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Table OF CONTENTS

Editorial

Bioceramic sealers: clinical applications and future perspectives Sandro Rengo

Original Articles

Cyclic fatigue resistance of WaveOne Gold instrument with different amplitudes of axial movement: a dynamic study Viviane Alves, Rafael Carvalh, Renê Silva, Adriana de Jesus Soares, Marcos Frozon

A survey on root canal obturation trends: warm versus cold obturation technique

Isabel Vasconcelos, Cátia Manilha, António Ginjeira

Influence of different root canal drying protocols on the bond strength of a bioceramic endodontic sealer

Ihan Vitor Cardoso, Maria Luisa Seixas-Silva, Gabriela Rover, Eduardo Antunes Bortoluzzi, Lucas da Fonseca Roberti Garcia, Cleonice Silveira Teixeira

Influence of operator experience on apical debris extrusion after endodontic instrumentation with different single-file systems

Carlos Eduardo Fontana, Carlos Eduardo da Silveira Bueno, João Daniel Mendonça de Moura, Gávila da Rocha Bastida Pinheiro, Victor Camelotti Trevensoli, Rina Andrea Pelegrine, Daniel Guimarães Pedro Rocha, Alexandre Sigrist De Martin, Sérgio Luiz Pinheiro

Residual dentin thickness at the apical third of mandibular first molar mesial root instrumented by nickel-titanium rotary and manual files with different tapers

Forough Farahi, Eshaghali Saberi, Shima Bijari

Interfacial Characterization of Hydraulic calcium silicatebased sealers by Scanning Electron Microscopy Jaime Abarca, Francisca Espinoza, Aldo Lizana, Paulina Muñoz, Karin Steinfort, Hector Monardes

Evaluation of the push out of bond strength of different bioceramic root canal sealers with different obturation techniques

Seda Falakaloğlu, Mustafa Gündoğar

Comparative evaluation of the effectiveness of different rotary systems in removal of root canal filling materials Neda Shekarchizade, Masoume Shahpouri, Mohammadreza Avalin Charsooghi, Gianrico Spagnuolo, Parisa Soltani

Effect of fentanyl on the success of inferior alveolar nerve block for teeth with symptomatic irreversible pulpitis: a randomized clinical trial

Neda Shekarchizade, Amir Shafa, Mohammad Javad Tarrahi, Niloufar Nafari, Marzia Maglitto, Alessandra Valletta

56

48

10

62

Table OF CONTENTS

Microbiological analysis of root canals of primary teeth with pulp necrosis 84 caused by caries or trauma Denise L Sousa, Rebecca BR Araújo, Brenda PFA Gomes, Juliana D Bronzato, Thais M Duque, Juliana O Gondim, Jose JS Moreira-Neto Application of artificial intelligence in a visual-based fluid motion estimator 92 surrounding a vibrating Eddy tip Harry Huiz Peeters, Faber Silitonga, Lavi Zuhal 101 The impact of composition, core metal mass and phase transformation behaviour on the dynamic cyclic fatigue of Ni-Ti files at different temperatures Mohamed Salah Abdel Aziz El-wakeel, Ahmed Abdel Rahman Hashem, Sarah Hossam Fahmy, Shehabeldin Mohamed Saber, Gianluca Plotino **Case Report** 110 Maintenance of vitality following horizontal root fracture of a lower central incisor Eros M. Villa, Andrea F.T. Tremolada, Roberto Fornara 116 Apicectomy and simultaneous obturation of root canals: a clinical case report Hebert Samapaio de Castro, Renata Nunes Cabral, Ricardo Kehrwald, Samira Salmeron, Ana Regina Casaroto, Polyane Mazucatto Queiroz One-year follow-up of endodontic retreatment in a patient with severe 123Hemophilia A: a case report Lucas Peixoto de Araújo, Mateus de Azevedo Kinalski, Lucas Pinto Carpena, Caio Cézar Randi Ferraz, Mateus Bertolini Fernandes dos Santos **Case Series** 132 Management of teeth with open apex and apical periodontitis using MTA and OrthoMTA: a case series and review Abhishek Parolia, Arlene Yu, Maya Feghali 150 Intentional replantation of severely compromised teeth Beatriz Pereira, Mariana Domingos Pires, Abayomi O. Baruwa, Jorge N.R. Martins, António Ginjeira **Review Article** 158 Intentional replantation in endodontics: review of literature Giuseppe Sangiovanni, Giuseppe Scelza, Francesco Giordano, Andrea Chiacchio, Roberto Ferrigno, Carlo Rengo, Massimo Pisano 170 Regenerative endodontic treatment in mature teeth: a systematic review and meta-analysis Afaf Y Al-Haddad, Nisreen M Al-Namnam

Vita Societaria

185 Lett

Lettera del Presidente Roberto Fornara



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Bioceramic sealers: clinical applications and future perspectives

ndodontic is a constantly improved area thanks to the technological progression of rotary instruments and to the development of recent materials that enhance the clinical procedures and increase the success rate over time.

The introduction of new calcium silicate hydraulic materials is changing the application of endodontic sealers. Among others features, such as insolubility biocompatibility, adequate setting-time, antimicrobial activity, dimension stability and adhesion, nanotechnologies have reduced the particles size within these cements allowing a high flow ability. In this view, bioceramic sealers may be drive along the entire root canal length, including the apical foramen, accessory canals, isthmus, voids and irregularities. Indeed, their aim is not only to fill gaps around of the gutta-percha but also to work as excellent obturation materials. They may be also applied in both cold (single cone) and warm (vertical and carriers) obturation techniques.

In the present issue several hydraulic calcium silicate-based sealers have been evaluated confirming their marginal adaptation and evaluating the limited interfacial gaps. Furthermore, different drying protocols of the root canal have been investigated in terms of influence on the bond strength between endodontic sealers and dentinal root thickness. Future studies are needed to confirm the clinical effectiveness and the potential properties of these biomaterials, with a particular attention to retreatability that may represent the main drawback to the clinicians.

In conclusion, it should be stress that, despite the technological development, the endodontic success is biologically-guided and strictly depends on the decontamination of the root canal system that represents the main goal of the therapy. In this scenario the canal obturation plays an important part in the endodontic success providing sealing and, basically, avoiding reinfection.

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10.32067/GIE.2021.35.02.57 Società Italiana di Endodonzia. Production and hosting by Ariesdue. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). ORIGINAL ARTICLE

Cyclic fatigue resistance of WaveOne Gold instruments with different amplitudes of axial movement: a dynamic study

ABSTRACT

Aim: Single-file reciprocating instruments were launched, aiming to diminish the risk of instrument separation. It operates in an in-and-out dynamic with an amplitude of approximately 3-4 mm. This study evaluated the impact of different amplitudes of axial movement in the dynamic cyclic fatigue resistance of the WaveOne Gold reciprocating file.

Materials and Methods: Forty-five WaveOne Gold 25.07 were divided into three different groups (n=15) according to the amplitude of axial movement of choice. In the G-2.5 group, the instruments were used in an in-and-out amplitude of 2.5 mm; in the G-5 group, the instruments were used in an in-and-out amplitude of 5.0 mm, and in the G-7.5 group, the instruments were used in an in-and-out amplitude of 7.5 mm. All instruments were tested in a simulated canal notched in metallic block, with 9.04 mm in its cervical portion, 13.3 mm in its apical portion, and curvature of 2.5 mm and 69°. The axial movement was applied with a back-and-forth speed of 2.5 mm/s during the dynamic cyclic fatigue test until the fracture was noticed. All of the tests were performed at a controlled temperature of 36 ± 1 °C and under oil lubrication. Then, the time to fracture (TTF/s), the number of cycles to fracture (NCF), and the length of the separated fragments were registered. The level of significance was set at 5%.

Results: The TTF/s was 11.40 ± 9.83 , 15.00 ± 7.46 and 22.33 ± 8.76 for G-2.5, G-5, and G-7.5, respectively. The NCF was 57.00 ± 49.13 , 75.00 ± 37.32 , and 111.67 ± 43.82 for G-2.5, G-5, and G-7.5, respectively. For both TTF/s and NCF, G-7.5 was higher than G-2.5, and G-5.0 was similar to both groups (P<.05). The lengths of the fragments were 10.27 ± 1.07 , 10.37 ± 0.66 , and 10.58 ± 0.77 for G-2.5, G-5, and G-7.5, respectively. There was no difference among the groups in regards to the length of the fragments (P>.05). The SEM images showed characteristics related to cyclic fatigue.

Conclusions: It can be concluded that in-and-out movements affect the dynamic cyclic fatigue resistance of the WaveOne Gold reciprocating instrument. Appropriate pecking motions in the root canals are recommended to prevent the breakage of NiTi reciprocating instruments.

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Introduction

he introduction of nickel-titanium in modern endodontics resulted in instruments with higher flexibility and cyclic fatigue resistance than the previous hand stainless steel (SS) instruments (1). Recently, the thermic treatment of this alloy resulted in increased resistance and the possibility of creation of controlled-memory instruments. Therefore, its use was indicated, mainly in curved canals (2).

The single-file reciprocating system was emphasized by Yared in 2008 (3), based on the concepts described by Roane et al. for use with SS hand files (4). The reciprocating kinematics reduced the possibility of fracture of the instruments when compared to the rotary kinematics (5). However, fractures in reciprocating instruments still might occur, despite the decreasing risk of instrument separation (6, 7). The removal of such instruments is not always a simple task and has the potential to compromise the outcomes of root canal therapy; therefore, efforts should be made to avoid such occurrence (8).

WaveOne Gold (Dentsply Sirona, Ballaigues, Switzerland) is a single-use instrument presenting a rectangular cross-section and a heat-treated alloy. As only 1 or 2 points of the instrument touch the root canal walls, the screw-in effect is attenuated. This aspect, in conjunction with the heat-treated alloy, decreases the risk of fracture (9). According to the manufacturer, the instrument should advance inside of the root canal in 2 to 3 passes of 3 mm of amplitude in each use.

