sealer decreased *E. faecalis* growth in vitro after 48 h of incubation in comparison to the group that used only the sealer. The MBC of 0.35 and 0.175 mg/mL of tt-farnesol reduced the CFU count after 48 and 72 h, respectively.

Clinical Relevance

An adequate endodontic treatment with a substance that promote microbial control mainly against *E. faecalis* strains can results in the greatest success of the treatmen.

Conflict of Interest

None.

Acknowledgements

None.

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ORIGINAL ARTICLE

Influence of photosensitizing agent and number of photodynamic therapy sessions on resistance of fiberglass posts to displacement within the canal

ABSTRACT

Aim: *To evaluate the influence of the type of photosensitizing agent and the number of photodynamic therapy (PDT) sessions on the resistance of cemented fiberglass posts to displacement within the root canal.*
Methodology: *Fifty bovine primary incisors were randomly divided into five experimental groups according to the type of photosensitizing agent and to the number of PDT sessions: CG without PDT (control); GF1M one PDT session with methylene blue; GF2M two PDT sessions with methylene blue; GF1T one PDT session with toluidine blue; and GF2T two PDT sessions with toluidine blue. Exacto® fiberglass posts were cemented with RelyX U200® in the root canal and kept for 15 days in distilled water. The specimens were sectioned with an average thickness of 1.56 mm at the cervical, middle, and apical root thirds and subjected to the push-out test. After the test, the fractured specimens were analyzed under a stereomicroscope to determine the fracture pattern. The data obtained were treated by one-way ANOVA (α=0.05).*
Results: *There was no statistical difference in the comparison of the proposed treatments and the analyzed root thirds (P>0.05).*
Conclusions: *The type of photosensitizing agent used and the number of PDT sessions do not influence the resistance of cemented intraradicular fiberglass posts to displacement.*

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Introduction

Two objectives are essential for achieving clinical success in endodontic treatment: control over root canal disinfection and placement of long-lasting restorations. The correct planning of the restorative treatment has provided high survival and restoration success rates of approximately 85% (1, 2). According to the degree of impairment of the dental crown structure, fiberglass posts have shown to be commonly used in direct restorative treatment, providing adequate support and retention for the restorations (3). However, the success of this procedure depends on dentin morphology, on the materials used during endodontic treatment, and on the adhesive cementation of intraradicular posts. Most of the failures occur in the adhesion between the root canal walls and the resin cement (4).

An attempt is usually made at eradicating bacteria from the root canal using chemomechanical preparation (5) associated with intracanal medication (6). However, these procedures cannot guarantee complete disinfection since the complex anatomy of the root canal system and the organization of microorganisms in highly complex biofilms contribute a lot to the persistence of the infectious process, with regions not accessible to instrumentation and irrigation (7). Thus, changes in therapeutic approaches with the associated use of other auxiliary resources, e.g. photodynamic therapy (PDT), have been tested to improve the treatment of endodontic infections.

PDT is performed with the aid of a low power laser at a wavelength between 630 and 980 nm and of a non-toxic photosensitizing agent that can eliminate endodontic pathogens through the formation of reactive oxygen species (8). Photosensitizers are heterocyclic light-absorbing molecules. They must have a resonant absorption band with the wavelength of the light source to be used (9). Photosensitizers derived from phenothiazines are the most widely used in PDT (10). Phenothiazines are tricyclic heteroaromatic compounds, such as toluidine blue and methylene blue. Photosensitizing agents absorb photons from the radiation source and conduct their electrons to an excitatory state. In the presence of oxygen, the energy transfer from the photosensitizing agent generates reactive oxygen molecules, such as singlet oxygen and free radicals, which cab damage cellular components such as lipids and nucleic acids through irreversible oxidation, causing bacterial death (11). PDT has shown to be a promising auxiliary resource for eradication of oral pathogenic bacteria that cause endodontic diseases, and periodontitis (12). On the other hand, as photosensitizing agents are viscous substances used in aqueous solutions, they can adhere to the root canal walls and dentinal tubules, forming a chemical smear layer, as described by Souza et al (13), which could influence the bond strength of intraradicular posts. It creates a favorable environment for microbial microleakage and inadequate adhesion of the root filling material to root canal dentin (14). According to Lima et al (15) and Akman et al (16), the photosensitizing chemical agents present a negative effect on the hybrid layer formation and on the adhesive interface between the fiber post cementation system and root dentin.

Therefore, the present study aims to assess whether the type of photosensitizing agent and the number of PDT sessions influences the resistance of cemented intraradicular fiberglass posts to displacement. The null hypothesis is that photosensitizers and the number of PDT sessions do not cause changes in the bond strength of fiberglass posts within the intraradicular dentin.

Materials and Methods

Sample selection and preparation

Fifty primary bovine incisors were selected and standardized to the initial apical diameter of the root canal, equivalent to a K-type #20 endodontic instrument (Dentsply/Maillefer Instruments S.A., Ballaigues, Switzerland). The root canals had circular sections. After cleaning, the dental crowns were sectioned at the cementoenamel junction with the aid of a low-speed silicon

Table 1
Experimental groups

Group	n	Photodynamic therapy
GC	10	Without PDT
GF1M	10	One PDT session+methylene blue
GF2M	10	Two PDT sessions+methylene blue
GF1T	10	One PDT session+toluidine blue
GF2T	10	Two PDT sessions+toluidine blue

carbide disc. The length of the root remnant was standardized to 17 mm and the working length (WL) was 1 mm below this standardized measurement (WL=16 mm). The apical foramina were previously sealed with composite resin to prevent leakage of the irrigating and photosensitizing agents. The restorative procedure was performed with the use of 37% phosphoric acid (FGM, Joinville, SC, Brazil) and Single Bond Universal® adhesive system (3M ESPE, St Paul, MN, USA), with later placement of Filtek Z250® composite resin (3M ESPE, St Paul, MN, USA).

Experimental groups
The teeth were divided into five experimental groups (Table 1) by the simple random sampling using Excel (Microsoft Excel, Microsoft, USA).

Endodontic preparation of samples
All samples were prepared manually with first and second series K-type stainless steel endodontic instruments (Dentsply/Maillefer Instruments S.A., Ballaigues, Switzerland). Chemomechanical preparation was carried out in the following sequence of K-type instruments: #20, #25, #30, #35, #40, and #45 (Dentsply/Maillefer Instruments S.A., Ballaigues, Switzerland). All instruments were used along the WL. At each instrument change, the canals were irrigated with the aid of a plastic syringe (BD Solumed, São Paulo, SP, Brazil) and 25 mm 30-gauge NaviTip needles (Ultradent, Indaiatuba, SP, Brazil), containing 2.5% sodium hypochlorite (Iodontec Indústria e Comércio de Produtos Odontológicas Ltda., Porto Alegre, RS, Brazil) in a standard amount of 2 mL.

After the preparation, the final toilet was made with 17% trisodium EDTA (Biodinamica, Ibiporã, PR, Brazil) for three minutes and with agitation of #45 instrument. The canals were then washed with distilled water (Iodontosul, Industrial Odontológica do Sul LTDA, Porto Alegre, RS, Brazil) and dried with absorbent paper points (Tanari Indústria Ltda., Manaus, AM, Brazil).
For the endodontic filling, the canals were filled with gutta-percha cones and AH Plus® epoxy resin-based cement (Dentsply/Maillefer Instruments SA, Ballaigues, Switzerland), using Tagger's hybrid technique and #60 McSpadden® compactor (Dentsply/Maillefer Instruments SA, Ballaigues, Switzerland).
After filling, all samples were provisionally restored with Cimpat® restorative material (Septodont, Saint Maur des Fosses, France) and immersed for two days in a flask containing distilled water, at 37 °C and 100% relative humidity, for complete setting of the endodontic sealer.
After that, the canals were cleared to prepare the space needed for the post to be cemented. The root canal filling was removed along 13 mm with the bur provided with the post kit and which corresponds to the diameter of the used post, leaving 3 mm of apical sealing.

Photodynamic therapy (PDT)
A flexible optical fiber with a diameter of 500 μm (MMOptics Ltda., São Carlos, SP, Brazil) coupled to the Duo® laser device (MMOptics Ltda., São Carlos, SP, Brazil) was used for PDT.
Initially, the dry canals were filled with the photosensitizing agent. The photosensitizing agents used were aqueous solutions of 0.01% methylene blue (Sigma-Aldrich, Sigma-Aldrich Brazil, SP, Brazil), 0.1 mg/mL, (Groups GF1M and GF2M) and toluidine blue (Sigma-Aldrich, Sigma-Aldrich Brazil, SP, Brazil) at 0.01%, 0.1 mg/mL, (Groups GF1T and GF2T). The teeth were filled with the photosensitizing agent, which was kept in the canal for 5 minutes (pre-irradiation period).
After the pre-irradiation period, the photosensitizing agent was activated by red

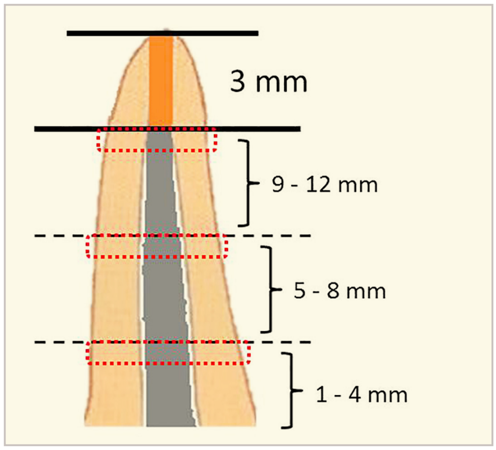

Figure 1
Schematic diagram of root slices.

visible light at 660 nm, 18 J of energy, for 3 minutes, with the aid of a flexible optical fiber, advanced 2 mm below the WL. The fiber was introduced in helical movements in the apical-cervical direction for uniform diffusion of light along the root canal length. The movements were repeated approximately 10 times/minute. Immediately after PDT, the root canals were finally irrigated with 10 mL of deionized water to remove the photosensitizing agent and subsequently dried with an aspiration cannula and absorbent paper points. In groups GF1M and GF1T, PDT was performed in a single session. This session took place after the final rinse and before the endodontic filling procedure. In specimens from groups GF2M and GF2T, PDT was performed in two sessions. The second session was carried out after unblocking and preparing the canal for post cementation.

Post cementation and specimen preparation

After the canals were cleared, the placement of Exacto® #1 or 2 fiberglass posts (Angelus, Londrina, PR, Brazil) followed the cementation protocol and the manufacturer's instructions. The posts were disinfected with 70% alcohol (Icarai, São Paulo, SP, Brazil) prior to use and subsequently dried. Single Bond Universal® adhesive was applied for 20 seconds and then dried with air jets for 5 seconds. The posts were luted with self-adhesive cement (RelyX U200®, 3M ESPE, St. Paul, MN, USA). The resin cement was applied to the root canal with the aid of a centrix syringe (DFL, Rio de Janeiro, RJ, Brazil) with a fine metal tip. The post was inserted into the root canal and filled with cement to the most coronal portion to hermetically seal the entrance and photoactivated with the aid of an EC450 device (ECEL, Ribeirão Preto, SP, Brazil), with light intensity greater than 400 mW/cm^2, for 20 seconds, and chemical polymerization for 6 minutes.

After 15 days of cementation and storage in distilled water, the roots were sectioned perpendicularly to the long axis, and three thick slices (1.56 mm±0.37 mm) were obtained with the aid of a cutting machine (Labcut 1010, Extec Corp., Enfield, CT, USA). The slices were obtained in a standardized manner at 4 mm (cervical third), 8 mm (middle third), and 12 mm (apical third) away from the cervical edge of the root (Figure 1), identified, and stored in an oven at 37 °C and 100% relative humidity for 7 days.

Push-out test

The specimens were placed on a stainless steel metal support with a 2 mm central hole. Given the conical shape of the posts, the load was applied in the apical-cervical direction from the apical surface, so that the post could be pushed towards the widest portion of the root canal.

The load was applied only on the post surface with a tip of approximately 1 mm in diameter coupled to the EZ-SX (Shimadzu Corp., Kyoto, Kyoto, Japan) universal testing machine. The selected load cell was 500 kg (50 N) and the loading speed was 0.5 mm/min. The values were recorded in N and displacement resistance in MPa.

To measure the area of the canal and calculate resistance, the diameter of the upper and lower circle of the canal and the thickness of the section (area of a cone trunk) were measured. After the push-out test, the fractured specimens were analyzed under an X20 stereomicroscope (Stemi 2000, Karl Zeiss, Germany) to determine the adhesive, cohesive, or mixed failure pattern.

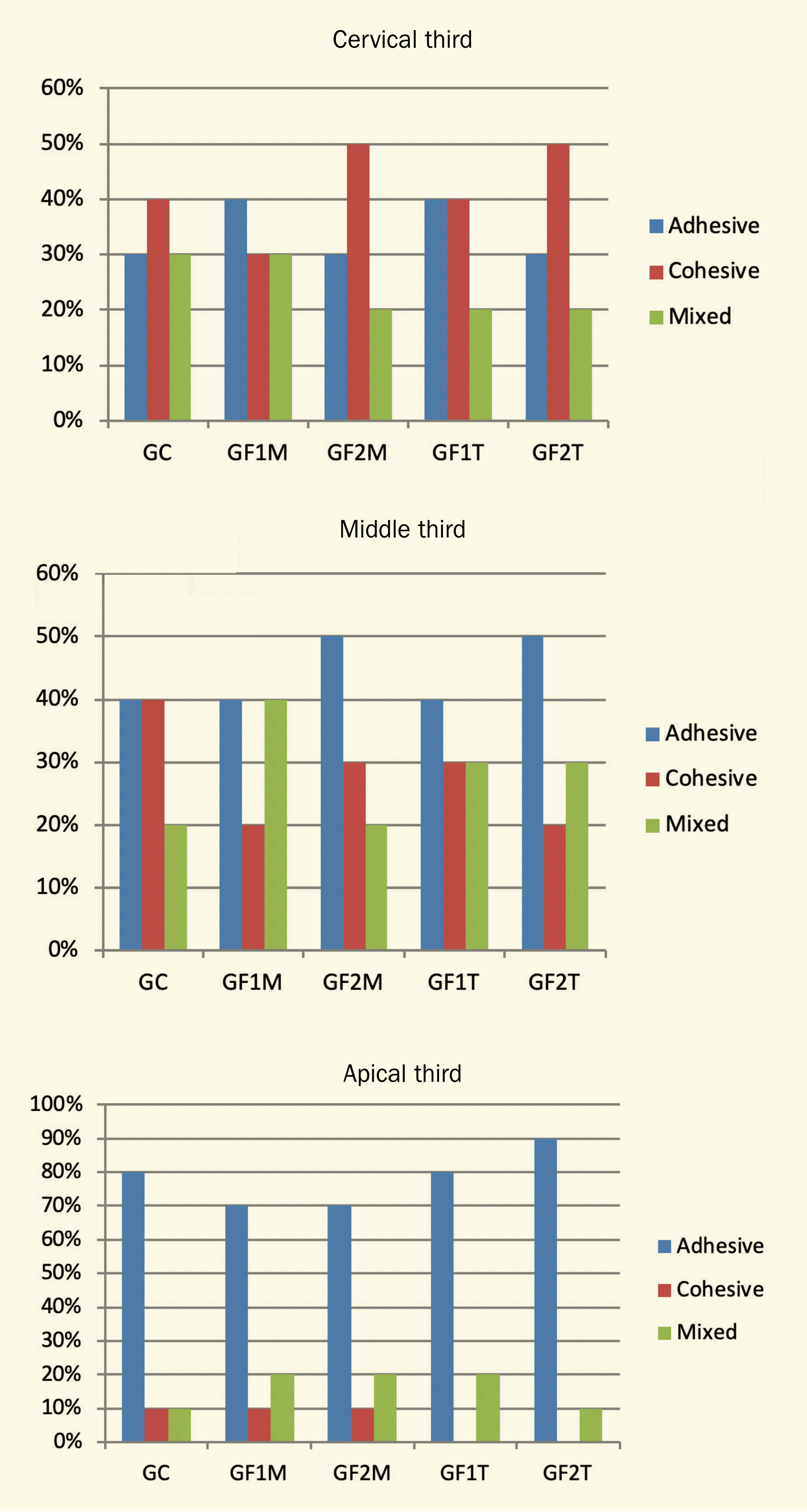

Figure 2 Failure patterns (%) after tested protocols.

Statistical analysis

The Shapiro-Wilk test was used to assess the normality of the data. One-way ANOVA was used to assess bond strength. The level of significance was set at 5% ($P \leq 0.05$). Statistical analysis was performed using GraphPad Prism 7 (GraphPad Software Inc., San Diego, CA, USA).

Results

The means of displacement resistance (MPa) for the different experimental groups in different regions of the canal are shown in Table 2. There was no statistical difference between the groups regarding the different root positions analyzed, that is, the number of PDT sessions and the type of photosensitizing agent used did not influence the bonding of the intraradicular post.

Graph in Figure 2 shows a homogeneous distribution of fracture patterns among the experimental groups in the different regions analyzed, with a higher rate for the adhesive pattern in the most apical region of the root canal.

Discussion

Intracanal preparation prior to cementation of an intraradicular retainer requires partial removal of the endodontic filling material. During this procedure, there could be breaches of the aseptic field, compromising endodontic success and/or rehabilitation treatment (17). Some substances, such as sodium hypochlorite and chlorhexidine digluconate, are used to clean and disinfect the dentin space prepared for the retainer. However, negative effects on the bond strength of resin cements to root dentin under these conditions have been described in the literature (15, 18, 19). Accordingly, the present study sought to analyze the behavior of PDT, used as an auxiliary resource for intraradicular disinfection, in the adhesive bond strength of fiberglass post on the dentinal wall. In the present study, only methylene blue and toluidine blue were tested, as they are commonly used in association with red low-intensity lasers, also used in our study,

Table 2
Bond strength in root segments in the push-out test

Experimental Group	Root thirds			P
	Cervical	Middle	Apical	
	MPa (±SD)	MPa (±SD)	MPa (±SD)	
GC	8.61Aa ± (4.32)	7.53Aa ± (5,23)	6.87Aa ± (4.30)	P=0.707
GF1M	10.39Aa ± (7.68)	8.96Aa ± (7.17)	7.15Aa ± (5.21)	P=0.574
GF2M	11.07Aa ± (4.92)	9.39Aa ± (3.27)	8.27Aa ± (3,56)	P=0.302
GF1T	10.52Aa ± (5.22)	9.83Aa ± (5.78)	6.96Aa ± (2.90)	P=0.230
GF2T	14.04Aa ± (5.32)	9.67Aa ± (5.65)	8.52Aa ± (7.05)	P=0.117
P	P=0.311	P=0.890	P=0.901	

Means followed by different uppercase letters in the row and means followed by different lowercase letters in the row differ significantly in the analysis of variance at the 5% significance level.

and because these associations with PDT have antimicrobial effects that have already been confirmed in the literature (20). The push-out test, one of the main resources for quantification of the bond strength between different materials and structures (21), according to the literature, was applied. A disadvantage of the push-out test is that voltage is not distributed evenly. To overcome this problem, sections should be prepared with a thickness of approximately 1 mm (22). The push-out test is still the most reliable and reproducible method when compared to microtensile, shear, and traction tests (22).
According to the results obtained, the null hypothesis of the present study was accepted, as PDT and the number of sessions did not influence the bond strength of the fiberglass post to the dentinal wall. These findings are consistent with the study by Ramos et al (23) who, regardless of the root third assessed, observed that PDT did not affect the bond strength of fiberglass posts cemented with the RelyX U200® self-adhesive system. On the other hand, in the study by Ramos et al (24), PDT negatively affected the bond strength of the cemented post with the conventional Relyx ARC® system. According to Konopka and Goslinski (25), the use of PDT within the root canal promotes the release of reactive oxygen species, mainly singlet oxygen, which have negative effects on the formation of the hybrid layer and on the polymerization and bonding of the adhesive system on the dentin surface. It is believed that the result obtained with RelyX U200® cement was different because of its bonding to the dentin substrate. For Pisani-Proença et al (26), the acidic monomers of RelyX U200® demineralize and infiltrate the dentin substrate, providing micromechanical retention. Simultaneously, the reaction between the acidic monomers of the cement and the hydroxyapatite of the dental substrate also leads to chemical retention. This, to some extent, explains our results. Another factor that might have influenced the results obtained in the study by Ramos et al (24) was the use of the optical fiber in a static position for 30 seconds; consequently, the irradiation might have been concentrated in only one region (cervical root third). Garcez et al (27) reported that light distribution and oxygen formation are uniformly generated when the optical fiber is used in spiral and non-static movements.
The type of photosensitizing agent also did not interfere with the adhesive bond strength of the posts to the root canal. According to Di Hipólito et al (28), methylene blue is a cationic substance that binds to anionic molecules, such as the phosphate present in hydroxyapatite. This reaction results in the formation of a precipitate that acts as a physical barrier and can thus influence the interaction between the resin cement and the dentin surface. Howev-

er, it is believed that because methylene blue and toluidine blue are hydrophilic compounds, the type of cement used (Relyx U220®) exhibits the same behavior which, in a way, may have led to the favorable results obtained in our study.

The failure pattern was also an interesting finding. Failures (adhesive, cohesive, or mixed) occurred homogeneously at the cervical and middle thirds. Only at the apical third did a higher percentage of adhesive-type failures occur, regardless of the group analyzed. The dentinal wall has a smaller amount of dentinal tubules in the most apical regions of the root canal when compared to the middle and cervical thirds (29), and cementation poses some challenges in regions closer to the apex of the tooth (30). Other studies, such as that by Rengo et al (31), found that this is due to the greater probability of cement accumulation in this area. Another factor that may be correlated with this type of failure is the difficulty in removing the photosensitizing agent from deeper regions of the root canal. Although deionized water was used with the aid of a plastic syringe coupled to a 30-gauge needle to remove methylene blue or toluidine blue from the root canal, the dentin surface still exhibited some pigmented areas at the apical thirds. According to Lima et al (15), the pigmentation of these chemical agents can have negative effects on the formation of a hybrid layer and on the adhesive interface between the fiberglass post and the root dentin surface. Ethylenediaminetetraacetic acid (EDTA) is considered the most effective chelating agent in endodontic therapy, showing the ability to very effectively remove the inorganic component, especially in the coronal and middle third of the canal (32). However, prolonged activity of chelating agents on the inorganic dentine structure may reduced tooth microhardness (33). The use of passive ultrasonic irrigation (PUI) instead of the traditional syringe irrigation method could be tested for more effective removal of photosensitizing agents from the root canal. PUI consists of the activation of the irrigating chemical solution within the root canal by means of a smooth ultrasonic tip that, when activated in a passive back-and-forth movement, respecting the WL (34), creates an acoustic flow of the irrigating solution with energy transmission through ultrasonic waves within the canal (35). This agitation of the irrigation solution by ultrasound waves improves its ability to dissolve tissues, also contributing to the removal of the smear layer (36) and promoting antimicrobial activity as a result of the physical disruption of bacterial aggregations, such as biofilm (37).

PDT can be a good alternative for promoting root canal disinfection prior to cementation of intraradicular posts and as a substitute for sodium hypochlorite and chlorhexidine which, according to the literature, have still questionable and deleterious effects on the root canal prior to the cementation of retainers (11). On the other hand, other clinical protocols should be investigated for a more effective removal of photosensitizing agents from the root canal.

Conclusions

The bond strength of cemented intraradicular fiberglass posts was not influenced by the type of photosensitizing agent used and by the number of PDT sessions.

Clinical Relevance

The methylene blue and toluidine blue do not influence intraradicular posts adhesion.

Conflict of Interest

The authors declares that there is no conflict of interest.

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ORIGINAL ARTICLE

In vitro cytotoxic effects of different intracanal medicaments on the co-culture of dental mesenchymal stem cells using the MTT assay

ABSTRACT

Aim: *This* in vitro *study aimed to assess the cytotoxic effects of different intracanal medicaments on the co-culture of dental mesenchymal stem cells.*
Materials and Methods: *The Periodontal Ligament Stem Cells (PDLSCs) and the stem cells of the apical papilla (SCAPs) were isolated from a human teeth. The cells were passaged and underwent mono-culture and co-culture. The cells were then exposed to different concentrations of calcium hydroxide/chlorhexidine, calcium hydroxide/distilled water, double antibiotic paste/ chlorhexidine, and double antibiotic paste/distilled water. The cytotoxicity and cell viability was evaluated and data were analyzed by independent t-test, ANOVA, and Tukey's test (alpha=0.05).*
Results: *The viability of PDLSCs in co-culture was higher than that in mono-culture following exposure to different concentrations of medicaments. However, when distilled water was used as the vehicle for intracanal medicaments, the viability of SCAPs in mono-culture was higher than that in co-culture. In use of DAP/CHX, the viability of SCAP in co-culture was higher than that in mono-culture (P=0.000). This difference was not significant in exposure of SCAP to DAP/CHX (P>0.05).*
Conclusions: *Generally, the viability of PDLSCs in co-culture was higher than that in mono-culture, and distilled water was a better vehicle than CHX for intracanal medicaments.*

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Introduction

Root canal disinfection by use of irrigating solutions and intracanal medicaments is a fundamental step in endodontic regeneration (1). Thus, intracanal medicaments play an important role in elimination of microorganisms (2), neutralization of endotoxins, preventing the entry of salivary microorganisms into the canal, reduction of pain and periradicular inflammation, and induction of mineralized tissue regeneration. Therefore, the byproducts of the medicaments should be biocompatible because they are in close contact with the periapical tissue (3). Otherwise, these products can trigger the release of many inflammatory factors such as histamines, kinins, and neuropeptides that can induce tissue destruction and slow down the healing process (4).
Several *in vitro* and in vivo studies have tried to optimize root canal disinfection in endodontic regeneration (5, 6). The optimization techniques have mainly focused on the survival and function of stem cells in the periapical region (7). Several chemical agents such as calcium hydroxide (CH), chlorhexidine (CHX), and antibiotics have been suggested to preserve and induce the dental mesenchymal stem cells (8-11). These products are all known for their optimal bacteriostatic and bactericidal effects; however, considering their potential side effects, some concerns exist regarding their cytotoxicity for host cells, especially dental mesenchymal stem cells (12-14).
CH can induce moderate angioblastic proliferation and formation of a fibrous capsule and moderate amounts of collagen fibers over time (15). Nonetheless, CH has lower cytotoxicity than other commonly used endodontic medicaments (16).
Evidence shows that CHX gluconate induces inflammatory reactions and tissue necrosis (17), delays the granulation tissue formation and tissue healing, inhibits the mitochondrial activity, and causes endoplasmic reticulum stress and cell death (18), brown extrinsic tooth and tongue staining, taste disturbance, dryness of mouth and burning sensation: these side effects limit its acceptability to users and its and long term use (19). Trevino et al. (9) evaluated the effect of 2% CHX on stem cells of the apical papilla (SCAP) by immunomagnetic separation, and showed that a combination of CHX/EDTA, and sodium hypochlorite/CHX/iodine potassium iodide/EDTA decreased the viability of the cells to 0%.
CHX is an effective agent against the microbial biofilms. Its main advantage over sodium hypochlorite is its lower cytotoxicity and no odor or bad taste. Although CHX has both bactericidal and bacteriostatic properties, it does not have tissue-dissolving capacity compared with other irrigating solutions (20). Another study used the methyl thiazolyl tetrazolium (MTT) assay and reported that CHX and saline had minimum cytotoxicity for SCAP (21). Kim et al. (22) demonstrated that double antibiotic paste (DAP) without EDTA significantly decreased the adhesion of dental pulp stem cells (DPSCs) to dentin, which can be due to the residual cytotoxicity of DAP. Alternatively, it may be due to changes in surface topography of dentin, which may interfere with the adhesion of DPSCs (22). Another study reported that 1 to 100 mg/mL DAP had direct adverse effects on SCAP and dental pulp fibroblasts (23, 24).
A noteworthy issue is that all the aforementioned studies were conducted on single-culture media; while, stem cells in the periapical region of immature teeth with an apical lesion are not independent of other cells under in vivo conditions. However, inter-cellular interactions in co-culture can be generalized to the in vivo conditions. In indirect co-culture, the cells are independently cultured in cell culture inserts on porous membranes with 0.4 µm pore size and $2x10^6$ pores/cm to allow cell proliferation. This technique has higher reproducibility and reliability *in vitro* (25). Since the cytotoxic effects of intracanal medicaments used in endodontic regeneration have not been evaluated in co-culture under *in vitro* conditions, this study aimed to assess the cytotoxic effects of different intracanal

medicaments on periodontal ligament stem cells (PDLSCs) and SCAP in co-culture and mono-culture using the MTT assay.

Materials and Methods

The PDLSCs and SCAP were isolated from the apical region of an immature impacted mandibular third molar of a 19-year-old patient after obtaining his written informed consent. The study was approved by the ethics committee of Zahedan University of Medical Sciences (IR.ZAUMS.REC.1398.150). The extracted tooth was immediately rinsed with sterile phosphate buffered saline (PBS; BRL, Grand Island, NY, USA) and stored in sterile saline. Using a scalpel, the periodontal ligament residues and the apical papilla were removed, and the tissues were diced and immersed in 5 mg/mL collagenase (Sigma Aldrich, France) at 37 °C for 1 h. The SCAP and the PDLSCs were isolated by enzymatic digestion. The cells were seeded on 25 cm^2 cell culture flasks containing Dulbecco's modified Eagle's medium (DMEM) supplemented by 15% fetal bovine serum and 1% penicillin/streptomycin, and incubated at 37 °C with 92.4% humidity and 5% CO_2. After a couple of days, adhesion of cells to the bottom of the flask was observed under an inverted microscope. After reaching 80% confluence, the cells were passaged. The contents of the flask were divided between three flasks. Third passage (or higher) cells were used for the experiment. The flasks were emptied from the culture medium and rinsed with 2-3 mL of FBS. Next, 700 λ to 1 mL of trypsin-EDTA was added to each flask. After 5 min, the adhered elongated cells transformed to round suspended cells. The cells were transferred into separate falcon tubes, and DMEM was added to neutralize trypsin. Next, 5 mL of DMEM was added to the flasks for the next passages.

Using a Neubauer chamber, the number of cells in each 1 mL of the suspension in falcon tubes was calculated. Next, PDLSCs were added to the 6 wells of a 24-well plate. SCAPs were added to another 6 wells. In the remaining 12 wells that had insert (SPL Life Science, Gyeonggi-do, South Korea), PDLSCs were placed at the bottom and SCAPs were added into the inserts. Each well contained 24,000 cells in 1 mL of culture medium. In the insert plates, 12,500 cells were at the bottom and 12,500 cells were placed in the insert along with 1 mL of the culture medium. After 24 h, the cells completely adhered to the wells and then the medicaments were added to the culture medium.

Preparation of specimens:
DAP composed of ciprofloxacin (Sigma Aldrich, France) and metronidazole (Sigma Aldrich, France) with 1:1 ratio was dissolved in distilled water. Also, combinations of DAP with 2% CHX (Cerkamed, Pawłowski, Poland) were prepared in 0.125, 0.250, and 1 mg/mL concentrations. CH powder (Sigma Aldrich, France) was also dissolved in distilled water and 2% CHX, and prepared in the abovementioned concentrations similar to DAP. According to ISO 1993, the medicaments were added to DMEM and incubated at 37 °C and 5% CO_2 for 48 h. They were then filtered through a 0.2 µm filter. The stem cells were treated with different concentrations of the medicaments. After 3 days, the plate was removed from the incubator and the inserts were placed in a different plate. The contents of the wells were emptied and replaced with 1 mL of the culture medium containing 10% MTT (5 mg/mL of MTT salt was dissolved in PBS and filtered using a 0.2 µm filter. It was then diluted 10 times by serum-containing culture medium). The plates were incubated for 4 h and then 1 mL of dimethyl sulfoxide (DMSO) was added to each well (the co-culture plates received 0.5 mL of DMSO since the number of cells at the bottom of the plate and in the insert was half). After several times of pipetting of each well to dissolve the formazan crystals, the optical density values were read by a spectrophotometer at 540 nm wavelength, and recorded.

Cytotoxicity was determined by estimating the percentage of viable cells, and the measurements were repeated in triplicate for each concentration. Single-cultured and co-cultured cells in DMEM without any medicament served as the control group.

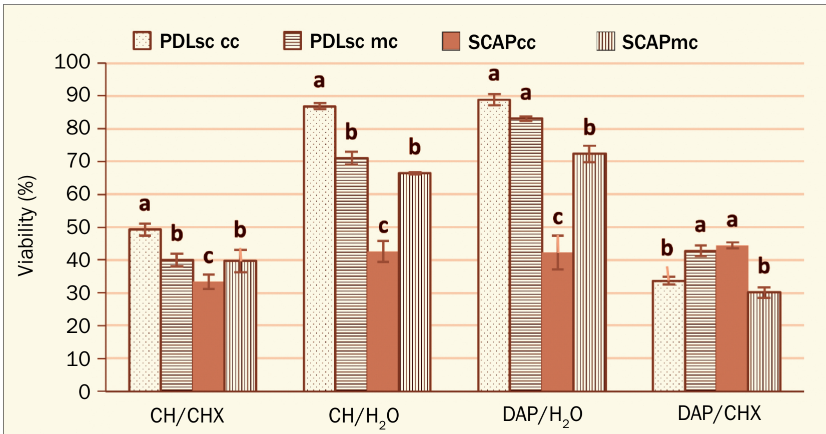
Statistical analysis

Statistical analysis of the data was performed by SPSS version 25. The mean and standard deviation of the percentage of viable PDLSCs and SCAP after exposure to different concentrations of medicaments were calculated and reported separately for the mono-culture and co-culture. Binary comparisons (stem cell types or culture types) were performed by independent t-test; while, multiple comparisons (different concentrations or different types of medicaments) were performed by one-way ANOVA. In case of significant results, pairwise comparisons were performed using the Tukey's post-hoc test. Level of significance was set at 0.05.

Results

The results showed 100% viability of both SCAP and PDLSCs in the control group in both co-culture and mono-culture. Diagrams in figures 1-3 show the viability of SCAP and PDLSCs treated with different concentrations of medicaments in co-culture and mono-culture forms.

Figure 1
Comparison of stem cell viability in co-culture (cc) and mono-culture (mc) following exposure to 0.125 mg/mL concentration of medicaments (CH calcium hydroxide, CHX chlorhexidine, DAP Double Antibiotic Paste).



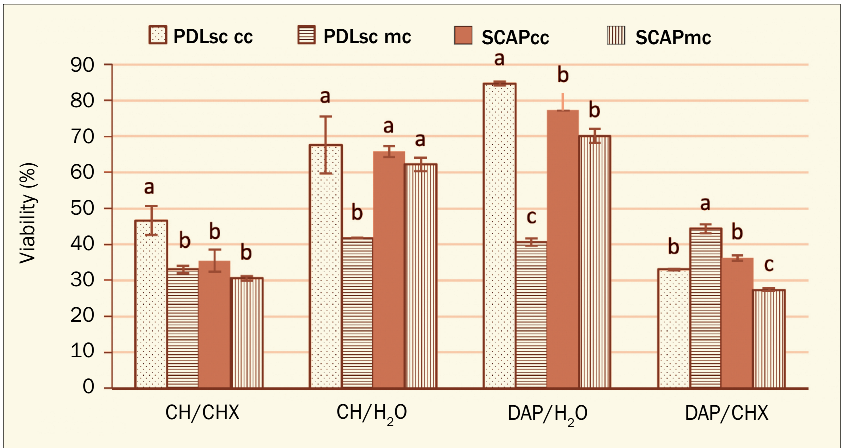
a,b,c: viability of stem cells in co-culture and mono-culture using Tukey's test. Similar letters indicate lack of a significant difference.

Co-culture

Increasing the concentration of CH/CHX in co-culture of PDLSCs significantly decreased their viability (P=0.047). However, this increase had no significant effect on the viability of SCAP (P=0.597). Also, in all concentrations, the viability of SCAP was significantly lower than that of PDLSCs (P=0.000). The current results showed that in co-culture, increasing the concentration of CH/distilled water had no significant effect on the viability of SCAP or PDLSCs (P>0.05). However, the viability of PDLSCs was higher than that of SCAP in all three concentrations (P=0.000). The current results showed that increasing the concentration of DAP/distilled water and DAP/CHX significantly decreased the viability of PDLSCs (P=0.004, P=0.003, respectively). However, this increase in concentration had no significant effect on the viability of SCAP (P>0.05). Both medicaments in all three concentrations significantly decreased the viability of SCAP compared with PDLSCs (P=0.000).

Mono-culture

In mono-culture, increasing the concentration of CH/CHX significantly decreased the viability of PDLSCs and SCAP (P=0.008 and P=0.003, respectively). Increasing the concentration of CH/distilled water significantly decreased the viability of PDLSCs only (P=0.016), and had no significant effect on the viability of SCAP (P>0.05). Also, the current results showed that increasing the concentration of DAP/distilled water significantly decreased the viability of both PDLSCs and SCAP (P=0.037 and P=0.021, respectively). However, a significant difference was noted in the viability of PDLSCs and SCAP only in 0.250 and 0.125 mg/mL concentrations (P=0.000 and P=0.01, respectively). This difference was not significant in 1 mg/mL concentration (P>0.05). Also, the current results showed that increasing the concentration of DAP/CHX significantly decreased the viability of PDLSCs (P=0.000) while it had no significant effect on the viability of SCAP (P>0.05). A significant difference was noted in the viability of



a,b,c: viability of stem cells in co-culture and mono-culture using Tukey's test. Similar letters indicate lack of a significant difference.

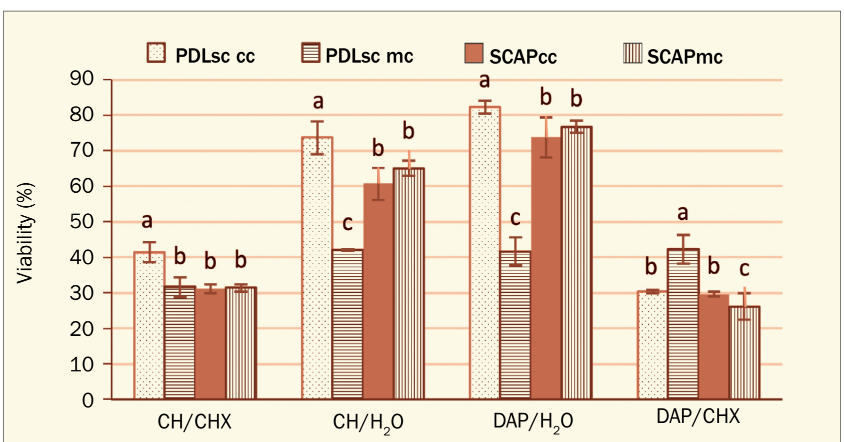
Figure 2
Comparison of stem cell viability in co-culture(cc) and mono-culture(mc) following exposure to 0.250 mg/mL concentration of medicaments (CH calcium hydroxide, CHX chlorhexidine, DAP Double Antibiotic Paste).