Usually, cyclic fatigue resistance essays are performed at room temperature (10). However, intracanal temperature is approximately 10 °C higher than room temperature, reaching around 35.1 °C. This difference in temperature might compromise the results of these studies (11). Another variable of interest is that dynamic tests are preferable to static tests for the assessment of cyclic fatigue resistance (12). While fracture of instruments is a complex phenomenon, it can be summarized in fracture by torsion or cyclic fatigue (13). Fracture by cyclic fatigue is particularly affected by the curvature and the location of this curvature within the root canal.

Engine driven files should be used inside of the root canal in an in-and-out or pecking motion fashion (14). Moreover, this dvnamic decreases the screw-in effect related to the full rotation of the instrument (10). Incorporating the pecking motion seems to increase the cyclic fatigue resistance because the stress is distributed in the whole extension of the file, rather than in a single spot. This study aimed to assess, at simulated body temperature, the cyclic fatigue resistance of WaveOne Gold instruments at different amplitudes of insertion -2.5 mm, 5.0 mm, and 7.5 mm in simulated metal blocks. The null hypothesis tested is that there is no difference in the cyclic fatigue resistance of the Wave-One Gold 25.07 at different amplitudes of axial movement.

Materials and Methods

Sample size calculation was based on a pilot study using the G*Power 3.1.9.2 software (Heinrich-Heine-Universität Dusseldorf, Dusseldorf, Germany). Considering the minimum difference (10) and the standard deviation of the difference (7.5) to compare the three groups, a minimum of 12 sample units was calculated to achieve the test power of 80% and significance of 5%. Therefore, with a sample number of 15, the study has power above 80%.

Forty-five new WaveOne Gold Primary (25.07) of 25 mm of length were inspected under 25x magnification to discard any signs of distortion. Then, the files were randomly assigned to the three different groups (n=15) according to the different amplitudes of axial movement assessed: G-2.5, 2.5 mm of amplitude; G-5, 5 mm of amplitude, and G-7.5, 7.5 mm of amplitude. A simulated canal was notched in a metallic block with the following features: a curved segment 2.15 mm long, 69° of curvature, 2.5 mm of radius, presenting 9.04 mm of length in its straight coronal



Figure 1

Schematic design of the metallic block simulating a 69° curvature in a root canal.



portion, as well as initial width of 2.0 mm decreasing apically until 1.54 mm of width and 13.3 mm of length in its straight apical portion, with initial width of 1.41 mm decreasing up to 1.00 mm in the apical portion was used for the experiment (Figure 1). The canal was covered with an acrylic plate to prevent the instruments from slipping out and to visualize the reciprocating files.

The metallic block was positioned vertically and kept on a heating plate (Fisatom Co., Sao Paulo, Brazil) that transmitted the temperature to the simulated canal and to the lubricated synthetic oil (Super Oil; Singer Co. Ltd., Elizabethport, NJ, USA) that filled the canal during the time of the experiment. The temperature inside of the simulated canal was kept stable at $36 \pm 1^{\circ}$ C, confirmed by the use of a digital laser infrared thermometer (Qingdao Tlead Internation, Shandong China) pointing to the inner of the canal; therefore, the synthetic oil was at the same temperature, and this temperature confirmation was performed for each file to be tested.

The instruments were used in a VDW Silver engine (VDW, Munich, Germany) and coupled to contra-angle with a 6:1 reduction (Sirona Dental Systems GmbH, Bensheim, Germany) at the WaveOne "Reciprocating ALL" setting. The handpiece was attached in a mobile unit powered by an electronically controlled motor (SAVOX SC-12 56T69; Savox, Taichung, Taiwan) that regulated the in-and-out amplitude of the insertion of the instrument in a perpendicular direction inside the simulated canal to allow a precise and reproducible continuous up-and-down pecking movement of each file. The inand-out speed was set at 2.5 mm/s.

All instruments were inserted 22 mm inside the simulated canal; a silicon stop was placed in each instrument to assure the exact 22 mm depth penetration. The mechanical system of axial movement was activated and immediately exerted a 2.5 mm retraction movement (G-2.5) so the file was inserted 19.5 mm into the metal canal (start position); immediately, the file initiated the axial movement 2.5 mm forward and 2.5 mm backward. Simultaneously, the file initiated the reciprocating movement. For the G-5.0 and G-7.5, the mechanical system exerted 5.0 or 7.5 mm of retraction movement, respectively, so the file was inserted with 17 mm or 14.5 mm depth in the canal (start position), respectively and start a 5.0 mm forward and 5.0 backward for G-5.0 and a 7.5 mm forward and 7.5 mm backward for G-7.5.

An IPhone X (Apple Inc, Cupertino, CA) using a 4K recording definition was used to record the movement of the files inside of the simulated canal. All of the films were assessed in the Movie Maker program (Microsoft Co., Redmond, WA), allowing the registration of the beginning of the movement up to the fracture of the instrument with 0.001 seconds precision. The time to fracture (TTF/s) was registered in





Figure 2

SEM images of the fractured surface of the separated fragments of the instruments in each group: G-2.5 (A, B, and C), G-5 (D, E and F), and G-7.5 (G, H and I). A, D and G: longitudinal surface of the fractured fragments, no torsional deformation on the helical shaft was noticed (magnification of 25x). B, E and H: cross section of the fractured fragments with ductile surface fracture morphology and with withe arrows indicating the crack initiation origin (magnification of 100x). C, F and I: higher magnification of **B**, **E** and **H**, respectively, the images show numerous dimples spread on the fractured surfaces, which constitute a typical feature of ductile fracture (magnification of 1000x).

> seconds, and the number of cycles to fracture (NCF) was calculated as follows: TTF (s) x 300/60. The apical separated portion of the instruments was collected, cleaned with an ultrasonic bath, and dried for 24 hours at 37 °C. Afterward, the length of the separated fragment was measured with a digital rule with a precision of ± 0.03 mm/0.001.

> Images from the fragment surfaces were obtained by Scanning Electron Microscopy (SEM, JSM-6010LA, JEOL, Tokyo, Japan) for the assessment of plastic deformation and the pattern of the fracture.

> Due to abnormal distribution of data for both TTF/s and NCF, verified by the Kolgomorov-Smirnov test, the Kruskal-Wallis (post-hoc Duncan) was used for statistical analysis. The significance level adopted was P<.05. All tests were performed using the Statistical Package for Social Sciences (SPSS), version 19.0.

Results

The initial inspection of the instruments showed no sign of distortion or fractures;

therefore, no instrument was removed from the test.

The time to fracture was 11.40 ± 9.83 sec, 15.00 ± 7.46 sec, and 22.33 ± 8.76 sec for G-2.5, G-5, and G-7.5, respectively. This result was different statistically only between G-2.5 and G-7.5 (P<.05) (Table 1). Similarly, the results of NCF were higher for G-7.5 (111.67±43.82 sec), followed by G-5 (75.00±37.32 sec) and G-2.5 (57.00±49.13 sec); the only statistically significant difference was noticed between G-2.5 and G-7.5 (P<.05) (Table 2).

There was no difference in the length of the fragments in the 3 different groups (Table 3). The SEM images showed no sign of plastic deformation of the fragments, suggesting no torsional fatigue. The cross-section of the fragments presented ductile characteristics and dimples formation related to cyclic fatigue (Figure 2).

Discussion

The study design herein adopted used a simulated canal in a metallic block mimicking a root canal with 69° curvature. In



order to diminish confound factors, the block was lubricated with synthetic oil, similarly as in previous studies (5). By using a heating plate, this study aimed to simulate the body temperature, thereby enhancing the similarities with the *in vivo* behavior of the instrument. The dynamic movement of the instrument at 2.5 mm/s in an axial direction is claimed to better simulate the movement of the instrument inside of the root canal (12).

The curvature was located in the middle portion of the simulated canal, and it can be considered severe (15). By doing so, the study aimed to simulate extremely difficult conditions that a file can be submitted *in vivo*. On the other hand, the use of a taper .07 file in severe curvature should be cautiously considered in a clinical setting. It is known that there is a greater challenge in the removal of separated instruments in the apical portion of the canal (16). However, there is a high incidence of curvatures located in the cervical and middle thirds in mesial roots of mandibular and maxillary molars (17). A previous study demonstrated that reciprocating instruments are more prone to fracture when the curvature is located in the middle or coronal third of the canal (18).

The Scanning Electron Microscopy (SEM) images of the apical portion of the instruments showed no signs of plastic deformation of the cutting blades. Moreover, there were clear signs of fatigue striations of the alloy. These features characterize the fracture to be promoted due to cyclic fatigue, which is the aim of the present study (19).

The results of the present study showed higher cyclic fatigue resistance with larger amplitudes of movement. Therefore, the

Table 1 Time necessary for fracture (TTF/s) of the fragments in each experimental group					
Group	Mean	SD	Median	Range	p-value
G-2.5	11.40ª	9.83	9.00	4-46	< 0.001
G-5	15.00 ^{a,b}	7.46	12.00	8-35	
G-7.5	22.33 [♭]	8.76	22.00	10-36	

Different superscript letters indicate statistically significant differences – Kruskall-Wallis (P<.05).

	Number of cycles	Table 2to fracture (NCF)	in each exper	imental group	
iroup	Mean	SD	Median	Range	p-v

Group	wean	SD	Median	Range	p-value
G-2.5	57.00ª	49.13	45.00	20-230	<0.001
G-5	75.00 ^{a,b}	37.32	60.00	40-175	
G-7.5	111.67 ^b	43.82	110.00	50-180	

Different superscript letters indicate statistically significant differences – Kruskall-Wallis (P<.05).