PDLSCs and SCAP only in 0.250 and 0.125 mg/mL concentrations (P=0.000) while this difference was not significant in 1 mg/mL concentration (P>0.05).

Comparison of co-culture and mono-culture

The viability of PDLSCs in different tested concentrations of medicaments was higher in co-culture than mono-culture. However, this difference in some concentrations was not significant (P>0.05). SCAP exposed to CH/distilled water and DAP/distilled water showed higher viability in mono-culture. However, this difference was not significant in exposure to CH/CHX (P>0.05). In exposure to DAP/CHX, cell viability in co-culture was higher than that in mono-culture (P=0.000) (Figures 1-3).

Figure 3
Comparison of stem cell viability in co-culture(cc) and mono-culture(mc) following exposure to 1 mg/mL concentration of medicaments (CH calcium hydroxide, CHX chlorhexidine, DAP Double Antibiotic Paste).



a,b,c: viability of stem cells in co-cultureand mono-cultureusing Tukey's test. Similar letters indicate lack of a significant difference.

Discussion

Tissue engineering is a multidisciplinary science aiming to generate new tissue and regulation of the immune response (26) or immunomodulatory activity (27, 28) in order to restore the function of organs and injured tissues due to a disease condition or trauma (29). In tissue engineering, attention must be paid to three components namely the scaffold, signaling factors, and cells. All the available *in vitro* studies on endodontic regeneration have used mono-culture for this purpose. In other words, the heterogeneity of the cells in the periapical region has not been considered. Thus, co-culture was used in this study.

A successful endodontic treatment depends on optimal efficacy of mechanical and chemical debridement of the root canal system. Since mechanical instrumentation of infected canals in immature teeth is contraindicated due to immature root development and fragility of dentinal walls, chemical debridement is the main disinfection technique in endodontic regeneration (30).

In designing studies on cytotoxicity of materials, biocompatibility of chemical agents used as intracanal medicament is particularly important due to their direct contact with the periapical tissue, since they can induce cell cytotoxicity and cell death. On the other hand, cell type is another important factor to consider (31, 32). In this respect, dental mesenchymal stem cells, especially SCAP are specifically important. It should be noted that a cellular heterogeneity exists in the apical papilla due to the presence of cells other than the SCAP (33, 34). This heterogeneity is also seen among the stem cells and the markers they express (34). SCAPs are among these cells, which have a mesenchymal origin. Under *in vitro* conditions, they can differentiate into different cell lines and show very good clonal expansion capacity (35). SCAPs are of particular interest in endodontic regeneration due to their anatomical location at the site of root development (i.e. right next to the root end). Also, they are beneficial in cell delivery strategies (36). They can produce relatively higher and more homogenous den-

Table 1

Comparison of viability of SCAP and PDLSCs in presence of different concentrations of medicaments in co-culture and mono-culture

Culture	Stem cells	Medicament	0.125 mg/mL		0.250 mg/mL		1.00 mg/mL		p value
			Mean	Std. deviation	Mean	Std. deviation	Mean	Std. deviation	
Co-Culture	PDLsc	CH/CHX	49.297^{aC}	1.827	46.653^{aC}	4.057	41.367^{cC}	2.804	0.047
		CH/H_2O	86.843^{B}	0.94	67.577^{B}	7.889	73.663^{B}	4.604	0.158
		DAP/H20	88.880^{aA}	1.677	84.667^{bA}	0.443	82.233^{cA}	1.859	0.004
		DAP/CHX	33.677^{aD}	1.137	33.030^{aD}	0.191	30.370^{bD}	0.426	0.003
		p value*	0.000		0.001		0.000		
	SCAP	CH/CHX	33.350^{B}	2.245	32.987^{C}	0.975	31.593^{B}	2.789	0.597
		CH/H_2O	42.597^{A}	3.19	41.707^{B}	0.152	41.983^{A}	0.255	0.838
		DAP/H_2O	42.260^{A}	5.173	40.633^{B}	1.101	41.660^{A}	3.877	0.871
		DAP/CHX	44.423^{A}	1.003	44.333^{A}	1.196	42.247^{A}	4.084	0.527
		p value*	0.013		0.000		0.008		
Mono-Culture	PDLsc	CH/CHX	40.047^{aC}	1.915	35.477^{bC}	3.116	31.117^{cC}	1.143	0.008
		CH/H20	70.933^{aB}	1.938	65.807^{bB}	1.496	60.720^{bB}	4.508	0.016
		DAP/H20	83.067^{aA}	0.726	77.263^{bA}	0.750	73.790^{bA}	5.627	0.037
		DAP/CHX	42.787^{aC}	1.651	36.210^{bC}	0.706	29.677^{cC}	0.59	0.000
		p value*	0.000		0.000		0.000		
	SCAP	CH/CHX	39.677^{aC}	3.394	30.560^{bC}	0.577	31.380^{bC}	0.994	0.003
		CH/H_2O	66.390^{B}	0.34	62.173^{B}	1.877	65.043^{B}	2.131	0.051
		DAP/H20	72.317^{bA}	2.558	70.123^{bA}	1.887	76.783^{aA}	1.744	0.021
		DAP/CHX	30.067^{D}	1.501	27.400^{C}	0.395	26.100^{C}	3.747	0.188
		p value*	0.000		0.000		0.000		

P value: One-way ANOVA for the comparison of different concentrations.
P value: One-way ANOVA for the comparison of different intracanal medicaments.*
a,b,c: comparison of concentrations regarding cytotoxicity by Tukey's post-hoc test. Similar letters indicate lack of a significant difference.
A,B,C: comparison of medicaments regarding cytotoxicity by Tukey's epost-hoc test. Similar letters indicate lack of a significant difference.

tin-like tissue (35). Also, they have higher proliferation rate, dentin regeneration capacity and cell motility than DPSCs (33). PDLSCs are a type of somatic stem cells that have the potential to differentiate into various cell types. Moreover, they have strong self-renewal capacity. Thus, they are considered a promising population of stem cells for periodontal regenerative treatments. Moreover, PDLSCs are proliferated easier and faster and are more efficacious than other somatic stem cells (37). Thus, two different types of stem cells namely SCAP and PDLSCs in co-culture and mono-culture forms were used in this study, which further adds to the uniqueness of this study (Tables 1 and 2).

Our results indicated that in co-culture, the concentration of CH/CHX had no significant effect on the viability of the two types of stem cells. However, at all three concentrations, SCAP showed significantly lower viability than PDLSCs. It has been demonstrated that addition of CHX to CH significantly increases the cytotoxicity of CH (38). Our findings confirmed this statement.

Our results indicated that in mono-culture, the viability of SCAP and PDLSCs treated with different concentrations of CH/CHX was not significantly different. In other

Table 2

Comparison of cell viability in co-culture and mono-culture following exposure to different intracanal medicaments

Medicament	Stem cells	0.125 mg/mL		0.250 mg/mL		1.00 mg/mL	
		Mean	Std. deviation	Mean	Std. deviation	Mean	Std. deviation
CH/CHX	PDLSCs cc	49.297	1.827	46.653	4.057	41.367	2.804
	PDLSCs mc	40.047	1.915	35.477	3.116	31.117	1.143
	p value*	0.007		0.004		0.002	
	SCAP cc	33.350	2.245	32.987	0.975	31.593	2.789
	SCAP mc	39.677	3.394	30.560	0.577	31.380	0.994
	p value*	0.051		0.680		0.999	
CH/H2O	PDLSCs cc	86.843	0.940	67.577	7.889	73.663	4.604
	PDLSCs mc	70.933	1.938	65.807	1.496	60.720	4.508
	p value*	0.000		0.995		0.007	
	SCAP cc	42.597	3.190	41.707	0.152	41.983	0.255
	SCAP mc	66.390	0.340	62.173	1.877	65.043	2.131
	p value*	0.000		0.091		0.000	
DAP/H_2O	PDLSCs cc	88.880	1.677	84.667	0.443	82.233	1.859
	PDLSCs mc	83.067	0.726	77.263	0.750	73.790	5.627
	p value*	0.165		0.000		0.084	
	SCAP cc	42.260	5.173	40.633	1.101	41.660	3.877
	SCAP mc	72.317	2.558	70.123	1.887	76.783	1.744
	p value*	0.000		0.000		0.000	
DAP/CHX	PDLSCs cc	33.677	1.137	33.030	0.191	30.370	0.426
	PDLSCs mc	42.787	1.651	36.210	0.706	29.677	0.590
	p value*	0.000		0.003		0.990	
	SCAP cc	44.423	1.003	44.333	1.196	42.247	4.084
	SCAP mc	30.067	1.501	27.400	0.395	26.100	3.747
	p value*	0.000		0.000		0.000	

P value: independent t-test for the comparison of the two culture types.*

words, both cell types showed similar response to the cytotoxic effects of these medicaments. A previous study showed that CH and CH reinforced with CHX in 0.016 to 0.00025 percent concentrations inhibited the proliferation of L929 fibroblasts. CH reinforced with CHX and CH powder in 0.016 percent concentration showed 75% and 45% cytotoxicity at 72 h, respectively (39).
This study showed that in mono-culture, the viability of PDLSCs treated with CH/distilled water significantly decreased by an increase in concentration. The difference in this respect was not significant between the two cell types. But, in co-culture, increasing the concentration had no significant effect on cell viability. However, PDLSCs showed significantly higher viability than SCAP in all concentrations. Increased proliferation of both DPSCs and PDLSCs in co-culture has been previously shown. Also, the expression of mRNA of dentin sialophosphoprotein increases in co-culture of PDLSCs and DPSCs. Immunohistochemical analysis showed overexpression of dentin sialoprotein in both cell types in co-culture compared with mono-culture. Prolonging the culture period further increased the expression of dentin

sialoprotein. The expression of osteopontin also increased in both cell types in co-culture (40).

Evidence shows that CH has cytotoxic effects on 3T3 fibroblasts after 48 h; this effect was cytostatic and reversible such that after 7 days of incubation, cell proliferation returned to normal (41). Another study demonstrated that after 3 days of incubation, all tested materials were cytotoxic for DPSCs compared with the control group. Mineral trioxide aggregate with 77% cell viability had minimum cytotoxicity while CH with 26% and Biodentine with 16% cell viability had the lowest cell viability compared with the control group. Also, microscopic assessments showed that a reduction in the number of cells and morphological changes in treated cells occurred with all tested medicaments, particularly CH compared with the control group (42). Moreover, in contrast to the results of this study, it was reported that CH/distilled water had the significantly lowest cytotoxicity for the mono-culture of SCAP (38). Although the exact mechanism of cytotoxicity of these medicaments has yet to be fully understood, primary release of calcium ions, ionic activity and release of toxic components, and changes in pH of the tested material can all affect cell behavior (43).

Furthermore, our results indicated that SCAP treated with DAP/CHX in mono-culture had the significantly lowest cell viability compared with the control group. A previous study showed that in WST-1 assays, all antibiotic dilutions except for 0.125 mg/mL concentration significantly decreased cell viability while in the lactate dehydrogenase (LDH) assays, minimum tested concentration of DAP (0.5, 0.25, 0.125 mg/mL) and minimum concentration of triple antibiotic paste (0.25, and 0.125 mg/mL) were not cytotoxic for DPSCs (44). Also, it was shown that addition of CHX to modified triple antibiotic paste significantly decreased the viability of SCAP (38). This difference in the results can be attributed to the methods employed for assessment of cytotoxicity and type of stem cells. Our results revealed that in co-culture, increasing the concentration of DAP/distilled water significantly decreased the viability of PDLSCs. However, in all concentrations, SCAP showed significantly lower viability than PDLSCs. But, in mono-culture, increasing the concentration of DAP/distilled water significantly decreased the viability of both cell types. In 0.250 and 0.125 mg/mL concentrations, the viability of PDLSCs was significantly higher than that of SCAPs. Sabrah et al. (44) evaluated the effect of DAP in 0.125, 0.25, 0.5, 1 and 10 mg/mL concentrations using the LDH and cell viability assays and concluded that in all antibiotic concentrations except for 0.125 mg/mL, the viability of DPSCs significantly decreased. Also, LDH assays revealed that low (0.5, 0.25 and 0.125 mg/mL) concentrations of DAP were non-toxic for DPSCs (44). Another study showed that DAP in 1, 10 and 100 mg/mL concentrations had cytotoxic effects on the viability of SCAP and significantly decreased their viability (23).

Our results showed that the viability of PDLSCs in co-culture was higher than that in mono-culture; although this difference was not significant following exposure to DAP/distilled water. In contrast, the viability of SCAP in mono-culture was higher than that in co-culture, except for exposure to DAP/CHX, where cell viability was significantly higher in co-culture. The indirect co-culture technique was employed in this study. Indirect co-culture techniques are systems in which, two or more distinct cell types are cultured in the same environment.However, the cell culture environments are physically apart. This physical separation is often performed by using a Transwell or a well insert/Boyden chamber. Indirect co-cultures are as simple as monolayer cultures and are easily controlled. Despite separation of cells, they allow inter-cellular interactions to continue. Thus, they are often used to study some particular aspects such as cellular interaction mechanisms and cell behavior. It seems that SCAP in co-culture may protect the PDLSCs by signaling via the soluble factors. However, this theory is in need of further investigations. Indirect co-culture allows the cells to reside in environments with the same architecture as the natural target tissue specific for each cell type and at the same time have efficient communication with other cells. Signaling in indirect co-culture between the cells occurs through paracrine signaling by use of soluble factors while physical separation does not allow the cells to have direct interactions (45). Also, paracrine signaling is an important factor for behavioral regulation of stem cells and terminally differentiated cells in the co-culture (46).

Conclusions

Despite the limitations of this *in vitro* study, the results showed that addition of CHX to CH and DAP significantly decreases the viability of PDLSCs and SCAP in both co-culture and mono-culture. Therefore, the use of low concentrations of intracanal medicament is highly recommended.

Clinical Relevance

Inftracanal medicaments and the viability of dental mesenchymal stem cells play a important role in endodontic regeneration, so in invitro studies, the use of co-culture media mimics more invivo conditions.

Conflict of Interest

None.

Acknowledgements

None.

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ORIGINAL ARTICLE

Comparison of various irrigation activation procedures after post space preparation on push-out bond strength of fiber posts: an *in vitro* study

ABSTRACT

Aim: *To compare the effectiveness of various irrigation activation methods applied after post space preparation on the push-out bond strengths of fiber posts to root canal dentin.*
Materials and Methods: *Twenty maxillary central incisor teeth were prepared and root-filled. Following the preparation of the post spaces, the teeth were randomly allocated to 5 groups (n=4) based on the irrigation activation method. Post spaces were irrigated with 2.5% NaOCl and 17% EDTA and various irrigation activation techniques were applied in each group as follow. Group 1: conventional syringe irrigation (CSI); Group 2: manual-dynamic activation (MDA); Group 3: negative apical pressure (EndoVac); Group 4: passive ultrasonic irrigation (PUI) and Group 5: Er,Cr:YSGG laser. Subsequent to fiber post placement, the samples were transversally sectioned and push-out tests were applied for measurement of the bond strength of fiber posts.*
Results: *Irrigation activation with Er,Cr:YSGG laser, EndoVac and PUI resulted higher push-out bond strength in comparison to MDA and CSI (P<.05).*
Conclusions: *Irrigation activation with EndoVac, PUI and Er,Cr:YSGG laser provides superior bonding strength for fiber posts.*

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Introduction

Bonding between post, dentin and adhesive material effectively makes contribution to longevity of restorations (1, 2). Nevertheless, owing to the complicated geometry of the root canal and physical properties of adhesive materials, efficient adhesion is still considered as a challenge (3). Post space preparation causes a smear layer consists of debris, sealer and gutta-percha residues that may hinder bonding to root-canal dentin (4, 5). Additionally, it has been shown that bond strength of filling material to root canal dentin may be strengthen with removal of the smear layer (6). To remove the smear layer, irrigation of the root canal is a critical procedure of the root canal treatment. Sodium hypochlorite (NaOCl) and EDTA are two of the contemporary irrigants being used for removal of inorganic and organic contents of the smear layer (7). EDTA is a chelating agent used at various concentrations and durations. It has been shown that, to avoid erosive effects of EDTA on dentin, low concentrations or shorter application times should be used (8, 9). Therefore 17% EDTA irrigation must not be applied for longer than 1 min for avoidance of dentinal erosion (8). Prolonged EDTA application, proportionally to its concentration, was demonstrated to cause collagen rearrangement (10) which is associated with shortened tooth longevity (11). For an effective irrigation procedure, irrigation solutions should come to direct contact with root canal walls (12). Conventional syringe irrigation (CSI) alone is not capable of delivering irrigation solutions three-dimensionally to microstructures of root canal system in effective elimination of the smear layer (13). Moreover "vapor lock effect", the phenomenon of entrapment of air bubbles in the apical third of root canals, might prevent the exchange of irrigation solutions and impair their debridement efficacy (14). Manual dynamic activation (MDA), which is recurring insertion motion of a well-fitted gutta-percha cone to the working length, were used initially for activation of irrigation solutions (15). Eventually, due to the introduction of new dental technologies including ultrasonic devices (Passive Ultrasonic Irrigation, PUI), apical vacuum devices (Negative Apical Pressure, EndoVac), and laser systems (Er:YAG, Er,Cr:YSGG laser), various irrigation activation methods have been claimed to improve the effectiveness of irrigation procedure and smear layer removal (16). Consequently, irrigation activation procedures after post space preparation and its relationship with bond strength of post systems are currently point of interest. However, few studies have been performed on the efficacy of the different irrigation activation procedures on the bond strengths of fiber posts to root canal dentin.

The purpose of this in vitro study was to compare the effectiveness of different irrigant activation techniques on the bonding strengths of fiber posts to root canal dentin.

Materials and Methods

Twenty freshly extracted human maxillary central incisor teeth with straight root canals were stored for this study. Teeth with any defects, curvature, cracks, and previous root canal treatment were excluded. Teeth were decoronated to attain a standard 17 mm root length using diamond burs in a high-speed hand piece. A working length of 1 mm above the radiographic apex was established and the root canals were prepared with ProTaper Universal system (Dentsply Maillefer, Ballaigues, Switzerland) to size F4 with 2.5% NaOCl (Wizard; Rehber Kimya, Istanbul, Turkey) irrigation after each instrument. Root canal irrigations were completed with 2.5% NaOCl and 17% EDTA (Meta Biomed, Cheongju City, Chungbuk, Korea) using conventional syringe irrigation. The root canals were dried with absorbent paper points and root canal treatments were completed with gutta-percha and AH Plus root canal sealer (Dentsply DeTrey GmbH, Germany) using lateral compaction technique. Afterwards, the samples were kept at an incubator at 37 °C 100% humidity for 7 days and subsequently embedded in self-curing acrylic resin (Imicryl SC; Imicryl, Konya, Turkey). Post spaces were prepared by removing the coronal gutta-percha with the help of size 1 post drills (White Post DC System; FGM, Joinville, SC, Brazil) to 12 mm of the filled root canals. The root canals were randomly divided into 5 groups (n=4) based on the irrigation activation procedures.

In group 1 (CSI) the post spaces were irrigated with 3 mL 2.5% NaOCl and 17% EDTA for 40 seconds for each solution. In the course of the root canal irrigation, a 30-gauge needle tip (Ultradent, South Jordan, UT) was located 1 mm short of the post space and manipulated up and down ~4 mm.

In group 2 (MDA), 3 mL 2.5% NaOCl and 3 mL 17% EDTA were used respectively for irrigation of the post spaces and each irrigant was activated with a size F4 (Dentsply Maillefer) gutta-percha cone throughout the post space with manual push-pull strokes. 100 strokes per minute was applied. The gutta-percha cone was renewed per post spaces.

In group 3 (ANP), NaOCl solution was delivered with a syringe at 20 s. The master delivery tip of EndoVac (Discus Dental, Culver City, CA, USA) was placed at the orifice to deliver irrigants into the pulp chamber. The microcannula (#32/0.00), which works with apical negative pressure (ANP), was located 1 mm above the apical end of the post space, and delivery of solution was completed in 20 s. Same irrigation and activation protocol was applied using 3 mL EDTA.

In group 4 (PUI), the post spaces were irrigated with 3 mL 2.5% NaOCl using syringe for 20 s. Irrigation solutions inside the post spaces were activated by placing the ultrasonic tip (Endo Soft Instrument, Electro Medical Systems, Nyon, Switzerland) 1 mm above the apical end of the post space for 20 s. The ultrasonic tip was attached to an ultrasonic device (Minipiezo, EMS, Milan, Italy) at the power setting of "½." 3 mL EDTA was delivered and activated with the same protocol. Irrigation and activation protocols were completed in 40 s per solution.

In group 5 (Er,Cr:YSGG laser) the post spaces were irrigated with 3 mL 2.5% NaOCl solution by using syringe for 20 s. The irrigants were activated by (Waterlase MD, Biolase Technology Inc., CA, USA) via 300 µm radial firing tip (RFT3). Laser settings were 1.5 watt output power, 140 millisecond pulse duration and 20 Hz frequency. Irrigation timing and activation was applied identical to the method with group 4. During the activation, fiber tip was placed 1 mm above the apical end of the post space. The air-water spray was set to "off".

During the irrigation of the post spaces, 3 mL distilled water applied between two irrigation solution and at the end of the irrigation procedure and dried with absorbent paper points. Cementation of the fiber posts (White Post DC, FGM) to the prepared post spaces were applied using Panavia F 2.0 (Kuraray Medical Inc, Tokyo, Japan) in line with the recommended procedure by the manufacturer. During cementation, equal amounts of ED primer II A and B were mixed and applied to the post space for 30 s and gently air dried. Excess primer was removed by paper points. Panavia F 2.0 paste A and paste B were mixed and the posts were coated with the mixture. The posts were seated and light-cured.

All samples were maintained at 37 °C 100% humidity for 24 h prior to the push-out tests.

Three consecutive horizontal sections with 1.00 ± 0.05 mm width from both the apical and coronal region of the post space were acquired from each specimen by the help of a precision saw (Micracut, Metkon, Bursa, Turkey) under water coolant. 24 slices (12 coronal and 12 apical) were collected for each group. Each slice was attached to a tensile test machine (Lloyd LF Plus, Lloyd Instruments, Leicester, UK). Push-out bond strength tests were applied to the apical surface of the sections with a metal rod at a crosshead speed of 0.5 mm/min until the failure.

Digital calipers were used to measure diameters of the coronal and apical post segments and width of the slice and to calculate bonding surface area the formula below was used.

Bonding surface area:
$\pi (r_1+r_2) \times (\sqrt{(r_1-r_2)^2 + h^2})$

In this formula r_1 and r_2 stand for the coronal and apical post radius respectively whereas h is the width of the slice.
The push-out bond strength (MPa) of fiber post was calculated by dividing the force needed to dislodge the fiber posts (N) by the bonding area of post segments (mm^2).
Push-out bond strength data were evaluated using Levene's variance homogeneity test. Statistical analyses were performed with SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA) using two way analysis of variance and post hoc Tukey test. The level of significance was set at 0.05.
Failure types of 120 post segments were examined under a stereomicroscope (Stemi 2000-C; Carl Zeiss, Göttingen, Germany) at ×40 magnification. Three type of failure mode were categorised, as adhesive failure between cement and dentin, cohesive failure between cement and post, and if both failure types are observed its considered as mixed failure.

Results

The mean and standard deviations of each group in different post space areas are presented in Table 1. In respect of the total bond strength, the highest and lowest values were observed in Er,Cr:YSGG laser and CSI techniques respectively. EndoVac, PUI and Er,Cr:YSGG laser demonstrated significantly higher push-out bond strength values compared to CSI and MDA (P<.05). Removing smear layer with special tools considerably improved the bonding strength of the fiber posts. There were no statistically significant differences between MDA and CSI and amongst Er,Cr:YSGG laser, EndoVac and PUI (P>.05).
With respect to the total bond strength values, a statistically considerable difference was observed between bond strengths in apical and coronal regions (P<.05). The bond strength values predominantly increased in apico-coronal direction. The lowest bond strength values in both coronal and apical regions were obtained in the CSI group. The highest bond strength in the coronal and apical region was observed in the Er,Cr:YSGG laser and EndoVac groups, respectively. When the groups were evaluated within themselves, there was a statistically significant difference between the apical and coronal bond strengths in all groups (P<.05), except the Endovac group.
Regarding the failure types, adhesive failure (50.83%) was the commonest, followed by mixed failure (28.33%). Cohesive failure (20.83%) was the least frequent failure type (Table 2).

Discussion

Pulpless teeth are considered more fragile due to reduced dentinal elasticity, deeper cavities and substantial loss of dentin compared to teeth with healthy pulps. Restoration of root-canal treated teeth with fiber posts, that have dentin-matched mechanical characteristics such as elastic modulus close to dentin is favorable due to their capability of distributing homogeneous stress. Thus, fiber posts have lower prevalence of root fracture. As a consequence of post cavity preparation with post drills, smear layer consists of debris, root canal sealer and gutta-percha is formed on root canal walls (17).
Adhesion of the dual-cure cement to dentin necessitates direct contact between cement and dentin through the micromechanical retention and chemical bond. Therefore, smear layer, gutta-percha and sealer debris must be removed by the help of effective irrigation protocol (18). However, some studies have demonstrated that irrigation solutions are not capable of removing root canal filling remnants even if they are activated by sonic or ultrasonic systems (19). Thus, incomplete removal of the remnants might be a reason for the adhesive failures in our study. Prati et al. have showed post-retreatment canal filling material remnants via environmental scanning electron microscopy/energy dispersive X-ray spectroscopy (ESEM/EDX) analysis (20). Micro-computed tomography (micro-CT) has been used in visualizing the remaining root canal filling material (21). A micro-CT study has showed that

Table 1
Mean and Standard Deviations (SD) of push-out bond strength values in groups and two regions of root canal

	Group 1 Mean ± SD	Group 2 Mean ± SD	Group 3 Mean ± SD	Group 4 Mean ± SD	Group 5 Mean ± SD	Total Mean ± SD
Coronal	3.93±0.49^{A,a}	4.34±0.39^{A,a}	5.34±0.66^{A,b}	5.46±0.52^{A,b}	5.82±0.39^{A,b}	4.98 ± 0.87^{A}
Apical	2.71±0.54^{B,a}	3.15±0.65^{B,a}	4.89± 0.75^{A,b}	4.26±0.78^{B,b}	4.67±0.74^{B,b}	3.94 ± 1.10^{B}
Total	3.32± 0.81^{a}	3.74±0.80^{a}	5.12±0.73^{b}	4.86±0.89^{b}	5.24±0.82^{b}	

Data with different superscript lowercase letters indicate significant differences within each row; data with different superscript uppercase letters indicate significant differences within each column (p<0.05).

Table 2
Type of failure modes in groups

	Group 1	Group 2	Group 3	Group 4	Group 5	Total
Cement-Dentine	15 (62.50%)	14 (58.33%)	11 (45.83%)	11 (45.83%)	10 (41.66%)	61 (50.83%)
Cement-Post	5 (20.83%)	5 (20.83%)	4 (16.66%)	5 (20.83%)	6 (25.00%)	25 (20.83%)
Mixed	4 (16.66%)	5 (20.83%)	9 (37.50%)	8 (33.33%)	8 (33.33%)	34 (28.33%)

despite significantly improving filling remnant removal, irrigation with laser activation fail to removal of epoxy resin-based sealer completely (22). Similarly, another micro-CT study has demonstrated incomplete removal of epoxy resin-based sealer by irrigation with sonic, ultrasonic and laser activation (23).
Several studies demonstrated that smear layer removal is a crucial factor for bond strength between post-luting cement-dentin interface (24, 25). NaOCl and EDTA combination is used in removing of the smear layer (26). Activation of these irrigant solutions could benefit post retention by increasing the efficiency of complete smear layer removal (27-29).
For evaluation of fiber post bond strength, push-out test was performed due to the fact that it provides more homogeneous stress distribution on the adhesive interface and few specimen losses (30). While dentin slices with various thickness were used in previous studies on push-out bond strength (31, 32), in this study, 1 mm-thick sections were prepared due to the lower friction areas and decreased likelihood of overestimated results compared to thicker slices. EndoVac system has been used as an irrigation method for more than a decade. EndoVac system comprises of a delivery tip which delivers irrigation solution in the pulp chamber and aspirates the excessive irrigant to prevent overflow and a tube attaches either a macro- or microcannula (33). In a study, higher mean push-out bonding strength values were achieved with EndoVac than CSI when the same NaOCl and EDTA irrigation regimen applied (29). Another study reported that NaOCl and EDTA delivery with EndoVac system resulted better smear layer removal than NaOCl and EDTA irrigation with CSI (34). Antunes et al. demonstrated that delivering 15% EDTA with EndoVac system results significantly higher push-out bond strength of gutta-percha than 15% EDTA irrigation with conventional irrigation (35). In our study, EndoVac system enhanced bond strength of fiber posts to root canal walls in comparison to conventional syringe irrigation and MDA. Effective smear layer removal in consequence of negative apical pressure could be rational for our results in this regard.
In this study, the use of PUI in irrigant

activation had an improving effect on the bond strength. Our finding is accordant with the findings of another study on bond strength of the fiber post to root canal (36). In another study it was found that ultrasonic activation of NaOCl has no positive effect on bond strength of fiber posts in any region of root canals in comparison to NaOCl irrigation alone (37). Contradiction between this study and our study might be attributed to the detrimental effect of NaOCl decreasing bonding ability of dentin by dissolution of collagens which is directly related to bond strength of fiber posts to root canal dentin (38-40). EDTA irrigation following NaOCl has a positive effect on bond strength of fiber posts. Therefore, more effective results were achieved with PUI method with NaOCl and EDTA irrigation.

Laser systems have been introduced to endodontics as a novel technology to improve treatment procedures including irrigation activation. Er,Cr:YSGG laser has been reported to be effective on smear layer removal when it is used for activation of irrigation solutions such as NaOCl and EDTA (28). Although there was no significant difference between Er,Cr:YSGG laser, EndoVac and PUI groups in our study, laser group demonstrated the highest bond strength in overall comparison. It can be asserted this result is correlated with smear layer removal capability of Er;Cr:YSSG laser when used with NaOCl and EDTA. Çökük et al. compared the bond strength of fiber posts after using different irrigation protocols. Results of their study showed that irradiating the post space with Er,Cr:YSGG laser using 1.5 W output power enhances bond strength of fiber post to root canal dentin and leds to higher push-out test values than NaOCI or chlorhexidine (41). In contrast, Kirmali et al. concluded that Er,Cr:YSGG laser application with various power settings did not enhance the bond strength of the fiber posts to the root canal dentin in their study which evaluated the effects of dentin surface treatment with Er,Cr:YSGG laser application. Besides, researchers mentioned that this outcome could be associated with the absence of endodontic irrigation regimes for smear layer elimination in the course of the post space treatment (42). The difference between the results of their study and this study is due to the use of Er,Cr:YSGG laser to activate irrigation solutions in this study, while Er,Cr:YSGG laser was used without irrigation solution in their study.

Araújo et al. compared the impact of Er,Cr:YSGG laser treatment and 5.25% NaOCl irrigation of post-space on push-out bond strength of fiber posts and reported that there were no considerable differences between two groups. While they used the same radial firing tip (RFT3) with this study, different laser settings and the fact that the laser in their study was used with air and water spray instead of irrigation solutions might result in contradicting findings to this study (43).

In this study, the push-out bond strengths were affected by the root region and increased in apico-coronal direction. This result is compatible with the outcomes of the studies investigated the push-out bond strengths of either fiber posts or obturation materials to dentin walls (29, 44, 45). Weaker bond strength of fiber posts in apical region might be associated with less efficient smear layer removal in apical region (28), void formation in resin cement, residues of gutta-percha (46). Smaller root canal diameter and fewer dentinal tubules in the apical part of the root canals can also be reasons for this result. When the groups were evaluated within themselves, there was a statistically significant difference between the apical and coronal regions in terms of bond strength in all groups apart from the EndoVac group. EndoVac was the only group resulted with similar bond strength in apical and coronal regions. This finding is in agreement with the study of Akyuz Ekim and Erdemir (29). The EndoVac system's effectiveness at apical level might be attributed to the apical negative pressure effect which is applied by placing the microcannula to apical portion of the canal. Thus, irrigant is constantly suctioned and replenished at apical region and smear layer might be removed as effectively as at coronal region (34).

EndoVac system showed the most favorable results in apical region. In numerous studies, EndoVac demonstrated better smear removal in apical region than CSI, PUI, MDA and laser assisted activation (34, 47, 48). As mentioned earlier, more efficient removal of smear layer, along with other factors, is correlated with higher push-out bond strength. Apical negative pressure principle and hence disruption of vapour lock effect and increased volume of irrigant delivery in the apical region might be the reason for superior result with EndoVac. In coronal region, Er,Cr:YSGG laser activation resulted highest bond strength mean value. It was reported that Erbium lasers were more efficient than CSI, PUI and EndoVac methods in smear removal from coronal segments of root canals (28, 34, 49). It is known that Erbium lasers, whether it is Er:YAG laser or Er,Cr:YSGG laser, have wavelengths that provide effective absorption in water and sodium hypochlorite (50). The Er,Cr:YSGG laser radiates laser energy in pulses and energy emitted is absorbed in liquid. This results with instant increase in heat of liquid to boiling temperature and vaporization consequently. Primary and secondary bubbles implode and this implosion creates shearing stress through the root canal walls which might be effective for smear layer removal (51). Thus, higher fiber post bond strength in coronal region in this study can be attributed to wavelength and mechanism of Erbium lasers.

None of the 120 specimens failed during specimen preparation before testing. The evaluation of failure types showed that dentine-cement failure was approximately 2 fold cement-post failure. This may be due to the formation of small gaps between dentine and cement due to polymerization shrinkage. In addition, while dual-cured resins have properties suitable for both self-cured and light-cured resins, it was showed that some dual-cured resins may not achieve sufficient degree of conversion in the absence of light (52). Therefore, insufficient light to reach the deep parts of the root canal may cause incomplete polymerization. The present study, however, has some limitations. Firstly, only four teeth per group were included which must be considered when interpreting the results. Although it may be seen as an advantage to use a smaller number of teeth in push-out tests, it can also be considered as a limitation of this study. Moreover, only teeth with straight canals were included in the study and the results might be not applicable to teeth with curved root canals. The use of irrigation activation techniques in curved canals may lead to different results.

Conclusions

Considering the limitations of this study, various irrigation activation protocols can be applied to enhance the bond strength between fiber posts and root canal walls. Removal of the smear layer with special tools such as EndoVac, PUI and Er,Cr:YSGG laser may provide a better push-out bond strength of fiber posts to root canal dentin. Further studies are required to investigate what procedure and settings would affect the smear layer removal from root canal walls by the help of EndoVac, PUI and Er,Cr:YSGG laser to achieve a more preferable bond strength.

Clinical Relevance

Improved smear layer removal with EndoVac, PUI and Er,Cr:YSGG laser before fiber post placement might led to better post retention.

Conflict of Interest

The authors declare no conflict of interests.

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ORIGINAL ARTICLE

Effect of different concentrations of propylene glycol on the physical-chemical properties of MTA

ABSTRACT

Aim: *This study evaluated the effect of different concentrations of propylene glycol (PG) on the dimensional change (DC), compressive strength (CS), solubility and pH of MTA.*
Methodology: *Groups were formed according to the proportion of distilled water (DW) and PG in MTA manipulation: G1 (100% DW); G2 (80% DW+20% PG); G3 (50% DW+50% PG); G4 (20% DW+80% PG). The tests were guided by ISO and BSI standards. DC and CS tests were performed after 24 h and 30 days of samples storage in water. In the solubility test, rings filled with the cements were weighed after setting and after 7 days. The pH was measured after 3 and 24 h, and 30 days. The data were analyzed with the ANOVA, Tukey's and Paired t tests (α=5%).*
Results: *After 24 h and 30 days, the DC and CS were similar among groups, except the CS for G3 in 30 days. After 7 days, G1 gained weight when hydrated, differing from G3 and G4 ($p<0.05$). When weighed dehydrated, G2 was different from the other groups ($p<0.05$). After 3 h, G1 had lower pH values in comparison with G2, G3 and G4 ($p<0.05$). At the 24-h period, G2 had higher pH values than G1 and G2 ($p<0.05$). After 7 days, there was no difference among groups.*
Conclusions: *PG did not affect the MTA dimensional change and compressive strength (except for G3). However, PG reduced MTA solubility (G2) and increased its pH after 3 hours.*

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Introduction

Mineral Trioxide Aggregate (MTA) was initially developed as a retrograde filling material, and to seal the communications between the root canal system and periodontium. Gradually, MTA was also used in pulp-capping, pulpotomies, apexification, apexogenesis and as filling material, mainly due to its biological properties (1). Despite its wide indication, MTA presents poor handling properties, extended setting-time and wash-out possibility (2, 3). In order to improve its physical-chemical properties, the use of several additives in MTA manipulation have been investigated, and propylene glycol (PG) is one of them (4-11).

PG is a non-carcinogenic and non-genotoxic alcoholic vehicle widely used in dermatology (12). It is considered ideal for many topical preparations, used as solvent, skin conditioner, fluidizing, and in numerous fragrances compositions (13).

In endodontics, PG is frequently used as a vehicle for calcium hydroxide intracanal dressing, allowing a slower hydroxyl and calcium ions dissociation because of its consistency (14). Considering the benefits of mixing PG to calcium hydroxide, Holland et al. (4) used it as an MTA vehicle for root canal obturation. These authors investigated the influence of distilled water (DW) or PG on the periapical response of dog's tissues. Both mixtures had similar biological behavior and were biocompatible. In addition, other studies have reported that PG improved several properties of MTA, such as the sealing ability in furcal perforations against bacterial leakage after 30 days (5) and dentin bond strength (7). In the ratio of 80% DW/20% PG, this vehicle improved several properties of MTA, such as setting-time, flowability, pH, and calcium ions release (6).

Given the studies found in the literature, using PG as a MTA vehicle seems to be promising (4-11). Although, different ratios of DW and PG may affect the physical-chemical properties of MTA, since crystal hydration is an important factor for its setting reaction (2). The addition of high PG ratios results in a longer setting-time, leading to higher solubility and greater pores formation, compromising MTA mechanical strength (6, 15). Therefore, this study aimed to evaluate the effect of manipulating MTA with different concentrations of PG on its dimensional change, compressive strength, solubility and pH. The null hypothesis tested was that the different concentrations of PG would not interfere on the MTA properties mentioned above.

Materials and Methods

White MTA Angelus (Angelus, Londrina, Brazil) was manipulated with different concentrations of DW and PG, and distributed into four groups, as follows: G1 (100% DW); G2 (80% DW and 20% PG); G3 (50% DW and 50% PG) and G4 (20% DW+80% PG). The DW-PG ratio was determined by volume, and the powder-liquid ratio was the same for all groups (1.0 g of MTA powder to 0.4 mL of liquid). After the cement was prepared as determined in each group, the physicochemical properties were evaluated according to the tests described below.

Dimensional Change

Twelve cylindrical samples for each group were fabricated in Teflon molds (3.58 mm in height, 3 mm in diameter), according to the methodology of Carvalho-Júnior et al. (16). The molds were placed on a glass plate (1 mm thick, 25 mm wide, 74 mm long) covered by a cellophane paper strip. Then, each mold was filled with the cements (MTA/different PG-DW ratios), leaving a slight material excess on the upper side. Another glass plate, also covered with cellophane paper strip, was placed on the top of each mold and the set was held tightly together by a "C" clip. All samples were transferred to a laboratory oven at 3± 1 °C and 95% relative humidity, five minutes after the manipulation has started. After 24 h, all samples were removed from the laboratory oven and their surfaces were polished with a 600-wet sandpaper (3M, Sumaré, SP, Brazil) under copious water irrigation. The initial length of each sample was measured with a thickness digital caliper (Mitutoyo America Corporation, Chicago, IL, USA). Next, the samples were immersed in individual flasks containing 2.24 mL of DW at 37±1 °C for 24 h and 30 days (16). Six samples from each group were used for both periods. After the experimental periods, they were removed from the flasks, dried with absorbent paper and measured again with the digital caliper. The dimensional change was calculated by the following formula:

$$[(C_{FINAL} - C/C)] \times 100$$

where C_{FINAL} is the final length of the sample (after the experimental periods), and C the initial length after setting (17).

Compressive Strength

The same samples (n=12) used in the dimensional change test were used to verify the mechanical strength of the cements. After 24 h and 30 days, the samples were removed from the flasks, dried with absorbent paper and the compressive strength was then evaluated using a Universal testing machine (Instron, Model 4444, Instron Corp., Canton, MA,USA) at a cross-head speed of 1 mm/min. The maximum load required to reach the failure point was recorded.

Compressive strength was calculated in MegaPascal (MPa) according to the equation: $C=4P/\pi D^2$, where P is the maximum load recorded by the testing machine in Newton (N), D is the sample diameter in millimeters (mm), and C is the maximum load in MPa (18).

Solubility

Twelve teflon rings molds (1.5 mm in thickness, 7.75 mm in internal diameter) were used following the methodology described by Carvalho-Júnior et al. (16). In each ring, a carbide drill nº 2 was used to create a through hole in the rings for posterior introduction of a nylon thread, which later allowed the suspension of the rings. Each ring was filled with the different MTA-like cements and kept in a laboratory oven at 37±1 °C and 95% relative humidity for a period equivalent to three times the setting-time. Then, the samples were polished with a 600-wet sandpaper, gently dried in absorbent paper and weighed (initial hydrated weight) on a precision balance (AND model GR-202, Tokyo, Japan). After 24 h in a desiccator with silica, the rings were re-weighed (initial dehydrated weight). The samples were suspended by a nylon thread and placed in flasks containing 7.5 mL of DW and placed in a laboratory oven at 37±1 °C for 7 days (16). Following, the rings were removed from the flasks, gently dried and weighed (final hydrated weight). Samples were placed in a desiccator for 24 h and then, re-weighed (final dehydrated weight). The solubility corresponded to the samples weight loss, expressed as percentage, in comparison with the original samples weight.

pH

The pH of the DW in which the samples were immersed in the dimensional change test was measured in triplicate at 3 and 24 h, and 7 days. The measurements were obtained using a pHmeter (Micronal, PH-1700, São Paulo, Brazil) calibrated according to the buffer solution (pH 7.0 and 9.0) at 25±2 ºC temperature, which was kept constant using an air conditioner.

Statistical Analysis

Data were analyzed for normal distribution and homoscedasticity using the Kolmogorov-Smirnov's and Levene's test, respectively, followed by parametric statistical tests. Data from the groups in each period was analyzed using ANOVA and Tukey's tests. Data from the same group over time were analyzed using the t-test. All tests used an $\alpha=0.05$ significance level and all analyses were carried out using the SPSS 21 software (IBM SPSS Inc.; Chicago, IL, USA).

Results

The dimensional change and compressive strength mean values are expressed in Table 1.

Regarding the dimensional change anal-

Table 1

Mean values and standard deviation (SD) for dimensional change (%) and compressive strength (MPa) of the different groups at the two periods of analysis

Test/Groups	100% DW	20% PG	50% PG	80% PG
Dimensional change*	Mean SD	Mean SD	Mean SD	Mean SD
24 h	0.05±0.03[Ab]	0.00±0.04[Ab]	0.11±0.03A[a]	0.11±0.11[Aa]
30 days	0.17±0.03[Aa]	0.24±0.02[Aa]	0.29±0.13[Aa]	0.25±0.06[Aa]
Compressive strength*				
24 h	27.75±19.05[Aa]	41.03±14.57[Aa]	29.75±8.40[Ab]	36.93±15.13[Aa]
30 days	42.67±3.06A[Ba]	42.72±6.19A[Ba]	52.52±7.57[Aa]	30.46±8.21[Ba]

**Capital letters represent a significant difference among the groups (ANOVA and Tukey's test) and lowercase letters between the periods (t-test) (α=5%).*
DW: distilled water, PG: propylene glycol.

ysis, there was no statistical difference among groups in each evaluated period (p>0.05). Over the course of time, the samples from all groups presented increase in their dimensions, mainly for G1 (100% DW) and G2 (20% PG), which had a significant expansion from 24 h to 30 days (p=0.0007 and p=0.0002, respectively).
At the 24-hour period, all groups have similar compressive strength values (p>0.05). After 30 days, there was significantly different among the groups. G3 (50% PG) had the highest mean value, differing from G4 (80% PG) (p=0.0008). When the analysis was performed between the experimental periods in the same group, G3 (50% PG) increased its resistance significantly at the 30-day period (p=0.0099).
The mean solubility values are shown in Table 2. After 7 days, G1 (100% DW) gained weight when hydrated, with statistically significant difference in comparison with G3 (50% PG) and G4 (80% PG), which lost weight (p=0.0194 and p=0.0221, respectively). When the dehydrated samples were compared among each other, it was observed that G2 (20% PG) gained weight, being statistically different from the other groups (p<0.05).
The pH mean values are expressed in Table 3. Statistical difference among groups after 3 and 24 h was observed (p<0.05). After 3 h, G1 (100% DW) had lower pH in comparison with G2 (20% PG), G3 (50% PG) and G4 (80% PG) (p=0.004, p=0.000, and p=0.000, respectively). At the 24-hour period, G3 (50% PG) had higher pH values, with statistical difference when compared to G1(100%) and G2 (20% PG) (p=0.002 and p=0.009, respectively). At the 7-day period, there was no statistical difference among groups.
When comparing the pH values among the experimental periods, at 7-days period, G1 (100% DW) presented significant higher pH mean values than in 24 h ($P = 0.0062$). G2 (20% PG) and G3 (50% PG) had higher pH values in the initial periods (3 and 24 h) than in the 7-day period of analysis (p<0.05).

Table 2

Mean values and standard deviation (SD) for solubility (%) of the different groups. Negative values mean weight loss

Groups	7 days (hydrated)		7 days (dehydrated)	
	Mean	SD	Mean	SD
100% DW	0.0160[Aa]	0.0097	-0.0032[Bb]	0.0017
20% PG	0.0074A[Ba]	0.0020	0.0048[Aa]	0.0020
50% PG	-0.0017[Ba]	0.0129	-0.0032[Ba]	0.0129
80% PG	-0.0013[Bb]	0.0039	-0.0057[Ba]	0.0039

The capital letters represent a significant difference among the groups (in each column, ANOVA and Tukey's tests) and lowercase letters between the conditions (hydrated and dehydrated, t-test) (α=5%).
DW: distilled water, PG: propylene glycol.

Table 3

Mean values and standard deviation (SD) for pH of the different groups at the three periods of analysis

Groups	3 h		24 h		7 days	
	Mean	SD	Mean	SD	Mean	SD
100% DW	10.62[Aab]	0.03	10.97[Ba]	0.06	10.18[Ab]	0.59
20% PG	10.79[Ba]	0.06	11.00[Ba]	0.16	9.93[Ab]	0.68
50% PG	10.85[Ba]	0.06	11.25[Aa]	0.07	10.07[Ab]	0.56
80% PG	10.86[Ba]	0.08	11.07[ABa]	0.08	10.42[Aa]	0.73

Capital letters represent a significant difference among the groups (in each column) and lowercase letters among the periods (in each line) (ANOVA and Tukey's tests, α=5%).
DW: distilled water, PG: propylene glycol.

Discussion

This study evaluated the dimensional change, compressive strength, solubility and pH of MTA prepared with different DW/PG ratios. Based on the obtained results, the null hypothesis tested was partially accepted, since the mixture of different DW/PG ratios to MTA affected the evaluated properties, except for dimensional change.
The PG dilutions in water and the powder-liquid ratio used in the present study followed those used by Duarte et al. (6). However, only four of the five proportions proposed by them were used. As the cement never reached its complete setting when it was manipulated with 100% PG (6), it was decided to exclude this group from the study.
Although the proportion 80%DW/20%PG in MTA is the most investigated in the literature (6, 8, 9), it may not yet be assert that it is the best choice, due to some negative effects (9). For this reason, more studies with different PG proportions should be carried out, and also other properties need to be evaluated.
In this study, the effect of PG on the dimensional change of MTA was investigated for the first time. The experimental groups manipulated with PG had similar dimensional change among each other and in comparison with G1 (100%DW) in both periods of analysis. Although no statistical difference among groups was found, it was noted that the groups with PG had higher expansion mean values. This result relies on the humectant ability of PG, which keeps the hydration process of MTA for a longer period, leading to a late expansion (2, 9). Camilleri (2) showed that after a few hours, the hydration process of mineral aggregate-based cements promotes a significant volumetric change in this type of materials. The hydration process also influences the setting-time, and it has been shown that the more PG is added to the mixture, the longer the setting-time of the MTA (6, 8).
There are no published data available on the the dimensional change of MTA when PG was used as a vehicle; therefore, direct comparison with other studies was not possible. However, in relation to G1, several studies have reported an average percentage of expansion for MTA mixed with DW of 0.08% after 24 h (19), and 0.28% (20) and 0.30% (21) after 30 days, differing from the findings of the present study, which were inferior (0.05% in 24 h and 0.1% in 30 days). The possible explanation for the controversial results relies on the use of samples of smaller dimension in the present study, in addition to the different MTA commercial brand used to perform the tests. To evaluate the expansion of MTA is an interesting issue, as this

property may increase the cement's sealing ability (21), and consequently, to reduce the possibility of leakage (20). However, an excessive expansion is undesirable, especially if the material is used as a root-end filling material, because it may lead to microcracks at the apical portion of the root (20).

ISO specification (17) states that the mean dimensional change in length of a sealer should not exceed 1.0% in shrinkage or 0.1% in expansion. In this study, G3 and G4 at the 24 h-period, and all groups after 30 days had greater dimensional change than the ISO recommendation (17).

When analysing the compressive strength test, at the 24-h period, PG groups were statistically similar to G1. After 30 days, G3 increased significantly its compressive strength (from 29.75 to 52.52 MPa) and had the highest mean value, differing only from G4 (30.46 MPa). Despite the statistical similarity with G1 (42.67 MPa) and G2 (42.72 MPa), G3 had the most appropriate mechanical performance. This finding is in agreement with the results of other studies, where the compressive strength of MTA significantly increased when the cement was manipulated with 50%PG (10, 11). It is valid to emphasize that a proper compressive strength is fundamental for MTA, as this material may be used for pulp-capping or as a coronal restorative material, because of the occlusal forces in which the cement is submitted (22).

The solubility of a specific material is the loss of mass during a period of immersion in water (16). Materials with high solubility may release irritant by-products into the medium and increase leakage risks (23). In the present study, all tested cements had a solubility percentage inferior than 3%, following the ISO recommendation (17).

When weighted hydrated after 7 days, G1 gained weight differing from G3 and G4, which lost weight. When the samples were weighted dehydrated, G2 gained weight being different from the other groups. These results were different from those found by Marciano et al. (9), where the group 20% PG had solubility of 0.25%. These findings might be explained by the different size of the samples used in each study. The authors of the mentioned study (9) used polytetrafluoroethylene rings (1.5 mm in thickness, 20 mm in internal diameter) filled with the cements and a nylon thread was placed inside the material. In the present study, Teflon rings with a smaller dimensions (1.5 mm, 7.75 mm) were used, as suggested by other studies (3, 16, 24). Also, the nylon thread passed through the holes created in the rings, and not within the material. The larger contact area between the material and the liquid, and the placement of the nylon thread inside the material probably induced the different results. The reduction of the dimensions of the Teflon rings aimed to decrease the amount of material used to perform the test, and showed to be compatible with the ISO standards (17).

ISO (17) also recommends the weight of the residues released by the cement's samples after the water in which they were immersed evaporates, not considering the possible absorption by the material. This feature is extremely significant for MTA, which has high water absorption capacity (2, 25). For this reason, samples were weighed before being placed in the desiccator, providing a weight value of the water absorbed (initial and final hydrated weight), showing the absorbent capacity of the cements (3, 25).

At the 3h-period, PG groups showed higher pH mean values than G1 (100% DW), and this result is in accordance with a previous study (6). After 24 h, the pH averages remained high, with emphasis on G3 (50%PG). At the 7-day period, there was no statistical difference among groups, in agreement with the results of previous studies (6, 9). When only the values were observed, this study showed higher pH values than those found by Duarte et al. (6). This variation might be attributed to the samples and solutions contact area. The authors used artificial resin teeth containing root-end preparations filled with the cements to perform the test. The methodology restricts MTA contact area with water, and consequently, lower pH values were found.

One of the main reasons for changing the MTA vehicle is to improve its consistency, which restricts it from being used as a filling material and also makes it difficult to insert it into cavities (3, 4). MTA sets by a hydration reaction to form a calcium silicate hydrate gel and calcium hydroxide. Calcium hydroxide will release Ca^{2+} ions and the decomposition of calcium silicate hydrate will generate an alkaline pH (2). Mixing PG to MTA reduces the amount of water available for the hydration reaction, resulting in a slower setting-time, and that process is probably sustained for a longer period (6, 8, 9). According to these authors, the interference with the setting-time might result in higher solubility, greater formation of pores and have a negative impact on the mechanical strength of the material. The present study has reported that the tested PG concentrations did not negatively affect the mechanical strength of MTA.

Conclusions

Considering the present results and the inherent limitations of the methodology used, it was concluded that the PG did not affect the MTA dimensional change and compressive strength (except for G3). However, PG reduced the MTA solubility (G2) and increased the pH of the medium after 3 hours.

Clinical Relevance

The ideal quantitity of propylene glycol to manipulate mineral aggregate-based cements still is a controversial point. This study investigated the effect of mixing different ratios of distilled water and propylene glycol on the physical-chemical properties of MTA in order to elucidate this matter.

Conflict of Interest

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

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ORIGINAL ARTICLE

Influence of operator's experience in three different instrumentation techniques: an *in vitro* study

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ABSTRACT

Aim: *To evaluate the influence of operator's experience comparing rotary and reciprocating shaping systems on simulated root canals.*
Methodology: *Ninety resin blocks with simulated root canals were distributed to six groups (15 for each group); three groups of instrumentation were assigned to undergraduate students and three to specialists in endodontics. For each category of experience, the shaping was performed with Protaper Gold™, WaveOne Gold™ and Stainless-steel K-File (Control). Resin blocks were inked, then pre- and post-instrumentation photographic images were taken to be superimposed by an imaging software (GIMP 2.10.10) and analyzed by ImageJ software. After the use of each instrument, a rinse with NaOCl 2,5% was made. The outcomes evaluated were the presence of apical zip, ledges, perforation, the centering ability and the amount of resin removed.*
Results: *The photographic analysis of the resin blocks showed a prevalence of apical zips within the undergraduate groups; the expert groups realized a higher number of ledges, while the number of perforations resulted to be higher in the inexpert Stainless-steel control group. The amount of resin removed was significantly higher in the undergraduate WOG group, as well as the centering ability.*
Conclusions: *The rotary and reciprocating systems provide valid operative standards; by the way, inexpert operators showed major difficulties managing the reciprocating system. More studies are required to assess this parameter.*

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KEYWORDS *apical zip, in vitro study, Protaper Gold, reciprocating, WaveOne Gold*

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Introduction

The shaping of the root canal is one of the most important steps of an endodontic treatment; its aim is to remove the necrotic tissues, pulpal debris and remnants, and to shape the canals in order to obtain a tridimensional obturation by preserving the integrity of the canal system (9). The introduction of the Ni-Ti alloy took to many evolutions and to an improvement both of techniques and instruments used in endodontics; nowadays, in fact, we can distinguish instruments for different materials, morphologies, taper and apical diameters. Anyway, they're all referable to two different cinematics: continuous and reciprocating.

The use of rotary instruments, introduced by McSpadden in 1992, has always been the best technique to obtain a predictable and repeatable shaping of the root canal, compared to the use of stainless-steel manual instruments (4). The occurrence of cyclic fatigue fractures (3), caused by the "taper lock" phenomenon, brought to the development of a new generation of instruments; they work through a reciprocating motion, in order to overtake the risk of this adverse event (18). The reciprocating motion allows to invert the direction of rotation of the instrument before the first cycle of rotation gets complete, reducing the engagement of the instrument in the root canal and, consequently, the stress it's exposed to. Protaper Gold™ and WaveOne Gold™ (Dentsply Maillefer-Chemin du Verger 3, 1338 Ballaigues, Switzerland) had a remarkably good response due to their innovative cutting sections and the increasing flexibility of the Gold alloy (16).

The Protaper Gold™ category includes seven flexible instruments, with a triangular convex section and different tapers along the working part: three shaping files SX (019.04), S1 (018.02) and S2 (020.04) for the coronal shaping and five finishing files for the apical shaping, F1 (020.07), F2 (025.08), F3 (030.09), F4 (040.06), F5 (050.05). WaveOne Gold™ includes a sequence of four instruments with different diameters and a progressive cross section: Small (020.07), Primary (025.07), Medium (035.06) e Large (045.05). The real advantage of using those instruments is to shape canals potentially with one file, reducing the working time; anyway, they are disposable and can't be sterilized.

Many reviews (5, 1, 11) compared the two different systematics, particularly in terms of cyclic fatigue resistance, shaping features, apical debris extrusion and dentinal cracks. The results were controversial and showed how, at the moment, there's no a significant difference in the centering ability, the apical debris transportation and the number of dentinal cracks. The main differences were found in the analysis the resistance to fractures, which was higher in the reciprocating categories; some studies (13), moreover, showed how the reciprocating endodontic treatment caused a worsening of patients *QoL* (quality of life) with a stronger post-operative pain due to the non-controlled apical debris extrusion.

The aim of this in vitro study is to compare three different shaping systems (stainless-steel K-File, Protaper Gold™ and WaveOne Gold™) and to evaluate the differences between the shaping made by undergraduate students and specialists in Endodontic.

Materials and Methods

The study presents three main groups of shaping: shaping with Protaper Gold™, with WaveOne Gold™ and with stainless-steel K-File. The influence of operator's experience will be evaluated for each category (6) and the shaping of the three groups above will be performed on simulation resin blocks (Endo Training Bloc, Maillefer).

The number of resin blocks was established in 15 blocks for each group of instruments, for both experience groups, for a total of 90 blocks (15) by *sample size calculation.* Every operator shaped three resin blocks, one for each category of instruments (Table 1).

The shaping with K-File (control) followed the steps below: WL detection and scouting with K-File 8-10 and, enlargement of the coronal third with Gates drills from #1 to

Table 1
Group division

Group 0 (Control)	K-File (inexpert)
Group 1 (Control)	K-File (expert)
Group 2	Protaper Gold™ (inexpert)
Group 3	Protaper Gold™ (expert)
Group 4	WaveOne Gold™ (inexpert)
Group 5	WaveOne Gold™ (expert)

#6, manual *preflaring* and *glide path* with stainless-steel K-File from 10 to 20; check of the patency with K-File 10, shaping of the root canal with K-File in sequence of sizes from 25 to 80, following an apical to coronal approach.
The shaping with Protaper Gold™ (group 2 and 3) was realized through the following steps: WL detection and scouting with K-File 8-10, manual *preflaring* and *glide path* with K-File 15-20, instrumentation of the root canal with Shaping Protaper Gold™ S1-S2, check of the patency with K-File 10, sequence of Finishing Protaper Gold™ F1-F2.
The shaping with WaveOne Gold™ (group 4 and 5) was realized through the following sequence: WL detection and scouting with K-File 8-10, manual *preflaring* and *glide path* with K-File 15-20, shaping with WaveOne Gold™ Primary 3 mm to the end of the canal system, check of the patency with K-File 10, shaping with WaveOne Gold™ Primary at the working length (WL).

A dedicated shaping program with parameters suggested by manufacturers has been selected for the utilization of each file (X-Smart; Dentsply Maillefer). Before use, each instrument was lubricated with Glyde (Dentsply Maillefer), whilst a rinse with 2,5% NaOCl was made after the use of each instrument (15).

Data recording
The endo training blocks were inked before the use, to enhance the canal. Pre and post- shaping photographic images were taken (Nikon D7200), at stable reference points and magnification. The camera was set on a tripod to guarantee the stability of the camera.
Then, resin blocks were positioned on a graph paper to obtain calibrated points and to ease the superimposition of images (Figure 1). The latter was realized through the utilization of GIMP 2.10.10; after that, the superimpositions were uploaded on ImageJ software to obtain the analysis of the shaping.

Outcomes
This study has the aim to evaluate two main outcomes: the centering ability and the amount of removed resin. The centering ability was evaluated by dividing the canal into nine parts of 1mm and subtracting at each point the amount of resin removed from the inner part to that removed from the outer aspect of the canal. The amount of resin removed was evaluated by adding the amount of resin removed from the inner and the outer part of the

Figure 1
Example of superimposition of the pre and post- instrumentation images.

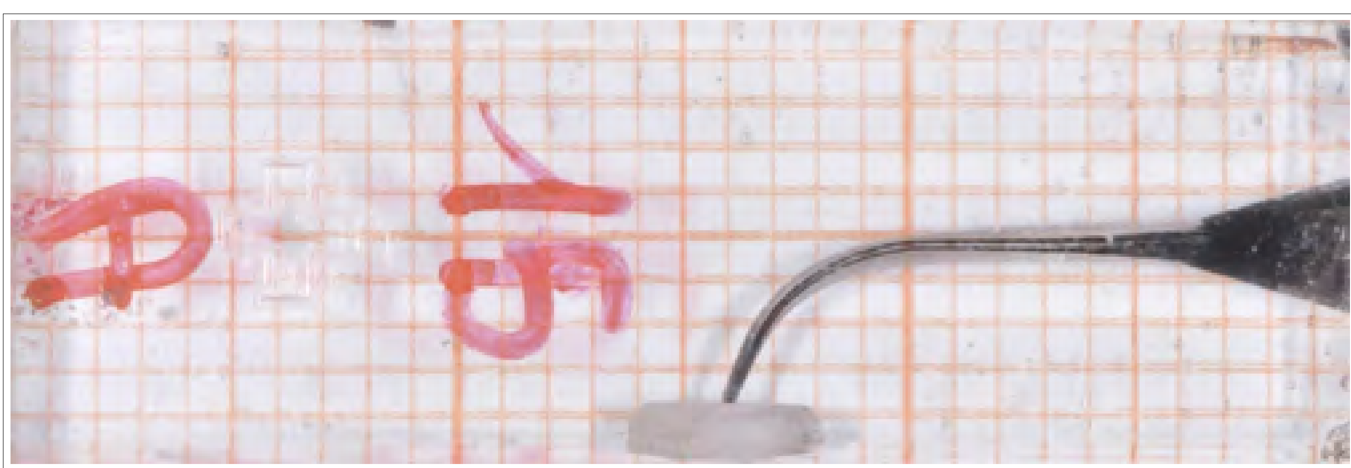

Table 2
Distribution of apical zip within the six groups

Apical zip	No	Yes	Total
Group 0	4	1	5
Group 1	3	0	3
Group 2	10	2	12
Group 3	14	0	14
Group 4	10	3	13
Group 5	9	0	9
Total	**50**	**6**	**56**

Group 0: Control (inexpert); Group 1: Control (expert); Group 2: Protaper Gold™ (inexpert); Group 3: Protaper Gold™ (expert); Group 4: WaveOne Gold™ (inexpert); Group 5: WaveOne Gold™ (expert).

Table 3
Distribution of ledges within the six groups

Ledges	No	Yes	Total
Group 0	11	3	14
Group 1	9	6	15
Group 2	13	0	13
Group 3	15	0	15
Group 4	13	1	14
Group 5	11	4	15
Total	**72**	**14**	**86**

Group 0: Control (inexpert); Group 1: Control (expert); Group 2: Protaper Gold™ (inexpert); Group 3: Protaper Gold™ (expert); Group 4: WaveOne Gold™ (inexpert); Group 5: WaveOne Gold™ (expert).

Table 4
Distribution of perforations within the six groups

Perforations	No	Yes	Total
Group 0	8	6	14
Group 1	11	4	15
Group 2	12	1	13
Group 3	15	0	15
Group 4	14	0	14
Group 5	15	0	15
Total	**75**	**11**	**86**

Group 0: Control (inexpert); Group 1: Control (expert); Group 2: Protaper Gold™ (inexpert); Group 3: Protaper Gold™ (expert); Group 4: Wave One Gold™ (inexpert); Group 5: WaveOne.

canal, for each of the nine millimeters (17). The study assessed three secondary outcomes: the presence of ledges, perforations and apical zips. For those parameters the measurements were made by dichotomous indexes (0=absent, 1=present).

Statistical analysis
The statistical analysis was performed by an operator who was unaware of group allocation. Descriptive statistics was provided, for continuous normally-distributed variables (centering and dentin removal), by means of mean values and relative standard deviations. Categorical variables were presented by frequency distributions. The comparison between groups was performed using Student's t-test. The level of significance was $p=0.05$.

Results

A total of 86 resin blocks were analyzed, while 4 were excluded due to problems during the superimposition of the images.

Presence of apical zip
The number of apical zips is summarized up in Table 2. Apical zips occurred 6 times of 56 considered, all in the undergraduate groups. Resin blocks in which ledges or perforations prevented a correct evaluation of the apex were excluded from the analysis.

Presence of ledges and perforations
Stainless-steel K-File showed a higher tendency to produce perforations compared to other groups (Table 3).
The distribution of ledges was uniform along the groups, except for the Protaper Gold™ groups in which none occurred (Table 4).

Amount of resin removed
The measurements of the amount of resin removed are shown in Table 5. The undergraduate control group removed a significantly higher amount of resin compared to the expert control group, as confirmed in literature (14).

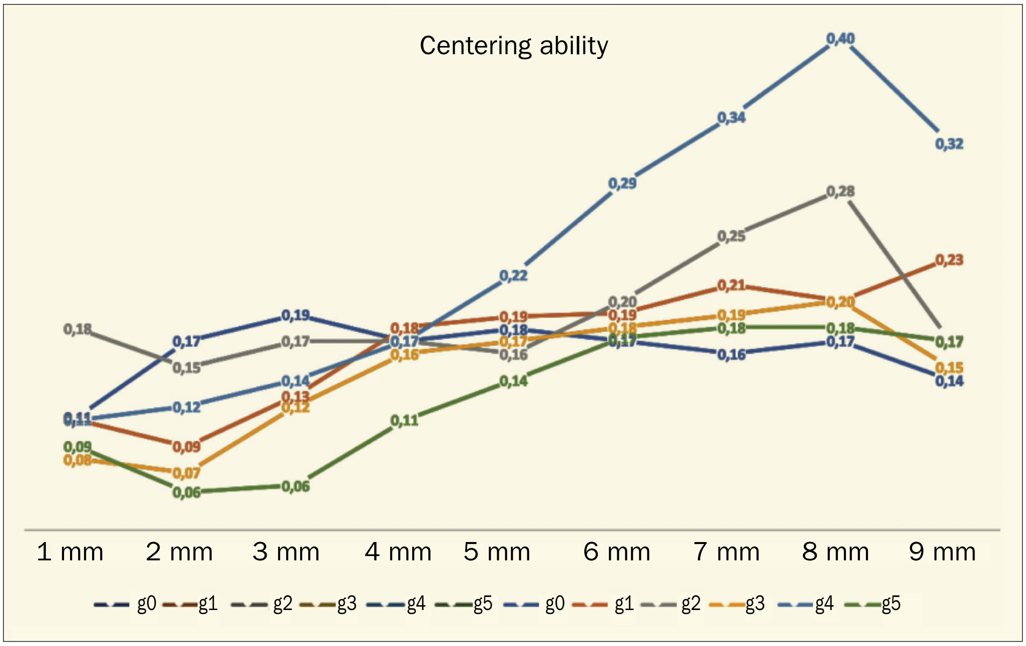

Figure 2
Centering ability within the six groups. Group 0: Control (inexpert); Group 1: Control (expert); Group 2: Protaper Gold™ (inexpert); Group 3: Protaper Gold™ (expert); Group 4: WaveOne Gold™ (inexpert); Group 5: Wave One Gold™ (expert).

The most significant data concerns the difference within the WaveOne Gold Groups™; a significantly higher amount of resin was, in fact, removed by inexpert operators. No statistically significant differences were found in the other groups.

Centering ability
The results are shown in Table 6. The most significant difference was found within the WaveOne Gold™ groups, in which inexpert operators removed more material than others; on the contrary, in the Protaper Gold™ expert group a higher amount of resin was removed, especially in the apical third. No differences were found in the other groups (Figure 2).

Discussion

Nowadays there's a lively debate over the best shaping system to use, in particular regarding the choice between continuous and reciprocating motions. The ideal shaping technique should guarantee an equally distributed dentine removal along the canals, anyway the presence of curvatures often compromises the result (8).
The introduction of the Ni-Ti alloy certainly contributed to the improvement of the

Table 5
Dentine removal

	1 mm	2 mm	3 mm	4 mm	5 mm	6 mm	7 mm	8 mm	9 mm
Group 0	0.28	0.3	0.36	0.35	0.35	0.37	0.4	0.45	0.46
Group 1	0.191	0.223	0.212	0.192	0.189	0.19	0.212	0.211	0.253
Group 2	0.36	0.33	0.37	0.42	0.42	0.43	0.47	0.515	0.437
Group 3	0.31	0.33	0.32	0.37	0.39	0.4	0.42	0.45	0.4
Group 4	0.42	0.36	0.39	0.44	0.44	0.46	0.51	0.53	0.45
Group 5	0.249	0.259	0.265	0.316	0.338	0.37	0.386	0.422	0.394

Amount of resin removed within the six groups. Group 0: Control (inexpert); Group 1: Control (expert); Group 2: Protaper Gold™ (inexpert); Group 3: Protaper Gold™ (expert); Group 4: Wave One Gold™ (inexpert); Group 5: Wave One Gold™ (expert).

Table 6
Centering ability

	1 mm	2 mm	3 mm	4 mm	5 mm	6 mm	7 mm	8 mm	9 mm
Group 0	0.11	0.17	0.19	0.17	0.18	0.17	0.16	0.17	0.14
Group 1	0.11	0.09	0.13	0.18	0.19	0.19	0.21	0.20	0.23
Group 2	0.18	0.15	0.17	0.17	0.16	0.20	0.25	0.28	0.17
Group 3	0.08	0.07	0.12	0.16	0.17	0.18	0.19	0.20	0.15
Group 4	0.11	0.12	0.14	0.17	0.22	0,.29	0.34	0.40	0.32
Group 5	0.09	0.06	0.06	0.11	0.14	0.17	0.18	0.18	0.17

Centering ability within the six groups. Group 0: Control (inexpert); Group 1: Control (expert); Group 2: Protaper Gold™ (inexpert); Group 3: Protaper Gold™ (expert); Group 4: Wave One Gold™ (inexpert); Group 5: WaveOne Gold™ (expert).

shaping method thanks to its properties: the shape memory and the superelastic effect. These features allow the material to have a better spring back and a higher self-centering ability in the root canal (2). Moreover, the development of new alloys contributed to enhance the performances of the instruments considered.
In this study the rotary and reciprocating systems were compared on the basis of the operator's experience, and shaping was performed on resin blocks (Endo Training Bloc, Maillefer). It was demonstrated that, although they're biologically different from teeth, resin blocks represent a valid tool for in vitro evaluations, allowing standardization and the comparison of different shaping methods (12).
The analysis of the *perforations* shown a prevalence within the control groups, in particular distributed in the undergraduate category. This result confirms the tendency of the stainless-steel instrument to straighten the canal curvatures because of their intrinsic rigidity, as already assessed in literature (20). For the same reasons the number of *ledges* was higher in the Control groups, with 9 cases on a total of 14.
The *apical zip* is defined by the American Association of Endodontics as "an elliptical shape that may be formed in the apical foramen during preparation of a curved canal when a file extends through the apical foramen and subsequently transports that outer wall" (19); the totality of them occurred in the undergraduate groups, highlighting the difficulty an inexpert operator finds in respecting the working length.
The evaluation of the *amount of resin removed* showed no significant differences either between the Protaper Gold™ groups or in the comparison between different techniques in the same groups of experience, even though the undergraduate operators realized less conservative shapings (15). The only significant difference was found within the inexpert WaveOne Gold™ group, with a relevant higher amount of material removed; this can be justified by the particularity of the reciprocating movement, as well as the parameter of the centering ability. We can hypothesize that reciprocating instruments, in fact, work more while entering the canal, in the most coronal portion, in contrast to the rotary instruments which work more during their comeback. This leads to a higher difficulty to control the pressure to apply during the shaping, resulting in an over instrumentation by a non-expert operator.
Finally, the *centering ability* was more respected by experts in particular in the apical third. Moreover, the WaveOne Gold™ group gained the highest results in the last millimeters of the canal system (10, 7), probably due to its apical taper (=7), smaller than the one of Protaper Gold (=8); this feature influences the amount of material removed, determining the ability of the instrument of "self-centering" inside the canal. The scientific literature only

offers few studies and only about the previous generation of the instruments considered, among which the most similar is the one written by Troiano G. et al. (15); our results are in contrast with some of those, and we can assume this is probably due to the differences of the alloy. Anyway, the results of this study can only give partial information about the parameters considered; an enlargement of the sample size and to the evaluation of other studies could lead to a better and more complete comprehension of those events.

Conclusions

This study easily allows to determine how the operator's experience influences, in particular, the manual shaping.
Regarding the comparison between continuous and reciprocating systems, there were no significant differences in terms of quality of shaping, except for the parameter of the *centering ability* which resulted more respected by expert operators, especially in the apical third. As expected, undergraduate students realized fewer conservative preparations, but the difference results significant only in a few millimeters of the canal.
Among the secondary outcomes the influence of the experience wasn't significant, even though some differences were found between categories.
Anyway, the present study was realized to analyze the primary outcomes; it would be appropriate to elaborate an *ad hoc* protocol for the evaluation of the secondary outcomes to reach more accurate results.

Clinical Relevance

Both rotary and reciprocating systems provide a valid instrumentation standard, with no significant differences resulting from the operator's experience.

Conflict of Interest

None.

Acknowledgements

None.

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ORIGINAL ARTICLE

Molecular microbiological analysis of ProTaper Next, XP-Endo Shaper and Reciproc Blue systems in severely curved canals

ABSTRACT

Aim: *This study evaluated the effectiveness of the mechanical reduction of intracanal bacteria produced by the endodontic systems Reciproc Blue (VDW GmbH), XP-Endo Shaper (FKG Dentaire) and ProTaper Next (Dentsply Sirona Endodontics) in severely curved canals by means of a molecular microbiological analysis.*
Methodology: *A total of 42 severely curved mesiobuccal canals of human permanent mandibular molars were selected and prepared. Then, canals were contaminated with* Enterococcus faecalis *strains (ATCC 29212) by incubation during 21 days at 37 °C for formation of a mature biofilm. After that, contaminated specimens were randomly divided in 3 groups (n=14): ProTaper Next (G1), XP-Endo Shaper (G2) and Reciproc Blue (G3). Microbial samples were obtained before (S1) and after root canal preparation (S2). Analysis of intracanal* E. faecalis *reduction was performed using quantitative polymerase chain reaction (qPCR), and the difference between groups was analyzed by Kruskal-Wallis test. Significance level was set at p<0.05.*
Results: *All systems presented effective bacterial reduction (p<0.05), but still had bacterial growth. No significant difference between the evaluated file systems was demonstrated (p>0.05).*
Conclusions: *ProTaper Next, Reciproc Blue and XP-Endo Shaper presented similar mechanical reduction of intracanal bacteria. No file system was capable of rendering severely curved canals completely free from bacteria.*

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KEYWORDS *bacterial reduction, molecular analysis, real-time polymerase chain reaction, root canal therapy, severely curved canals*

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Introduction

Success in root canal therapy relies on the reduction of microorganisms and their by-products from the root canal system (1). The mechanical action of endodontic files underscores high levels of bacterial decrease (2, 3). However, endodontic instruments are frequently unable of reaching all root canal walls; thus the complete elimination of microorganisms is not achieved and the untouched areas may remain colonized (1, 4). Aiming to increase the treatment prognosis, endodontic instruments undergone a considerable evolution with progressive generations of NiTi files presenting modifications mainly as regards files design, manufacturing process, alloy processing and heat treatment (3, 5).