Table 3

Mean length of the apical separated fragment (mm) in each experimental group

Group	Mean (mm)	SD	Median	Range	p-value
G-2.5	10.27ª	1.07	10.00	8.06-12.14	0.154
G-5	10.37ª	0.66	10.13	9.85-11.95	
G-7.5	10.58ª	0.77	10.37	9.58-12.05	

Different superscript letters indicate statistically significant differences - Kruskall-Wallis (P<.05).



null hypothesis was rejected. Despite the lack of statistically significant difference when G-5 is compared with G-2.5 and G-7.5, the higher resistance of the instrument in G-7.5 when compared to G-2.5 led to the conclusion that there is an influence in the amplitude on the results. These findings, in a manner, concur with Li et al., as that study also found no difference in cyclic fatigue resistance with variations in pecking motion in the order of 3 mm (10). Another study also found no difference in the forces generated when the variation in the amplitude of movement was as short as 2 mm (20). One possible explanation for this result is that the larger amplitude of movement prevents the narrower area of the instrument from being submitted to tension and compression in the curved area of the block (21). However, as those studies were performed with rotary instruments, care should be taken with the comparisons in relation to the findings of the reciprocating instruments assessed in this study. On the other hand, in the case of shorter in-and-out movements, the stresses are concentrated in a small area of the instrument, which reduces both the TTF/s and the NCF (10). A recent study assessed the resistance of reciprocating instruments Reciproc Blue (VDW, Munich, Germany), WaveOne Gold, and Prodesign R (Easy Co., Belo Horizonte, Brazil), and found an average time for fracture of 876.5 s; 409.3 s; and 2099.8 s, respectively (22). These results are significantly higher than the findings of the present study. Also, the length of separated instruments ranged from 4.98 to 5.01 mm without statistically significant differences among the files, which differs from the present findings. It is worthwhile to mention that despite the similarities in the curvature -60° for that study and 69° for the present study – in that study, the curvature was located 5 mm from the apical portion of the simulated canal. This comparison corroborates the hypothesis that curvatures in the middle portion are riskier than apical ones.

The mean length of the fragments in the present study differs considerably from the findings of previous studies, which found 4.51 mm for Edge Taper Platinum, 4.99 mm

for ProTaper Gold, and 7.51 mm for Hyflex EDM (19, 23). Also, the mean length found by Alcalde et al. was significantly shorter than the results of the present study (22). While the differences among the files and methods used prevent a straightforward comparison, these findings are clinically relevant. This aspect might be explained by the design of the simulated canal. Moreover, the in-and-out movement is likely to have promoted the fracture in a thicker portion of the file (13). Interestingly, the length of fragments was the same regardless of the experimental groups. Although the different amplitude ranges that resulted impacted the TTF/s and NCF, the area in which the files are submitted to the higher stress remains the same. Considering the characteristics of the simulated canal, and the fact that all files were inserted up to 22 mm, the approximate length in the straight portion of the canal was 10.81 mm, resulting in the fractures occurring in similar areas (means ranging from 10.27 to 10.58), meaning that the separation occurred at the end of the curvature, in the highest area of tension.

Conclusions

While the results of the present study concluded that the amplitude of movement impacts the cyclic fatigue resistance of the instruments, this procedure should be carefully assessed prior to being applied clinically. Further in vitro studies should evaluate, for instance, the possibility of this larger amplitude increasing the debris extrusion, especially in treatments of teeth presenting necrotic pulps.

Within the limitations of an in vitro model, it can be concluded that larger amplitudes of in-and-out movements increase the cyclic fatigue resistance of the WaveOne Gold reciprocating instrument.

Clinical Relevance

Reciprocating kinematics is claimed to diminish the risk of fracture of instruments. These instruments are recommended to be used in an in-and-out fashion with 3-4 mm of amplitude. This study showed



that the variation of the amplitude of this movement impacts the risk of fracture of these instruments.

Conflict of Interest

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

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None.

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ORIGINAL ARTICLE

A survey on root canal obturation trends: warm versus cold obturation technique

ABSTRACT

Aim: The purpose of this study was to obtain information about the current trends on obturation between members of endodontic societies, affiliated with the European Society of Endodontology, specifically the trends of using calcium silicate-based sealers and the preferred use of the warm versus cold obturation technique related to years of practice, additional training in endodontics and working situation.

Methodology: A questionnaire was distributed, and data from Portugal, Italy and Turkey societies was collected for demographic and professional information, and also about material and techniques commonly used in endodontic obturation. Statistical analysis comprised descriptive statistics, presented as frequencies (n) and percentage (%). A chisquare test of homogeneity and a Z Test were conducted between obturation technique (warm versus cold) and years of practice, additional training in endodontics and working situation. A significance level of 0.05 was considered.

Results: The proportion of users of warm obturation technique was 58.7% and of cold obturation technique was 41.3%. The most selected sealer was the epoxy resin-based sealer (52.3%). Within the calcium silicate-based sealers, BioRoot was the most selected (40.3%). When calcium silicate-based sealers were selected, the majority of participants answered not to use the bioceramic gutta-percha cones (65.4%). The years of practice, the additional training in endodontics and the working situation influenced the selection of warm or cold obturation technique (p<.001).

Conclusions: The choice of warm or cold obturation technique showed association with the years of practice, the additional training in endodontics and the working situation.

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Introduction

complete sealing of the root canal system after cleaning and shaping is critical to prevent oral pathogens from colonizing and re-infecting the root canal system and periapical tissues (1). The aim of root canal obturation is to prevent coronal leakage, prevent influx of periapical fluids and entomb remaining bacteria in the root canal (1). A root canal obturation with no voids, extending to 2 mm within the radiographic apex and a satisfactory coronal restoration were found to improve the outcome of primary root canal treatment (2).

Over the years, new materials are being developed and a variety of sealers have been employed in root canal obturation. The requirements for materials to fill the root canal system are: biocompatibility, dimensional stability, ability to seal, insolubility, non supportive of bacterial growth and radiopacity (3). Various kinds of endodontic sealers are available, including sealers based on glass ionomer, zinc oxide-eugenol, resin, calcium hydroxide, silicone, and recently calcium silicate-based sealers, which has been associated to a reconsidered single cone technique, as an easier and faster obturation technique. When introduced into the market, calcium silicate-based sealers were claimed as an advantage for use in a cold obturation technique.

The lateral compaction is also a cold obturation technique, which is taught and practiced worldwide, serving as the gold standard against which new techniques must be compared (4). But the well-known shortcomings of the lateral compaction technique might decrease the effectiveness of root canal obturation, and several filling technique variations have been developed to incorporate the use of thermal or frictional heat, with the aim of thermoplasticizing the gutta-percha (5). The complex canal anatomy with accessory and lateral canals, isthmus and bifurcations, or with oval-shaped canals can make obturation a challenge. In the 1960's, the warm obturation technique was introduced in endodontics with the objective of thermoplasticizing the gutta-percha in order to adapt it to the irregularities of the root canal system. The idea of three-dimensional root canal obturation gained popularity among the specialists in endodontics with warm vertical compaction (6). Modifications to the technique have been applied. The System B endodontic heat source unit (EIE/ Analytic, Redmond, WA, USA) was designed to thermoplasticize the apical gutta-percha with a single continuous wave (7). However, the root canal complex areas may be unfilled, even when termoplasticized gutta-percha is applied (5). Since there are multiple techniques and materials available, and as there are no reports only focused on the warm or cold obturation preferences, the aim of this study was to clarify the tendency of selection of these two techniques associated on years of practice, additional training in endodontics and working situation.

Materials and Methods

An invitation to participate in the study was sent by email to all the country representatives of all national endodontic societies of the European Society of Endodontology (ESE), requesting collaboration from their registered endodontic practitioners. The email was sent directly to the societies representatives, who then forwarded the invitation to their members, which included instructions and details regarding the study's purpose. Three contacts to the societies were made in order to obtain the maximum compliance. The first was in December 2018, then March 2019 and finally in May 2019.

The questionnaire comprised nine questions divided in two parts (figure 1). The first part included social and professional data with five questions: gender, years of experience as endodontic practitioner, country where the respondent works, type of additional training in endodontics, and working situation. The second part included four questions related to obturation (techniques and materials): obturation technique more often used by the respon-



Questionnaire

1. Gender

() Female () Male

2. How many years have you been practicing as an endodontic practitioner?

() <5 years () 5-10 years () >10 years

3. In which country do you practice?

() Austria () Belgium () Denmark () Estonia () Finland () France () Germany () Greece () Israel () Italy () Latvia () Lithuania () Luxembourg () Norway () Portugal () Republic of Ireland () Spain () Sweden () Switzerland () The Netherlands () Turkey) United Kingdom () Other_____

4. What type of additional training do you have?

() Full-time post-graduation () Part-time post-graduation () Non- Structured training () None

5. Which of the following describes your working situation?

() Full-time private practice () Part-time private-practice/Part-time academic () Academic

() National Health Service

6. Which obturation technique do you use more often?

() Lateral compaction () Carrier-based techniques (gutta- percha) () Continuous Wave Compaction () Warm Vertical Condensation () Thermoplasticized injection technique () Thermomechanical compaction with rotary () Single Cone () Other _____

7. Which sealer do you routinely use?

() Zinc oxide eugenol () Epoxy resin () Calcium hydroxide () Glass ionomer () Calcium Silicate-based Sealer () Silicone Based-Sealer () Other _____

8. If you use a "Bioceramic Sealer", which one do you prefer?

() Endosequence BC Sealer () Endosequence BC Sealer Hiflow () TotalFill () BioRoot BC Sealer () GuttaFlow () Other

9. If you use a "Bioceramic Sealer", do you use Bioceramic coated gutta-percha cones?

() Yes () No

Figure 1 The survey questionnaire. A survey on obturation trends

Gender Years of Practice Country 25.1% 30.4% 33.9% 40.6% 47.3% 59.4% 44.5% 18.7% Portugal Italy Male Female <5 5 10 >10 Turkey Additional Training Working Situation 6.4% 19.1% 16.3% 48.1% 51.9% 25.4% 32.9% Non Structure PG Part-Time PG Full-Time PG Full-Time PP Part-Time PP Academic Academic NHS

Figure 2

Demographic and professional data of the respondents according to gender, years of practice, additional training in endodontics and working situation. PG-Post-Graduation; PP-Private Practice; NHS-National Health Service.

dents, root canal based sealer routinely used by the participants, the preferred calcium silicate-based sealer, and if the practitioner uses a "bioceramic" coated gutta-percha when a calcium silicate-based sealer is the choice. Related to "obturation technique", the different techniques were clustered into two major groups: warm and cold obturation technique. Carrier-based gutta-percha technique, continuous wave compaction, warm vertical condensation, thermoplasticized injection technique were included in warm obturation technique. Lateral compaction, thermomechanical compaction with rotary, and single cone were included in cold obturation technique.

The questionnaire was introduced into a Google forms® to ensure anonymity of the respondents. The questions were formatted as dropdown or selection options.