Culture analysis by means of counting colony-forming units is the most common method used in literature to evaluate the bacterial reduction produced by endodontic instruments (2, 4). However, this method presents limitations such as not considering low bacterial amounts and detecting exclusively viable and cultured microorganisms (1). Molecular microbiological methods, such as quantitative polymerase chain reaction (qPCR), are capable of overcoming these issues; but, to date, very few studies used molecular assays to analyze the effectiveness of mechanical reduction of intracanal bacteria produced by different files systems (6-9). Multi-files systems, such as ProTaper Next (Dentsply Sirona Endodontics, York, PA, USA), are broadly used in root canal therapy since wide evidence support these instruments (2, 3). Nevertheless, large interest towards single-file systems has emerged resulting from treatment optimization and a growing body of studies that also indicate their use (4, 5, 10). XP-Endo Shaper (FKG Dentaire, La Chaux-de-Fonds, Switzerland) is a newly developed snake-shaped single-file rotary system with high flexibility, which is expected to produce minimal stress on dentinal walls (4, 5). Moreover, Reciproc Blue (VDW GmbH, Munich, Germany), a single-file reciprocating system with a S-shaped cross-section, was also introduced (11). This instrument presents a new heating process that increase flexibility and resistance to cyclic fatigue (5, 10). Previous reports have shown some advantageous properties of Reciproc Blue as regards dentinal microcracks formation (5), cyclic fatigue resistance (12), removal of root canal filling materials and regaining apical patency (10). However, the capacity of these newly developed files in mechanical bacterial reduction, which is a relevant aspect for the treatment success, still scarce in literature. Therefore, the purpose of this study was to evaluate the mechanical reduction of intracanal bacteria produced by ProTaper Next, XP-Endo Shaper and Reciproc Blue systems in severely curved canals by means of the molecular microbiological analysis.

Materials and Methods

Specimens' selection and preparation

This study was approved by the University Ethics Committee (2.705.981). The mesiobuccal canal of 48 permanent human mandibular molars with complete root formation, extracted for periodontal reasons not related to this study, were selected. Initially, specimens were maintained in 4% sodium hypochlorite solution (NaOCl) during 2 hours and cleaned by periodontal curettes. Periapical radiographs were taken from each tooth in a buccolingual and a mesiodistal direction. This stage aimed to select only similar radiographic morphology specimens with one isolated mesiobuccal canal and severely curved (20-35°) mesial roots, according to Schneider's method (5, 13). Then, the crown of each tooth was sectioned near the cementoenamel junction, and the mesial roots were standardized to a length of 13 mm from the anatomic apex, using a diamond disc. The following features excluded teeth from this study: dental caries, previous root canal treatment, root resorption, root canal calcification, initial apical diameter larger than a size 15 K-file, dentinal crack or root fracture. The exclusion criteria were detected using periapical

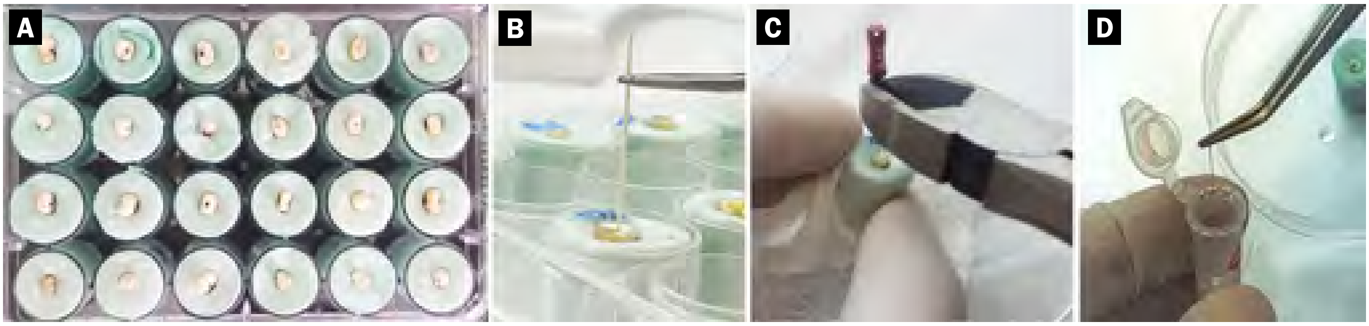

Figure 1
Representative images of bacterial samples collected for quantitative polymerase chain reaction (qPCR).
A) Individual models of each specimen.
B) Collection of root canal sample by a sterile paper point.
C) Sectioning of Hedstroem file with the aid of sterile tweezers for post root canal preparation quantification of bacterial contamination (S2).
D) Insertion of sectioned Hedstroem file into a sterile Eppendorf containing sterile saline solution.

radiographs, a clinical microscope (10x magnification), and a size 15 K-file (Dentsply Sirona).
Firstly, 5 mL of 2.5% NaOCl was used and root canals were explored with a size 10 K-file (Dentsply Sirona Endodontics, York, PA, USA). The working length was established in the total root length, 13 mm. Next, the intracanal contents were removed with a size 15 K-file (Dentsply Sirona), and specimens were newly irrigated with 5 ml of 2.5% NaOCl. The smear layer was removed using 3 mL of 17% EDTA during 3 minutes and a final irrigation with 5 mL of sterile saline solution was obtained. Root canals were dried with the aid of capillary tips (Ultradent products, South Jordan, UT, USA) and sterile paper points in the working length. The apical foramen of each specimen was sealed with Z 100 composite resin (3M, Saint Paul, MN, EUA) in order to prevent apical bacterial leakage and to create a closed-end channel, producing the vapor lock effect (6). Afterwards, the external apical surface was sealed with nail varnish.
In laminar flow chamber, individual models of each specimen were prepared using silicone impression material (Zetaplus, Zhermack, RO, Italy) with the aim to simplify root handling and shaping (Figure 1A). Teeth were prepared vertically up to the cervical third with the produced models, inserted into individual wells in 24-well cell culture plates (Costar, Washington DC, USA) and sterilized in an autoclave at 134 °C for 15 min.
Following this, aseptic conditions were confirmed after retaining teeth at 37° during 24 h, in which no bacterial growth was detected.

Root contamination with Enterococcus faecalis
Pure culture suspension of *Enterococcus faecalis* strains (ATCC 29212) was obtained by cultivation in brain heart infusion agar (BHI; Difco, Detroit, MD, USA) and then standardized on the McFarland nephelometric scale $30x10^8$ bacteria/mL. From the total sample, 6 specimens were selected as control, being filled solely with sterile BHI. At the same time, 42 roots were contaminated with 10 µl of *E. faecalis* suspension using sterile micropipette tips. Samples were incubated at 37 °C for 21 days in CO_2, during which the BHI was removed and replenished in 20 µL every 24 h (2, 14). This procedure was performed under laminar flow by means of sterile micropipettes.
Bacterial viability and intracanal sampling purity was checked every week by a selection of 2 random samples. For this, a sterile paper point #15 was maintained into the root space during 1 min, and immediately spread in BHI and incubated at 37 °C and 5% CO_2 for 24 hours. After growing, Gram staining and colony morphology on Columbia Agar with 5% Sheep Blood (CA-SB) (Becton Dickinson GmbH, Heidelberg, BW, Germany) was performed.

Initial quantification of bacterial contamination (S1)
After incubation period, root canals were rinsed with 1 mL sterile saline solution. Then, the initial sample (S1) was obtained by the sequential use of 2 sterile paper points #15 placed inside the root canal during 1 min each (Figure 1B). Following this, paper points were transferred with sterile tweezers to sterile polypropylene flasks containing 500 µL of sterile saline solution, and vortexed for 30 seconds.

Root canal preparation

The process of endodontic treatment and bacterial samples collection are represented in Figure 2. The 42 contaminated specimens were randomly assigned to the following groups, according to the file system used (n=14): ProTaper Next (G1), XP-Endo Shaper (G2) and Reciproc Blue (G3). The working length (WL) was established in the total root length.

- **G1 ProTaper Next (Dentsply Sirona).** Files were driven in continuous rotation in a crown-down technique using a gentle up-and-down motion. Firstly, the X1 file (17.04) was used for cervical and middle thirds shaping. This file was reused until reaching the WL. Following this, the X2 file (25.06) was used in the same manner as previously described.
- **G2 XP-Endo Shaper (FKG Dentaire).** XP-Endo Shaper file (30.01 as initial taper; however, during use, this instrument expands to a minimum taper of 0.04) was activated in continuous rotation at 800 rpm and 1.0 Ncm. Long and light up-and-down movements were applied inside root canals until reaching the cervical and middle thirds. After that, the file was reused in the same manner until reaching the WL.
- **G3 Reciproc Blue (VDW GmbH).** Reciproc Blue R 25 (25.08) was activated in reciprocating motion. The file was gently inserted with an up-and-down pecking motion that presented a maximum amplitude of 3 mm until the shaping of cervical and middle thirds was completed. After 3 up-and-down movements, when superior pressure was necessary to advance the instrument along the canal, the file was removed for cleaning of the flutes. Then, the file was reused in the same manner until reaching the WL.

Root canal preparation was performed by a single operator, an endodontist, who had been previously trained for each system. All files had single use. The protocol of each system followed manufacturer's instructions as previously explained. All systems were powered by a torque-controlled motor (X-Smart Plus; Dentsply Sirona Endodontics, York, PA, USA), set at the designated function according to the used system.

Root canals were irrigated during and after finishing instrumentation with a total volume of 10 mL sterile saline solution. Solution was delivered using a 24-G needle (Ultradent products, South Jordan, UT, USA) by means of a peristaltic pump (LAP-101-3; MS Tecnopon, Piracicaba, SP, Brazil) and a flow rate of 5 mL/min. For this, irrigation method was standardized. Initially, root canals were irrigated with 2.5 mL of saline solution. Next, after cervical and middle third shaping, 2.5 mL was used for root canal irrigation while instruments were cleaned with sterile gauzes. After completing preparation of specimens in the WL, a final irrigation with 5 mL was performed. During the use of all instruments, when a resistance requiring more apical pressure was detected, the file was removed and the flutes were cleaned.

Six non-contaminated root canals were instrumented for each file system (*n*=2) and used as negative controls.

Post root canal preparation quantification of bacterial contamination (S2)

Finishing endodontic instrumentation and irrigation, a post root canal preparation sample (S2) was obtained. For this, a size 25 sterile Hedstroem file (Dentsply Sirona) was introduced in the WL with circumferential strokes on all root canal surfaces (2). The file was then sectioned below the handle and, with the aid of sterile tweezers, dropped into a sterile Eppendorf containing 500 µL of sterile saline solution (Figure 1C, 1D). Following this, a sequential use of 2 sterile paper points #25 placed inside the root canal during 1 min each was performed. Collected paper points were stored in the same tube as the file.

Quantitative analysis by qPCR

Collected samples were vortexed for 30 s and subjected to DNA extraction for further qPCR analysis. DNA was extracted by the use of the QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer's directions. Aiming to maximize DNA extraction, a pre-incuba-

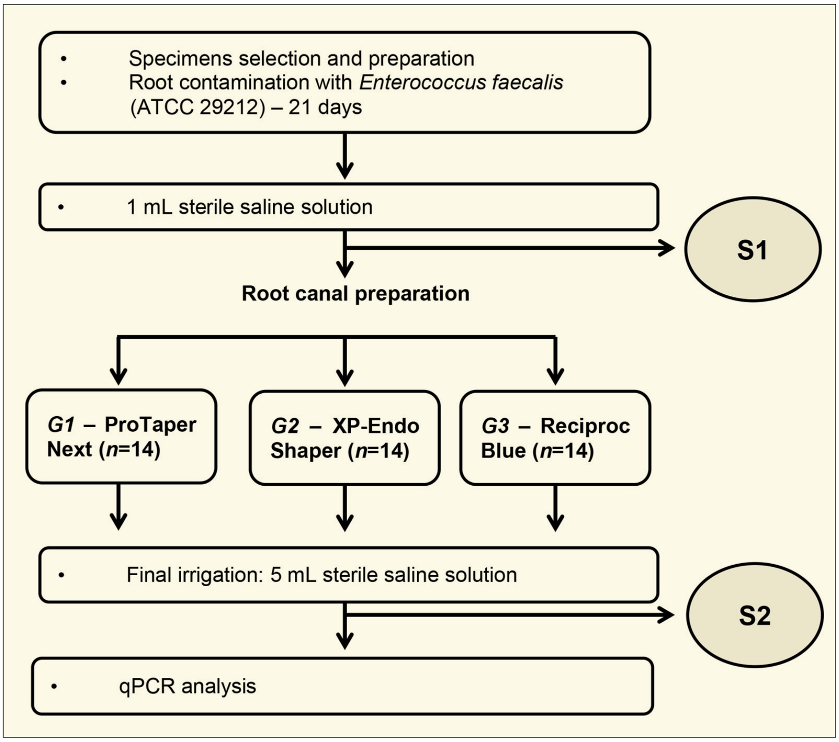

Figure 2
Flow chart of endodontic procedures and bacterial samples for quantitative polymerase chain reaction (qPCR) analysis. Root canal samples were taken before treatment (S1) and after root canal preparation according to each group (S2).

tion stage was included using lysozyme during 30 min. Obtained DNA extracts were stored, frozen at 20 °C, until qPCR analysis.
Afterwards, *E. faecalis* cells levels were quantified in root specimens by a 16S rRNA gene-based qPCR, using Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) on an ABI 7500 real-time PCR instrument (Applied Biosystems). A total reaction volume of 20 µL was applied. Species-specific primers for *E. faecalis*, qPCR conditions, standard curve construction, controls, and data analyses were performed as previously described (6, 10). Primers in a concentration of 0.5 µmol/L each and DNA extract volume of 2 µL were added to the PCR master mix in MicroAmp Optical 96-well reaction plates. The conditions for the qPCR amplification cycling were: 95 ºC for 10 min; 40 repeats of the following stages: 95 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min. Double-stranded DNA product was measured at 78 °C. At each cycle, accumulation of PCR products was verified by monitoring the increase in fluorescence of the reporter dye (dsDNA-binding SYBR Green).

All measurements were performed in duplicate for samples and triplicate for standards. In order to detect and exclude any possible contamination or carryover, triplicates of appropriate negative controls containing no template DNA were subjected to the same procedures. After amplification, melting curve analysis of PCR products was performed to determine the specificity of the amplified products.
Melting curve was obtained from 60 to 95 °C, with continuous fluorescence measurements taken at every 1% increase in temperature. Data acquisition and analysis were performed using the abi 7500 software v2.0.4 (Applied Biosystems).
E. faecalis cell counts were accomplished based on obtained standard curves. *E. faecalis* ATCC 29212 was used to create a 10-log-fold standard curve for direct bacterial quantification. DNA was isolated from a fresh pure culture of this strain using the QIAamp DNA Mini Kit (Qiagen) and quantified using a spectrophotometer (BioPhotometer, Eppendorf, Hamburg, Germany). Knowing the genome size of *E. faecalis* (3.2 Mb, http://www.cbs.dtu.dk/services/GenomeAtlas-3.0/) and the average molecular weight of one base pair (660 Da), the measured DNA value could then be converted into target genomic copy levels per microlitre using the formula

$$m=n\,[1\ \text{mole}/6\cdot10^{23}\ (\text{pb})]\,[660\ (\text{g})/\text{mole}] = n\,[1.096\cdot10^{-21}(\text{g/bp})]$$

where "m" is the genomic mass of a single cell and n the genome size. Genome copy levels were considered as numerically equivalent to bacterial cell levels. The standards were then 10-fold diluted from 10^7 to 10^2 cells in TE buffer and used for standard curve construction (6).

Statistical analysis
Obtained values were transformed to log10 and statistically analyzed using SPSS 23.0 (SPSS Inc., Chicago, IL, USA). Differences in bacterial counts before (S1) and after treatment (S2) were analyzed in each group by the Wilcoxon test. Comparisons between groups were performed by

Table 1

Intracanal E. faecalis quantification [Mean (standard deviation - SD)] before (S1) and after root canal preparation (S2) and total bacterial percentage reduction for qPCR analysis, observed in all groups

Groups	Sample		p**	Total bacterial reduction
	S1	S2		
G1 ProTaper Next	$5.96 x 10^5$ ($1.13 x 10^6$)[a]	1.52x103 ($1.15 x 10^3$)[b]	p=0.003	$8.02 x 10^5$ ($1.36 x 10^6$)[A]
G2 XP-Endo Shaper	$8.26 x 10^5$ ($1.02 x 10^6$)[a]	5.92x104 (1.68x104)[b]	p=0.001	$4.42 x 10^5$ ($6.19 x 10^5$)[A]
G3 Reciproc Blue	$9.36 x 10^5$ ($7.06 x 10^5$)[a]	8.16x103 (1.23x104)[b]	p=0.009	6.54x105 ($7.81 x 10^5$)[A]
p*	-	-	-	p=0.176

p: comparison between groups. Different uppercase letters indicate statistically different values ($p<0.05$) between groups.*

*p**: comparison between S1 and S2 within each group. Different lowercase letters indicate statistically different values ($p<0.05$) between S1 and S2 samples.*

Kruskal-Wallis test. Significance level for all analyses was p<0.05.

Results

Tables 1 reveals the bacterial percentage reduction qPCR analysis before (S1) and after root canal preparation (S2), observed in all groups. All endodontic systems presented effective bacterial reduction. However, despite that bacterial levels were reduced after preparation with ProTaper Next (p=0.003), XP-Endo Shaper (p=0.001) and Reciproc Blue (p=0.009), all groups still had bacterial growth. Protaper Next was the most effective system, whereas preparation with XP-Endo Shaper showed the highest post-preparation bacterial levels. Nevertheless, no significant difference between the evaluated groups was demonstrated (p=0.176).

Discussion

In the present study, it was revealed similar bacterial decontamination values promoted by ProTaper Next, XP-Endo Shaper and Reciproc Blue systems preparation. Therefore, despite presenting considerable differences (e.g. cross-sectional design, tapers, kinematics, manufacturing process, alloy processing and heat treatment), all systems were individually effective in mechanical preparation of severely curved root canals. In addition to that, although all file systems showed efficient decrease of E. faecalis counts [ProTaper Next (p=0.003), XP-Endo Shaper (p=0.001) and Reciproc Blue (p=0.009)], which can be translated into adequate mechanical action of instruments in dentinal walls, none of them was able to render severely curved mesiobuccal canals of mandibular molars completely free from bacteria. This finding is in accordance with previous researches (1, 2, 4, 15) and highlights the need for the development of new instruments capable of increasing the cleaning of root canal complexities and irregularities.

The comparison in bacterial reduction of ProTaper Next, XP-Endo Shaper, and Reciproc Blue in severely curved canals had not been ranked in literature yet. Nevertheless, this result consists with the findings of recent studies that concluded that Reciproc Blue and XP-endo Shaper instrumentation presents no differences with respect to the bacterial reduction in oval-shaped canals (9, 16). Also, the disinfecting abilities of Reciproc Blue and XP-endo Shaper also showed similar results in an association between micro-computed tomographic and histobacteriologic approaches (11).

ProTaper Next is a multi-file system fabricated with a NiTi M-Wire alloy that also presents a rectangular cross-section design with an increasing and decreasing tapering. This configuration permits to mold root canals asymmetrically by a continuous asymmetrical rotational kinematics, similar to snake movements, which improves the modeling effectiveness of root canals (17). In XP-Endo Shaper system, the Max-Wire alloy is used so that files can achieve greater flexibility and resistance to cyclic fatigue. In addition to that, this file has a reinforcing tip (Booster Tip) that allows to start shaping the root canals with an initial diameter smaller than the original diameter (5). According to the manufacturer's instructions, at 37 °C, the instrument is capable of expanding from an initial taper of 30/0.01 to a final canal preparation of a minimum of 30/0.04, adapting to the morphology irregularities of the root canal system. Lastly, Reciproc Blue system presents a S-shaped cross-section that allows deeper cutting and favors the removal of smear layer and debris (12). Its NiTi wire processing method uses a visible layer of titanium oxide that results in a distinct blue color that changes its molecular structure to generate greater flexibility and resistance to cyclic fatigue (10). The methodology applied in the current study presents some features that should be addressed. The intrinsic heterogeneity of root canals morphology between specimens is largely known as a notorious biological bias. It is important to emphasize, however, that efforts by radiological analysis and anatomy classification were undertaken to ensure a reliable comparison of groups. This stage reduced anatomical biases and allowed to increase the internal validity of this study (5, 6, 15). Furthermore, severely curved mesiobuccal canals from mandibular molars were selected for this study due to the considerable challenge that they represent for proper cleaning and disinfection (18, 19). The high prevalence of isthmus in mesial root canals of mandibular molars should also be highlighted (20).

The number of bacteria in an endodontic infection is restricted, predominating facultative or strictly anaerobic microorganisms (21). For this study, only one bacteria specie, *E. faecalis*, was used. This isolated standard strain was selected based on its survival characteristics associated with its prevalence in cases of endodontic failure, and for being widely used in previous studies with similar aim (4, 6, 21, 22).

In contrast to bacterial culture-dependent analysis, molecular assays such as qPCR present high sensitivity, since they allow the amplification of bacterial DNA in low amounts (1, 6). Also, qPCR method presents the ability to detect bacteria in their stationary stage (1). Under stress, the resistant bacteria selected for this study, *E. faecalis*, is capable of entering into stationary phase; being viable but undetectable in conventional CFU counts (1, 23). To date, a limited number of works used qPCR for the evaluation of mechanical bacterial reduction of root preparation methods (6-8).

Finally, the limitations of this study should also be pointed. Considering that only mesiobuccal canals were used in this study and the presence, size and volume of isthmus were not considered, this anatomy can influence the results in a certain way. Isthmus represents a real system with connections between roots canal (20), what may reflect in the remaining contamination of each group. Also, aiming to isolate the action of instruments from the chemical action of irrigating solutions, solely sterile saline solution was used during root canal preparation (2, 4, 22). This permitted the observation of isolated mechanical action of endodontic files, but did not reflect the clinical conditions of endodontic treatment. Further studies could use using molecular microbiological analysis for investigating the bacterial decontamination results of other endodontic systems and/or using different irrigating solutions.

Conclusions

ProTaper Next, Reciproc Blue and XP-Endo Shaper presented similar mechanical reduction of intracanal bacteria. No file system was capable of rendering severely curved canals completely free from bacteria.

Clinical Relevance

This study evaluated by a molecular microbiological analysis for the first time the mechanical reduction of intracanal bacteria produced by ProTaper Next, XP-Endo Shaper and Reciproc Blue in severely curved canals. Despite considerable differences, endodontic systems demonstrated similar results.

Conflict of Interest

The authors affirm that this paper has been submitted solely to Giornale Italiano di Endodonzia and it is not published, in press, or submitted elsewhere. The authors deny any other conflict of interest.

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ORIGINAL ARTICLE

Surface tension and wetting ability comparison of sodium hypochlorite and ethylenediaminetetraacetic acid with and without surfactants

ABSTRACT

Aim: *To evaluate the surface tension and wetting ability (contact angle) of endodontic irrigants as 5.25% sodium hypochlorite (NaOCl) and 17% ethylenediaminetetraacetic acid (EDTA) with and without addition of surfactants.*
Methodology: *Eighty halves of single-root teeth were randomly distributed into five groups (n=16) to investigate surface tension and wetting ability of: 5.25% NaOCl; 5.25% NaOCl containing surface-active agents (Hypoclean); 17% EDTA; 17% EDTA with surfactants (EDTA Plus) and freshly obtained distilled water MilliQ as the control group. The surface tension was calculated by the "pendant drop method" and the wettability of the surface by the "sessile drop method". All measurements were taken at room temperature (20 °C). Data were analyzed by one-way analysis of variance (ANOVA).*
Results: *The highest surface tension and the least wetting ability were observed for distilled water and 5.25% sodium hypochlorite (p>0.05). EDTA with or without surfactants and Hypoclean showed a significant lower surface tension and a higher wetting ability than distilled water at room temperature (p<0.001). The addition of surfactants reduced the surface tension and the contact angle of sodium hypochlorite (as in Hypoclean, p<0.001), but not those of EDTA (as in EDTA Plus, p>0.05).*
Conclusions: *Addition of surfactant agents reduces the surface tension and increases the wetting ability of the sodium hypochlorite; surfactants did not affect surface tension and wetting ability of EDTA.*

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Introduction

Success and prognosis of root canal therapy have both been shown to be dependent on many factors, including the quality of instrumentation and irrigation (1). Mechanical debridement failure may occur due to residual organic tissue, not completely removed from the root canal walls. Therefore, instruments and chemical irrigants are used in conjunction to achieve endodontic debridement especially in cases of teeth presenting complex anatomy in which the morphological irregularities are commonly untouched by instrumentation (1, 2).
The irrigation effectiveness depends on the working mechanisms of the irrigants and the ability to bring the irrigants in contact with microorganism and tissue debris in the root canal (1, 3). The intimacy of this contact is associated to the wettability of the dentin surface where the drop of irrigant is applied. The term "wetting ability" refers to the ability of a liquid to wet a solid surface while the term "wettability" is defined as the ability of the surface of a material to be wet in a uniform and stable way by a liquid substance (4, 5). The wetting ability of a solution influences its penetration ability into the main and lateral canals as well as into the dentinal tubules. It strictly correlated to its surface tension (6, 7) which could be considered as "the force between molecules which produces a tendency for the surface area of a liquid to decrease" (8), limiting the capacity of a liquid solution to penetrate into a capillary hole. Thus, a low surface tension and a high wetting ability represent ideal properties for an endodontic irrigant (8, 9).
The sequential protocol for irrigation includes the use of sodium hypochlorite (NaOCl) during mechanical preparation to dissolve the organic matter and kill microorganisms followed by a strong chelating agent like the ethylenediaminetetraacetic acid (EDTA) solution to remove the inorganic components of the smear layer and leave an adequate substrate for optimal efficacy of the final irrigant (9-12). However, both solutions have high surface tension, which could limit their penetration into dentinal tubules as well as into irregularities of the root canal system such as fins and isthmuses (9).
The effectiveness of an endodontic irrigant could be improved by reducing its surface tension and enhancing its wetting ability using surfactants (13) which act as detergents, wetting and foaming agents, emulsifiers and dispersants (6). Thus, surfactants improve wetting properties of irrigants by diffusing in water and adsorbing interfaces between air and water (dentinal fluid), reducing water's surface tension and increasing their wetting ability on surface. Moreover, surfactants should induce a greater protein solvent power in lateral canals and dentinal tubules due to their capacity to improve wetting ability (14).
Hypoclean (Ogna Lab Srl, Muggiò, Milan, Italy) is a commercial available 5.25% NaOCl solution added with two surface-active agents (1) which presents an optimized ability to kill bacteria compared to a pure 5.25% NaOCl solution and a greater penetration into inaccessible areas of the root canal system and dentinal tubules (15). EDTA Plus (Essential Dental Systems, S. Hackensack NJ, USA) is a 17% ethylenediaminetetraacetic acid sodium salt solution in water with surfactants, presenting, according to the manufacturer, an improved canal diffusion due to the reduced surface tension (16).
No study has examined the effectiveness of surface-active agents in reducing both surface tension and wetting ability of two common irrigants, as NaOCl and EDTA, at the most common concentration of 5.25% and 17%, respectively. Therefore, aim of this study was to evaluate the surface tension and wetting ability of sodium hypochlorite and ethylenediaminetetraacetic acid with and without surfactants. The null hypothesis tested was that surfactants did not affect the surface tension and wetting ability of 5.25% NaOCl and 17% EDTA solutions.

Materials and Methods

Four endodontic irrigant solutions were tested:

- 5.25% NaOCl (Niclor 5, Ogna Lab Srl, Muggiò, Milan, Italy);
- 5.25% NaOCl containing surface-active agents (Hypoclean, Ogna Lab Srl, Muggiò, Milan, Italy);
- 17% ethylenediaminetetraacetic acid (17% EDTA, Ogna Lab Srl, Muggiò, Milan, Italy);
- 17% ethylenediaminetetraacetic acid with surfactants (EDTA Plus, Essential Dental Systems, S. Hackensack NJ, USA).

Freshly obtained distilled water MilliQ (MilliQ 18 MOhm, Millipore Corporation, Billerica, MA, USA) was used in the control group.

The study is in accordance with principles of Declaration of Helsinki and it was approved by the Institutional Review Board. A pilot study was performed to establish the sample size calculation. The minimum sample size was fixed at 16 samples for each group (n=16) to ensure a test power of 0.80 (G*Power 3.1.9.2 software, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) with α=0.05.

Therefore, forty fully formed human single-root teeth extracted for periodontal or orthodontic reasons were assessed under 20 magnification using a dental operating microscope (OPMI pico Dental Microscope; Carl Zeiss, Oberkochen, Germany) to exclude those with open apices, resorptive defects, and longitudinal fractures. All included teeth were stored in a 0.2% thymol water solution at 6 °C and used within 30 days of extraction (17).

After cutting off the crown and the apical third of each tooth, the remaining root was longitudinally split using a low-speed saw (Isomet, Buhler, Ltd, Lake Bluff, NY, USA) with a diamond disc (Ø 125×0.35×12.7 mm at 330 °C) under cooled distilled water to prevent overheating. Eighty sectioned root halves were obtained. Each cut surface was polished using a series of abrasive papers (CarbiMet; Buehler, Lake Bluff, IL, USA) in the following sequence: 180/P180, 240/P280, 320/P400, 400/P800, and 600/P1200 to obtain a flat wide dentin surface (1, 18). Five root halves were discarded because were damaged during the longitudinally split face and were replaced with other samples. Thus, the eighty root halves were used as substrate for the wetting ability measurements and randomly divided (n=16) in 1 control group and 4 test groups on the basis of the tested liquid:

Group 1 (Control) = freshly produced MilliQ water (MilliQ, 18 MOhm)
Group 2 = 5.25% NaOCl (Niclor 5)
Group 3 = Hypoclean
Group 4 = 17% EDTA
Group 5 = EDTA Plus

All measurements of surface tension and wetting properties were carried out at 20 °C, in order to replicate the conditions of previous assays, as this value is often described as equivalent to "room temperature" (19).

The tested liquids were contained in glass vials which were opened immediately before the experiments.

Measurement of the surface tension

An optical method was performed to calculate the surface tension of endodontic irrigants, the so called "pendant drop method" (8), using the Phoenix 300 system (goniometer and software by SEO-Surface Electro Optics; Suwon City, Gyunggido, South Korea) following the manufacturer's instructions (19, 20-22).

The test liquid was automatically expelled (computer controlled) out of a syringe needle until it almost separated from the tip. Afterwards, the drop shapes were taken by a self-contained digital charge-coupled device camera just before the gravitational force exceeded the interfacial force that caused the drop release from the tip. The surface tension of every tested irrigants and fresh distilled water was automatically evaluated by the apparatus. Pure water was used for calibration before the assays (22).

The equations explaining the drop profile are obtained from the Young-Laplace equation. Using the selected plane method, the surface tension is rapidly derived. This method estimates the surface tension by measuring the equatorial diameter, d_e, and the diameter d_s of a pendant drop in a plane located at a distance d_e from the tip of the droplet. Surface tension (ST) can be mathematically determined by the following equation:

$$ST=(\Delta\varrho \cdot g \cdot d_e^{\,2})/H \qquad [1]$$

where $\Delta\varrho$ is the fluids density difference, g is the gravitational acceleration, and H is the correction factor. Distilled water was used for calibration prior to the assays. Sixteen measurements per test solution were made.

Measurement of wetting ability

The method used for the measurement of the angles of wettability is called "sessile drop" (4, 5); it consists in measuring the contact angle "θ" between a solid interface and the tangent to the profile of a drop.

Contact angles of the tested irrigants or distilled water (control) were measured by depositing 1l drops on the dentinal surface of root halves using a calibrate syringe. The drop images were captured by a webcam (Philips CamSuite 2.0, Milan, Italy) aligned to the eyepiece of an optical microscope which records video images and pictures of the system formed by the solid sample (root dentine) and drop (Figure 1). The eyepiece resolution was of 10× while the lens one could vary between 4× and 2×. The images were scanned in color with a resolution of 640×480 pixels and processed by a computer with an image editing software (Capture One Pro 7, PhaseOne, Cambrige, MA, USA) and appropriate software for the angles measurement (Image measurement, Klonk SmBA, Ringsted, Denmark) (23). Dental samples were dried with compress air before their use for wettability measurement. Sixteen measurements were carried out at room temperature (T=20 °C, UR=35%, P=1 atm) for every tested solution and distilled water, one for each root halve. The average was calculated; for each liquid one microliter was tested. The droplet of the liquid was distributed in thermal equilibrium on the horizontal surface of the samples. Contact angles values less than 90° indicate that the wettability of the surface is favorable (hydrophilic), while angles greater than 90° means that the wettability of the surface is unfavorable (hydrophobic) (Figure 2).

The contact angle θ (degree) was calculated from the height h (mm), the base diameter d (mm), the arctangent (arctg) and the arccosine ($\cos^{-1}$) of the deposited drop on the surface, according to the relations:

$$\theta=2 \text{ arctg } (2h/d) \qquad [2]$$

$$\text{or } \theta=90° + \cos^{-1} (4\,hd/4h^2+d^2) \qquad [3]$$

Equation [2] was used for θ<90 °C (hydrophilic), while equation [3] was used when θ>90 °C (hydrophobic).

Statistical analysis

After verifying the normality of the data through the Shapiro-Wilk test, the parametric one-way analysis of variance (ANOVA) followed by Tukey's post hoc was applied to identify significant differences among the irrigant solutions, using a statistical software (Prism 7.0; GraphPad Software, Inc, La Jolla, CA). The significance level was set at $p<0.05$.

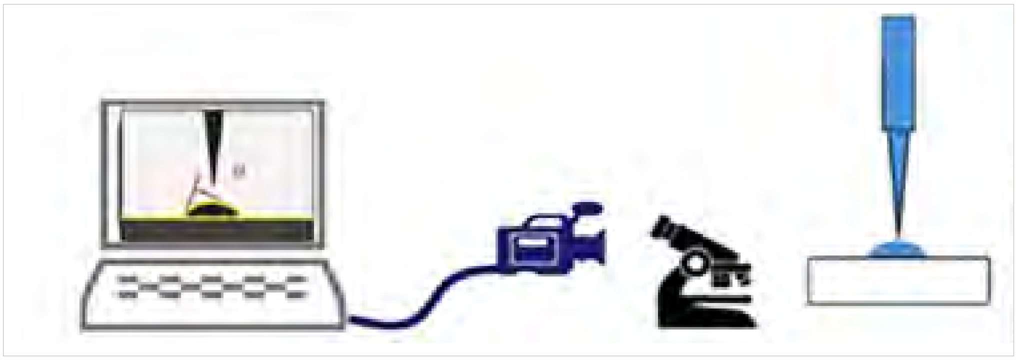

Figure 1
Setup used to record the wetting ability measurements: sketch of the experimental setup.

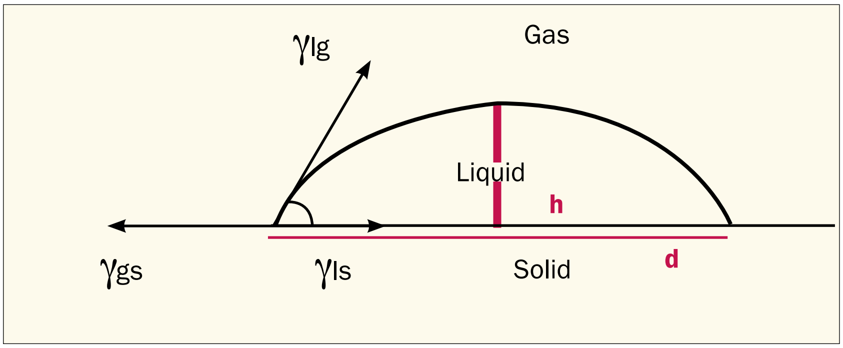

Figure 2
Sketch of the θ angle. This angle is an index of wetting ability of a liquid on a surface.

Results

Table 1 shows the mean, the standard deviation and the median values of surface tension and contact angle on dentin observed for the different irrigants and distilled water. Inferential analysis showed no significant difference between distilled water and sodium hypochlorite for the parameters analyzed (>0.05). EDTA with or without surfactants and Hypoclean showed a significant lower surface tension and contact angle (which means higher wetting ability) than distilled water and sodium hypochlorite ($p<0.001$).
The addition of surfactants agents to sodium hypochlorite, as in Hypoclean, reduced the surface tension and the contact angle significantly ($p<0.001$). Instead, EDTA Plus (17% EDTA with surfactants) not showed a significant reduction of its wetting ability and surface tension than 17% EDTA without surfactants ($p>0.05$).

Discussion

This study compared the superficial tension and the wetting ability (evaluated by means of the contact angle θ) of different endodontic irrigants with and without surfactant agents. On the basis of the present outcomes, the null hypothesis was partially rejected because the results showed that in sodium hypochlorite, the surface tension was reduced and the wetting ability was increased by the addition of surfactants, while these parameters did not significantly change in 17% modified EDTA (EDTA Plus). Even the purest water incurs in contamination on storage, consequently fresh MilliQ water was chosen as control liquid avoiding the interfacial adsorption of ubiquitous hydrocarbons from the atmosphere that could decrease its surface tension (24). Sodium hypochlorite and EDTA, as Niclor 5 and EDTA 17% respectively, were chosen because they are the most common irrigants used in endodontic clinical practice. More specifically, Hypoclean and EDTA Plus were selected because they have the same concentration of Niclor 5 and 17% EDTA respectively but they are added with surfactant agents. There are different surfactants

Table 1
Mean, standard deviation and median of surface tension (DINE/cm²) and contact angle (wetting ability) alues (grades) for tested irrigant solutions and distilled water

Group	Liquids tested	n	Surface tension			Contact angle (wetting ability)		
			Mean	*Standard Deviation*	*Median*	*Mean*	*Standard Deviation*	*Median*
1	MilliQ water	15	70.80^{a}	1.75	69.75	82.72^{a}	7.31	82.84
2	5.25% NaOCl	15	67.65^{a}	1.31	67.88	77.99^{a}	8.91	78.84
3	Hypoclean	15	27.60^{b}	1.81	27.89	57.31^{b}	7.53	61.64
4	EDTA 17%	15	30.57^{b}	1.01	30.13	67.32^{b}	5.43	66.12
5	EDTA Plus	15	32.94^{b}	1.69	33.47	65.95^{b}	5.78	65.94

Liquids with same superscript letter are not statistically significant (p>0.05).

that could be added to endodontic irrigant solutions: Hypoclean uses cetrimide (cationic surfactant) and polypropylen glycol (nonionic polymer of propylene glycol) in an unknown concentration (2) while the surface-active agent of EDTA Plus is not declared.