Participation in the study was voluntary. The survey was available online from the 26th of December to the 26th of May. *Statistical Analysis*

Data were collected and analyzed with SPSS® (version 24.0, IBM Corporation, Chicago, IL, USA). Descriptive statistics were given as frequencies (n) and percentages (%). A chi-square test of homogeneity and the Z Test were used to compare proportions of users of warm obturation technique or cold obturation technique associated with years of practice, additional training in endodontics, and working situation. A significance level of 0.05 was considered.

Results

The 32 countries represented within the 35 ESE full member societies were: Austria,



Belgium, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Republic of Ireland, Israel, Italy, Kosovo, Latvia, Lebanon, Lithuania, Norway, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spanish, Sweden, Swiss, The Netherlands, Turkey, United Kingdom. Belgium, Greece and Italy have each one two representative societies.

After the questionnaire was being available online for five months, 424 answers were obtained from 19 countries: Belgium, Czech Republic, Denmark, Estonia, Finland, France, Greece, Italy, Lebanon, Norway, Portugal, Republic of Ireland, Serbia, Slovakia, Slovenia, Sweden, The Netherlands, Turkey, United Kingdom. Most of the answers collected came from Italy (n=133), followed by Turkey (n=87) and Portugal (n=72). In order to do a reliable statistical analysis with comparable sub-groups, only the questionnaires from Portugal, Italy and Turkey were considered. A total of 292 answered were analyzed. Within these total participants, 9 of them answered not having any additional training in endodontics, and then were discarded. So, a total of 283 participants were considered to the statistical analysis.

The demographic and professional data of the respondents are represented in figure 2. The data came from three countries. From Portugal was registered 71 participants (25.1%), 126 from Italy (44.5%), and 86 from Turkey (30.4%). Within the respondents, 168 were male (59.4%) and 115 were female (40.6%). According to years of practice, 96 of the endodontic practitioners had been practicing for less than 5 years (33.9%), 53 of the respondents had been practicing for 5 to 10 years (18.7%) and 134 for over 10 years (47.3%). Considering the type of additional training in endodontics, 136 of the respondents had a full-time post graduation course in endodontics (48.1%), 93 had a part-time post graduation in endodontics (32.9%), and 54 had a non-structured training in endodontics (19.1%). Related to the working situation, more than half of the respondents (n=147) had been working full-time in private practice (51.9%). The other working situations were in descending order of frequency: part-time private practice/part-time academic (25.4%), academic (16.3%), and national health service (6.4%).

The preferences related to obturation (techniques, sealers, fillers) are represented in figure 3.

The warm obturation technique was practiced by 166 respondents (58.7%) and 117 practitioners preferred cold obturation technique (41.3%). Epoxy resin-based sealer was used by 148 of the respondents (52.3%), followed by zinc oxide eugenol (36.7%), calcium silicate-based sealer (8.1%), calcium hydroxide (1.4%), glass ionomer (1.1%), and silicone-based sealer (0.4%). Considering the question if the respondent used a "Bioceramic Sealer", which one was chosen, 114 (40.3%) of the respondents selected the BioRoot RCS (Septodont, Saint-Maur-des-Fossés, France), 79 (27.9%) selected the Endosequence BC Sealer (Brasseler, Savannah, GA, USA), 19 (6.7%) selected the TotalFill BC Sealer (FKG, La Chaux-de-Fonds, Switzerland), 10 (3.5%) selected the Endosequence BC HiFlow (Brasseler, Savannah, GA, USA), 18 (6.4%) answered "Others", and 40 (14.1%) answered not use at all the calcium silicate-based sealers. Within the respondents that used calcium silicate-based sealers, only 98 (34.6%) used bioceramic coated gutta-percha cones.

Years of Practice

A chi-square test of homogeneity was conducted between obturation technique (warm versus cold) and years of practice. All expected cell counts were greater than five. The practice of warm or cold obturation technique is dependent on years of practice as endodontic practitioner $[\chi^2(2)=40.7; p<0.001]$. The percentage of users of warm obturation technique was higher between the users with more than 10 years of practice (77.6%), and lower between the users with less than 5 five years of practice (36.5%) (figure 4). Inversely, the percentage of users of cold obturation technique was higher between the users with less than 5 years of practice (63.5%), and lower between the users with more than 10 years of practice (22.4%).



Figure 3

The preferences related to obturation (techniques, sealers, fillers) used by the practitioners. HCSS-Hydraulic calcium silicate sealers; CaOH-Calcium hydroxide-based sealer; ZOE-Zinc oxide eugenol-based sealer; BC-Gutta- Bioceramic gutta-percha; ES_BC-Endosequence BC Sealer; ES_HF-Endosequence Hiflow.

Additional Training

A chi-square test of homogeneity was conducted between obturation technique and additional training in endodontics. All expected cell counts were greater than five. The practice of warm or cold obturation technique was dependent on the type of additional training [$\chi^2(2)$ =35.5; p<0.001]. The differences were statistically significant within the 3 categories of additional training. Then, the choice of warm or cold obturation technique was dependent by additional training, even considered non-structure, part-time or full-time post graduation in endodontics.

The proportion of users of warm obturation technique was higher (83.3%) within the users that received a non-structured post graduation in endodontics, and lower (41.2%) between the users with a full-time post graduation as additional training in endodontics (figure 5). The percentage of users of cold obturation technique was higher between the endodontic practitioners with a full-time post graduation (58.8%), and lower within the respondents with a non-structured post graduation (16.7%).

Working Situation

The chi-square test of homogeneity was conducted between obturation technique and working situation. All expected cell counts were greater than five, and was showed association between all categories of working situation and the choice of warm or cold obturation technique $[\chi^2(3)=83.9; p<0.001]$, except for part-time private practice with partial academic working situation, when the choice of warm or cold obturation technique was not dependent by the working situation.



The full-time private practice was the working situation with higher proportion of warm obturation technique users (78.2%), and the academic working situation had a lower proportion of warm technique users (4.3%). Within the academics, 95.7% selected cold obturation technique (figure 6).

Discussion

Several studies have investigated the attitudes of general dental practitioners towards various aspects of endodontic treatment, for example, in United Kingdom (8), Belgium (9), Nigeria (10), Sudan (11), Australia (12), Denmark (13), USA (14), Sweden (15), Turkey (16), Iran (17), and Saudi Arabia (18). However, none of them only focused on the obturation preferences. This way, this study was important to clarify the obturation trends, specifically the preferred use of the warm obturation technique versus the cold obturation technique related to years of practice, additional training in endodontics and working situation.

One of the limitations of this survey is related to a representation of only three European countries, and then cannot be a truly representative of filling trends in Europe. Within the country representative from all ESE societies we assisted of an uneven low number of respondents for



Figure 4

Proportion of participants (%) who perform warm obturation technique or cold obturation technique within each category of years of practice: <5 less than 5 years of endodontic practice; 5-10 five to ten years of endodontic practice; >10 more than 10 years of endodontic practice.

Different letters show statistical significant difference (p<0.001).

Figure 5

Proportion of participants who perform warm obturation technique or cold obturation technique related to additional training in endodontics. PG=Post-Graduation. Different letters show statistical significant difference (p<0.001).



Giornale Italiano di Endodonzia June 2022; 36(1)



Figure 6

Proportion of participants who perform warm obturation technique or cold obturation technique related to working situation. *PP=Private Practice; NHS=National Health Service. Different letters show statistical significant difference (p<0.001).*

each country. Although the 32 countries represented within the 35 ESE full member societies were asked to participate, some countries didn't attend based on the explanation that don't have the policy to deliver this kind of initiatives among its members. If all the answers would be considered, an unbalance statistical analysis in the groups would be present. Then, just answers collected from the most reliable countries representation were considered. From Portugal were collected 71 answers, and from Italy and Turkey, 126 and 86, respectively. In 2019, the Portuguese society had 230 endodontic practitioners associated. The Italian societies and the Turkish society had about 1001 and 450 endodontic practitioners associated, respectively. Although these numbers may present variability over the years, related to 2019, the response rate from Portugal was 30.9%, and from Italy and Turkey was 12.6% and 19.1%, respectively.

The strategy applied in this work was a web-based survey, introduced into a Google forms® to ensure anonymity of the respondents. The increase in the use of web-based surveys can improve response rates by achieving higher levels of participation and enhancing the statistical power of the results (19). Investigators have reported that web- or internet-based surveys have greater effectiveness over traditional methods of data collection when used by specific populations who regularly use the internet in their daily lives (20). However, the main drawback of this webbased survey was the low response rate relative to the large number of professionals who receive the questionnaire. A low response rate to a web-based survey does not entail a nonresponse error; however, a reduction in the sample size could result in a higher sample error (21, 22).

This questionnaire comprises a specialized cohort, with additional training in endodontics. Almost half of the respondents had a full-time post graduation in endodontics (48.1%). The warm obturation technique was the most used among the participants (58.7%). The cold obturation technique had a proportion of 41.3%. The various obturation techniques presented in questionnaire were clustered into two major groups: warm versus cold technique. This allowed a more balanced groups, in order to do a more reliable statistical comparisons. Within the warm obturation technique, the continuous wave of compaction represented the most used warm technique (72.3%). Within the cold obturation technique, the 2 techniques most used were the lateral compaction (57.3%) and the single cone technique (34.7%). Considered the totality of participants (N=283), the single cone technique had a proportion of users of 15.2%. A study conducted between the Flemish general



dentists (9), described the single cone as the technique used by 16.0% of the respondents, which it's corroborated with this work.

In the majority of surveys with general dental practitioners, the lateral compaction was the most popular obturation technique. Indeed, the obturation trends could change among the general dental practitioners. Another study, understanding the adoption of new endodontic technology amongst 692 Danish general dental practitioners, registering that 65% never used warm gutta-percha (13). The percentage of use of lateral compaction varies among the studies: 81% (18), 65.8% (9), 40% (14). Current trends in endodontic treatment by general dental practitioners were also reported in a United States national survey (14). In that study, 40% of general practitioners reported using cold lateral compaction; however, 54% used various warm obturation techniques. This last percentage corroborates the percentage of warm obturation technique registered in the present study.

Over the years, numerous methods have been advocated to filling the prepared root-canal system, each with its own claims of easy, efficiency or superiority. However, there are few data on the influence of obturation technique on treatment outcomes. A meta-analysis reported that a higher rate of overextension was associated with warm gutta-percha obturation compared with cold lateral compaction, but other factors such as postoperative pain prevalence, long-term outcomes, and obturation quality were not different (23). Few studies have been concerned with factors that influence the quality of root canal treatment, like factors related to root canal obturation. It might be assumed that such factors will be related not only to the individual endodontic practitioner (knowledge, attitudes and skills), but also to the context in which the practitioner works. For example, the remuneration system, time pressure, working conditions and patient expectations.

This could explain the result in this study, where practitioners who are working in the public service revealed more practice of cold technique (66.7%), contrasting with 78.2% of endodontic practitioners with a full-time private practice, which adopt warm obturation technique most frequently. Surprisingly, regarding a full-time academic situation, 95.7% of endodontic practitioners selected a cold obturation technique. Indeed, lateral compaction is taught and practiced worldwide, serving as the gold standard against which new techniques must be compared (4).

This could explain that probably the most taught technique could be the one most applied by the academic endodontic practitioners themselves. Indeed, the working situation had a strong association with the type of obturation technique selected (warm versus cold). When it was observed a statistical parameter likes the Phi nominal measure, the value of association between warm or cold obturation technique and years of practice, additional training in endodontics, and working situation, 0.379, 0.354, 0.545 was obtained, respectively.

Within the three values, the stronger association is related to working situation (0.545), which revealed a high dependence on obturation technique and working situation. This could explain the surprisingly proportions of warm technique users when a full-time post graduation was done. Indeed, when the endodontic practitioner had an additional training like a full-time post graduation, 41.2% of participants selected warm obturation technique, contrasting with 83.3% of practitioners with a non-structured post graduation, who selected warm technique. Probably, in a more deeply and complete additional training, like a full-time post graduation, it will be expected to be associated with a more proportion of warm technique users, which are more technique sensitive, and with a higher learning curvature. However, a possible explanation could be done crossing the information between the additional training and the working situation.

Within the participants who did non-structured post graduation training, 94.4% had a full-time private practice. However, participants who did full-time post grad-



uation training, just 28.7% had a full-time private practice. The participants who did a full-time post graduation more frequently were associated with an academic working situation or other public service, where financial resources could be a limitation to select a more expensive equipment like the one applied to warm obturation technique.

The association between the years of practice as endodontic practitioner, the additional training in endodontics, and the working situation have a dependent relationship with the choice of a warm or a cold obturation technique (p<0.01). Practitioners with more than 10 years of practice in endodontics selected most often the warm obturation technique (77.6%). This percentage dropped to 36.5% between the respondents who had graduated less than 5 years. Not because the "young" endodontist has less skills and less academic differentiation (participants who answered that have less than five years of practice, 55.2% have a full-time post graduation endodontic training), but probably because the "young" endodontist are likely to have less economic autonomy to invest to their clinical private practice. Indeed, the lateral compaction is a relatively simple and versatile technique that does not require expensive equipment like continuous wave technique does. Also, the single-cone technique, which is becoming increasingly popular since it is being associated as an easy handling and straightforward technique, low cost, less operator-dependent and associated with a short procedure time (24, 25). In this technique more emphasis is placed on the sealer and not primarily on the gutta-percha cone. However, a higher sealer volume inside the root canal space may negatively influence the seal, as most available sealers tend to shrink upon setting (26).

As a result, single cone technique combined with conventional sealers was deemed inappropriate, and up until now, it was recommended to maximize the gutta-percha volume and minimize the sealer thickness (27), using thermoplasticized gutta-percha obturation techniques. However, in this study, the epoxy resin-based sealer was the most used sealer (65.1%), even when the single cone technique was applied. Just 16.3% of the practitioners used calcium silicate-based sealers in a single cone technique.

The principal reason of using single cone technique by a more specialized endodontic practitioner could be more related to association between the single-cone technique and the biologic benefits of calcium silicate-based sealers. Since mineral trioxide aggregate development, silicate-based materials are widely used in endodontic procedures because of their excellent biological properties (28). Considering the preference of calcium silicate-based sealers, in this study the BioRoot RCS sealer was the most chosen (40.3%), followed by Endosequence BC Sealer (27.9%), and TotalFill BC Sealer (6.7%). Even with a more easily way of use, like a premixed sealer in a syringe with a capillary tip, easy to introduce into the canal, a manual mixing of calcium silicate sealer, like BioRoot, was preferred. Probably related to a high disposability in a European endodontic market. Endosequence BC and TotalFill have the same composition (29). However, the first is most commercialized in United States and TotalFill in Europe. If we grouped together the use of Endosequence BC and TotalFill, the proportion corresponded to 34.6%. The Endosequence Hiflow represented a low proportion (3.5%) of calcium silicate-based sealers used, probably because it's the most recently calcium silicate sealer launched to the market, which has yet a few independent studies (30-32).

This study corroborates the finding of some clinical inconsistency on using of calcium silicate-based sealer. In this work, even considering just the single cone practitioners, epoxy resin-based sealer was preferably chosen (65.1%). Also, the practitioners who selected the calcium silicate-based sealers only 34.6% selected bioceramic coated gutta-percha cones. Indeed, a recently international survey was published to gain insight on the current clinical usage of "bioceramic" root canal sealers by general dental practitioners and endodontic practitioners, and



to determine if "bioceramic" root canal sealer clinical application is in accordance with the best available evidence (33). The authors highlighted wide variation in the clinical practices, which are not often in accordance with the current literature on "bioceramic" root canals sealer. This inconsistency implies to provide further clarifications and better standardization on "bioceramic" root canals sealer clinical application.

Other studies about obturation trends should be carried out to reach more representation from each European country. It is hoped that this baseline information providing a snapshot of current endodontic practice by some members of the endodontic societies, and can serve as a launching point for further, more in-depth investigations of particular topics of interest. It will be interesting in the future, to check whether the single-cone technique and trends of calcium silicate-based sealers will increase in popularity in Europe, the correct clinical application of these types of sealers and the possible impact on the outcome.

Conclusions

The results of this survey found that standard techniques and materials remain to be used by the practitioners, like a cold lateral compaction, although the warm obturation technique was the most preferred within the participants. However, new materials and techniques, like calcium silicate-based sealers and the reconsidering single cone technique, are growing in popularity amongst endodontic practitioners. Individual factors like years of practice, additional training in endodontics, and working situation influenced the obturation technique choice.

Clinical Relevance

Among endodontic practitioners who participated in this study, the warm obturation technique is still the most used. However, the selection between warm or cold obturation technique is dependent by years of clinical practice, additional training in endodontics and working situation. The single cone associated with calcium silicate-based sealers is an emerging technique. However, when it is adopted, the core material most selected is gutta-percha non bioceramic.

Conflict of Interest

The authors deny any conflict of interest.

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ORIGINAL ARTICLE

Influence of different root canal drying protocols on the bond strength of a bioceramic endodontic sealer

ABSTRACT

Aim: This study aimed to evaluate the influence of different root canal drying protocols on the bond strength of a bioceramic sealer, in comparison with an epoxy resin-based sealer. **Methodology:** Sixty-six mandibular premolar teeth had their crowns sectioned and their canals prepared with Reciproc (VDW GmbH). Next, the roots were randomly distributed into three groups (n=22), according to the different drying protocols: absorbent paper points (G_{pp}); irrigation with 95% ethanol and drying with paper points (G_{gsc}); and irrigation with 70% ethanol and aspiration with a 30-gauge needle (G_{70E}). Each group was then redistributed into two subgroups (n=11), according to the sealer used in the single cone technique: Sealer Plus BC (MK Life) or AH Plus (Dentsply). After 7 days of obturation, the roots were sectioned into 1-mm thick slices and submitted to the push-out test. Bond strength was calculated (MPa) and data were analyzed by Kruskal-Wallis test, complemented by Games-Howell post-hoc test and Mann-Whitney U test (a=5%).

Results: There was no statistical difference among the drying protocols in the specimens filled with AH Plus, irrespective of the root third (P>0.05). For the Sealer Plus BC, no significant difference was observed for the drying protocols, however, there was statistical difference among the root thirds in $G_{g_{5E}}(P=0.017)$. When the sealers were compared to each other, no statistical difference was observed, regardless of the drying protocol evaluated: G_{pp} (P=0.447), $G_{g_{5E}}(P=0.687)$ and G_{70F} (P=0.132).

Conclusions: The different drying protocols of the root canal did not influence the bond strength of both endodontic sealers.

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Introduction

n order to reestablish the health of the periapical tissues, it is necessary to effectively perform the cleaning, shaping and obturation of the root canal system (1). Ideally, the obturation should promote efficient filling and sealing along the entire root canal length, including the apical foramen, accessory canals, isthmus, voids and irregularities (2, 3). For this reason, it is essential that the sealers used in the root canal obturation have certain characteristics, such as insolubility to the tissue fluids, biocompatibility, flow ability, adequate setting-time, antimicrobial activity and adhesion to the dentine walls (4-6).

Among the root canal sealers disponible in the market, calcium silicate based-sealers, also called "bioceramic sealers" by most manufacturers for marketing purpose, have stood out mainly for their adequate physical, chemical and biological properties (7). One of them is the Sealer Plus BC (MK Life Medical and Dental Products, Porto Alegre, RS, Brazil), which is a material based on nanoparticulate di- and tricalcium silicate. This sealer has biocompatibility and bioactive potential (8, 9), in addition to antimicrobial activity (10). This activity has been associated with a large release of calcium hydroxide and an increase in the pH of the medium, when this sealer is used for root canal obturation (11). Unlike to conventional root canal sealers, calcium silicate-based sealers are hydraulic cements and need water to start their setting reaction. Under hydration conditions, would produce calcium silicate hydrate gel (C-S-H), which leads to calcium hydroxide formation, contributing to their biological properties (7).

Another sealer commonly used in endodontics, AH Plus (Dentsply De Trey, Konstanz, Germany), is an epoxy resin-based material and has been commonly used as gold standard sealer (12) due to its low solubility, adequate radiopacity, resistance and flow; besides having dimensional stability and high bond strength to dentine (2, 5, 8, 9, 11, 13).