The methodology used to assess the superficial tension as well as the wetting ability of endodontic irrigants tested is currently accepted in the literature and straightforward to reproduce (5, 8). Several methods have been developed to measure surface tension such as the "ring method" of Du Noüy adopted in a recent study (18), the Wilhelmy plate, the capillary rise, the shape analysis of pendant drop, the maximum bubble pressure, the drop weight. However, when different methods are used, values are not necessarily comparable (22). The pendant drop method using a goniometer and the same apparatus have previously been employed and consequently validated in endodontic research (19-22).

In this study, the detected values of irrigants wetting ability were obtained on dentin in order to simulate the surface that takes contact with the irrigants in clinical practice. A possible limit of this methodology, however, is that the dentin surface was gridded and dry according to Stojicic et al. (25). As a consequence, the tested surfaces are not completely comparable to the dentin surfaces found in clinical practice. Moreover, dentin has a complex structure: it possesses roughness and dentinal tubules and could present chemical interactions with some of the tested liquids in vivo (1, 26). In addition, the results obtained could be affected by the type and concentrations of irrigants tested. Therefore, considering these limitations, the results must be applied in clinical practice with extreme caution.

According to our results, the NaOCl solution with surfactants tested (Hypoclean) showed a significant lower surface tension and higher wetting ability than sodium hypochlorite alone (Niclor 5), as previously reported (6, 25, 27). Regarding EDTA, the solution with surfactant tested (EDTA Plus) showed values of the wetting ability (contact angle's values) and superficial tension not significantly different from the 17% EDTA alone. Nevertheless, it is previously reported the ability of surfactants to reduce the surface tension and increase dentine wetting ability of 17% EDTA (2). These contrasting findings could be due to the possible differences in type and concentration of surface-active agents used in the solutions with surfactants tested in the different studies (27-30). Consequently, the present results showed that the surfactants reduce the surface tension and contact angle (wetting ability increased) of sodium hypochlorite, but not that one's of 17% EDTA. It is possible to hypothesize that this result is due to the different chemical composition of two solutions as well as to the different kind and/or concentration of surfactants agents employed.

Moreover, EDTA with or without surfactants and Hypoclean showed a significant lower surface tension and contact angle values than distilled water. These outcomes are in agreement with some of the previous studies (25, 28-30).

The penetration of irrigating solutions into the complex anatomy of the root canals and dentinal tubules could be increased by the addition of surfactants that reduce the surface tension and increase the wetting ability of irrigating solutions as sodium hypochlorite and EDTA (1, 24, 29). Improving wetting ability of an irrigant solution is clinically important because it could increase its solvent capability and consequently enhance its bactericidal ability through a better penetration into the non-instrumented areas of the root canal system (1, 6). Anyway, in literature, there is no common consensus on the higher dissolution capability and antibacterial action of the sodium hypochlorite with surfactants than the sodium hypochlorite without them. Some studies showed that the addition of surfactants did not significantly improve these capabilities (6, 21, 30) while other authors have shown a significant improvement (17, 18, 31-33). Interestingly, Estevez et al. (17) suggested that these contrasting results can be explained by several methodological differences regarding the irrigation protocol. More specifically, the greater irrigation

times and irrigant volumes could compensate for the effects associated to the lowered surface tension (17).
Due to their low surface tension and increased contact with dentinal walls, the new irrigants have the potential to penetrate more readily into instrumented areas of root canal system (34) as well as allow a more rapid exchange with fresh solution, enabling greater antimicrobial effectiveness and enhanced pulp tissue dissolution ability (29).
There is also disagreement on the better clinical efficacy of the EDTA with surfactants than that one without surface active agents (12, 27). These differences could be related to the different study designs, type of commercially available irrigant solution tested and kind and/or concentration of surface-active agent added to the irrigants (28). Moreover, some previous ex-vivo studies reported that a reduced chelating power was detected when EDTA was associated with a wetting agent (21, 35). Despite the chelating agents could differ for type and concentration and thus a generalization is not possible, extreme caution is necessary to clinically evaluate these in vitro results.
Further research into the chelating ability of different irrigating solutions used in combinations and/or with the incorporation of surfactants would be needed to take the advantage of maximum irrigant penetration into the uninstrumented areas of the root canal system with maximum chelating efficiency.

Conclusions

Sodium hypochlorite solution modified with surfactants showed a lower surface tension and a higher wetting ability than sodium hypochlorite alone and distilled water.
However, the addition of surfactants to EDTA (as in EDTA Plus) did not result in better wetting ability and lower surface tension compared with EDTA alone.
Further investigations into biological and physical experimental models should be performed to demonstrate higher penetration, protein solvent power and bacterial decontamination in instrumented areas of root canal system of endodontic irrigants with surfactants than that one's without surfactants.

Clinical Relevance

Improving wetting ability of an irrigant solution could be clinically relevant because it may increase its solvent capability and ability penetration into instrumented areas of root canal system.

Conflict of Interest

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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ORIGINAL ARTICLE

Influence of operator experience on vertical force during instrumentation using Neoniti rotary files

ABSTRACT

Aim: *This study aimed to measure the vertical force developed during canal instrumentation with Neoniti rotary files at predetermined torque in relation to the operator's experience.*
Methodology: *The research was performed on 60 human maxillary and mandibular incisors extracted for periodontal reasons. Each group of 20 teeth (10 maxillary and 10 mandibular incisors) was prepared by three different operators with different levels of experience in endodontics: a fresh dental school graduate, a postgraduate student training in endodontics and an endodontic specialist. Maxillary incisors represented the experimental model of wide root canals while mandibular incisors represented a model of narrow root canals. Root canals were prepared employing Neoniti files and TCM Endo unit at speed and torque recommended by the manufacturer. Vertical force measurement was performed utilizing device constructed for this purpose. Statistical analysis was accomplished using Mann-Whitney U test, Kruskal-Wallis test and Spearman's rank correlation.*
Results: *The amount of vertical force was significantly higher in narrow than in wide root canals (P=0.001). Comparison of vertical forces developed by three different operators demonstrated significant differences (P<0.001). Median vertical force developed by postgraduate student was significantly lower than in other two operators (both P<0.01). In postgraduate student, a significant positive correlation between number of shaped root canals and vertical force was demonstrated (ρ=0.490; P=0.003).*
Conclusions: *The postgraduate with limited experience in rotary instrumentation had on average lowest values of vertical force as opposed to other two operators. However, these values increased with the number of shaped root canals.*

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Introduction

Shaping of the root canal space is one of the most challenging issues of endodontic treatment, particularly in narrow and curved root canals. It ensures proper mechanical debridement, facilitates irrigation and provides a suitable shape for the placement of the root canal filling material. Over the past few decades, motor driven instrumentation systems have become a standard in root canal shaping due to flexibility and mechanical properties of the nickel- titanium (NiTi) files, decreased operating time in comparison to hand instruments and application simplicity (1). NiTi alloys enabled construction of superelastic instruments, a feature that ensures flexibility and facilitates preparation of curved canals. In recent years, new manufacturing processes and alloys have been introduced to improve conventional NiTi systems (2). The Neoniti rotary files (Neolix Neoniti, Evron, France) are manufactured through the Wire-Electric Discharge Machining (wire-EDM). According to the manufacturer, this specific process of manufacturing is responsible for high flexibility and surface hardness which together with a rectangular cross-section ensure cutting efficacy and optimal flexibility (3).

However, despite improvements in the mechanical properties, NiTi instruments are still prone to fracture if certain conditions are met. Among many factors associated with failure of the NiTi instruments are their construction design, cyclic fatigue and torsional stress, rotational speed, curvature angle of the canal and operator's experience (4). The occurrence of cyclic fatigue and torsional stress in NiTi instruments is directly influenced by vertical force and torque and may result in instrument separation (5-8). Methods used to reduce instrument separation include use of torque-controlled electrical motors that identify when torsional limits are reached, modification of the instruments' cross-sectional geometry to reduce contact areas and increase cutting efficiency and introduction of reciprocating files with increased cyclic fatigue resistance (2, 9, 10). The experience of the operator has been identified as an important factor in the success of the endodontic therapy. Differences between the experienced and inexperienced operator are observed in the number of procedural errors, fractured instruments and time required to prepare a root canal. Several studies observed a statistically higher occurrence of canal transportation in the group of inexperienced operators (11, 12). Munoz et al (11) reported more frequent instrument separation among the inexperienced operators in comparison to the experts. However, studies regarding operator's experience and instrument separation are not unanimous in their finding. A study performed by Generali et al (13) demonstrated that operator's experience was not significant in relation to instrument fracture. Since it has been advocated that vertical force is one of the factors that can directly influence instrument separation, it is necessary to explore if a particular motor driven instrumentation system can be used by beginner and experienced operator with the same degree of safety.

The aim of this study was to measure the vertical force developed during canal instrumentation with Neoniti rotary files at predetermined torque in relation to the operator's experience. The null hypothesis tested was that operator's experience does not affect the amount of the vertical force developed during canal shaping.

Materials and Methods

This study received approval from the Institutional Ethical Committee (No.818101218). The research was performed on 60 human maxillary and mandibular incisors extracted for periodontal reasons. Following extraction, the teeth were stored in 0.1% thymol solution up to 2 months to prevent bacterial growth. Two initial radiographs of each tooth were taken from bucco-lingual and mesio-distal projection to assess the shape and cross-sectional diameter of the canals as well as to detect their number and possible obstructions. The radiographs were obtained utilizing X-ray unit (Trophy Elitys, Trophy Radiologie, Marne-la-Vallee, France) and intraoral sensor (One, Owandy Radiology, Roslyn, NY, USA). Canals with morphological abnormalities, obstructions, curvatures or multiple canals were excluded from the study. Only incisors with a single straight root canal, oval cross-section in the coronal and middle third and round cross-section in the apical third were included in the study.

Access cavity was prepared using the Cavity Access Set (Dentsply Sirona, Ballaigues, Switzerland). The root canal length was determined utilizing a size 10 K-file (Dentsply-Sirona, Ballaigues, Switzerland) in mandibular and 15 K-files (Dentsply-Sirona, Ballaigues, Switzerland) in maxillary incisors group, depending on the initial apical diameter of the canal. Files with rubber stops were inserted into the canal until their tips became just visible through the apical foramen. The position of the file in each canal was confirmed by digital radiographs. To standardize root lengths, all teeth were shortened coronally to a length of 19 mm. The working length of 18 mm was set by subtracting 1mm from the adjusted length of the root.

The apical diameter of all root canals was standardized by manual instrumentation up to a size 20 K file (Dentsply-Sirona, Ballaigues, Switzerland) in mandibular incisors and size 30 K-file (Dentsply-Sirona, Ballaigues, Switzerland) in maxillary incisors. During manual preparation 3 ml of 3% sodium hypochlorite (NaOCl) per canal was used for irrigation. Final irrigation was performed with 2 mL of 17 % ethylenediaminetetraacetic acid (EDTA; Vista Dental Products, Racine, WI, USA) for 1 min followed by irrigation with 6 mL of 3% NaOCl for 3 min and 2 mL sterile saline solution for 1 min (14).

The prepared teeth were randomly divided into three experimental groups. Each group consisted of 10 maxillary and 10 mandibular incisors. Maxillary incisors represented the experimental model of wide root canals while mandibular incisors represented a model of narrow root canals. The cervical portion of teeth was immersed into auto acrylate mold (Polirepar S, Polident, Volčja Draga, Slovenia) to secure specimen placement into the measuring device (Figure 1). Canal orifices were shaped and enlarged with Neoniti C1 orifice opener nº25/.12 (Neolix Neoniti, Evron, France). The narrow root canals were prepared employing Neoniti A1 nº25/.06 shaping files (Neolix Neoniti, Evron, France) with new files used for every specimen. Wide root canals were instrumented utilizing Neoniti A1 nº40/.04 (Neolix Neoniti, Evron, France) with files discarded after each specimen preparation. Before use, each rotary instrument was lubricated with RC Prep (Well-Prep,Vericom Co, Anyang, Korea), whilst a rinse with 3 ml of 3% NaOCl solution was made after the use of each instrument. Apical patency was obtained using a size 10 K-file. The rotational speed was preset at 300 rpm and the shaping files were introduced in pecking and upward circumferential brushing motions from the bottom up according to the manufacturer's instructions. The preparations were performed utilizing the TCM Endo unit (Nouvag, Goldenstein Switzerland) at a preset torque value of 1.5 Ncm according to the manufacturer's recommendations.

Each group of 20 teeth was prepared by different operator depending on their experience. Group 1 was shaped by beginner, a fresh dental school graduate. Group 2

Figure 1
Tooth immersed into auto acrylate mold and placed into the measuring device.

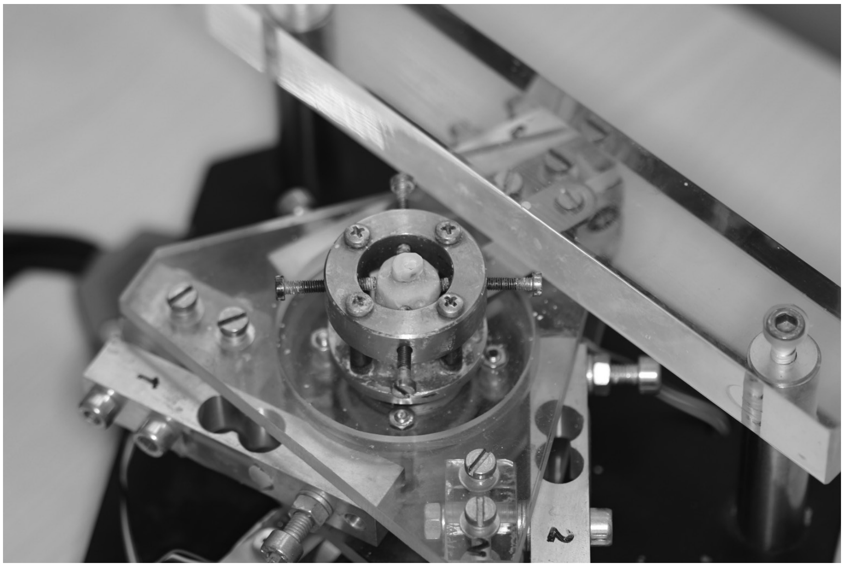
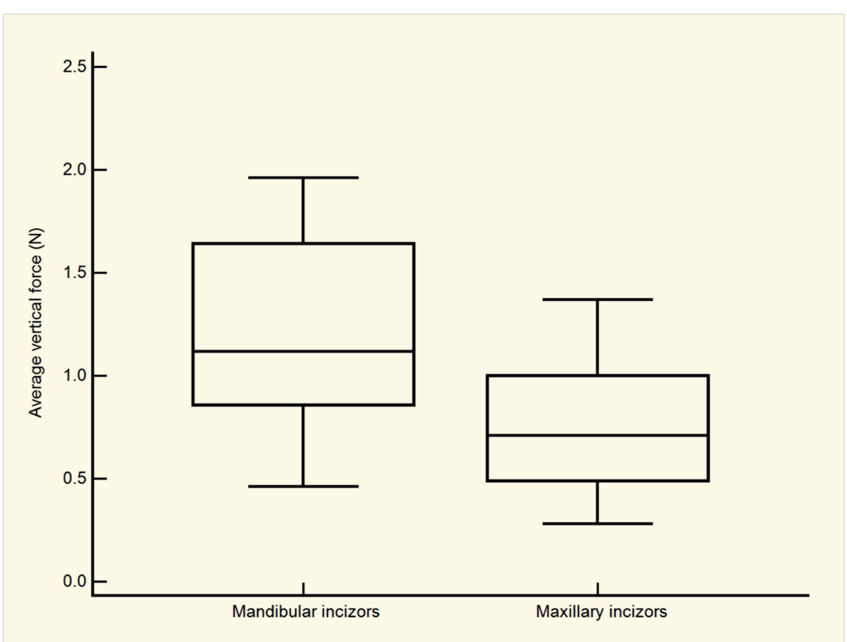


Figure 2
Vertical force measuring device.

was prepared by a postgraduate student training in endodontology. Group 3 was prepared by an experienced operator, an endodontic specialist with more than 10 years of experience. None of the operators had previous experience using Neoniti rotary files. They had only theoretical knowledge about the system.

Vertical force measuring device
The constructed device had a platform for tooth placement, which was wedged using a vertical shaft into a membrane sensor with eight gauges (1-Ly11-10/120, Hottinger, Baldwin, Messtechnik, GmbH, Darmstadt, Germany) to measure vertical forces (Figure 2). The construction allowed free passage and rotation of the vertical shaft in a radial bearing while being wedged into the membrane sensor trough axial-radial bearing.
Prior to instrumentation, device calibration was performed using 50 g and 100 g standardized weights. Measured data were recorded with a two-channel oscilloscope (ADC-216, Pico Technology Limited, St Neots, UK) every 0.1 s. They were presented in a form of diagrams and tables on the computer monitor and entered into Microsoft. Excel data sheet. The recorded variables were expressed in newton (N) units for vertical force variable. The measuring device was described in a previously published paper (15).

Figure 3
Difference in vertical force regarding tooth type.



Statistical analysis
Statistical analysis was performed using the commercial software MedCalc 14.8.1 (MedCalc Software bvba, Belgium) at level of statistical significance $P<0.05$. Kolmogorov-Smirnov test was used to test data for normal distribution. Since distribution of the data was not normal, as a measure of central tendency and dispersion, a median and interquartile range values were used. Testing the differences between the groups was accomplished using Mann-Whitney U test and Kruskal-Wallis test. Given the large number of tests Bonferroni correction concerning statistical significance was applied in the post hoc analysis. To explore relationship between number of shaped root canals and vertical force a Spearman's rank correlation was used.

Results

The median vertical force measured during instrumentation was 1.12 N (0.86-1.64) in mandibular incisors and 0.71 N (0.49-1.00) in maxillary incisors. The amount of vertical force was significantly higher in mandibular incisors ($P=0.001$, Figure 3). Significant differences in vertical forces developed by three operators were found

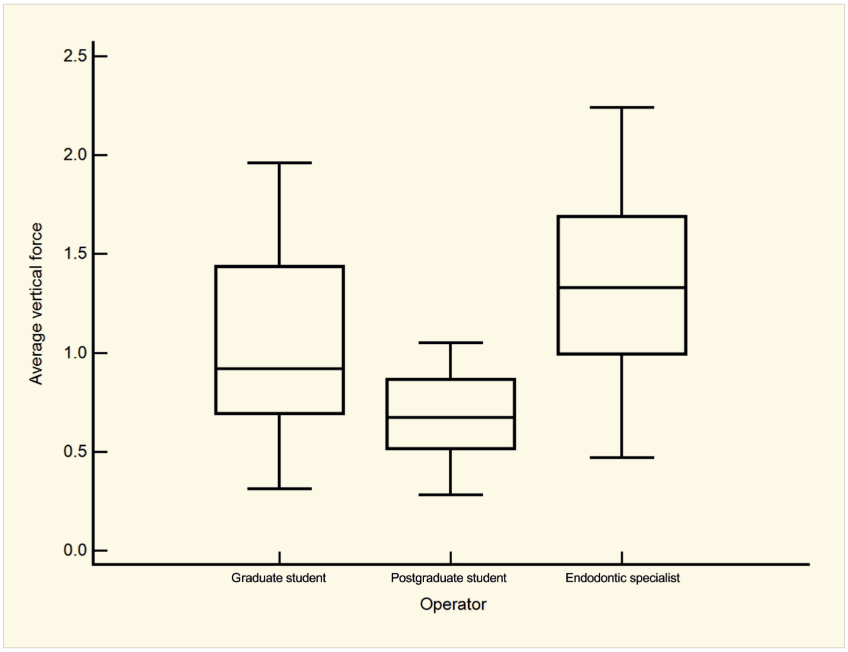
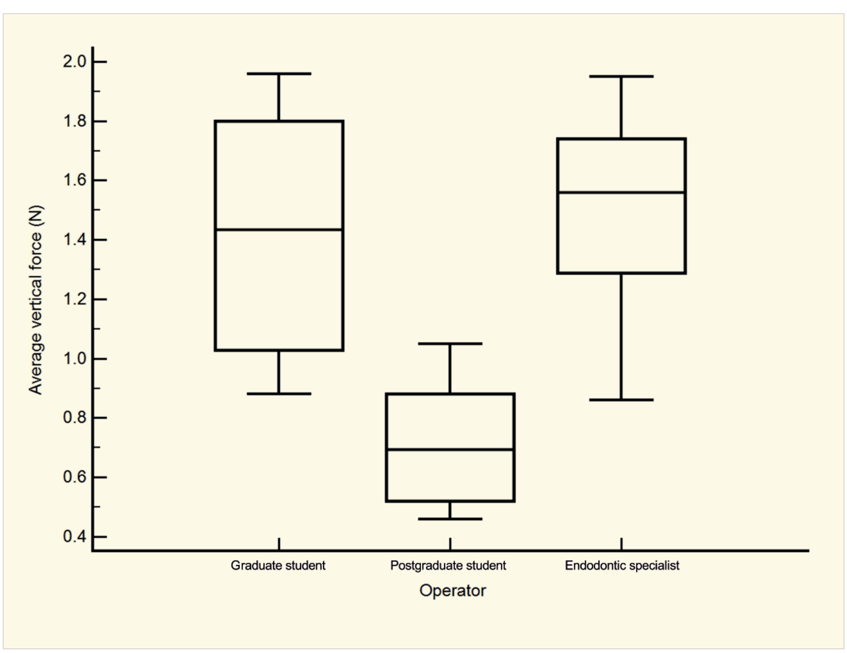


Figure 4
Difference in vertical force in all teeth regarding operator's experience.

Figure 5
Difference in vertical force in mandibular incisors regarding operator's experience.

(*P*<0.001). Average vertical force developed by postgraduate student was 0.68 (0.52-0.87) N while graduate student and endodontic specialist developed 0.92 N (0.70-1.44) and 1.33 N (1.00-1.69) , respectively (Figure 4). Post hoc analysis revealed that median vertical force developed by postgraduate student was significantly lower than in other two operators (both *P*<0.01). The figures 5 and 6 show median, minimal and maximal values as well as interquartile range of vertical force applied by three operators during instrumentation of mandibular and maxillary incisors. Significant differences in amount of vertical force during instrumentation between different operators were observed in both mandibular and maxillary incisors. (*P*<0.001 and *P*=0.008, respectively). In mandibular incisors, the average amount of vertical forces developed by graduate student and endodontic specialist were 1.44 N (1.03-1.80) and 1.56 N (1.29-1.74), respectively. Force developed by postgraduate student was on average 0.70 N (0.52-0.88). Post hoc test revealed that force developed by postgraduate student was significantly lower than in other two operators (*P*<0.01, Figure 5).
Significant differences in amount of vertical force during instrumentation of maxillary incisors between operators were also observed (*P*=0.008). The average amount of vertical force developed by endodontic specialist was 1.05 N (0.89-1.37), and it was significantly higher than average forces developed by graduate and postgraduate student (*P*<0.01, Figure 6). A significant positive correlation between number of shaped root canals and vertical force was demonstrated for postgraduate student (ρ=0.490, *P*=0.003). The amount of vertical force increased with the number of shaped root canals. A negative correlation was found for graduate student (ρ=-0.143, *P*=0.548) and endodontic specialist (ρ=-0.340, *P*=0.143), however it was not statistically significant.



Discussion

Only a few studies evaluated the influence of operator's experience on the shaping of the root canals (11, 16, 17). While some of them used simulated root canals in resin blocks, others used natural extracted teeth. In the present study extracted teeth were used as samples. There are several disadvantages when resin blocks are used. First, the physical properties such as texture, hardness, and cross section of simulated root canals differ from natural teeth. Second, heat generated during instrumentation of simulated root canals, may soften the resin material and lead to binding of

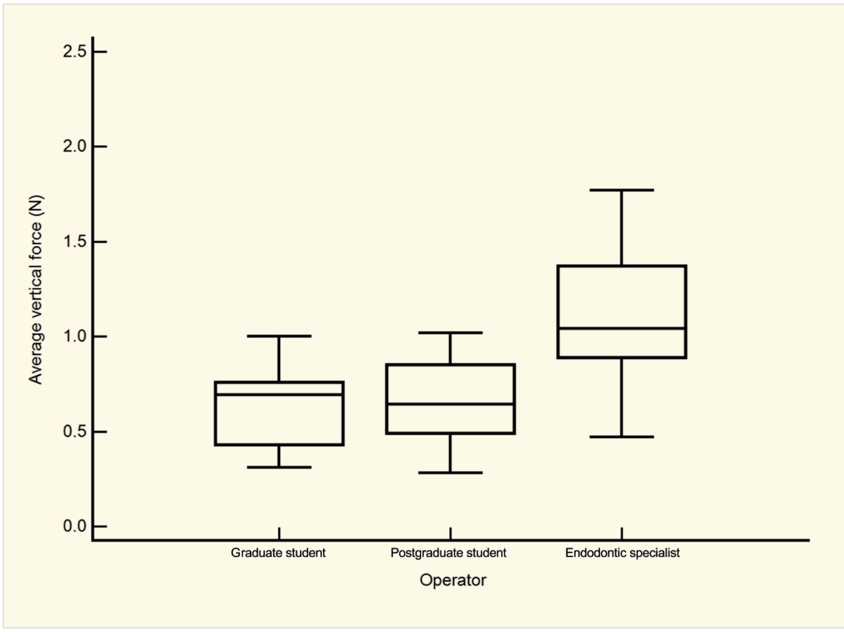


Figure 6
Difference in vertical force in maxillary incisors regarding operator's experience

cutting blades (16). However, it can eliminate morphological variations of root canal as a source of measurement variations (18, 19). Although the skills required for preparation of plastic block canals and canals in natural teeth are not identical (16), the experience and tactile skills of operator remain important issues of investigation. The results of this study demonstrated significant differences in vertical force developed by operators with different levels of experience.

An operator with extensive experience in rotary instrumentation (endodontic specialist) had on average highest vertical force values between the operators, but this difference was significant only in the model of wide root canals. On the other hand, the postgraduate with limited experience in rotary instrumentation had on average lowest values of vertical force as opposed to other two operators.

Statistical analysis revealed that this was significant in total sample and in the model of narrow root canals. This finding can be attributed to the "learning curve" of the less experienced operator. Blum et al (20) compared vertical forces developed by a group of students and a group of endodontists during root canal instrumentation utilizing an endograph. The results of their study showed that the vertical forces of students were lower than those of the endodontists in the beginning of the experiment. However, with the progression of the study, the amount of forces increased in the group of students and became similar to the values of experienced operators. This could indicate their advancement in correct manipulation of the rotary instruments (20). This trend can be observed in the present study as well and confirms the positive correlation between progression of operator's experience and an increase in instrumentation force. Our results demonstrated that the vertical force values of the postgraduate student increased with the number of shaped root canals, possibly reflecting an increase in experience and tactile skills of the operator.

Instrument separation is an unpleasant complication, which can often be prevented through correct instrument manipulation. Care is advised especially during instrumentation of the narrow root canals. Previous study performed by Peters et al confirmed a correlation of vertical force to preoperative canal volume (21). It was observed that the apically directed force increased during preparation of narrow root canals. Torsional fractures and "taper lock" effect were induced through forced manipulation of the rotary file into the apical portion of the narrow root canal (21, 22). Similar finding was observed in this study. Regardless of the operator experience, the average amount of vertical force was significantly higher in the model of narrow root canals.

Regardless of the recorded vertical force values, no instrument separation was recorded in any of the operator groups. This could be attributed to the single use of rotary files, maintenance of the glide path and manual enlargement of the root canals prior to rotary instrumentation. During the last two decades, NiTi instruments have gained popularity and are widely used by both general practitioners and endodontic specialists (23, 24).

A study performed on general dentist and endodontists in Tehran revealed that the most common procedural fault associated with NiTi instruments was file separation

due to over-use and application of excessive pressure (25). Aside from instrument separation, increased instrumentation force is associated with formation of dentinal defects on the canal walls. These structural defects can increase the risk of root fracture and lead to tooth loss (22). One of the major shortcomings of many comparative studies conducted in ex vivo conditions, including the present study, is a lack of anatomical matching of teeth prior experiment (26).
In the present study, after applying the exclusion criteria and standardization of the root length and apical diameter, teeth were randomly divided to create experimental groups. Although several previously conducted studies created experimental groups by randomization (14, 27), there are concerns that the results may reflect the root canal anatomy rather than the variable of interest (26). To overcome this problem several studies measured buccolingual and mesiodistal root canal diameter at 5 mm from the apex and calculated long: short cross-section diameter ratio to discern oval from round root canals (28-30). Recently, a study by De-Deus et al (26) proposed a micro-computed tomographic (micro-CT) technology as a method to remove variations in root canal anatomy as a confounding variable in experiments with pair-matched design.
Other limitations of the present research include a relatively small sample of teeth analyzed in each operator group. Instrumentations were performed by only one representative of the operators.A more generalized representation of each operator group would have been achieved through multiple operators in each experience group. Finally, we cannot exclude the possibility that differences in vertical force may be rather attributable to individual operator characteristics than operator's experience. Several investigations have demonstrated that utilizing machine-driven Ni-Ti instruments by inexperienced users results in more confidence and an improved sense of security in performing endodontic treatment (31, 32). Therefore, it seems rational to introduce the use of machine-driven Ni-Ti files into their pre-clinical curriculum.

Conclusions

Under the present experimental conditions, the postgraduate with limited experience in rotary instrumentation had on average lowest values of vertical force as opposed to other two operators. However, these values increased with the number of shaped root canals.

Clinical Relevance

The present study emphasized the importance of preclinical training to acquire experience and tactile skills for correct manipulation of the rotary endodontic instruments.

Conflict of Interest

None.

Acknowledgements

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ORIGINAL ARTICLE

Percentage of Gutta-percha filled area in canals shaped with Nickel-Titanium instruments and obturated with GuttaCore and Conform Fit gutta-percha cones

ABSTRACT

Aim: *The purpose of this study is to compare continuous wave with conform fit gutta-percha point and the core-carrier system with GuttaCore assessing the percentage of gutta-percha filled areas (PGFA), sealer filled areas (PSFA) and voids (PVA) in extracted teeth.*
Methodology: *Seventy-five extracted single rooted premolars were assigned to 5 groups, groups A and C were shaped with WaveOne Gold medium, groups B and D with ProTaper Next X3, and group E with manual instruments #35 K-file. Obturations were managed with GuttaCore in groups A and C; with Conform fit gutta-percha points in groups B and D; and with ISO-sized 35 gutta-percha master cones and cold lateral compaction technique in group E. The teeth were sectioned at 2, 4, 6, and 8 mm from the apex and for every section the percentages of gutta-percha and sealer filled, and void areas with respect to the total area were calculated. Data obtained were analysed for each variable at each level by using a one-way ANOVA with group as dependent variable (α=0.05). Multiple comparisons between the five experimental groups were conducted by using the Student's t-test with Šidák alpha correction.*
Results: *At all levels group E produced significantly less PGFA and higher PSFA and PVA than all the other experimental groups. At 2 mm from the apex, group A showed significantly higher PGFA than group C and a significantly lower PSFA than C and D. At 4 mm from the apex groups A and B produced significantly better results in terms of PGFA than groups C and D. the PSFA and PVA were significantly lower in group D with respect to groups A and B. PVA were statistically significant lower in group C than in group B. At 8 mm from the apex group A showed significantly higher PGFA than groups B and D, a significantly lower PSFA and PVA than group B.*
Conclusions: *Independently of the instrument used for preparation (WaveOne gold versus ProTaper Next) Conform Fit point and GuttaCore produced very homogeneous obturations with high PGFA. The association of WaveOne Gold file system shaping and the GuttaCore obturation technique produced better results in terms of PGFA, PSFA and PVA than all the other techniques especially in the apical portion of the root canals. Manual instrumentation and the cold lateral obturation technique yield poor quality root canal obturations.*

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Introduction

Preventing bacteria from entering the endodontic system is a fundamental biological principle for achieving a successful treatment (1). A recent systematic review and meta-analysis concluded that both adequate root canal treatment and restoration are fundamental for saving functional teeth (1). These results strengthen findings of previous reviews.

A satisfactory endodontic obturation without voids is one of four factors with a significantly positive effect on the outcome of primary root canal treatment (2).

Research is aiming at matching engine-driven nickel-titanium (NiTi) instruments with an appropriate obturation technique that will seal all ports of entry to prevent bacterial contamination. A 3-dimensional (3D) adaptation of the filling material to the root canal space could be accomplished with the core-carrier system or single-cone gutta-percha technique with the same taper and diameter of the last instrument used (3-5).

Both methods are based on the use of thermoplasticized gutta-percha. The dimensional stability of the obturation is directly dependent on filling the canal with gutta-percha using as little sealer as possible and leaving no void areas (6-8). The quality of the obturation can then be evaluated in terms of the percentage of gutta-percha filled areas (PGFA), sealer filled areas (PSFA), and voids (PVA) in filled straight-root canals using digital images (9-12).

In the single-cone gutta-percha technique, the adaptation to the endodontic space was achieved with the warm vertical compaction or continuous wave compaction technique. In 2013 Schäfer et al. found that the variable taper of root canal instrumentation negatively influenced obturation quality with single-cone gutta-percha (4).

Recently, new Conform-Fit gutta-percha points (Dentsply, Sirona, Canada), that closely matched the variable taper of relative Ni-Ti instruments (ProTaper NEXT and WaveOne Gold), were introduced. Previous studies stressed the importance of correspondence between the diameters and tapers of the files and the gutta-percha cones (13, 14).

The manufacturer described the Conform Fit Gutta-Percha point as a micronized gutta-percha formula that permits thermoplastic adaptation at low temperatures (105-180°) minimizing any negative effect on the periodontal ligament (https://www.dentsplysirona.com).

Until recently core-carrier were either metal or plastic (Thermafil thecnique) now, the core can be made of cross-linked gutta-percha. However, the cores are always coated alpha-phase gutta-percha.

The most popular core carrier systems with cross-linked gutta-percha are Gutta-Core (Dentsply Maillefer, Ballaigues, Switzerland) and GuttaFusion (VDW, Munich, Germany). These systems seem to guarantee a high quality of obturation with respect to lateral and Single-Cone techniques even with the use of different instruments with varied and constant tapers (9).

The purpose of the study was to compare the quality of obturation, in terms of percentage of gutta-percha filled area (PGFA) sealer (PSFA) and voids (PVA), in straight root canals instrumented with two varied taper NiTi instruments (Wave One Gold and ProTaper NEXT) and filled with single-cone gutta-percha (Conform Fit point) or the Gutta-Core carrier system.

The tested null hypothesis was that there is no difference in the quality of obturations between two techniques.

Materials and Methods

Seventy-five straight root canal (curvature <5°) mandibular premolars, extracted for periodontal disease, were selected for this study. The study was approved by the ethics committee of the Tuscany Region, University of Florence, Italy N17877_BIO. In order to prevent bacterial growth, the teeth were stored at 4 °C until use, in formalin solution. The remaining were then examined with stereomicroscope under X20 magnification; those with root canal cracks were also excluded. The coronal access was realized in all root canals, apical patency was checked and working length measured with a size 10 k-file (Dentsply Maillefer, Ballaigues, Switzerland). The distance between the cementoenamel junction and the apex was recorded in order to assign the canals to five similar groups (n=15 teeth for group).

After placing the teeth in an endodontic simulator (Protrain; Simit Dental, Mantova, Italy), the electric motor with a 16:1 reduction hand-piece (X-Smart Plus; Dentsply Maillefer, CH-1338 Ballaigues, Switzerland) was set for each file with torque and rotational speed as suggested by manufacturer. The glide path was performed using Wave One Gold Glider in groups A and C and with ProGlider in groups B and D, until working length was reached. In group E manual instrumentation with stainless steel K-File (Dentsply, Maillefer) size 10-35 were used.

In the experimental groups shaping was accomplished using two different NiTi energy driven instruments: in groups A and C Wave One Gold medium (WOGM) (Dentsply, Maillefer); in groups B and D ProTaper Next X3 (PTNX3, Dentsply, Maillefer) until the working length was reached.

After using each instrument, the root canals were irrigated with 5% NaOCl and 17% EDTA using a 30-gauge monojet irrigation needle for a total of 12 ml and 9 ml, respectively; the final rinse was done using 3 ml of saline solution for 1 minute. All the roots were dried with the paper point corresponding to the last instruments used.

Groups A and B were obturated by using the core-carrier system: GuttaCore (GC) obturator (Dentsply Sirona Ballaigues, Switzerland), matching the WOGM and PTNX3 respectively, were heated in a ThermaPrep 2 oven (Dentsply Sirona), on heat setting 35-40 °C. Groups C and D were obturated by using the continuous wave technique: Conform Fit (CF) gutta-percha cone (Dentsply Sirona), matching WOGM and PTNX3 respectively, using the System B (EIE Analytic Technology, Orange, CA); backfilling was accomplished using an Obutra II syringe (Obtura II, Obtura Corporation, Fenton, MO, USA). Finally, group E was obturated with the cold lateral compaction technique using ISO-sized 35 gutta-percha master cones. In all the samples pulp-canal sealer EWT (Kerr, Salerno, Italy) was used as endodontic sealer. Teeth were stored for 14 days at 37 °C and 100% humidity to allow the sealer to set completely.

The teeth were embedded in resin blocks (Technovit; Heraeus-Kulzer, Wehrheim, Germany) and sectioned horizontally, with a 0.1 mm low-speed saw (Leitz, Wetzlar, Germany) under permanent water-cooling at 2, 4, 6, and 8 mm from the apex. All slices were observed from a coronal to apical direction under a digital stereomicroscope (SMZ-10 Nikon Corporation, Tokyo. Japan) at X40 magnification and digital pictures were taken of each section. The total area of each canal segment and the areas of gutta-percha, sealer, and voids were measured in pixels using ImageJ Software (National Institutes of Health, public domain) for all sections. The areas of gutta-percha, sealer, and void areas, PGFA, PSFA, and PVA respectively, were converted to percentages with respect to the total area.