The bond strength of a sealer to the root dentin is an important property for maintaining the integrity of the filling material (14). The drying protocol performed prior to root canal obturation may directly influence the adhesion of the sealer to the dentinal walls and reflect on the success or failure of the endodontic treatment (15, 16). Several studies have reported that residual moisture in the root canal modify the sealing ability and the adhesion of endodontic sealers (15-17). On the other hand, the complete absence of moisture may also lead to unsatisfactory results (18). Moisture plays a fundamental role on the bioceramic endodontic sealers setting and hardening (19). The complete absence of moisture might be associated to alteration in setting reaction of this type of endodontic sealer (19). To the best of our knowledge, there is no scientific accordance regarding the most appropriate protocol for root canal drying prior to obturation with bioceramic sealers (16, 19-22). Therefore, the present study evaluated the influence of different root canal drying

protocols on the bond strength of a bioceramic endodontic sealer (Sealer Plus BC, MK Life), in comparison with an epoxy resin-based sealer (AH Plus, Dentsply De Trey). The null hypothesis tested was that the adhesion of both endodontic sealers to the root dentin would not be influenced by the different levels of moisture, irrespective of the root canal third.

Materials and Methods

Sample Size Calculation

The sample size was estimated based on previous studies comparing the bond strength of teeth filled with different endodontic sealers (17, 18). Accordingly, with the aid of the sealed Envelope software (Sealed Envelope Ltd., 2018, https:// sealedenvelope.com), for the analysis with α =0.05 and considering an effect size=0.80, at least 10 teeth should be allocated in each testing group.

Sample Selection and Preparation After Institutional Ethics Committee approval, (Protocol no. 035475), sixty-six

freshly extracted human mandibular premolars were selected for this study. To confirm the existence of a single and straight root canal with a fully formed apical foramen, the teeth were radiographed (Spectro 70X Selectronic X-ray machine, Dabi Atlante, Ribeirão Preto, São Paulo, Brazil) in both mesial-distal and buccal-lingual directions. The roots with large oval canals were substituted in order to provide canals with round section after endodontic preparation. After examination in stereoscopic lens under ×4 magnification (Illuminated Magnifying Glass, Tokyo, Japan), the teeth with caries, restorations and signs of cracks were discarded from the final sample. Then, the selected teeth were cleaned with periodontal curettes (SM 17/18, Hu-Friedy, Rio de Janeiro, RJ, Brazil), followed by disinfection in 0.5% chloramine T solution at a temperature of 4 °C for 48h, and washing under running water for 24h.

Next, the teeth were positioned, in their long axis, on a surface parallel to the ground and had their crowns sectioned below the cement-enamel junction by a double-sided diamond disc (Brasseler Dental Products, Savannah, GA, USA), under air/water spray copious cooling, in order to provide root length of approximately 16 mm. The root canal length was verified by introducing a size 15 Flexofile instrument (Dentsply Maillefer, Tulsa, OK, USA), until its tip reached the apical foramen. lished by subtracting 1 mm from the root canal length.

All root canals were prepared with Reciproc R40 (40/0.06) instrument (VDW GmbH, Munich, Germany), driven by an electrical motor (VDW Silver, VDW GmbH), according to the manufacturer's instructions. At each removal of the instrument for cleaning, the root canals were irrigated with 2 mL of 1% sodium hypochlorite (NaOCl) solution using a syringe with NaviTip 30-gauge needle (Ultradent, South Jordan, UT, USA) inserted up to 2 mm from the apical foramen. As final irrigation, 3 mL of 2.5% NaOCl was also used for 3 minutes, followed by 3 mL of 17% EDTA and rinsing with 5 mL of distilled water.

Drying Protocols

The roots were randomly assigned to the following three experimental groups, according to the drying protocols performed (n=22).

 $G_{_{PP}}$ (control group): root canals were dried with size 40 absorbent paper points (Dentsply Maillefer), until complete dryness of the last paper point used.

 $G_{_{95E}}$ (lower moisture): the excess of distilled water was removed with size 40 absorbent paper points (Dentsply Maillefer). Root canals were then dried with the application of 3 mL of 95% ethanol using a syringe with a NaviTip 30-gauge needle inserted up to 2 mm from the apical foramen. Ethanol was left in the root canal for 10 seconds, and removed with absorbent paper points, as described in G_{pp} .

Then, the working length (WL) was estab-

Table 1

Sealers used in the experimental procedures, with their respective composition and manufacturers

Sealer	Composition	Manufacturer
AH PlusTM	 AH Plus Paste A: Epoxy Resin with Bisphenol-A, epoxy resin with bisphenol-F, calcium tungstate, zirconium oxide, silica, iron oxide pigments. AH Plus Paste B: Dibenzildiamina; aminoadamantane; triciclodecano diamine, calcium tungstate, zirconium oxide, silica, silicone oil. 	Dentsply DeTrey, Konstanz, Alemanha
Sealer Plus BC	Zirconium oxide, tricalcium silicate, dicalcium silicate, calcium hydroxide, propylene glycol.	MK Life, Porto Alegre, RS, Brazil



Figure 1

Representative SEM images of the failure mode analysis for the AH Plus (A-B, E-F, I-J) and Sealer Plus BC Groups (C-D, G-H, K-L). Adhesive-type failures in low magnification (A, C). Note in higher magnification the dentine surface free of sealer and the exposure of dentinal tubules after displacement of the filling material, specially at the AH Plus group (B). In the group filled with the calcium silicate-based sealer, a white surface with a lesser number of open dentinal tubules was observed (D). Low magnifications of cohesive-type failures showing the fracture of the filling material (E, G). Details of the cohesive failures in higher magnification (F, H). In the AH Plus group, it is possible to observe the sealer still adhered to the dentine surface (white arrows) and fractured inside material (black arrows) (F). Higher magnification of the area demarcated in G showing the calcium silicate-based sealer fractured and partially adhered to the dentine (H). Mixed-type failures showing the simultaneous occurrence of adhesive failure at the dentine and cohesive failure of the filling material in both groups (I, K). Higher magnification showing details of the mixed failure mode, with the presence of the epoxy-resin based sealer fractured and adhered to the dentine (J), and the presence of sealer's particles adhered to the dentine surface showing structural cohesive failure of the calcium silicate-based sealer (L).

d: dentin; g: gutta-percha; s: sealer.

 $G_{_{70E}}$ (higher moisture): the excess of distilled water was removed with size 40 absorbent paper points (Dentsply Maillefer). Root canals were dried with the application of 3 mL of 70% ethanol using a syringe with a NaviTip 30-gauge needle inserted up to 2 mm from the apical foramen. Ethanol was left in the root canal for 1 minute, and then, it was aspirated using a syringe with Capillary tip (Purple, Ultradent, South Jordan, UT, USA).

Each group was then randomly redistributed into 2 subgroups (n=11), according to the endodontic sealer used, as follows: Sealer Plus BC (MK Life) and AH Plus (Dentsply De Trey). Each sealer used in the experimental procedures, with the respective composition and manufacturers are shown in Table 1.

Root Canal Obturation

The endodontic sealers were manipulated according to the recommendations of their respective manufacturers. Root canal obturation was performed by the single cone technique, using a master gutta-percha cone of the Reciproc system (R40, VDW GmbH). The sealer was applied to canal walls using a lentulo spiral (Dentsply, Maillefer). The master cone was coated



with sealer and placed within the canal up to the WL. After gutta-percha excess removal with a heated plugger (Odous de Deus, Belo Horizonte, MG, Brazil) and vertical compaction, the root canal entrance was restored with a temporary restorative material (Citodur, Dorident, Austria) and the specimens were stored in an oven at 37 °C and 100% relative humidity for 7 days, to allow sealers setting.

Push-Out Test

The roots were transversely sectioned in relation to their long axis with a diamond saw blade (South Bay Technology, San Clement, CA, USA), coupled to a metallographic cutter (Isomet 1000, Buehler, Lake Forest, IL, USA), obtaining 1 mm-thick slices. The first and last slices were discarded from the final sample. Six slices per root were selected to perform the pushout test.

The root slices were individually attached to the lower portion of the Universal Testing Machine (Model 4444, Instron, Canton, OH, USA). A compressive load was applied by a cylindrical plunger (0.6-1.0 mm in diameter) attached to the upper portion of the Universal Testing Machine. A crosshead speed of 0.5 mm/min was applied, until bond failure has occurred.

The maximum force required for the filling material displacement was measured in Kilonewtons (KN), transformed into Newtons (N) and converted into Megapascal (MPa) by force division by the lateral area (SL) of the obturation. SL was calculated by the following formula:

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

where SL=lateral bonded surface area, R=measure of the radius of the root canal in its coronal portion, r=measure of the radius of the root canal in its apical portion, h=height/thickness of the root cross section.

Failure Mode Analysis

After the push-out test, the fractured slices were submitted to a careful visual examination in a stereomicroscope (SteREO Discovery V12, Carl Zeiss, Jena, Germany) at \times 50 magnification. The failure mode was classified, according to the following criteria: adhesive failure (dentine surface free of sealer); cohesive failure (filling material fracture, with the dentine surface covered by sealer); or mixed failure (part of the dentine surface covered by sealer).

Representative samples of each group were selected and prepared for analysis under Scanning Electron Microscope (SEM) (JEOL JSM 6390 LV, Peabody, MA, USA). The samples were dried, mounted on aluminum stubs, placed in a vacuum chamber and sputter-coated with a gold layer of 300

Table 2

Bond strength Mean Values (MPa) and Standard Deviation for AH Plus, considering the different root canal thirds and drying protocols (G_{pp}, G_{qsp} and G_{70p})

	AH Plus				
Drying Protocol	Cervical	Medium	Apical		
G _{PP}	0.69 ±1.21 ^{Aa}	0.87±1.29 ^{Aa}	0.97±1.28 ^{Aa}		
G _{95E}	0.79 ±1.07 ^{Aa}	0.91±1.18 ^{Aa}	1.06±0.90 ^{Aa}		
G _{70E}	1.19 ±1.49 ^{Aa}	0.71±0.89 ^{Aa}	1.16±0.91 ^{Aa}		

*Equal uppercase letters in the lines indicate no significant statistical difference among the root thirds (Kruskal-Wallis test, p>0.05). *Equal lowercase letters in the columns indicate no significant statistical difference among the drying protocols (Kruskal-Wallis test, p>0.05). G_{pp} : control group, drying protocol with paper points; G_{gse} : 95% ethanol and paper points; and G_{roe} : 70% ethanol and aspiration.