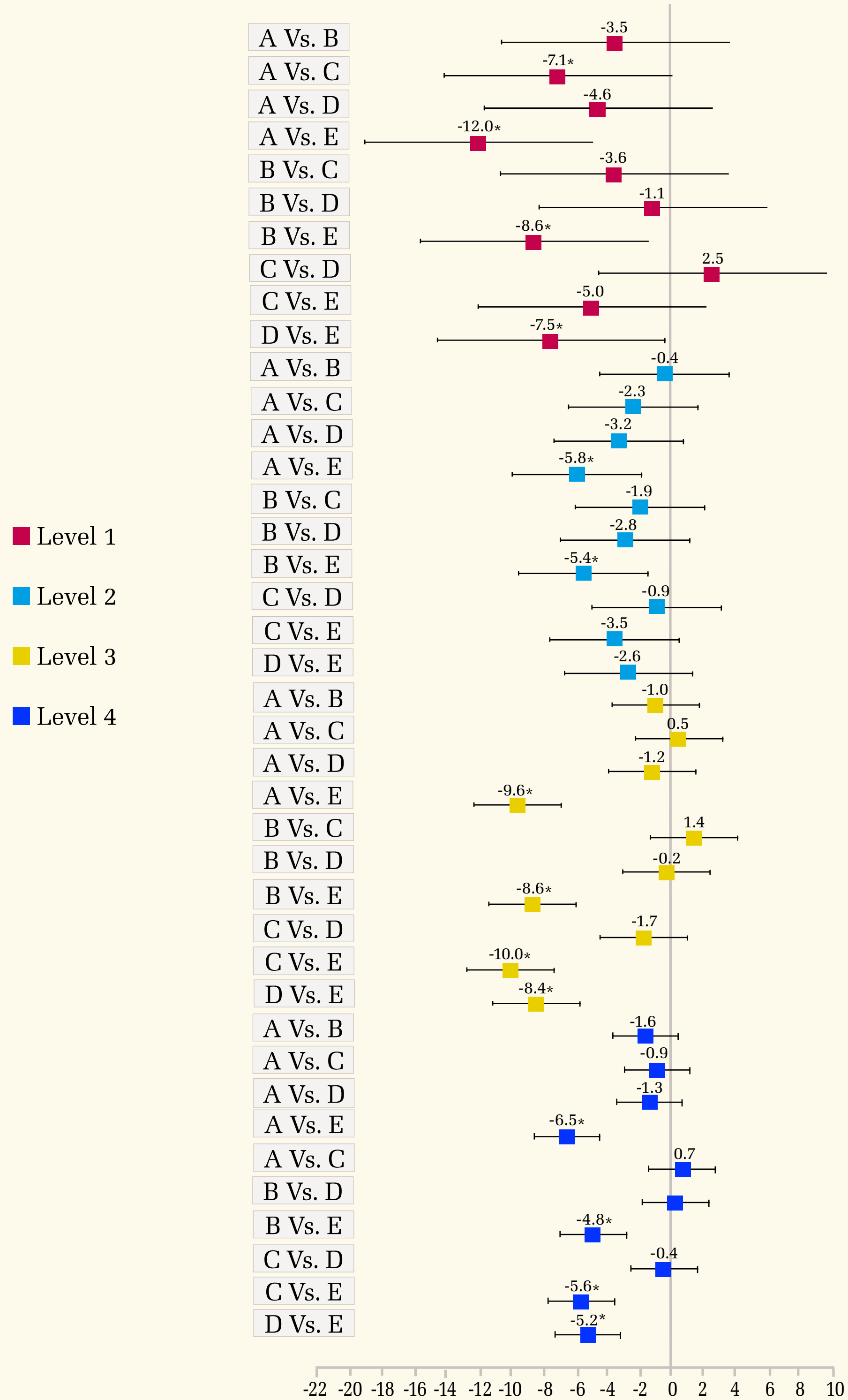
These sections were analyzed by an examiner blinded to all the experimental groups and sections.

Statistical Analysis

The sample size was determined considering the comparison of main interest (i.e. PGA at first level, experimental group A vs. all the other experimental groups). Assuming that the most similar percentages of PGA were observed in the experimental groups A and B (about 94% and 90%, respectively) and considering a common SD of 3 and an alpha level of 0.0127 (i.e. Šidák correction [i.e. $1-(1-\alpha)^{1/m}$] applied to the four contrast of main interest), a sample size of 15 teeth for each experimental group is sufficient to guarantee study power of at least 80%.

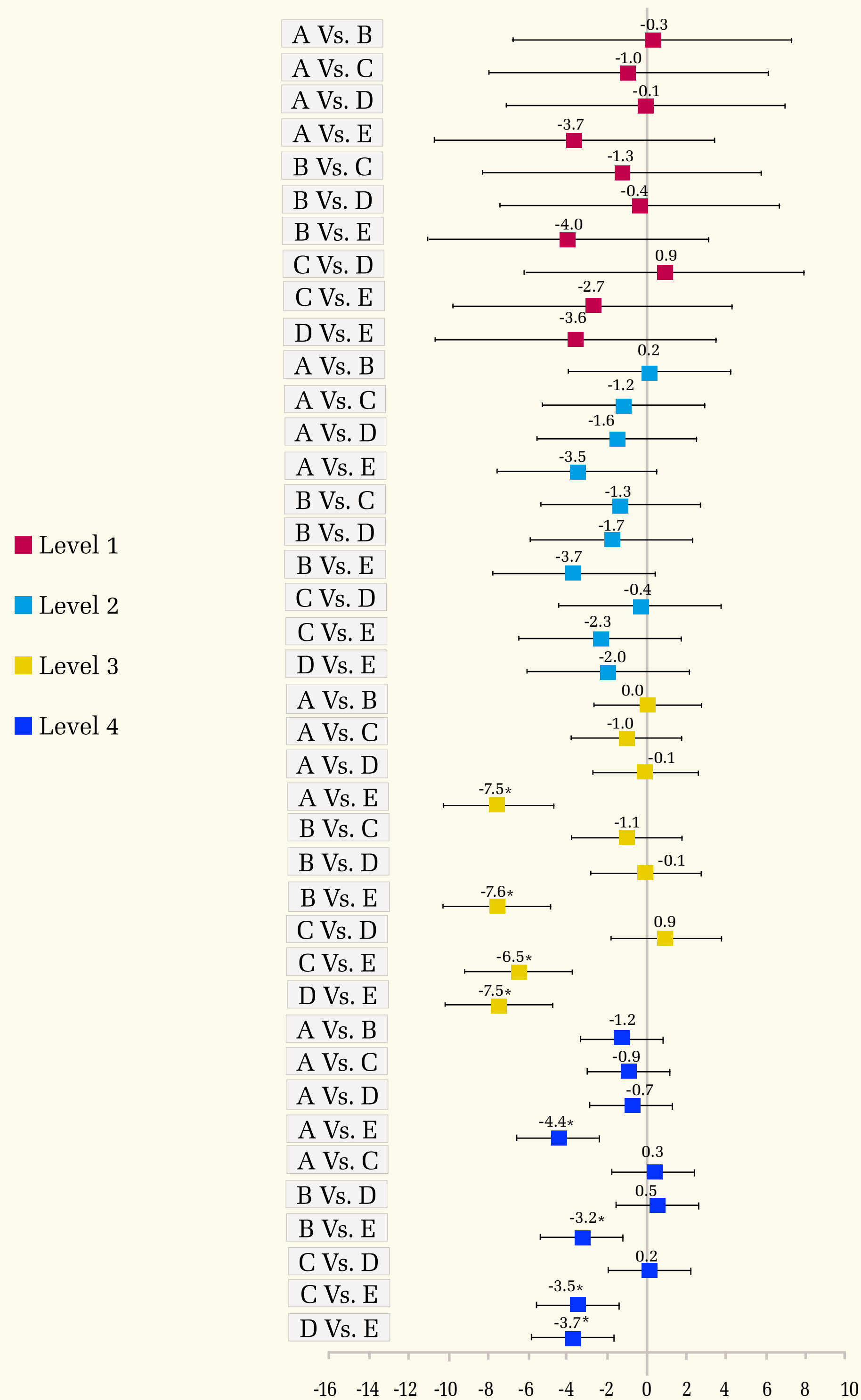
Statistical analyses of PGFA, PSFA and PVA were performed for each level (i.e. 2, 4, 6, and 8 mm), using the one-way ANOVA with group as dependent variable. Multiple comparisons between the five experimental groups for each variable at each level were also performed by using t-Test. The overall study statistical significance level

Figure 1
PGFA contrasts by level.



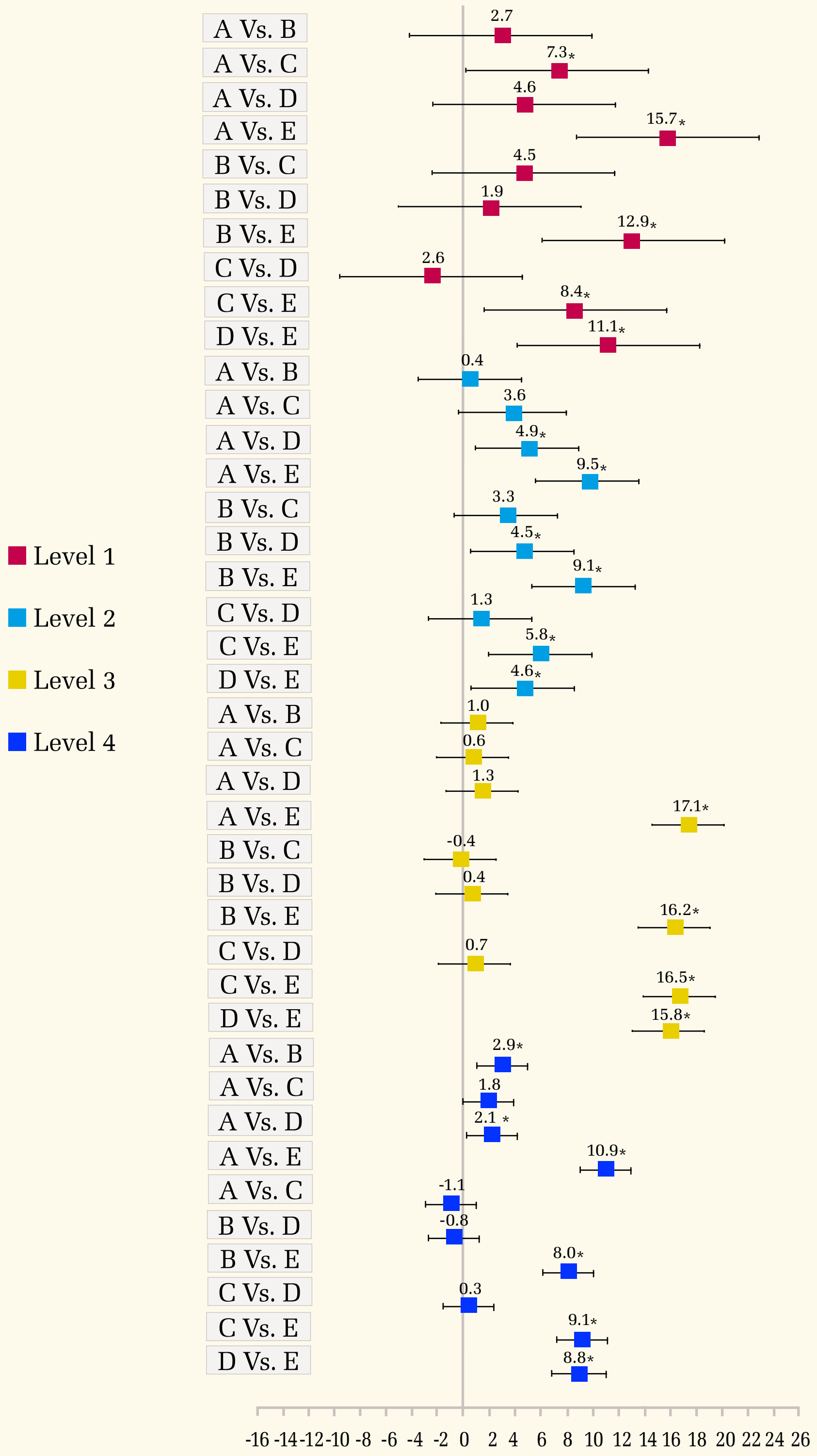
*Statistically significant (Šidák correction)

Figure 2
PSFA contrasts by level.



*Statistically significant (Šidák correction)

Figure 3
PVA contrasts by level.



*Statistically significant (Šidák correction)

Table 1
Descriptive Statistics

			PGFA		PSFA		PVA	
Level	Group	N	Mean (St. dev)	Min-Me-Max	Mean (St. dev)	Min-Me-Max	Mean (St. dev)	Min-Me-Max
1	A	15	94.7 (3.04)	89.4-95.2-99	3.8 (2.07)	1-3.9-7.4	1.5 (1.65)	0-0.8-6
1	B	15	91.9 (7.5)	67.2-94.9-97.6	7.3 (6.99)	1.4-5.1-30.5	1.2 (1.36)	0-0.5-4.6
1	C	15	87.4 (8.52)	64.3-88.9-97.2	10.9 (7.52)	2.5-10.9-32.4	2.4 (1.73)	0.2-1.9-5.3
1	D	15	90.1 (6.84)	80.2-90.2-99	8.4 (5.88)	0.7-8.3-18.4	1.5 (1.26)	0.1-1.4-3.7
1	E	15	79 (6.16)	60.7-79.4-89.3	15.9 (5.54)	5.7-16.3-29.4	5.1 (2.51)	1.8-4.6-11
2	A	15	96.1 (1.97)	93-96.3-99.2	3.2 (2.25)	0.5- 2.7-6.7	0.9 (0.56)	0-0.9-2
2	B	15	95.7 (3.53)	88-96.8-99.7	3.6 (3.22)	0.5- 2.6-11	0.7 (0.96)	0-0.4-3.7
2	C	15	92.4 (4.24)	83-92.9-98.2	5.5 (3.88)	0-5- 12.6	2.1 (2.37)	0-1.3-9.1
2	D	15	91.2 (5.71)	76-91.8-98.5	6.4 (5.51)	1.1-4.9-24	2.4 (2.27)	0-2.-5.8
2	E	15	86.6 (2.41)	82.6-86.3-91	9 (1.58)	5.3-9.3-11	4.4 (1.7)	1.3-4.8-7
3	A	15	98.1 (1.09)	96-98.3-99.2	1.4 (0.98)	0-1.2-3.1	0.5 (0.48)	0-0.6-1.3
3	B	15	97.1 (3.73)	87.9-98.6-100	2.3 (3.24)	0-1.2-11.9	0.5 (0.92)	0-0.2-3.6
3	C	15	97.5 (1.01)	95.4-97.8-98.8	0.9 (0.95)	0-1-3	1.6 (1.16)	0-1.6-4.6
3	D	15	96.8 (2.01)	92.5-97.6-99	2.6 (1.48)	1-2.1-5.3	0.6 (0.7)	0-0.6-2.2
3	E	15	81 (3.66)	74-81.1-86.3	11 (2.69)	7.2-10-16.3	8.1 (3.43)	4.7-7.6-16.6
4	A	15	98.8 (0.84)	97.1-98.9-100	1.1 (0.79)	0-1-2.9	0.2 (0.28)	0-0-0.7
4	B	15	95.9 (2.09)	92.1-95.6-100	2.7 (1.75)	0-2.5-6.8	1.4 (1.89)	0-1-7
4	C	15	97 (1.25)	95-97.3-99	1.9 (1.26)	0-1.7-4.5	1.1 (1.13)	0-0.6-4
4	D	15	96.7 (2.06)	92.9-97-99	2.4 (1.29)	1-2.4-4.9	0.9 (0.97)	0-0.6-3
4	E	15	87.9 (2.79)	83.2-87.7-92.6	7.5 (1.93)	3.7-7.9-10.3	4.6 (1.73)	1.5-4.7-7.7

was set at $\alpha=0.05$ for each variable within each level. Multiple comparisons were conducted by applying the Šidák alpha correction. No formal tests were performed for data distribution since, for small sample size, the tests for normality could lead to misleading conclusions. Instead, a graphical exploration of the symmetry of the data was performed.

Results

Table 1 shows the mean and standard deviation values of PGFA, PSFA and PVA at four different levels for the five experimental groups. At all levels significant differences were found among the five groups in term of pooled PGFA, PSFA and PVA (Table 2, 3, 4). At all levels group E showed a significantly lower PGFA value and a significantly higher PCFA and PVA than all other groups ($p<.05$).

At the more apical level (2 mm from the apex) group A has a significantly higher PGFA than group C and a significantly lower PSFA than groups C and D ($p<.05$).

At 4 mm from the apex groups A and B presented a significantly higher PGFA than groups C and D, for PSFA. A significant difference was observed between groups D and A and between D and B, for PVA as well. Finally group B had lower PVA than groups C and D ($p<.05$).

At 6 from the apex no significant difference

Table 2
Analysis of variance for PGFA by level

Level	Effect	Num DF	Den DF	F Value	Pr>F
1	Group	4	70	12.10	<.0001
2	Group	4	70	15.41	<.0001
3	Group	4	70	121.18	<.0001
4	Group	4	70	72.74	<.0001

Table 3
Analysis of variance for PSFA by level

Level	Effect	Num DF	Den DF	F Value	Pr>F
1	Group	4	70	8.61	<.0001
2	Group	4	70	6.60	0.0001
3	Group	4	70	59.24	<.0001
4	Group	4	70	45.22	<.0001

Table 4
Analysis of variance for PVA by level

Level	Effect	Num DF	Den DF	F Value	Pr>F
1	Group	4	70	12.96	<.0001
2	Group	4	70	11.03	<.0001
3	Group	4	69	54.07	<.0001
4	Group	4	70	24.97	<.0001

was observed in terms of PGFA, PSFA and PVA. At 8 mm from the apex group A had a significantly lower value of PSFA and PVA than group B, and a significantly high PGFA than groups D and B ($p<.05$).
All the contrasts between experimental groups by levels are fully shown in figure 1, 2, 3.

Discussion

The obturation of root canal systems is generally accomplished using biocompatible and removable material such as gutta-percha and sealer in order to reduce the presence of voids in so far as possible prevent recontamination of the system (3, 15, 16). Since gutta-percha is a dimensionally stable material over time the PGFA was largely used to evaluate root filling quality (4, 9, 17). In the present study the root canal sections of group E (manual instrumentation and cold lateral obturation technique), at all levels analysed, showed the worst performance: maximum amount of voids and cement and minimum amount of gutta-percha. This widely used obturation technique (18, 19) has been considered a control in several studies (4, 9, 20).
The other four experimental groups were instrumented with two different NiTi system (Wave One Gold and ProTaper Next), both with have a variable taper, a different cross sectional design (parallelogram versus rectangule) and a different motion (reciprocating versus continuous respectively).
Previous studies have investigated the quality of root canal obturation using different techniques (core carrier system versus warm continuous condensation). In general no statistical differences have been found between the two systems (20-22, 10) either in extracted teeth or in simulated C-shaped canals. In a recent *ex vivo* study carrier-based techniques produced a statistically significant difference in terms of PGFA at 4 mm from the working length in oval canals; the authors emphasized the role of both hydraulic and mechanical condensation in order to obtain a tridimensional root canal filling (23).
In the present study, the main difference between the four experimental groups was observed at 4 mm from the apex. At 4 mm, sections of group A resulted in a significantly higher gutta-percha filled area and less of cement compared with groups C and D. With GuttaCore there were greater amounts of gutta-percha, and less sealer and voids than in root canals filled with the Conform fit cone and continuous wave of condensation technique.
As suggested in the literature, using the cold continuous wave obturation system the apical portions of the root canal are more prone to having larger amounts of

sealer and voids. Furthermore apical anatomical variations are more difficult to manage using this technique as opposed to the other (17, 22).
The results did not reveal any significant difference between groups C and D for any of the analysed parameters (PGFA, PSFA and PVA) when the conform fit cone was used. Type of mechanical instruments used to shape the canals did not seem influence the performance of the cold continuous wave technique with the Conform fit gutta-percha cone. Likewise, when a core carrier system technique was used, the type of instruments did not affect the quality of obturation (group A versus group B), concurring with the results obtained by Schäfer (9). Except at 8 mm from the apex, in the present study, no significant difference was documented in terms of PGFA, PSFA and PVA between groups A and B. At this level the canal shaped with Wave-One Gold resulted in a significantly higher better root filling quality than those shaped with ProTaper NEXT. These results could depend on the instrument design, alloy type and rotation motion (continuous or reciprocating): all of these characteristics lead to a rotational phenomenon known as "swagger" (24). Despite the use of the ProGlider glide path, the loss of centralization preparation could not ensure a good fit of the GuttaCore to the canal walls (25).
Finally, in the apical portion of the canals, when WaveOne Gold was used for shaping, GuttaCore insured better root canal obturation in terms of PGFA and PSFA than Conform fit gutta-percha cone and System B. These findings could be due to the various phases of the continuous wave technique (17).
Regarding the detection of voids the present study supports previous findings, root canals filled with the GuttaCore carrier system showed the best results, close to 0 (9, 20).
Within the limitations of the present study, the association of Wave-One and corresponding GuttaCore yielded high quality root filling in extracted teeth, therefore the null hypothesis has been rejected. The quality of obturations in manually instrumented root canals filled using the cold lateral technique was very low.

Clinical Relevance

The association of reciprocating motion file system shaping and the core carrier system obturation technique produces highly affordable results in terms of PGFA, PSFA and PVA especially in the apical portion of the root canals.

Conflict of Interest

The authors deny any conflict of interest.

Acknowledgements

None.

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ORIGINAL ARTICLE

Effect of glass fiber on the restorative procedure in relation to fracture strength of endodontically treated molars

ABSTRACT

Aim: *To evaluate whether the restorative procedure using glass fibers influence the fracture strength of endodontically treated molars with class II mesio-occlusal (MO) preparation.*

Methodology: *Fifty human maxillary third molars were selected and randomly assigned to five groups (n=10). MO cavity preparation and endodontic treatment were standardized, except for the positive control group (S, sound teeth). The other groups were classified as: ET, no restoration (negative control); SF, restoration with SonicFill 2® system; SFB, restoration with braided glass fiber and SonicFill 2® system; and SFP, restoration with transfixed glass fiber post and SonicFill 2® system. The specimens were subjected to fracture strength testing on a universal testing machine. Fracture site – either pulp chamber floor or cusp – was inspected. Statistical analysis was performed using ANOVA, followed by Tukey's multiple comparison test (α=5%).*

Results: *Means followed by the same letter did not show statistical difference in Tukey's test (P>0.05). S: 3563^{A} ± 780.7; ET: 1001^{D} ± 237.6; SF: 1689^{C} ± 280.7; SFB: 2256^{B} ± 289.2; and SFP: 2493^{B} ± 364.*

Conclusions: *The glass fiber, regardless of composition, increases the fracture strength of endodontically treated teeth. The use of a glass fiber post attached to the dental crown seems to provide more favorable rehabilitation when the fracture position is determined.*

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Introduction

Fractures continue to be a major cause of loss of endodontically treated teeth (1, 2). The restorative procedure aims not only to promote coronal sealing, preventing contamination of the endodontic filling material, but also to strengthen the teeth by restoring lost structures. According to Faria et al (3), there is a direct relationship between the number of missing walls and fracture strength. Plotino et al (4) also observed that the absence of one or two marginal ridges results in considerable loss of tooth stiffness, around 46% and 63%, respectively.

Composite resin restorations are commonly the first clinical rehabilitation alternative. The incremental technique has been the most common placement method for the use of composite resin (5). However, when large cavities are filled, this clinical protocol may result in a higher risk of air bubble formation between increments, greater polymerization stress, and an increase in consultation time (6-8). In an attempt to address these drawbacks, the industry has developed a new type of resin-based restorative material that is applied in single increments of approximately 4 mm in thickness and with reduced shrinkage (9, 10).

SonicFill 2® is a system that uses a specific sonic handpiece to carry resin into the cavity. This sonication feature transmits energy to the composite, reducing its viscosity (11) in order to better adapt the material to the cavity (12). According to Agarwal et al (13), the mechanical properties of this new composite are similar to those of a hybrid composite resin.

Studies have described the application of glass fiber to reinforce composite resin restorations. For Freilich et al (14), the use of fibers associated with the restorative procedure has reduced the risks of permanent deformation and tooth fracture. Belli et al (15) also placed a polyethylene fiber together with a flowable composite resin into mesio-occluso-distal (MOD) cavity preparations and found that there was greater fracture strength than in teeth filled with composite resin alone.

An alternative researched in the literature, in order to increase the fracture strength of endodontically treated teeth, is the use of flexible fiberglass posts horizontally transfixed in the buccal and palatal/lingual surfaces. These prefabricated aesthetic posts have an elasticity module very close to that of dentin (16). According to studies by Favero et al (17), Karzoun et al (18), and Mergulhão et al (19), the groups that received post transfixation and restoration with composite resin showed a significant increase in fracture strength when compared to the groups restored only with resin. In addition, a lower degree of impairment of the dental structure in the face of the fracture can be observed.

The presented study aimed to evaluate whether the use of glass fiber associated with the sonic-resin placement system as restorative material influences the fracture strength and type of fractures in endodontically treated molars with class II mesio-occlusal (MO) preparation. The initial null hypothesis was that there is no statistically significant difference between endodontically treated molars restored with glass fiber (post or braided).

Materials and Methods

This study was approved by the Research Committee of the UFRGS School of Dentistry and its Research Ethics Committee (Process CAAE 06753019.6.0000.5347).

Sample selection and preparation

Fifty human maxillary third molars, free from carious lesions, restorations, or cracks were used in this study. The buccopalatal (11 mm ± 0.5 mm) and mesiodistal (9.5 mm ± 0.5 mm) widths of the selected crowns were measured with a digital caliper (Mitutoyo, Suzano, SP, Brazil) at the most prominent point of the respective surfaces. We calculated sample size on the basis of a pilot study and considered the following parameters: type I error probability of .05, nominal test power of 0.8, difference be-

tween groups of 230 newtons, and average standard deviation of 90 N. The minimum sample size was of 10 specimens per group.
After the cleaning procedures, the teeth were disinfected in 0.5% chloramine solution (Seachem Laboratories, Madison, GA, USA) for 48 hours. The teeth were randomly assigned to five experimental groups (Table 1).

Preparation of specimens
The teeth were inserted individually in self-curing acrylic resin, centered inside a PVC cylinder (height: 2 cm, diameter: 3 cm) so that the anatomical neck of the tooth was exposed 2 mm above the edge of the acrylic.

MO cavity preparation
A piece of equipment was used for MO cavity preparation to standardize the inclination and movements performed by diamond bur #2143 (KG Sorensen, São Paulo, SP, Brazil) during the procedure.
Cavity preparations followed the methodology described by Cötert et al (20) and Beltrão et al (21), in which a line was delimited from the central groove to allow the buccal and palatal walls to be equidistant from the measurement of two thirds of the intercuspal distance. This line, over the central groove, was extended to the mesial surface, passing over the marginal ridge, going towards the dental neck until it reached a height of 4 mm. This was the depth established for the preparation. The predetermined bucco-palatal width on the occlusal surface was extended to the mesial surface and likewise established for the proximal box. Diamond bur #2143 was initially positioned on the mesial surface over the centerline to the extent of the predetermined length. Next, a mesial box was made towards the center of the dental crown, preserving a 2 mm wide distal crest structure. From this preparation, the buccal and the palatal walls were set to the predetermined limits so that the gingival floor was joined to the pulp floor of the occlusal box. The bur was changed every five preparations. The cavosurface enamel margin received manual finishing with margin trimmer #28 and #29 (SS-White Art. Dental Ltda., Rio de Janeiro, RJ, Brazil). No cavity preparation was performed in Group 1.

Endodontic treatment
Carbide burs #02 and #04 (KG Sorensen Ind. And Com Ltda., Barueri, SP, Brazil) were used at high speed and under water cooling for access to the pulp chamber. The convenience form was obtained using the Endo Z bur (Dentsply Ind. E Com Ltda., Petrópolis, RJ, Brazil).
Initially, the cervical preparation was performed with the La Axxess® bur (SybronEndo, Glendora, USA) #35 taper 0.6 at a depth of 5 mm from the entrance to the canal, under irrigation with 2.5% sodium hypochlorite. The working length for canal preparation was 1 mm below the outlet of the foramen. Chemomechanical preparation followed the serial technique using K files #15, #20, #25, #30 and #35 (Dentsply/Maillefer, Ballaigues, Switzer-

Table 1

Experimental group design

Groups	n	Description
S	10	Sound teeth (positive control)
ET	10	MO preparation + endodontic treatment and no restoration (negative control)
SF	10	MO preparation + endodontic treatment + SonicFill 2® restoration system
SFB	10	MO preparation + endodontic treatment + braided glass fiber + SonicFill 2® restoration system
SFP	10	Tooth with MO preparation + endodontic treatment + transfixed fiberglass post + SonicFill 2® restoration system

land). Irrigation with a hypochlorite solution was delivered using a 10 mL plastic syringe (Plastipak Indústria Cirurgica Ltda., Curitiba, PR, Brazil) and 0.30 mm Navitip® needle (Ultradent Products, Inc South Jordan, Utah, USA).

After completion of the chemomechanical preparation, the canals received a final rinse with 17% EDTA solution for three minutes under agitation of instrument #35, prior to filling. The canals were filled using epoxy resin cement - AH Plus® (Dentsply/Maillefer Instruments SA, Ballaigues, Switzerland) and by Tagger's hybrid technique using McSpadden® #60 compactor (Dentsply/Maillefer Instruments SA, Ballaigues, Switzerland). After the gutta-percha plastification and removal of the activated McSpadden® from the canal, the vertical condensation of the gutta-percha was carried out using the Paiva instrument No. 2 (SS White, Rio de Janeiro, RJ, Brazil), leaving the material at the entrance level of the root canal.

Demarcation and perforation for post transfixation

Reforpost® (Angelus, Londrina, PR, Brazil) glass fiber posts with 1.1 mm in diameter were placed in the SFP group. Perforations for horizontal transfixation of the posts were made on the buccal and palatal walls with a diamond bur #3145 (KG Sorensen, Sao Paulo, SP, Brazil), at high speed under water cooling. Bur #3145 has a diameter of 1.2 mm. The perforation of both buccal and palatal surfaces was done simultaneously on the same axis of insertion of the tip. The perforations were performed at the coronal middle third of the two dental surfaces at a distance of 2 mm from the mesial border. The bur was changed every five preparations.

Bonding of glass fiber post in transfixed position

The following procedures were performed according to the manufacturer's instructions:

- cleaning of the posts with 70% alcohol and drying with air jets.
- Application of a silane layer (FGM Dental Products, Joinville, SC, Brazil). Drying at room temperature followed by spraying air at a distance of 15 cm for 1 minute.

Figure 1
Schematic drawing with occlusal view of the transfixed post in the dental crown.

Figure 2
Schematic drawing with occlusal view of the position of the braided glass fiber inside the MO cavity preparation.

- Application of a thin layer of Singlebond Universal adhesive (3M ESPE, St. Paul, MN, USA) and photoactivation with LED light unit (Bluephase, Ivoclar) for 20 seconds.
- Eetching of transfixation holes and cavosurface enamel margin of the cavity preparation with 35% phosphoric acid (Dentisply Ind and Com. Ltda, Petrópolis, RJ, Brazil) for 20 seconds, washing for 20 seconds, and drying with air jets.
- Application of Singlebond Universal adhesive to the transfixation holes, pulp chamber, and in the whole cavity preparation, drying for 5 seconds, and photoactivation for 20 seconds.
- Insertion of Bulkfill flow resin (3M ESPE, St. Paul, MN, USA) into transfixation holes, post placement into transfixation holes, and photoactivation for 40 seconds (Figure 1).

Braided glass fiber placement

In the SFB group, a braided glass fiber, Interlig® (Angelus, Londrina, PR, Brazil), was cut according to the internal anatomical design of the MO cavity preparation. The fiber should extend throughout the inner walls: buccal, distal, lingual, and mesial (absent wall), thus having a circular shape (Figure 2).

The following procedures were performed according to the manufacturer's instructions:
- etching of the internal dentin walls of the buccal, lingual, and distal surfaces with 35% phosphoric acid for 20 seconds, air-water spray cleaning for 20 seconds, and air drying for 5 seconds.
- Application of a thin layer of Universal Singlebond adhesive and light curing for 20 seconds.
- Insertion of a thin layer of Bulkfill flow resin on the inner surface of these walls for braided glass fiber placement, and light curing for 40 seconds.

Restorative procedure

All teeth, except those in the S and ET groups, were restored with Single-Fill TM Bulk fill resin (Kerr Corporation, Orange, CA, USA). The restorative procedures were performed as follows: single-Fill TM Bulk fill resin was inserted into the cavity with the SonicFill 2® handpiece (Kerr Corporation, Orange, CA, USA), standardizing resin insertion speed at the "3" level. The cavity was completely filled, starting from the mesial proximal box.

The resin was spread with the aid of a spatula. After spatulation, each surface (buccal, lingual, mesial, and occlusal) was photoactivated for 20 seconds.

After the restorative procedure, the specimens were placed in distilled water and kept at 37 °C in an oven (Fanem, Model 002-CB, São Paulo, SP, Brazil) for 48 hours.

Mechanical fracture testing

The specimens were initially thermocycled at 5 °C to 55 °C for 500 cycles before being subjected to mechanical fracture testing.

The fracture strength testing was performed on an EMIC DL 2000 universal testing machine (São José dos Pinhais, PR, Brazil). A 10 kN load cell and 0.5 mm/min speed were selected. A 6.5 mm steel ball was placed for contact of the inclined planes of the occlusal surface in the intercuspal position with the cusps (buccal and lingual) and not with the restorative material. Compressive stress was applied parallel to the long axis of the tooth until its fracture. The maximum force to fracture (rupture) was recorded in Newtons (N).

Analysis of tooth fracture site

After fracture strength testing, the teeth were visually examined with a magnifying glass (4X magnification) to assess the site of the tooth fracture: 1) pulp chamber floor fracture associated or not with cusp fracture; or 2) cusp fracture only. Floor fracture was considered when the fracture line split the tooth into two parts at the pulp floor level of the cavity, regardless of whether it was buccal/palatal or mesial/distal. Cusp fracture was considered when the fracture line totally or partially involved the cusp, regardless of the presence or absence of its displacement.

Statistical analysis

The Shapiro-Wilk test was used to assess

Table 2

Fracture strength (Newtons (N)), coefficient of variation (CV), strength recovery in relation to group S, and pulp and cusp floor fracture in different experimental groups

Groups	Mean ± SD (N)	CV	Strength recovery	Pulp floor fracture	Cusp fracture
S	3563[A] ± 780.7	22%	——	——	100% (10)
ET	1001[D] ± 237.6	24%	-72%	40% (4)	60% (6)
SF	1689[C] ± 280.7	17%	-52.6%	20% (2)	80% (8)
SFB	2256[B] ± 289.2	13%	-36.7%	20% (2)	80% (8)
SFP	2493[B] ± 364	15%	-30.1%	——	100% (10)

Means followed by different uppercase letters differ significantly in one-way ANOVA and Tukey's test (p <0.05)

normality of the data. ANOVA, followed by Tukey's multiple comparison test, was used to assess fracture strength. The significance level was set at 5% ($P \leq 0.05$). Statistical analysis was performed using GraphPad Prism 7 (GraphPad Software Inc., San Diego, CA, USA).

Results

In Table 2 the healthy teeth group showed greater mean fracture strength, differing statistically from ET, SF, SFB, and SFP. The group restored with transfixed fiberglass post (SFP group) had a similar pattern of failure distribution to healthy teeth group.

Discussion

The initial null hypothesis was rejected since there was statistical difference between teeth restored and not restored with glass fibers. When compared to sound teeth, endodontically treated teeth are more susceptible to fracture because there is substantial tissue loss (4), and reduced dentinal elasticity (22). The irrigating and chelating substances used in endodontics (Ethylenediaminetetraacetic acid and citric acid) can act on the inorganic structure of dentin, compromising the microhardness of the structure (23). The study simulated an unfavorable clinical scenario with class II MO cavities, in which the cusps lost support from the pulp chamber and the mesial marginal crest. The teeth were under deflection when an occlusal load was applied. The method of occlusal loading during the fracture test is another important factor. In this in vitro study, axial forces were applied to the center of the occlusal surface. Clinically, axial forces, in addition to lateral forces and fatigue loading, should be considered. The use of a approximately 6 mm steel sphere for resistance to fracture testing by Dietschi et al (24), and Soares et al (25) was shown to be ideal for molars, because it contacts the functional and nonfunctional cusps in positions close to those found clinically. Although fracture strength was statistically lower in the restored experimental groups than in the positive control group (sound teeth), the results were very impressive. Other studies such as Belli et al (15) and Taha et al (26) also observed that restored teeth, regardless of the technique or direct material used, did not present fracture strength similar to that of sound teeth, although the correct filling (three-dimensional obturation) of root canals combined with a good coronal marginal seal, allows obtaining a long-term high clinical success rate in teeth with a periapical lesion (27).

Regardless of glass fiber composition, the results reveal that, the association of this material with the restorative system presented satisfactory fracture strength. Placement of braided glass fiber (Interlig®)

on all the internal surrounding surfaces of MO cavity preparation is framed under the concept that the presence of this braided mesh could change the stress dynamics generated during the compressive test, promoting a better stress distribution at the tooth/restoration interface as a whole (28). One possible explanation would be the reduction in cusp deflection caused by anchorage and fixation of all surrounding walls with glass fiber (28). This was somewhat confirmed in the results, as fracture strength was higher when glass fiber was used than when it was not. Belli et al (15) used a polyethylene strip, but in the buccal to lingual direction, in a MOD preparation of molars, and observed a significant increase in fracture strength. On the other hand, there was no difference in the site of fracture in teeth restored with or without association of braided glass fiber strips.

A sonic composite resin system (SonicFill 2®) was used because it has a good flow and, consequently, better tooth/restoration bonding (29). SonicFill® organic matrix consists of bis-GMA, TEGDMA (5%), EBPDMA, and inorganic fillers that react to sonic energy that, in turn, decreases its viscosity. This process reduces shrinkage stress to 2.05%. Alrahlah et al (30) evaluated the polymerization depth of numerous single increment resins using the Vickers hardness test and found that SonicFill® presented the best result among the tested materials. Of the Bulk Fill resins tested (Venus Bulk Fill®, SDR®, Tetric N-Ceram Bulk Fill®, and SonicFill®) in the study by Kim et al (31), SonicFill® presented the highest microhardness values. This finding was justified by the high amount of inorganic fillers present in the material.

The teeth restored with glass fiber post transfixation showed considerable increase in fracture strength. This result was also found in the studies by Beltrão et al (21), and Scotti et al (32). The use of a transfixed post in the buccal to lingual dental crown promoted reinforcement of the cusps, thus minimizing their deflections. In addition, the transfixed post served somewhat as a threshold for the level at which fractures occurred. All fractures occurred at the level at which the post was transfixed, restricted to the dental cusp and without involvement of the pulp floor (Figure 3), favoring, to some extent, a better prognosis and survival in relation to a new rehabilitation of the fractured tooth. Bromberg et al (33) also observed high results of fracture strength in molars with transfixed fiberglass posts when compared to direct restoration with composite resin only or the inlay indirect technique. In fact, there was no statistical difference when compared to onlay indirect restoration with cusp coverings, reinforcing the area of the cusps and preventing their deflections. Performing the post transfixation does not present any clinical or technical difficulties. According to Kim et al (34), this is a relatively fast and simple

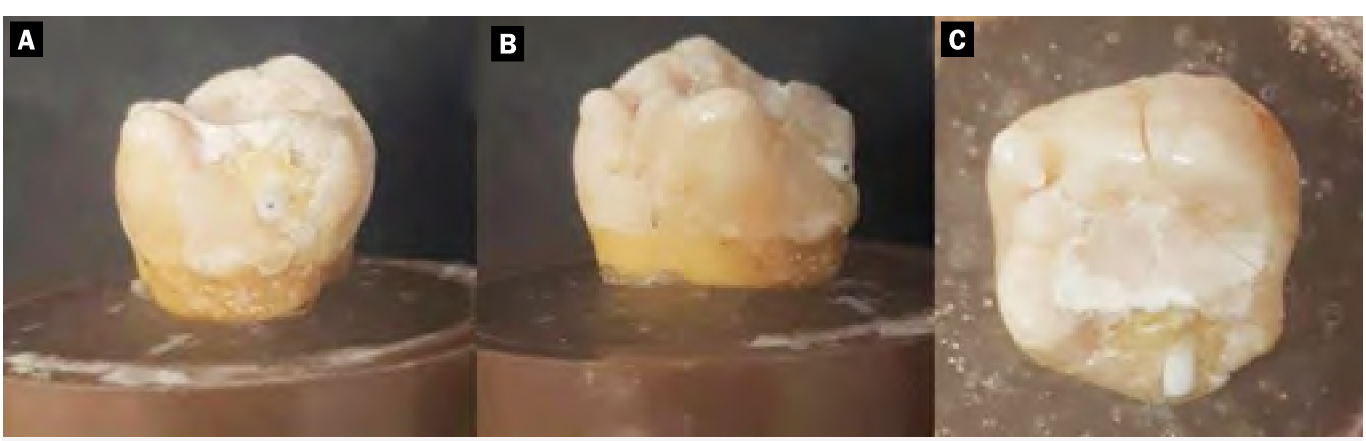

Figure 3
Tooth fracture above transfixed post level after compression test (A=lingual view; B=distal view; C=occlusal view).

procedure (30 minutes) and can be performed by the endodontist or general dentist at a low cost.
It is possible to speculate that fixation of the post closest to the occlusal surface could result in less catastrophic tooth fracture. Thus, it is of great importance to have a careful restorative planning in which it is possible to predict or induce the site of a future tooth fracture.
Considering the results of the presente study, other parameters need to be investigated, such as force vectors in different directions, checking the type of fault that occurred and the power of hermetic sealing on the fiberglass/resin interface, to evaluate the advantages and disadvantages of these restorative protocols. Given the limitations of in vitro tests and the experimental conditions of this study, the combination of sonic-resin placement system and glass fiber tends to increase the dental fracture strength.

Conclusions

It can be concluded that the glass fiber, regardless of the composition of the latter, increased the fracture strength of endodontically treated teeth. However, the use of transfixed glass fiber posts in the dental crown seems to influence the occurrence of fractures with more favorable rehabilitation than that provided by the other protocols tested.

Clinical Relevance

Fractures continue to be a major cause of loss of endodontically treated teeth. The glass fiber, regardless of composition, tends to promote an increase in fracture resistance of teeth.

Conflict of Interest

The authors declares that there is no conflict of interest.

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ORIGINAL ARTICLE

Direct pulp capping using Simvastatin and MTA in dogs' teeth: marginal adaptation SEM study

ABSTRACT

Aim: *To investigate the marginal adaptation of Simvastatin (Smv), Mineral Trioxide Aggregate (MTA), and the combination of the two materials after direct pulp capping (DPC) in dogs' teeth after three months.*
Methodology: *DPC was performed at random on 18 maxillary and mandibular incisors of two dogs. The pulpal exposures in class V cavities were capped with either Smv or Smv+MTA or MTA. All cavities were restored with Intermediate Restorative Material (IRM). After a 90-day follow-up period, the dogs were euthanised and the incisors were sectioned into two halves and studied under the scanning electron microscope (SEM). The interface between the DPC materials and pulp as well as dentine is examined for gap mean percentage to the total area of the DPC material.*
Results: *There was a statistically significant difference between Smv and Smv+MTA groups (P<0.05). Statistically, significant difference was neither observed between MTA and Smv nor between MTA and Smv+MTA groups (P>0.05). The highest mean gap area percentage value was recorded in the Smv+MTA group (3.750±1.802) followed by the MTA group (2.121±1.166) while the lowest gap percentage was recorded in the Smv group (1.339±1.271).*
Conclusions: *Simvastatin showed a good marginal adaptation property that encourages its use as DPC material.*

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Introduction

Direct pulp capping (DPC) is a procedure where a biocompatible material is applied on a vital exposed pulp to seal it preventing aggression of micro-organisms (1). Mineral Trioxide Aggregate (MTA) is a bioactive and cytocompatible material displaying consistent outcomes for pulp capping (2-4). It encourages pulp cell differentiation into dentinoblast-like cells as well as increasing angiogenic factors secretion (5). Comparing MTA to calcium hydroxide as a DPC agent, MTA exhibits a higher incidence of dentine bridge formation and a minor extent of pulpal inflammation (6). MTA yields great alkalinity of pH. Former studies of MTA physical properties have supported its effective marginal adaptation, sealing ability and low or no solubility. But still, its high cost and tooth discoloration are some of the main drawbacks (5, 6).

Simvastatin (Smv) is a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor used as a drug to lower cholesterol. It is well established that Smv has multiple influences on the induction of angiogenesis and bone formation (7-9):

-has a beneficial effect on repair and regeneration especially when an implant is needed (10);

-showed good results in the treatment of periodontal diseases (11);

-reduces periodontal ligament spaces subjected to the induction of periapical disease of rats' teeth (12).

Previous trials have confirmed that dental pulp stem cells (DPSC) handled with Smv at 1 µmol/L displayed increased growth factors angiogenesis and dentinoblastic differentiation in addition to improved Alkaline Phosphatase activity and development of mineralized nodes (13-16). Similarly, an animal experiment obtained an enhancement of DPSC induced pulp regeneration after pulpotomy with Sim at 1 µmol/L (14).

A recent study investigated the dentine thickness and continuity after direct pulp capping using Smv and MTA. There was no statistically significant difference between MTA and Smv at 0.5% and 1.5% (17). Moreover, Smv has a strong anti-inflammatory action preventing the inflammatory process induced by lipopolysaccharide (LPS) (18).

Marginal adaptation is one of the essential physical properties of a proper DPC material to have to resist the microleakage throughout its entire thickness. Consequently, the biological response of the pulp towards the DPC will be improved (19). Numerous studies analyzed the MTA marginal adaptation as a retrograde filling using a Scanning Electron Microscope (SEM), where it showed a good adaptation (19-21). Some researchers examined the dentinoblastic activity of MTA using SEM in DPC or in pulpotomy procedures. MTA showed the highest biological response (22, 23). Smv sealing ability was tested in the case of furcal perforation using dye extraction method and showed poor results (24). Several histological studies have been conducted to examine the histological response of pulp tissues to MTA and Smv but no immunohistochemical assessment of the dentine formed was done. No published data investigated the marginal adaptation of using Smv and MTA as pulp capping agents, especially in an animal model. So, we have adopted one animal model, where DPC procedure was performed on dogs' teeth.

Later, the work was divided into two parts according to the point of assessment. In the first part, the marginal adaptation of either Smv to pulp and dentine was assessed in comparison to MTA or their combination using SEM. In the second part, the biological behavior of the same tested materials using a histopathological and immunohistochemical evaluation was accomplished.

This paper is concerned about the marginal adaptation of the DPC materials. The rationale of this study is to find out a material that is comparable to MTA regarding the minimum micro-gaps at the interface between Smv and dentine as well as the pulp.

Our null hypothesis that there is no significant difference among the three tested DPC agents; Smv, Smv+MTA and MTA. The present study was designed to compare the marginal adaptation of using Smv, Smv+MTA and MTA as DPC materials in dogs' incisors, over three months using SEM.

Materials and Methods

The present part of the animal study started after the approval of Research Ethical Committee (REC), Faculty of Dentistry, Suez Canal University, Ismailia, Egypt (Registration No. 212/2019). We worked according to the ethical guidelines and regulations of the International Guiding Principles for Biomedical Research Involving Animals (Geneva, 2012). This study was carried out on 2 Mongrel dogs at the age of 2 years old and with a weight of 15-18 kg having permanent dentition. Housing, operative procedures, and sacrificing were done at the Department of Veterinary Surgery, Faculty of Veterinary Medicine, Suez Canal University.

Sample size determination

G* power statistical analysis software was used to determine sufficient sample size (25). The sample size was determined on a sample population of 18 incisors with an α error probability of 0.05, effect size f of 0.82 , and a 0.8 power (1-β). Five maxillary and four mandibular incisors were capped for a total of nine incisors in each dog (total sample size=18) (26).

Operative procedure

Dogs were kept under the same managemental and nutritional regimens during the experiment. Food and water were withheld 6-8 hrs before anesthesia. Each dog was premedicated with I/M injection of chloropromazine hydrochloride (Misr Co. Pharm. Industries, S.A.A, Cairo, Egypt) in a dose of 1mg /kg. The site of operation was aseptically prepared, and then general anesthesia was conducted by I/V injection of thiopental sodium (Sandoz GmbH, Kund, Austria) 2.5% solution until the main reflexes disappeared. After ensuring dryness of the field using cotton rolls and separating the jaws by a modified plastic syringe, the teeth were subjected to a class V preparation on their labial surface coronal to the gingival margin. Inverted cone bur size 1 (Dentsply Maillefer, Tulsa, Oklahoma, USA) at high speed (30,000 rpm) contra-angle handpiece (NSK, Tokyo, Japan) was used under a water coolant until the pink color was noticed at the floor of the cavity but without exposure. Later, the exposure was achieved by a sharp probe to standardize the size. Bleeding was controlled after a few seconds with a moistened cotton pellet by 2.5 % sodium hypochlorite NaOCl (Clorox; Household Cleaning Products of Egypt, Cairo, Egypt) and sterile cotton pellets pressed over the exposure site (17). Rinsing the cavity was done using normal saline (El Fath, Cairo, Egypt) and later the cavity was dried and prepared to receive the DPC material.

Grouping of teeth according to the experimental DPC material

Samples randomization

One of the co-authors who was not involved in the clinical procedure performed the blind allocation of the teeth after running randomization that was done for grouping by Microsoft Excel. Incisors were randomly and equally divided into three groups according to the DPC material applied to the exposure site (n=18) according to the following:

Group A (n=6): using Smv (Sigma-Aldrich, St. Louis, MO, USA); tablet was ground and weighted to obtain 1.5 mg (13, 17, 18) and then mixed with distilled water to produce a creamy mix.

Group B (n=6): using Smv+ MTA (Angelus, Londrina, Brazil); the mixture was prepared where the ratio of the powder of ground tablet Smv (1.5 mg) to MTA to the distilled water is 1.5:1.5:1 to obtain a creamy mix.

Group C (n=6): using MTA; it was prepared where the ratio of the powder to the distilled water is 3:1, according to the manufacturer's instructions.

The DPC material was then applied to the exposure site using a plastic instrument and compacted with a hand plugger. Later, final restoration was placed using Inter-

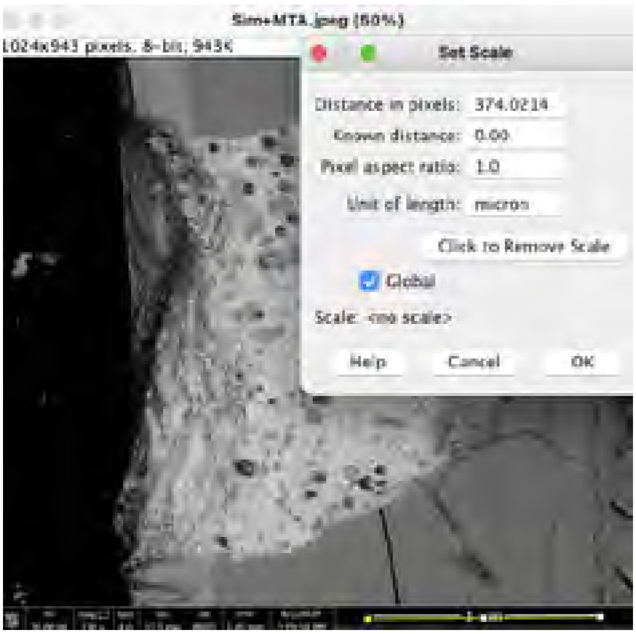

Figure 1
Photograph showing image analysis by image J software for setting image scale.

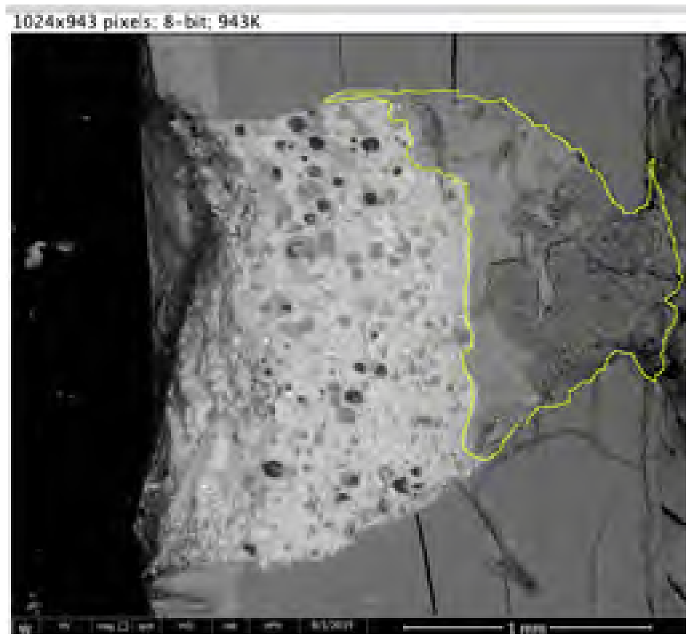

Figure 2
Photograph showing image analysis by image J software for total area selection of the DPC material.

mediate Restorative Material (IRM) (Dentsply, Charlote, U.S.A).

Euthanasia and jaw sectioning
Dogs were clinically observed daily for a follow-up period of 90 days during the study period for recording any postoperative complications. They were then euthanised by thiopental sodium overdose. The teeth contained within bone pieces were removed and reduced in size to fragments measuring approximately 3 mm of coronal height and 3 mm of root height. The samples were stored in buffered 10% formalin at 4 °C for 72 h. Then teeth were then dried before the SEM evaluation.

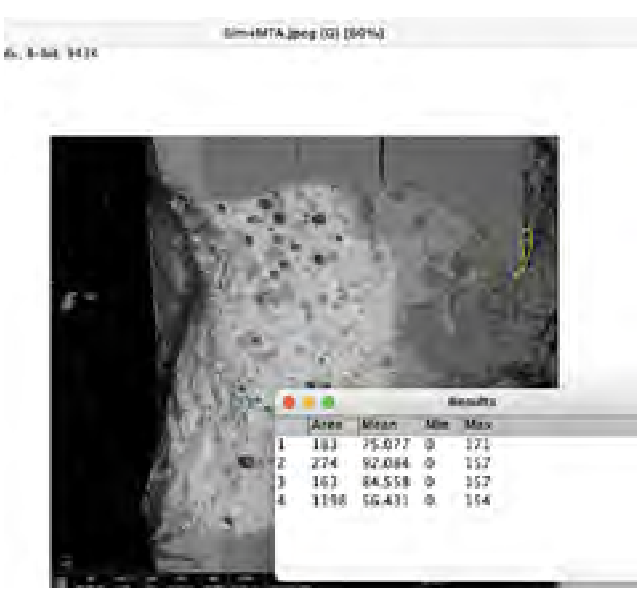

Figure 3
Photograph showing image analysis by image J software for measuring and analysis of gap area.

SEM evaluation
<u>Sectioning of the teeth</u>
The teeth were carefully notched in a labiolingual direction by a diamond disc to obtain a crack. Afterward, by using an isomet low-speed the samples were cut into two halves. The samples were washed using saline and only the better half was used for the evaluation.
<u>Preparation of samples for SEM evaluation</u>
The samples were dehydrated in sequence with 80% alcohol for 15 minutes, 90% alcohol for 15 minutes, and 100% alcohol for 20 minutes. The coronal portion of the samples was viewed under SEM (Model Quanta, FEI, Eindhoven, Netherlands) at 20 kV with 120-180X, 1000X, and 4000X magnifications. The marginal

adaptation of the DPC material to the pulp and dentine was evaluated using an image analysis software (Image J version 1.53e; National Institutes of Health, Bethesda, MD, USA). The scale measurement was calibrated for each image to micron (Figrure 1). Each photomicrograph was analyzed by measuring the total area of the DPC (Figure 2), then the gap percentage was measured to the total area after adding all areas of gaps at the interface of the DPC material to the pulp and dentine (Figure 3) according to the following equation (Gap%=addition of the gap areas at the interface between the DPC material and pulp and dentine/total area of the DPC material X100). Two calibrated examiners assessed readings blindly, where each one of them repeated the assessment two times within a week to check the intra-examiner reliability. Additionally, comparing both examiners showed the reliability between them (inter-examiner reliability).

Statistical analysis

The raw data were subjected to preliminary testing to verify the normality of the results using Kolmogorov-Smirnov and Shapiro-Wilk tests. The mean and standard deviation (SD) values were calculated for each group. Comparison among the 3 groups was conducted using the One-Way Analysis of variance (ANOVA) test at a significance level of $P \leq 0.05$. Then multiple pairwise comparisons between groups were performed using Tukey's post hoc test using SPSS software version 26 (IBM, Armonk, NY, USA).

Results

One-way ANOVA test displayed a statistically significant difference between groups at p-value ≤0.05 (p=0.032). Analysis of Tukey's post hoc test revealed a significant difference between Smv and Smv+MTA groups (p=0.028). Whereas there was no statistically significant difference between the MTA and Smv+MTA groups (p=0.157). Additionally, no significant difference between MTA and Smv groups (p=0.624). The highest mean gap area percentage value was recorded in Smv+MTA group (3.750±1.802) followed by MTA group (2.121±1.166) while the lowest gap percentage was recorded in Smv group (1.339±1.271) (Table 1, Figure 4).

Discussion

The prognosis of DPC depends upon many factors. One factor is the quality of the DPC material adaptation to prevent microbial ingress; accordingly pulpal healing predictably happened (27). Poor adaptation of DPC materials to dentine results in gaps and causes fluid fluctuations into dentinal tubules (28). Whenever the DPC material is capable of providing the biological seal, the result will be towards dentine bridge formation and regeneration (26, 27, 29). Dogs were selected as an animal model due to the similar dentine synthesis as human beings. Despite the difference in the rate reparative dentine formation, dogs' pulps are equivalent to that of humans. Interestingly, the size of the pulp offers a proper sample for the histopathological

Table 1

Mean and standard deviation of gap area percentage values in the three tested groups

Groups	No. of samples	Mean±SD	P-value
Smv	6	1.339^{b}±1.271	0.032*
Smv+MTA	6	3.750^{a}±1.802	
MTA	6	2.121^{ab}±1.166	

Mean values with the same superscript letters are not statistically significant at P≤0.05.
Mean values with different superscript letters are statistically significant at P≤0.05.

evaluation. Furthermore, dogs afford a wide range of teeth that can permit the comparison of different materials in the same dogs (22, 30). DPC was conducted and then the assessment was done in two parts. The first part was completed to evaluate the marginal adaptation of the tested materials. The second part was completed to examine the biological effect of the tested materials through histopathological and immunohistochemical analysis.
This article is concerned about the marginal adaptation of Smv, a mixture of Smv+MTA and MTA using SEM after 90 days to assess the long-term adaptation of the tested materials and hence the advanced pulpal response. Adaptation of Smv as a DPC material was not investigated previously. That is why we have chosen this point of investigation in an animal model to simulate the clinical situation. MTA is considered the gold standard DPC material of choice (5, 6).
In a recent study by Dianat et al. they recommended that the addition of Smv to MTA may enhance the pulpal repair (17). Proper partial isolation was followed in the study before starting the cavity and it was ensured through the procedure (30). Class V cavity in anterior teeth was chosen rather than Class I in posterior teeth to avoid any variation in the occlusal force that might affect the results (22). Mechanical Pulpal exposure was achieved by a sharp explorer to prevent pulpal damage that might be

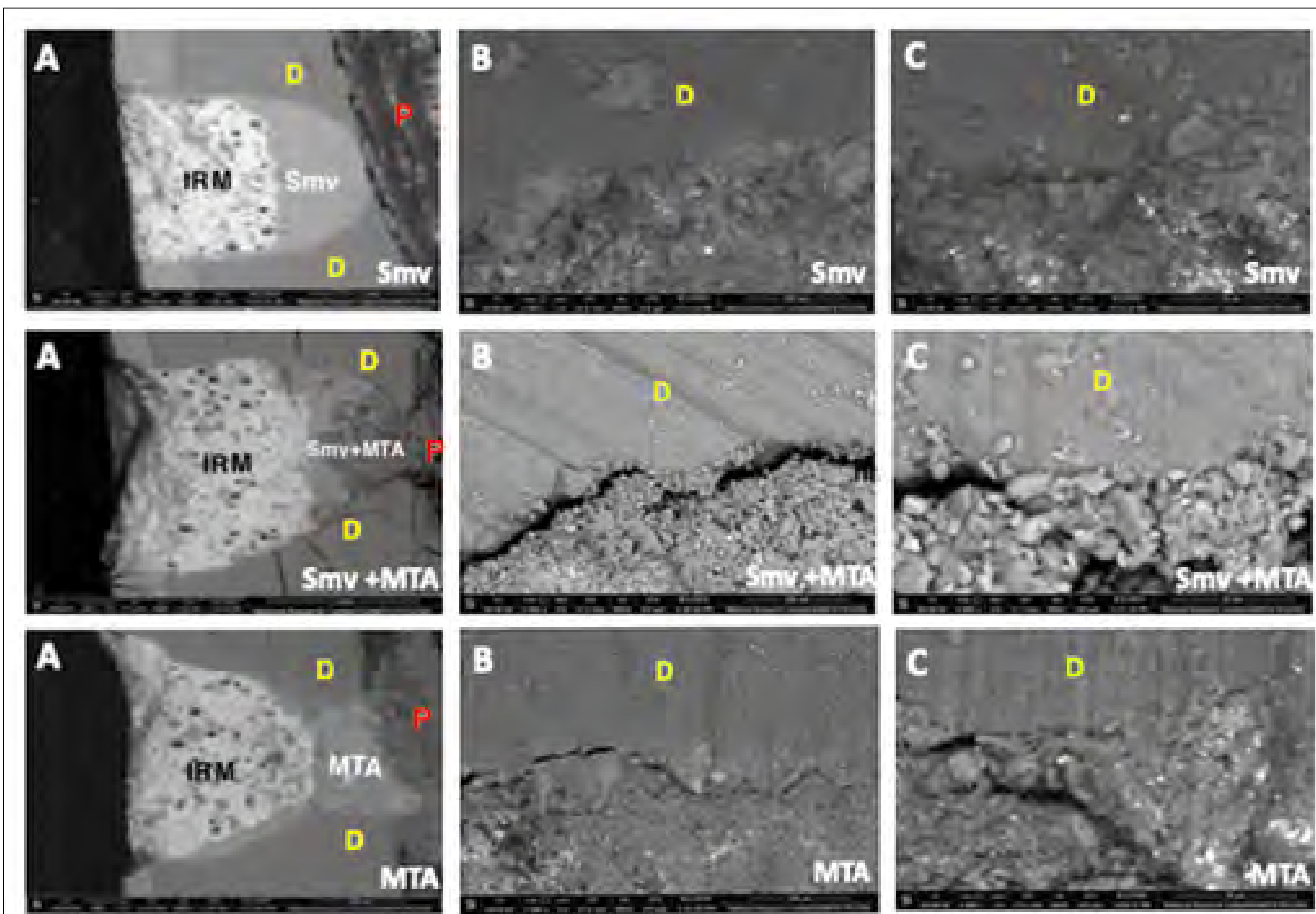

Figure 4
Representative SEM micrographs displaying cavities filled with Smv, Smv+MTA, MTA then IRM at A) 120X, B) 1000X, C) 4000X representing the interfaces between DPC materials and dentine as well as pulp. Where D stands for Dentine and P for Pulp.

happened by using a bur, to ensure that pulpal exposure would be consistent in size (29). IRM was used as a coronal restoration as it was recommended due to its acceptable compressive strength and hardness that could be placed for a year (31). SEM was used for evaluation because it is considered the main tool to measure the tooth structures/material interface (19, 20, 22, 23, 27, 28). However, using a non-destructive tool such as micro-computed tomography (μCT) could be beneficial. This might be a limitation in our study, but the use of μCT is costly and difficult to work with.

We partly rejected the null hypothesis, as we found a significant difference between Smv+ MTA and Smv groups. On the other hand, a significant difference was neither obtained between Smv and MTA, nor between MTA and Smv+MTA groups. The highest mean of gap area percentage value was obtained in the Smv+MTA group. Previously, combining Smv+MTA showed lower cell growth, decreased Alkaline Phosphatase activity , and decreased levels of mineralization markers than combining Smv + αTricalcium phosphate cements (32). On the other hand, Dianat at al. demonstrated that 1.5% Smv gel below MTA showed active pulpal repair with well-formed dentine when placed as a DPC material in all of the samples treated with this combination (17). Different study designs, concentrations, and forms of the Smv combination might be the reason for such discrepancies in results. High gap (%) in this group might be because of the lack of any chemical or mechanical bond to both dentine and pulp. Smv group revealed the lowest mean of gap area percentage value. Many studies showed the beneficial action of Smv on DPSC differentiation and its potent anti-inflammatory action that can enhance pulpal regeneration (13-18). Likewise, its valuable effect on apical periodontitis adds to its advantages (12). Nevertheless, a recent study exhibited an adverse effect of Smv on osteoblast differentiation but it was a time-dose dependent reduction in cells (33). They found a decrease in cell viability and a significant increase of mineralization in a late mineralization stage while the alkaline phosphatase turnover was unaltered (33). Higher concentrations of Smv have caused increased rates of cell death. The use of simvastatin as a pulp capping material requires a thorough evaluation of the optimum dose (13, 17, 34, 35). That is why we used 1.5 mg of Smv in the current study. Although there was no statistically significant difference between MTA and Smv, MTA group showed a higher mean of gap area percentage than Smv of mean 2.12 μm, but lower than the previous study where the mean value was 4.92 μm (36). MTA has a slow setting reaction that might participate in leakage and surface disintegration that causes loss of marginal adaptation (31).

In accordance with the mean of gap area percentage we detected in the MTA group, Torbinejad et al. noticed a gap equal to 2.5 μm (20). A study emphasized the superior sealing ability of MTA due to 1.5 μm MTA particles which are smaller than the diameter of some dentinal tubules (2-5 μm) (37). They added that formation of a hydraulic seal after hydration is one of the main causes of sealing ability (37). Likewise, an insoluble barrier might be formed against microleakage (20). All the previously mentioned studies were carried out to assess MTA marginal adaption as a retrograde filling material. Smv showed a comparable marginal adaptation as good as MTA. Further long-term studies are required to support our findings with larger sample size. More physical properties are required to be studied such as push-out bond strength, compressive strength, and hardness of Smv.

Conclusions

Under the limitation of this study, taking into consideration Smv's low price, superior Smv marginal adaptation in the present study favors its use as a DPC material.

Clinical Relevance

This study emphasizes that simvastatin has a good marginal adaptation in comparison to the gold standard direct pulp capping agent (MTA). Thus, Smv can be a promising material to be used in direct pulp capping treatment with favourable outcome. It is important to find an inexpensive alternative to MTA which displays good biological and physicochemical properties.

Conflict of Interest

The authors declare that there is no conflict of interest.

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ORIGINAL ARTICLE

Clinical reproducibility of Tri Auto ZX2 dedicated motor and electronic foraminal locator in determining rootcanal working length

ABSTRACT

Aim: *To evaluate the clinical reproducibility in determining the working length of Tri Auto ZX2 electronic foraminal locator (EFL) with instruments activated in OGP function by comparing the results obtained by this new device with those obtained by Root ZX II EFL.*

Methodology: *One hundred twenty-five teeth (72 vital and 60 non-vital pulps) were measured by Root ZX II EFL and Tri Auto ZX2 with instruments activated in OGP function to determine their respective working length, which was defined as a zero reading on the EFL. The instrument length was fixed with a rubber stop and measured with a caliper to an accuracy of 0.1 mm. The values obtained by Root ZX II and Tri Auto ZX2 were statistically compared by student t test with 5% of significance. The agreement between the different devices were determined in percentage. The statistical correlation was also used to determine the agreement between the two EFLs.*

Results: *There were no significant differences between tested EFLs measurements at "0.0" in vital, non-vital and in the overall analysis (P>0.05). Considering the agreement between devices, the results revealed 98.66% and 100% of concordant values in vital and non-vital pulps, respectively. The R_2 coefficient obtained was close to 1 in cases of vital pulp, non-vital pulp and in overall analysis, denoting a strong agreement between the EFLs.*

Conclusions: *The clinical reproducibility of Tri Auto ZX2 was confirmed when compared to Root ZX II.*

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Introduction

Accurate root canal length determination is an essential step in root canal therapy. The working length (WL) determination has a direct influence on root canal preparation and filling, and is considered a significant predictor of successful outcome in root canal therapy (1, 2). Root canal length can be determined using periapical radiographs, considering the radiographic apex as a reference for establishing the tooth length. To this, it is recommended a shortening regarding the radiographic apex in order to approach the apical constriction position. Even so, in most cases, the apical foramen does not coincide with the radiographic apex, which may lead to incorrect WL compared to the real position of the apical foramen (1, 2).
The use of electronic devices to determine the WL has become common. Several studies have been conducted on various commercially available electronic foramen locators (EFLs) and reported their accuracy and efficiency, even in adverse situations, such as the presence of blood or in cases of wide foramens (3-5). Additionally, the radiographic method has limitations that include image distortion, superimposition of roots and anatomical structures, and higher exposure to ionizing radiation when compared to EFLs methods (5).
Endodontic motors with integrated apex locators have been developed to continuously measure the WL during canal preparation. Root ZX II (J. Morita, Tokyo, Japan), Tri Auto ZX (J. Morita) and VDW Gold (VDW, Munich, Germany) are examples of such type of motors. These devices can be configured to have the EFL used isolated, like the standard EFLs, or a mode which the EFL and motor are activated simultaneously, with the first influencing the motor during the mechanical preparation. These motors have the capability to stop or reverse the activation of the nickel-titanium instrument as the estimated end point of the root canal is reached. Recently, the Tri Auto ZX2 motor with integrated EFL (J. Morita) was launched featured by the “optimum glide path (OGP)” mode, which is a combination of watch-winding-like and balanced-force-like reciprocating motions. The OGP motion is intended for safe glide path preparation, whereas scant information is available regarding the how such a movement influences the EFL readings during glide path preparation. Although the EFL of such motors seems to work on the same way that EFL not integrated to motors, the motion can have a direct influence on the working length determination.
Therefore, the aim of this study was to evaluate the clinical reproducibility in determining the working length of Tri Auto ZX2 EFL with instruments activated in OGP function by comparing the results obtained by this new device with those obtained by Root ZX II EFL. The null hypothesis tested was that there are no differences in the WL measures obtained by Tri Auto ZX2 and Root ZX II EFL.

Materials and Methods

This study was approved by the Local Ethics Committee (protocol number #550.788) and included an informed written consent in compliance with ethical principles obtained from each patient before the treatment was initiated. One hundred twenty-five teeth with fully formed apices, without root resorption and apical radiolucency in initial periapical radiographs were included. Teeth with previous endodontic treatment, internal or external root resorption, and/or intracanal calcification were excluded. Patients with active systemic disease and physical or mental disability were also excluded. Pulp tests revealed 72 vital and 60 non-vital pulps.
All clinical procedures and measurements were performed by a single experienced operator. Local anesthesia was administrated in all cases. Access cavities were prepared with round diamond burs and refined with an Endo-Z bur. When present, all metallic restorations were completely removed prior to conducting the measure-

ments. Then, rubber dam isolation of the tooth was performed. Root canals were irrigated during all endodontic treatment using 2.5% sodium hypochlorite. Excess fluid from the pulp chamber was removed, but canal was not dried before electronic measurements. Cervical root canal preparation was performed using a 40/.10 Race instrument (FKG Dentaire, La Chauxde-Fonds, Switzerland) inserted up to 2/3 of root canal length measured from initial radiograph. Then, electronic WL was performed using the Root ZXII EFL and a #15 C-Pilot instrument (VDW) until the device reach the "0.0" level, according to the manufacturer´s instruction. Measurements were considered to be valid if the reading remained stable for at least 5 s. The silicon stop was adjusted, and the distance between the silicon stop and the instrument tip was measured with a 0.1 mm precision digital caliper (Mitutoyo, Suzano, SP, Brazil). This length was recorded as Root ZX II Length (RZL). After that, 15/.02 Scout Race instruments (FKG Dentaire) were used in OGP function driven by the Tri Auto ZX2 device with automatic apical-stop activated until the zero reading was indicated by "Apex" or "0.0" in device display. At this time, radiographs were performed using a digital sensor (Kodak RVG 6100; Carestream, Rochester, USA). Then, instruments were removed, and the length was measured as described previously and recorded as Tri Auto ZX2 Length (TAL).

Statistical analysis

The values obtained by RZL and TAL were statistically compared by student t test with 5% of significance. The agreement between the different electronic WL devices were determined in percentage. The statistical correlation was also used to determine the agreement between the two EFLs. Statistical analysis was performed using SPSS (SPSS Inc., Chicago, IL, USA).

Results

There were no significant differences between tested EFLs measurements at "0.0" in vital, non-vital and in the overall analysis (P>0.05) (Table 1). Considering the agreement between devices, the results revealed 98.66% and 100% of concordant values in vital and non-vital cases, respectively. Figure 1 presents the statistical correlation of the WL determination of the two EFLs in cases of vital pulp, non-vital pulp and overall. The R_2 coefficient obtained was close to 1 in all conditions, denoting a strong agreement between the EFLs.

Table 1

Mean and standard deviation of measurements of tooth length using Root ZX II and Tri Auto ZX2

	Vital	Non-vital	Overall
Root ZX II	22.10±2.10^A	22.16±2.17^A	22.13±2.13^A
Tri Auto ZX2	22.11±2.10^A	22.16±2.17^A	22.14±2.13^A

The same capital letters represent no statistically significant difference between EFLs (P>0.05).

Discussion

Several studies have been conducted to evaluate the accuracy of EFLs in determining the WL with satisfactory results, especially after the advent of impedance based devices (3-6). These studies validated EFLs clinical use as a simple and effective alternative to the dubious determination of WL using periapical radiograph. Even the hybrid devices, which combine a dedicated electric motor to an EFL, seems to be precise when inserted until the apex foramen (7-10). However, to the best of the author's knowledge no study evaluated the recent launched Tri Auto ZX2 hybrid device under the OGP function clinically. Therefore, the present study assessed the clinical agreement of Tri Auto ZX2 EFL by comparing the results obtained with this new device with those obtained by Root ZX II EFL.