Table 3

Bond strength Mean Values (MPa) and Standard Deviation for Sealer Plus BC, considering the different root canal thirds and drying protocols (G_{PP}, G_{95E} and G_{70E})

Drying Protocol	Sealer Plus BC			
	Cervical	Medium	Apical	
GPP	0.65±0.71 ^{Aa}	0.47±0.43 ^{Aa}	1.08±0.87 ^{Aa}	
G95E	0.50±0.54 ^{Aa}	1.04±1.55 ^{ABa}	1.06±0.59 ^{Ba}	
G70E	1.24±1.61 ^{Aa}	1.21±1.72 ^{Aa}	1.51±1.23 ^{Aa}	

*Different uppercase letters in the lines indicate statistical difference among the root thirds (Kruskal-Wallis test and Games-Howell post-hoc, p<0.05). *Equal lowercase letters in the columns indicate no significant statistical difference among the drying protocols (Kruskal-Wallis test, p>0.05). GPP: control group, drying protocol with paper points; G95E: 95% ethanol and paper points; and G70E: 70% ethanol and aspiration.

> A° (Bal-Tec SCD 005, Bal-Tec Co., Balzers, Liechtenstein). The failure mode analysis was performed under SEM operated at accelerating voltage of 15 kV, at ×20, ×40, ×80, ×500 and ×1000 magnifications (Figure 1).

Statistical Analysis

The normality of the data was verified by the Kolmogorov Smirnov test (p<0.05) and non-parametric tests were used in the data analysis (α =5%). When the drying protocols and different root canal thirds were considered in the analysis, the bond strength data were submitted to Kruskal-Wallis test, complemented by the Games-Howell posthoc test. The comparison between the endodontic sealers was performed by the Mann-Whitney U test, when the root canal thirds were not considered. The statistical tests were performed with the aid of the GraphPad Prism 4.0 Software program (GraphPad Software, La Jolla, CA, USA).

Results

Table 2 and 3 show the bond strength mean values of AH Plus and Sealer Plus BC, respectively, considering the different root canal thirds. In samples obturated with AH Plus, there was no significant difference between the experimental groups (drying protocols), irrespective of the root canal third assessed (P>0.05). For Sealer Plus BC, no significant difference between

Table 4

Bond strength Mean Values (MPa) and Standard Deviation for AH Plus and Sealer Plus BC considering the drying protocols (G_{PP}, G_{95E} and G_{70E})

Drying Protocol	Sealer			
Drying Protocol	AH Plus	Sealer Plus BC		
G _{PP}	0.84±1.23 ^{Aa}	0.71±0.65 ^{Aa}		
G _{95E}	1.24±0.94 ^{Aa}	0.85±0.69 ^{Aa}		
G _{70E}	1.16±1.25 ^{Aa}	1.34±1.38 ^{Aa}		

*Equal uppercase letters in the lines indicate no significant statistical difference between the sealers (Mann-Whitney U test, p>0.05). *Equal lowercase letters in the columns indicate no significant statistical difference among the drying protocols (Kruskal-Wallis test, p>0.05). G_{pp} : control group, drying protocol with paper points; G_{gse} : 95% ethanol and paper points; and G_{roe} : 70% ethanol and aspiration.



Table 5

Distribution of Failure Modes (%) according to the sealers and drying protocols

		Sealer				
Drying Protocol	AH Plus		Sealer Plus BC			
	Adhesive	Cohesive	Mixed	Adhesive	Cohesive	Mixed
$G_{_{PP}}$	22.23	44.44	33.33	17.16	31.42	51.42
G _{95E}	22.23	41.66	36.11	20.58	29.41	50.01
G _{70E}	48.49	12.12	39.39	21.42	39.30	39.28

 G_{pp} : control group, drying protocol with paper points; G_{app} : 95% ethanol and paper points; and G_{70p} : 70% ethanol and aspiration.

the drying protocols was observed (P>0.05), however, there was statistical difference between the cervical and apical root canal thirds for G_{95E} (P=0.017). When the root canal thirds were not considered in the analysis, no statistically significant difference was observed between the drying protocols for AH Plus (P=0.446) and Sealer Plus BC (P=0.497) (Table 4).

Table 5 shows the distribution of the failure modes (adhesive, cohesive or mixed). AH Plus presented predominantly cohesive failures for $G_{\rm PP}$ (44.44%) and $G_{\rm 95E}$ (41.16%), and adhesive failure for $G_{\rm 70E}$ (48.49%). For the Sealer Plus BC, the majority of specimens presented mixed failure ($G_{\rm PP}$ 51.42% and $G_{\rm 95E}$ 50.01%). $G_{\rm 70E}$ presented similar percentages of cohesive (39.30%) and mixed (39.28%) failures.

Discussion

The integrity of root canal sealing after obturation is directly related to the bond strength of the endodontic sealer to the dentinal walls (23). However, the moisture and the presence of fluids prior to this clinical step may influence the adhesiveness of sealers (21, 22). Other studies also have showed the effect of intracanal moisture on the bond strength of endodontic sealers to root canal dentin (16, 17, 20, 21, 24, 25). On the other hand, until now, no studies have evaluated the influence of different drying protocols on the bond strength of Sealer Plus BC (bioceramic endodontic sealer) to the root canal walls. Therefore, the purpose of the present study was to evaluate the influence of different root canal drying protocols on the bond strength of a bioceramic endodontic sealer, in comparison with an epoxy resin-based sealer. Based on the results obtained, the null hypothesis was accepted, as none of the assessed drying protocols affected the bond strength of the endodontic sealers to the root dentin, when root canals were obturated with the single cone technique.

The bond strength of the endodontic sealers may be measured by several methods, and the push-out test is one of the most used due to its easy reproducibility and interpretation of the results (26). In the present study, the resistance of the filling material to displacement was evaluated using tips with different diameters (0.6 to 1.0mm), specifically selected for use according to the diameter of the root canal in each root portion. The use of this apparatus allowed the force to be applied to the material in a more homogeneous way, making the results more reliable (27). The epoxy-resin based sealer chosen for comparison to the bioceramic has been widely used in previous studies and is consid-



ered a gold-standard sealer, since it has excellent physicochemical properties (28) and adequate bond strength to dentin (4). In this study, to perform different moisture conditions of the root dentine, the canals were irrigated with different concentrations of alcohol and the content was dried with absorbent paper points or aspirated using a syringe with Capillary tip. This protocol has already been used in previous studies, with the aim of evaluating the influence of different moisture conditions on dentine and its relationship with different types of endodontic sealers (15, 17, 20).

Our results shows that the presence of greater or lesser moisture in the root canal, after the use of the different drying protocols, did not interfere in the bond strength of the tested sealers to root dentin. In addition, there was no statistically significant difference between both endodontic sealers, regardless of the drying protocol evaluated. These results differ from the literature, which has shown significant difference with the use of different root canal drying protocols, especially with respect to bioceramic sealers (18, 20-22, 24). It is important to point out that in our study we performed root canal filling simulating what happens in clinical reality, in which the endodontic sealers are used in conjunction with gutta percha cones for root canal filling. Many studies use only sealers to check the influence of moisture on its bond strength to root canal dentin (16-20). However, it is necessary to check the behavior of these sealers according to their clinical application, regardless of existing laboratory results.

The use of the single cone technique may be associated with the low bond strength values exhibited by the AH Plus sealer in our experiment. While some studies have shown higher bond strength values with the use of the single cone in root canal filling, others have observed lower adhesion to dentin with the use of this technique (26, 29), mainly in the obturation of oval root canals (27).

In another study, Araújo et al. (29) obtained results of bond strength to dentin (0.77 MPa), after root canal preparation with a reciprocating system and obturation with AH Plus by the single-cone technique, lower than the group in which the specimens were obturated by the lateral condensation technique.

These results are similar to the bond strength values obtained in the present study (0.84 MPa, Table 4), in the experimental group where the root canals were dried with paper points and sealed with epoxy-resin sealer.

According to Rached-Júnior et al. (26), during the execution of the single cone technique, the sealing forces are exerted mainly in the apical direction, which may reduce the frictional resistance of the filling material against the root canal walls and decrease the bond strength to dentin. In addition, the anatomic variations of the root canal may increase the occurrence of non-touched areas by the instruments during preparation, which may negatively affect the adaptation of the master cone (26). Also, according to the study of Pereira et al. (27), root canals with circular section have higher bond strength in the cervical and middle thirds than those with more oval section. The latter have 3 to 4 times more sealer in the cervical and middle thirds than circular canals, making these areas more susceptible to failure, due to larger sealer accumulation (27). In the apical third, the presence of less sealer may explain similar values of bond strength in all groups, including in the bioceramic sealer groups (27). However, the assessment of this condition was not included in the present study and should be evaluated in the future studies with the use of other methodologies.

The most appropriate dimensional stability of calcium silicate-based sealer is often highlighted as the main reason for allowing their use with the cold hydraulic condensation, especially the single-cone technique (7). In the single-cone technique, greater emphasis is placed on the sealer used than on gutta-percha (concept of sealer-based filling) (7). Thus, especially in non-vital teeth, the use of a calcium silicate-based sealer, such as Sealer BC, due to its more adequate biological properties, may induce a mild


inflammatory reaction and favor repair when in close contact with the periapical tissue (9).

Several studies have shown that the level of root canal moisture may affect the adhesiveness of the endodontic sealers, and that their composition also influences their behavior in the presence of higher or lower moisture (16, 17, 20, 22, 30). Nagas et al. (17) evaluated the bond strength of the iRoot SP, AH Plus and MTA Fillapex sealers inserted in root canals with different levels of residual moisture. These authors observed that, regardless of the moisture level, the bioceramic iRoot SP demonstrated the highest adhesive potential, which may be explained by the material composition. This class of sealer have particles of reduced size, which allows better flow between the gutta-percha cones and possible irregularities inherent to the root canal (30).