Considering the periapical radiograph limitations to adequately determine the WL, the present study adopted the Root ZXII EFL as a reference of comparison. The choice for Root ZX II was based on an extensive amount of *in vitro* and *in vivo* studies that have shown a good efficacy and accuracy of this EFL (11-13). In fact,

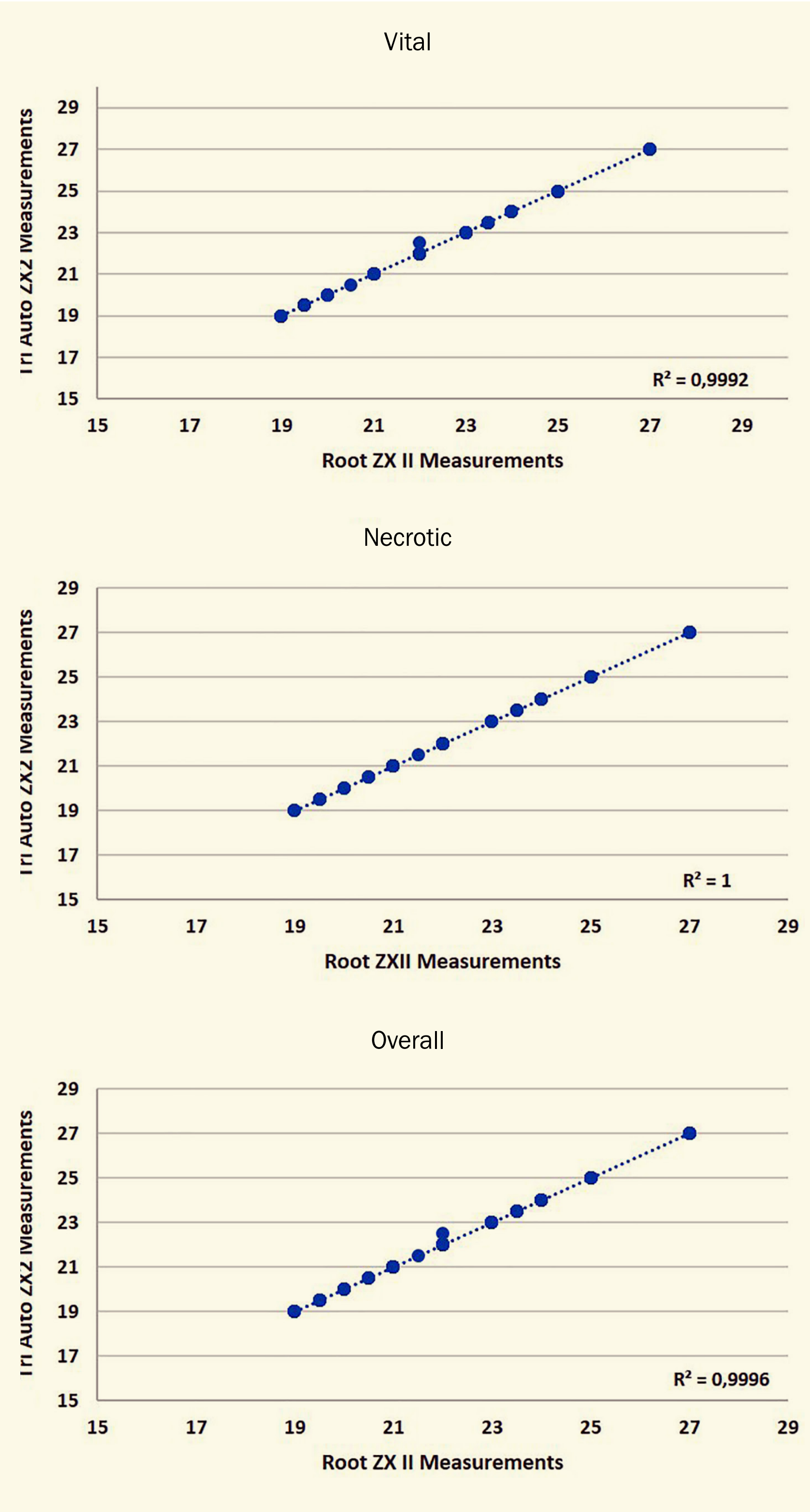

Figure 1
Statistical correlation of the working length determination between EFLs in vital, non-vital and overall cases.

several authors consider this EFL as the gold-standard. To check Tri Auto ZX2 accuracy and reproducibility, both devices were used until reach the apical foramen, at the “0.0” landmark, considered as the major foramen which is a position that can be consistently located (1, 5, 14).
The results of the present study point out that the coincidence of accuracy at the “0.0” landmarks of Tri Auto ZX2 and Root ZX II were 98.66% for vital pulp and 100% for non-vital pulps, with no differences between the two devices. This was also confirmed by the R_2 coefficient obtained which was close to 1 in all conditions, denoting a strong agreement between the EFLs. Therefore, the null hypothesis tested was upheld. Although this is the first study evaluating Tri Auto ZX2 device, previous studies demonstrated similar results when comparing EFL measurements with the measurements provided by electric motor combined to EFL with activated instrument (7-10). Although some previous studies reported that the accuracy of EALs was lower in the presence of non-vital canal content compared with the vital pulp tissue (5, 15), our results showed similar accuracy for both pulp conditions, which is in agreement with other reports (16, 17).
It is important to emphasize that comparisons of results should take in account the adoption of the same parameters of apical limits and the use of similar methods. In the present study, the standardization of the WL length measurements involved the irrigant concentration, coronal preparation, reference point and also the use of teeth serving as their own controls. This allows certain variables to be controlled in the clinical setting. Even so, some differences were present in the current experimental design, such as the use of different instruments (#15 stainless-steel C-Pilot, for the Root ZXII EFL and 15/0.02 NiTi Scout Race for Tri Auto ZX2). However, previous studies did not point out differences in foraminal length measurement when these different alloys were compared (18, 19). It is also important to emphasize that, although a direct comparison and the reproducibility between Root ZXII EFL and Tri Auto ZX2 were per-

formed, it is not possible to confirm the efficiency of both EFLs within the current study setup. However, it is well known that Root ZXII EFL is considered a gold-standard for foraminal length determination (4, 5, 11, 14), and in consequence it is suggested that Tri Auto ZX2 has a good efficiency. Even so, future *in vitro or in vivo* studies with different setups should be performed to confirm Tri Auto ZX2 clinical efficacy.

Conclusions

The clinical reproducibility of Tri Auto ZX2 EFL with instruments operated under OGP motion was confirmed when compared to Root ZX II.

Clinical Relevance

Tri Auto ZX2 EAL with instruments activated in OGP function seems to have a similar clinical performance of Root ZX II EFL.

Conflict of Interest

The authors declare that they have no conflict of interest.

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None.

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ORIGINAL ARTICLE

Efficacy of XP-endo Finisher-R in enhancing removal of bioceramic sealer from oval root canal: a micro CT study

ABSTRACT

Aim: *To investigate the efficacy of the XP-endo Finisher-R (XP-FR) and manual H-filing in enhancing the removal of Totalfill BC sealer from oval root canals.*
Methodology: *Forty mandibular incisors were prepared using ProTaper Next up to file X3 then filled with warm vertical condensation using Totalfill BC sealer. The volume of the root canal filling post-obturation and the remaining volume post-retreatment using Mani NRT-GPR system were assessed using Micro-CT (μ-CT). The total volume in 3 mm was calculated from the cervical line till the root apex using the CT-an software. Specimens were randomly divided into two equal groups n=20 according to the supplementary approach of gutta percha removal used; where Group I: XP-FR was used for 1 min, and Group II: size 30 H-file was used in a filing motion for 1 min. Specimens were again scanned using μ-CT and the remaining gutta percha volume was calculated. Data were statistically analyzed using Wilcoxon, Man-Whitney, Friedman tests with significance level of 5%.*
Results: *Significant reduction of the remining filling material was recorded post-retreatment with percentage of reduction of 63.34%, 78.53%, and 66.21% at the apical, middle, and coronal thirds respectively using Mani NRT-GPR system. Supplementary removal approaches significantly improved filling material removal (P<0.05). XP-FR removed significantly more filling material than manual H-filing with percentage of filling material reduction of 52.22%, 34.92%, and 40.60% compared to 21.89%, 18.43%, 31.72% in the apical, middle, and coronal thirds (Man-Whitney test, P<0.001).*
Conclusions: *Rotary retreatment files failed to totally remove the root canal filling material. Supplementary methods have improved root canal filling material removal; where XP-FR significantly removed more filling than manual H-filing.*

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Introduction

Persistent intracanal microorganisms following primary root canal treatment are causative factors of treatment failure (1, 2). Root canal system disinfection following total elimination of the root canal filling material is the main objective of nonsurgical root canal retreatment. Several techniques have been advocated for intracanal filling material removal using manual and engine-driven files (3). Moreover, supplementary techniques were introduced as lasers and ultrasonics with or without solvents (4-7).
Gutta percha and sealer removal from oval canals is quite challenging (8) as most endodontic files fail to touch all the root canal walls. Therefore, supplementary techniques following retreatment procedures would be beneficial.
Recently, bioceramic sealers (BCS) have been introduced as root canal sealers. BCS showed superior biocompatibility, physical properties, biomineralization, antimicrobial activity, and sealing ability (9-12). Totalfill BC sealer (FKG Dentaire SA, La Chaux-de-Fonds, Switzerland) is a premixed, injectable, calcium silicate-based, containing zirconium oxide, calcium phosphate monobasic, calcium silicates, calcium hydroxide, as well as various filling and thickening agents. BCS use moisture within the dentinal tubules to complete its setting by forming hydroxyapatite resulting in dentin-sealer bond. Retrieval of BCS is considered a difficult task due to the formation of a strong chemical bond with hydroxyapatite (13-16).
Many engine-driven file systems were designed for gutta percha removal. Mani NRT-GPR system (Mani Inc, Tokyo, Japan) includes four rotary files with 0.04 taper. Two stainless steel files 1S and 2S with tip size 0.7 mm and 0.5 mm respectively designed to remove the root canal filling material from the coronal two thirds of the canal. Two NiTi files 3N and 4N with tip size 0.4 mm and 0.3 mm respectively used to the full working length of the root canal. Recently, XP-endo Finisher-R (XP-FR) (FKG Dentaire, La Chaux-de-Fonds, Switzerland) was introduced as a new variation of the XP Endo Finisher "MaxWire" Martensite-Austenite Electropolish Flex (FKG Dentaire, La Chaux-de-Fonds, Switzerland) to aid in the removal of gutta percha and sealer from the root canals. It has a tip size of 0.3 mm and a zero taper. The manufacturer claims it has the ability to expand in the root canal at body temperature which enables it to abrade the root canal walls (17). Micro-CT (μ-CT) allows for three-dimensional evaluation of the remaining intracanal filling material (18). The current study aimed to investigate the ability of XP-FR and manual H-filing in enhancing the removal of Totalfill BC sealer from oval root canals using μ-CT. The null hypothesis tested is that there is no difference between XP-FR and manual H-filing in removal of Totalfill BC sealer from oval root canals.

Materials and Methods

Sample selection
After approval from the local Ethics Committee (Protocol No 037-01-19), forty human mandibular mature incisors were selected. Teeth with oval root canals, completely formed apices, tooth length ranging from 19 to 21 mm and root curvature <20° calculated using Schneider method (19) were included. Teeth were cleaned then immersed in 2.5% sodium hypochlorite (NaOCl) for 24 hours then kept in 10% formalin till use. Teeth were subjected to digital radiographs in mesiodistal and buccolingual directions. The canal is considered oval when its buccolingual dimension is at least double its mesiodistal dimension. Teeth with more than one root canal, calcification, accessory root canals, or previous root canal treatment were excluded.

Root canal preparation
One operator (the first author) performed all the procedures. Diamond round burs mounted on high-speed handpiece were used to prepare access cavities. Working length (WL) was calculated by subtracting

0.5 mm from the tooth length. Teeth were placed in silicone mount to facilitate positioning for the µ-CT scan. Root canals were prepared using ProTaper Next (Dentsply Tulsa Dental; Tulsa, OK, USA) with rotational speed of 300 rpm and torque 2 N/cm using X-Smart endodontic motor (Dentsply Tulsa Dental; Tulsa, OK, USA) till X3 (30, 0.07). Root canal irrigation was done using 3 ml of 2.5% NaOCl. Final irrigation was done using 10 ml of 2.5% NaOCl followed by 1 ml of 17% Ethylenediamine Tetraacetic Acid (EDTA) MD-cleanser, (MetaBiomed, Chungcheongbuk-do, Republic of Korea) to remove the smear layer. Final rinse using 5 ml of distilled water was done followed by canal drying using paper points X3 (Dentsply Tulsa Dental; Tulsa, OK, USA).

Root canal obturation

Warm vertical condensation technique was used. Thin layer of TotalFill BC sealer was applied using paper point X2. Master gutta percha cone X3 (Dentsply Tulsa Dental; Tulsa, OK, USA) was selected and inserted into the canal, tug-back was ensured, and down packing was performed using System B (Analytic Technology, Redmond, WA). Fine medium plugger was selected for the down pack procedures. Temperature was set at 200 ºC and power at 10. Gutta percha was cut off at the root canal orifice. Plugger was reactivated at the same temperature to compact the gutta percha till reaching 5 mm of the WL. Gentle pressure was maintained for 10 seconds. Backfill of the canal was performed to the root canal orifice level.

Access cavities were temporized using Coltosol (Coltene, Altstätten, Switzerland). Obturation quality was evaluated using periapical radiographs in mesiodistal and buccolingual projections. Teeth were kept in incubator at 37 ºC and 100% humidity for 30 days to ensure maximum setting of the root canal sealer.

Post-obturation micro-CT scanning

Following obturation, teeth were scanned using micro-CT (SkyScan 1172 Kontich, Belgium). Teeth were placed in silicone mold to allow each tooth to be scanned during all stages of evaluation in the same position. During acquisition, teeth were rotated 360 degrees around the vertical axis with 0.6 step-size rotation, with 100 kV,100 mA, and 13.7 µm voxel resolution, and a cupper aluminum filter. Scanning time was 40 minutes in average for each tooth. Images were saved as 16-bit raw data TIFF files. Raw data were reconstructed to bitmap files using software (NRecon v.1.6.7.2; Bruker-microCT, Kontich, Belgium). Total volume in mm3 of gutta percha was calculated from the cementoenamel (cervical) line till the root apex by integration of the regions of interest in all cross sections using the CT-an software (Bruker-micro CT).

Root canal retreatment procedures

Following Coltosol removal, gutta percha was removed using Mani GPR Files. These files were operated at 1000 rpm and no torque control, as recommended by the manufacturer. Gutta percha was removed using the 1S file 3 mm from the canal orifice followed by the 2S instrument in the coronal third for 1 to 2 seconds. The 3N instrument was used in the coronal two thirds of the canal followed by the 4N instrument to the WL and operated for 1 to 2 seconds. Root canal preparation was then expanded using X3 (30, 0.07) and X4 (40, 0.06).

All files were used in 3 mm vertical strokes with light apical pressure. Retreatment was considered complete when the WL could be reached and there was no gutta percha or sealer on the instruments. All instruments were used for 5 root canals and then discarded. Root canals were irrigated using 2.5% NaOCl during the procedure.

Post-retreatment µ-CT scanning

Teeth were scanned post-retreatment using the same initial parameters for scanning, reconstruction, and calculation of the remaining gutta percha volume in the root canals. Total volume in mm3 of filling material was calculated from the cementoenamel (cervical) line till the root apex by integration of the regions of interest in

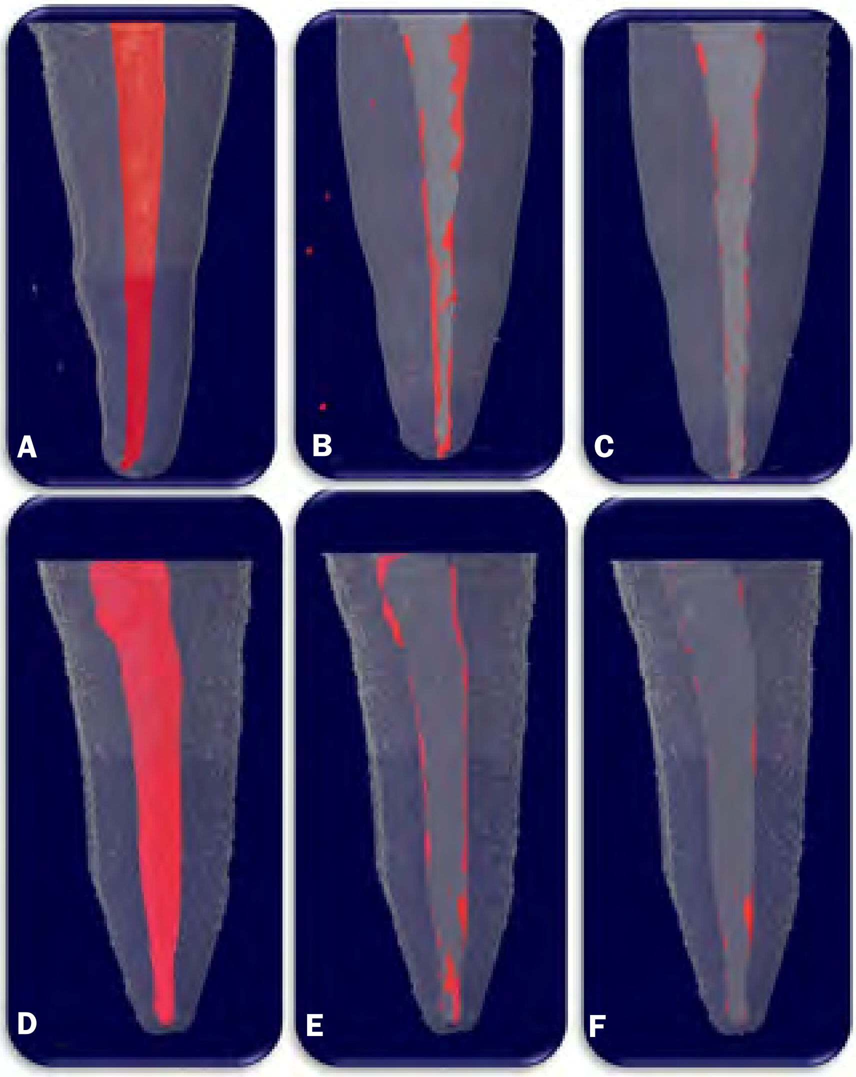

Figure 1
Micro-CT images of representative samples before (**A** & **D**), after re-treatment procedure (**B** & **E**), after supplementary approach XP-FR (**C**) and H-file (**F**).

all cross sections. Volume (mm 3) of root filling material removed was calculated as the difference between initial (post-obturation) and final readings (post-retreatment). Percentage of reduction in filling material was also calculated. Teeth were randomly divided into two equal groups (n=20) according to the supplementary root canal cleaning method used.

Supplementary cleaning methods
<u>XP-endo Finisher-R</u>
For group I, XP-FR was removed from the plastic tube in rotation motion by lateral movement.
Speed was set at 800 rpm and torque 2 N/cm and a 16:1 reduction-gear contra-angle handpiece was used. Each root canal was filled by 2 ml of 2.5% NaOCl and XP-FR was used in a gentle vertical motion 7-8 mm strokes up to the WL. XP-FR was used in a brushing motion against the walls for one minute inside the canal. XP-FR instrument was discarded after preparation of 4 canals.
<u>Manual filing</u>
For group II, Hedstrom file number 30 was placed to the WL and used in a brushing motion against the walls for one minute. Final irrigation was performed using 2 ml of 2.5% NaOCl.
<u>Post-supplementary retreatment μ-CT scanning</u>
Teeth were scanned with μ-CT using the same parameters to evaluate the volume of the remaining filling material. Volume (mm3) of filling material removed after using both supplementary approaches was calculated. Percentage of reduction in the filling material was also calculated.

Statistical analysis
Primary observation for the normality of recorded data took place using Kolmogorov-Smirnov and Shapiro-Wilk tests. Data failed to pass normality test and presented non-parametric (not-normal) distribution. Based on this, non-parametric tests were used for analysis namely; Wilcoxon and Man-Whitney.
Wilcoxon test was used to evaluate the assumption that the baseline volume (post-retreatment) is similar between tested groups. Man-Whitney test was used to evaluate whether the difference of volume of root canal filling material is similar after both supplementary approaches (post-supplementary retreatment).
Additional intra-group analysis was performed using Friedman test to evaluate and compare the amount of root filling material removed from root canal thirds (coronal, middle, and apical) by each supplementary approach.
The significance level was set at P≤0.05. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

Results

Remaining filling material was observed post-retreatment and after both supplemen-

Table 1

The mean, median, and standard deviation (SD) values of root filling material volume in mm 3 post-obturation, post-retreatment, and percentage of reduction in each anatomical third

	Apical			Middle			Coronal			Total		
	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
Post-obturation	0.81	0.73	0.25	1.94	1.57	0.72	4.16	3.6	1.03	6.86	5.91	1.77
Post-retreatment	0.32	0.24	0.21	0.42	0.34	0.21	1.37	1.29	0.21	2.12	1.86	0.62
% reduction	63.34	66.09	12.28	78.53	79.61	5.90	66.21	65.52	4.42	69.13	70.01	4.37
p-value	<0.001*			<0.001*			<0.001*			<0.001*		

significant (p<0.05)

tary approaches as shown in Figure 1. While Table 1 shows the means, medians and standard deviations of the initial volume of root canal filling material (post-obturation), volume of remaining gutta percha (post-retreatment) and percentage of reduction at root canal thirds after retreatment. Significant reduction in the amount of filling material was shown when compared to the baseline (post-obturation) (Wilcoxon test, P=0.001), Also, significant reduction was recorded (P<0.001) with a median of 66.09%, 79.61%, and 65.52% at the apical, middle, and coronal thirds respectively.

Comparing the supplementary removal methods, XP-FR was more effective than H-filing (Man-Whitney test, P<0.001), as it removed a median of 43.83% compared to 22.28% for manual H-filing (Table 3).

Additional intra-group analysis revealed that XP-FR significantly reduced the remaining filling material at all root canal thirds (Freidman test, P=0.004). It removed 57.27%, 38.75 % and 42.86% from the apical, middle, and coronal thirds respectively, compared to H-filing which removed 22.31%, 19.23%, and 32.09 % from the apical, middle, and coronal thirds respectively. Mean, median and standard deviation values of the root filling material volume and the percentage of reduction post-supplementary approaches are presented in Tables 2 and 3.

Discussion

BCS retrieval is not easy due to the strong chemical bond formed with hydroxyapatite (13-16). Although BCS could be used in obturation with a single cone (20), continuous wave of condensation was used in this study to achieve maximum contact with dentin. Oval-shaped canals represent another challenge for intracanal filling material removal due to minimal contact between the endodontic file and the root canal wall (21). Therefore, the aim of this study was to compare two supplementary methods used in the removal of gutta

Table 2

The mean, median, and standard deviation (SD) values of percentage of gutta percha reduction following use of supplementary method for both groups

	Apical			Middle			Coronal			Total		
	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
Group I	0.18	0.09	0.2	0.28	0.19	0.2	0.81	0.72	0.2	1.28	1	0.6
Group II	0.27	0.28	0.18	0.37	0.38	0.2	0.96	0.91	0.22	1.72	1.57	0.65
p-value	0.004*			0.005*			0.003*			0.004*		

significant (p<0.05)

Table 3

The mean, median, and standard deviation (SD) values of remaining gutta percha volume in mm³ post-supplementary approaches for both groups

	Apical			Middle			Coronal			Total		
	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
Group I	52.22	57.27	22.74	34.92	38.75	11.77	40.6	42.86	5.02	40.21	43.83	8.75
Group II	21.89	22.31	9.51	18.43	19.23	9.18	31.72	32.09	4.01	22.31	22.28	5.24
p-value	<0.001*			<0.001*			<0.001*			<0.001*		

significant (p<0.05)

percha and BCS from oval root canals. Microcomputed tomography is a good, accurate, reliable, and noninvasive method for evaluation of the root canal system qualitatively and quantitatively (22). The data can be represented as 2D or 3D images (23). Microcomputed tomography has already been shown to be the gold standard for testing filling material retrieval (17). This technique enables the 3D calculation of the remaining root canal filling material without splitting the root which may cause loss of some of the gutta percha (24). In our study, the retreatment procedure was expanded using X3 (30, 0.07) and X4 (40, 0.06) in an attempt to achieve better removal of intracanal filling material. Yet, Mani GPR files failed to completely remove intracanal filling material from oval root canal, with the median total percentage of reduction recorded of 70.01%. This comes in agreement with Rubino et al. who showed 74% reduction in intracanal filling material using Mani GPR Files (25).

In order to achieve complete removal of root canal filling material, two supplementary approaches were compared; engine-driven XP-FR and manual H- filing. Results showed significant reduction in the total amount of remaining filling material using both techniques. XP-FR removed significantly more intracanal filling material with reduction percentage of 43.83% compared to only 22.28% for manual H-filing. Therefore, the null hypothesis was rejected in this study.Our results are in full agreement with Alves et al. (26), Machado et al. (27), De-Deus et al. (17), Campello et al. (28), and Silva et al. (17) who also showed significant reduction of remaining intracanal filling material using XP-FR. This could be attributed to expansion of the file within the oral cavity temperature together with its spiral movement intracanal which promotes remaining filling material displacement (26). Yet, these studies have tested XP-FR on gutta percha and resin-based sealers. Silva et al. showed that XP-FR decreased the total volume of remaining intracanal filling material by 59.4% using resin-based sealer compared to 43.83% in the current study using bioceramic sealer. This is a logic finding as bioceramic sealers are more difficult to remove than resin-based sealers due to the strong chemical bond they form with hydroxyapatite (13, 14, 29).

Manual H-filing is capable of removing intracanal filling material (30, 31); however, it was used as a reference for comparison as a supplementary technique in the current study. Borges et al. used H-filing in an oscillatory motion and compared it to XP-endo Shaper. XP-endo Shaper removed significantly more intracanal filling material due to the ability of Max-Wire novel files to expand and contract touching and abrading the walls more effectively compared to the linear motion of H-filing (32).

The use of different supplementary approaches for intracanal filling removal is deemed mandatory. Especially, XP-FR that showed superior ability of intracanal filling material removal. However, being an in-vitro study, extrapolation of these results into the clinical setting needs further investigation as identification of the remaining intracanal filling material using µ-CT is not applicable in the clinical setting.

Preoperative, intraoperative and postoperative factors lead to root canal treatment failure (33). Persistence of microorganisms is considered one of the major causes of root canal treatment failure (34). Removal of root canal filling material is considered the key of success for root canal retreatment. Presence of root canal filling material may harbor microorganisms that affects the disinfection procedures (35). Numerous studies have reported that complete removal of intracanal filling material, especially the apical third, is nearly impossible (36-39). Therefore, the use of supplementary techniques has been advocated to allow more contact with dentin and forcing irrigation laterally to reach inaccessible areas (40, 41).

Conclusions

Based on this in-vitro study, it could be concluded that rotary files are not efficient in complete removal of bioceramic sealer and gutta percha from oval root canals. The use of supplementary removal methods has improved intracanal filling material removal. XP-FR significantly removed more intracanal filling material than manual H-filing.

Clinical Relevance

Supplementary methods following rotary retreatment files improve bioceramic sealer and gutta percha removal from oval root canals.

Conflict of Interest

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

Acknowledgments

None.

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Lettera DEL PRESIDENTE

Cari Soci,
eccomi a Voi per la prima *Lettera del Presidente* della nostra amata rivista.
In questa missiva vorrei porre la Vostra attenzione proprio sul **Giornale Italiano di Endodonzia** e sulla grande importanza che questa Rivista ricopre non solo per la nostra Società ma anche per i clinici, i ricercatori e le università non solo italiane.
In tanti anni di militanza nel Consiglio Direttivo della SIE mi sono spesso confrontato con molti di Voi raccogliendo dubbi o addirittura poca rilevanza per la nostra Rivista, come se il GIE fosse qualcosa di scontato e per questo motivo di scarso valore, ma non è così.
Il Nostro Giornale è una parte fondamentale della Società, della quale è **l'organo ufficiale**, gode fama di essere una delle più apprezzate riviste del settore in campo internazionale, sia per i contenuti degli articoli pubblicati, sia per la grafica. È diventata motivo di vanto negli ultimi anni, per i bellissimi risultati ottenuti in un crescendo di attività e iniziative. Per questo motivo, in questa mia prima lettera, vorrei condividere con Voi cosa significhi e cosa si nasconda dietro le quinte di ogni singola uscita del GIE.
Come ben saprete, negli ultimi anni, la Rivista ha subìto molti cambiamenti che ne hanno permesso un'importante riqualificazione.
Gli articoli pubblicati in **lingua inglese** consentono di allargare la nostra platea che sempre di più ha connotati internazionali. La Rivista è stata completamente digitalizzata e quindi resa **"eco-friendly"**. So quanto possa essere gradevole sfogliare una rivista cartacea, ma il ruolo di una rivista scientifica non è quello di prendere polvere sugli scaffali, ma di essere **rapidamente fruibile**, di **consentire una diffusione il più capillare e veloce possibile**, così che articoli e ricerche possano essere **prontamente a loro volta citati in altri lavori scientifici su altre riviste**.
Un importante tassello è stato il **cambio di editore**. Non è stato né semplice né scontato, ma alla fine abbiamo stretto la partnership con **Ariesdue**, nostro nuovo editore dal 2019. L'intento è quello di far crescere il GIE ogni anno di più e Ariesdue è l'unica Casa Editrice italiana che ha portato una rivista scientifica del settore odontoiatrico a ottenere un riconoscimento importante come l'**Impact Factor**.
Il GIE parte da un'indicizzazione su **Scopus ed Embase**, importanti banche dati scientifiche che permettono al nostro giornale di essere apprezzato in tutto il mondo e i dati in nostro possesso lo dimostrano ampiamente.
Il Giornale Italiano di Endodonzia è pubblicato online su **ScienceDirect** e **www.giornaleitalianoendodonzia.it,** come una sorta di diario aperto che lo rende sempre consultabile.
Abbiamo approntato la piattaforma affinché la Rivista possa garantire l'analisi delle citazioni degli articoli pubblicati, in tempo reale, con metodo **Early View**, e senza aspettare come negli anni passati l'uscita dei due numeri a giugno e novembre.
Altro elemento di crescita importante è rappresentato dalla suite per le **Submission degli articoli:** dal 2019 avvengono completamente online come per le riviste "più quotate".
Infine, ma non per questo meno importante, la grande squadra composta da **Editor in Chief e Associate Editor**, che si impegna costantemente nella ricerca di nuovi articoli e nella pianificazione del lavoro quotidiano che consente alla nostra rivista di uscire con **regolarità semestrale** ormai da tanti anni.
Quindi, cari Soci, sappiate che appartenere alla Società Italiana di Endodonzia e far parte di questo gruppo significa anche **sostenere il GIE leggendolo**, **divulgandolo e condividendo i Vostri articoli,** perché teniamo davvero molto alla Nostra Rivista, fiore all'occhiello dell'Endodonzia Italiana, e per mantenerla operativa e per incrementare la sua visibilità sono stati fatti dei grandi sacrifici da parte di tutti Noi.
Per concludere voglio citare il nostro attuale **Editor in Chief, Prof. Sandro Rengo**, ricordando proprio con grande piacere una sua Lettera del Presidente del lontano 2005, che già allora mi aveva colpito positivamente. ***"È proprio sugli obiettivi che intendo soffermarmi: la progettualità, in una Società Scientifica, deve rappresentare il punto di partenza, il presupposto fondamentale di qualunque discorso".***
Per questo motivo **facciamo crescere ancor di più il nostro Giornale Italiano di Endodonzia!**
Non diamo nulla per scontato, non pretendiamo dalla Società quello che Noi per primi non siamo in grado di dare, perché per ricevere, prima dobbiamo imparare a donare.

Un caro saluto a tutti Voi e arrivederci al prossimo numero!

Dott. Roberto Fornara
Presidente SIE
Società Italiana di Endodonzia

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COME DIVENTARE SOCIO ATTIVO/AGGREGATO

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

SOCIO AGGREGATO

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

DOCUMENTAZIONE NECESSARIA PER DIVENTARE SOCIO AGGREGATO

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

SOCIO ATTIVO

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

DOCUMENTAZIONE NECESSARIA PER DIVENTARE SOCIO ATTIVO

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

MODALITÀ DI DOCUMENTAZIONE DEI CASI CLINICI

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

CRITERI DI VALUTAZIONE

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

ADEMPIMENTI DEL CANDIDATO

La domanda di ammissione allo status di Socio Aggregato/Attivo, rivolta al Presidente della SIE, dovrà pervenire, insieme alla documentazione di seguito elencata, alla Segreteria 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 2021-2022

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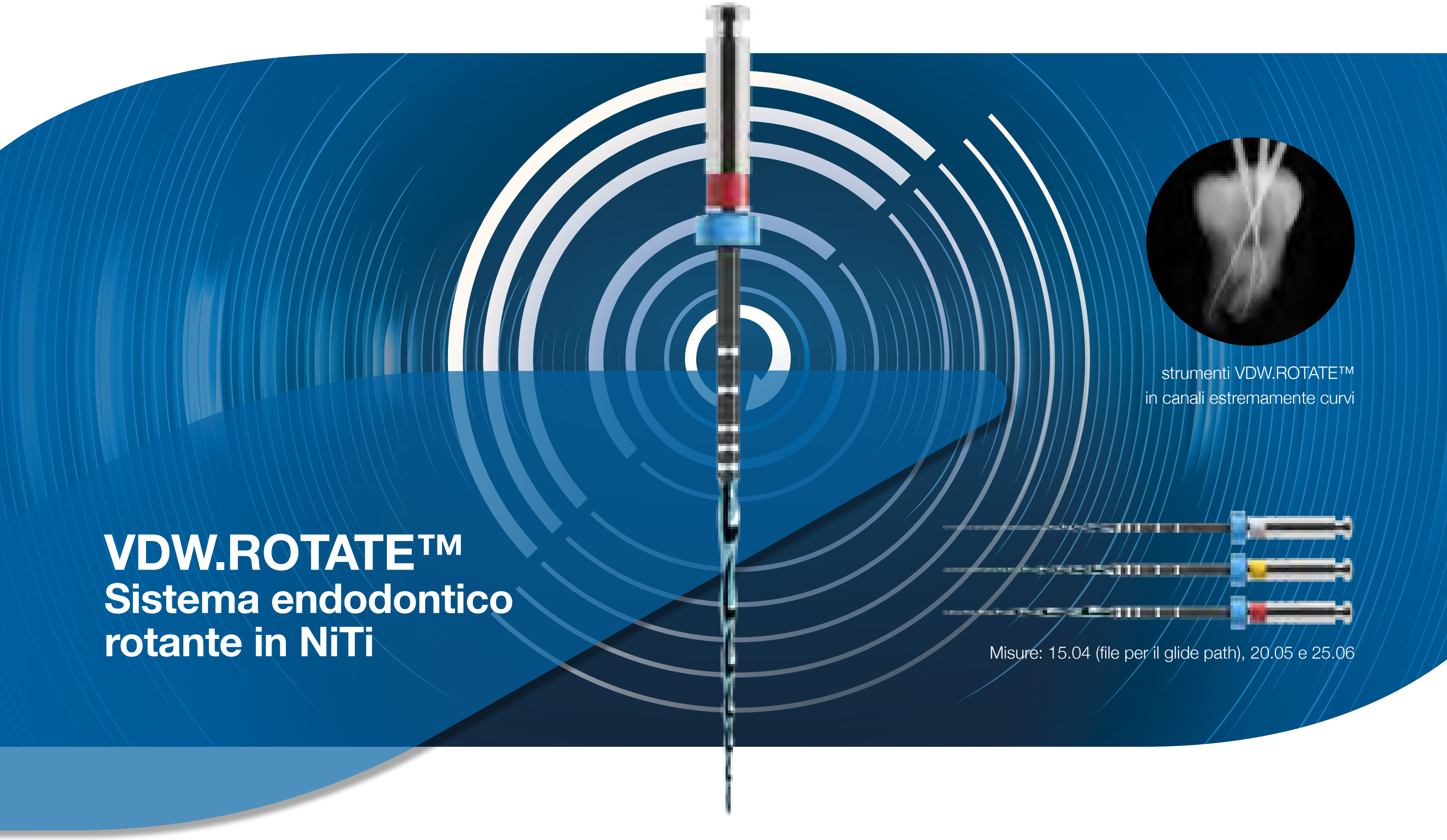

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GUIDELINES FOR AUTHORS

Giornale Italiano di Endodonzia (GIE)
was founded in 1987 and is the official journal of Società Italiana di Endodonzia, SIE (Italian Society of Endodontics) https://www.endodonzia.it/
It is a peer-reviewed journal, only available in electonic format and publishes original scientific articles, reviews, clinical articles and case reports in the field of Endodontology. Scientific contributions dealing with health, injuries to and diseases of the pulp and periradicular region, and their relationship with systemic well-being and health. Original scientific articles are published in the areas of biomedical science, applied materials science, bioengineering, epidemiology and social science relevant to endodontic disease and its management, and to the restoration of root-treated teeth. In addition, review articles, reports of clinical cases, book reviews, summaries and abstracts of scientific meetings and news items are accepted. Please read the instructions below carefully for details on the submission of manuscripts, the journal's requirements and standards as well as information concerning the procedure after a manuscript has been accepted for publication in Giornale Italiano di Endodonzia. Giornale Italiano di Endodonzia is indexed in Scopus, Science Direct, Embase and published online by Ariesdue, Milan, Italy and hosted by PAGEPress, Pavia, Italy. All articles are available on www.giornaleitalianoendodonzia.it.
We publish, monthly, new articles in the Early View section while the full Journal is issued twice a year, in June and November.
Authors are encouraged to visit **www.giornaleitalianoendodonzia.it** for further information on the preparation and submission of articles and figures.

Ethical guidelines

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

Authorship and Acknowledgements

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

Manuscript preparation

Manuscripts should be uploaded as Word (.doc) or Rich Text Format (.rtf) files (not write-protected) plus separate figure files: TIF, EPS, JPEG files are acceptable for submission.
The text file must contain the **abstract, main text, references, tables and figure legends**, but no embedded figures or title page. The title page should be provided as a separate file. In the main text, please reference figures as for instance **figure 1, figure 2** etc to match the tag name you choose for the individual figure files uploaded.
Please note that **manuscripts must be written in English**. Authors whose native language is not English are strongly advised to have their manuscript checked by a language editing service or by a native English speaker prior to submission.

Manuscript Types Accepted

Original Scientific Articles must describe significant and original experimental observations and provide sufficient detail so that the observations can be critically evaluated and, if necessary, repeated. Original Scientific Articles must conform to the highest international standards in the field.
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.
Case Reports or **Case Series** illustrating unusual and clinically relevant observations are acceptable, but they must be of sufficiently high quality to be considered worthy of publication in the Journal. On rare occasions, completed cases displaying nonobvious solutions to significant clinical challenges will be considered. Illustrative material must be of the highest quality and healing outcomes, if appropriate, should be demonstrated.
Case reports should be written using the **Preferred Reporting Items for Case reports in Endodontics (PRICE) 2020 guidelines.** A PRICE checklist and flowchart (as a Figure) should also be completed and included in the submission material. The PRICE 2020 checklist and flowchart can be downloaded from: http://pride-endodonticguidelines.org/price/. It is recommended that authors consult the following papers, which explains the rationale for the PRICE 2020 guidelines and their importance when writing manuscripts:

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

Manuscript Format

The **official language** of the publication is **English**. It is preferred that manuscript is professionally edited. All services are paid for and arranged by the author and use of one of these services does not guarantee acceptance or preference for publication.
Authors should pay special attention to the **presentation** of their research findings or clinical reports so that they may be communicated clearly.
Technical **jargon** should be avoided as much as possible and clearly explained where its use is unavoidable. **Abbreviations** should also be kept to a minimum, particularly those that are not standard. Giornale Italiano di Endodonzia adheres to the conventions outlined in *Units, Symbols and Abbreviations: A Guide for Medical and Scientific Editors and Authors.* If abbreviations are used in the text, authors are required to write full name+abbreviation in brackets [e.g. Multiple Myeloma (MM)] the first time they are used, then only abbreviations can be written (apart from titles; in this case authors have to write always the full name).
If names of equipments or substances are mentioned in the text, brand, company names and locations (city and state) for equipment and substances should be included in parentheses within the text.

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

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

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

Abstracts should be no more than 250 words giving details of what was done.
Abstract for Original Scientific Articles should be no more than 250 words giving details of what was done using the following structure:

- **Aim:** give a clear statement of the main aim of the study and the main hypothesis tested, if any.
- **Methodology:** describe the methods adopted including, as appropriate, the design of the study, the setting, entry requirements for subjects, use of materials, outcome measures and statistical tests.
- **Results:** give the main results of the study, including the outcome of any statistical analysis.
- **Conclusions:** state the primary conclusions of the study and their implications. Suggest areas for further research, if appropriate.

Abstract for 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:

- **Aim:** give a clear statement of the main aim of the report and the clinical problem which is addressed.
- **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.
- **Key learning points**: provide up to five short, bullet-pointed statements to highlight the key messages of the report. All points must be fully justified by material presented in the report.

Abstract for Clinical Articles should be no more than 250 words using the following structure:

- **Aim:** give a clear statement of the main aim of the report and the clinical problem which is addressed.
- **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.
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Corporate author
British Endodontic Society - Guidelines for root canal treatment. Giornale Italiano di Endodonzia 1979;16:192-5.

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

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

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

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

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

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

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