Paula et al. (20) assessed different drying protocols (70% isopropyl alcohol, paper points, EndoVac and 95% ethanol) prior to obturation with MTA Fillapex, AH Plus and Sealapex, and correlated the high polarity of the ethanol molecules with the canal dehydration, and consequent decrease of the adhesive potential of the sealers. In another study (25), the authors found that the iRoot SP sealer presented lower bond strength when the root canals were dried with paper points (3 to 4 units) and ethyl alcohol. This result might be explained by the fact that this material, similarly to other bioceramic sealers, has in its composition calcium silicate and calcium phosphate, whose main compounds require moisture to carry out the hydration reaction, allowing the sealer setting and hardening (31). Furthermore, the excessive drying promoted by the mentioned protocols removed the water present in the dentinal tubules, which may hinder the penetration of hydrophilic sealers and compromise the adhesion quality of them (15).

Such evidence corroborates with a recent study (16), which concludes that maintaining moist dentin, but not dry or wet dentin, may be advantageous before the filling root canals with bioactive sealers. However, in our study, there was no significant difference among the tested drying protocols when Sealer Plus BC was used for root canal filling. The methodological differences of the studies may have contributed to the achievement of different results, since in the study by Tasdemir et al. (24), for instance, the obturation was performed only with the iRoot sealer, without the presence of gutta-percha cones.

In the present study, Sealer Plus BC presented significant difference among the root canal thirds in $G_{0.5F}$ (drying with 95%) ethyl alcohol and paper points, lower moisture). The apical third had significant difference when compared to the middle and cervical thirds. In a study conducted by Dias et al. (18), the residual moisture was evaluated after root canals drying with paper points or paper points associated with irrigation with 70% isopropyl alcohol. The authors also reported differences in bond strength values among the root canal thirds, and related their results to the difficulty in standardizing the moisture along the root canal, due to the difference in density of the dentinal tubules and the difficulty of accessing and drying the solutions in the more apical portions (32). According to the manufacturer of the Sealer Plus BC, this sealer is an insoluble, radiopaque and non-aluminum compound, which contains calcium silicate and requires the presence of moisture to set. It may be assumed that in the groups obturated with Sealer Plus BC, the apical third presented a greater content of residual moisture than the cervical and middle thirds, optimizing the setting reaction of the sealer. However, new studies need to be performed to assess other obturation techniques associated to other drying protocols.

Studies have reported that water removal almost completely decreases the bond strength of calcium silicate-based endodontic sealers to root dentine (17,20,24). However, the excess of water may also be a detrimental factor for this class of materials (21). In a study conducted by Razmi et al. (21), the decrease of bond



strength to dentin was verified when the sealer EndoSequence was inserted in an intracanal environment with presence of excessive humidity. The hydrophilic characteristics of the sealing materials will never be sufficient to displace the water in a fully wet root canal, leading to a trap of water droplets at the dentine-cement interface, which results in a decrease in adhesiveness (15).

Despite the results presented in other studies, the occurrence of extreme situations, which include dehydrated or excessively hydrated root canals, negatively interferes with the quality of sealer adhesion to the dentinal walls (17, 21). Sealer Plus BC and AH Plus, did not show significant statistical difference in relation to the drying protocols performed. Sealer Plus BC has good physicochemical properties, such as pH, calcium ion release, flow, radiopacity and setting-time, however, this new calcium silicate-based endodontic sealer showed higher solubility than recommended by ISO 6876:2012 (11). Also, as it is a premixed bioceramic sealer, it has some advantages, such as the reduced working time, and it is not influenced by manual mixing and any change in the powder-liquid ratio that may affect its physicochemical properties (33-35). When compared to the powder-liquid presentation form, this sealer may have better physicochemical properties, such as viscosity, solubility and bond strength to root dentine, however, as it was not the objective of the present study, further studies are needed to evaluate this comparison.

It is important to highlight that the present study has some limitations, such as the non-comparison with other bioceramic sealers and the non-use of a wet chamber to simulate a wet root canal, which would be more similar to the clinical situation than the use of an oven in this experiment. Therefore, it is necessary to carry out further laboratory and clinical studies in order to verify the physicochemical and biological properties of this sealer, comparing it with other calcium silicate-based endodontic sealers (premixed or not) and its use with different techniques of root canal filling.

Conclusions

The different levels of moisture did not influence the bond strength of the tested endodontic sealers to the root canal walls, when the single cone technique was used to perform the root canal filling. The bioceramic and the epoxy resin-based sealers presented similar results regarding bond strength, except for the apical third, when the root canal was dried with absorbent paper points and 95% ethanol prior to obturation with the bioceramic sealer.

Clinical Relevance

Moisture has a fundamental role in the hydration kinetics of bioceramic sealers. It is important that clinicians know the most appropriate root canal drying protocols when using bioceramic sealers for root canal obturation.

Conflict of Interest

None.

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Influence of operator experience on apical debris extrusion after endodontic instrumentation with different single-file systems

ABSTRACT

Aim: This study evaluated whether operator experience interferes with the amount of apically extruded debris and actual instrumentation time.

Methodology: Seventy-five mesial roots of extracted mandibular first molars were randomly allocated to 6 groups (n=15 each) according to operator experience and instrumentation system used (HyFlex EDM, WaveOne Gold, or Reciproc Blue). Each root was secured in a preweighed Eppendorf tube for collection of debris extruded during instrumentation. Actual instrumentation time (the timer was started when the instrument was set in motion within the root canal and stopped when the instrument was removed) and the total amount of extruded debris were recorded. The Shapiro-Wilk test was used to assess data distribution normality, followed by descriptive analysis and the Kruskal-Wallis test with Dunn's post hoc test. Spearman's correlation analysis was done too.

Results: There was no significant difference in the amount of apically extruded debris between experienced and inexperienced operators or between the instrumentation systems used. Regarding instrumentation time, a significant difference was observed: HyFlex EDM-inexperienced > WaveOne Gold-experienced. No correlation between extruded debris, instrumentation time, and operator's experience was found.

Conclusions: All instrumentation systems produced extrusion, with no difference between them or between operator's experience. Instrumentation time differed only between 2 groups.

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Introduction

ain is an unpleasant experience associated with actual or potential tissue damage and one of the main reasons why a patient seeks endodontic treatment. Undeniably, a more significant amount of debris extruded apically during endodontic treatment increases the likelihood of postoperative pain, flare-ups, and even treatment failure (1).

Factors such as working length (WL), kinematics, apical diameter, amount and/or type of irrigant, and instrumentation systems have been evaluated in several studies investigating the amount of apically extruded debris (2-4).

The HyFlex EDM system uses continuous rotation kinematics and is manufactured by electric discharge machining, which, according to the manufacturer, provides flexibility and fracture resistance to the instruments. The system consists of a 25/.12 orifice opener (optional), a 10/.05 glide path file, and a 25/~ modeling instrument called HyFlex OneFile (https://www.coltene.com/fileadmin/Data/EN/Products/Endodontics/Root_Canal_Shaping/Hy-Flex_EDM/31328A_HyFlexEDM_Brochure_US.pdf).

The WaveOne Gold and Reciproc Blue systems use reciprocating kinematics and are manufactured by a "gold" and "blue" heat treatment process, respectively, which provides high flexibility and fatigue resistance to the instruments (5). The WaveOne Gold system consists of 20.07 (Small), 25.07 (Primary), 35.06 (Medium), and 45.05 (Large) files. They have a parallelogram-shaped cross-section with an 85° cutting angle and a semi-active tip (Dentsply Tulsa Dental Specialties. Wave One Gold. Available at: https://www.dentsplysirona.com/content/dam/dentsply/pim/ en GB/Endodontics/Obturation/Paper Points/WaveOne Gold Absorbent Points/ WaveOne%20GOLD%20Brochure%20 2015.pdf). The Reciproc Blue system consists of 25.08 (R25), 40.06 (R40), and 50.05 (R50) files with an S-shaped cross-section and a non-cutting tip. Both should be used in reciprocating motion with a three inand-out movements (pecks) with a stroke amplitude of 3 mm and performed in each third of the canal (cervical, middle, and apical) until the WL is reached (2, 6).

It is known that all instruments available on the market promote apical extrusion of debris (7). However, to our knowledge, no study has investigated to date whether the amount of apically extruded debris varies according to operator experience, considering the importance of teaching mechanical instrumentation to dental undergraduates without generating risk for patients (8).

This ex vivo study's primary objective was to evaluate whether operator experience interferes with the amount of apically extruded debris. As a secondary objective, we evaluated actual instrumentation time to evaluate difficulties encountered in reaching the WL. The null hypothesis tested was that there would be no significant differences in the amount of apically extruded debris or time required for instrumentation between different instrumentation systems, whether used by experienced or inexperienced operators.

Materials and Methods

Tooth selection and specimen preparation After approval by the local Research Ethics Committee (approval number 2,379,268), 75 extracted human mandibular first molars, indicated for extraction for periodontal or ortodontic reasons, were selected for this study. Only teeth with fully formed apices showing independent foramina, curvature angles of 10-15° (9), no calcifications, no resorption, and no prior endodontic treatment were included in the study. Specimens were immersed in 0.5% chloramine-T trihydrate solution for one week for disinfection.

The sample size was calculated using G*Power statistical software, version 3.1.9.4. To detect a difference of 0.0024 (standard error of 0.0025) between the experimental groups, which is in agreement with the study of Uslu et al. (10), with a 5% significance level and a power of 80%, a sample size of 15 specimens per group was necessary.

Standard access cavities were made by



sectioning the crowns at the cementoenamel junction with a round diamond bur (Horico Dental Hpf; Ringleb, Berlin, Germany) mounted on a low-speed handpiece powered by a micromotor under water cooling, thus generating specimens of 13 mm in length, as confirmed by a digital caliper (500 series, DIN 862; Mitutovo, São Paulo, SP, Brazil). The initial diameter of the mesiobuccal canal was determined by introducing a #15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) into the canal until it fits snugly within the canal and its tip was visible at the apical foramen under a dental operating microscope at 12.5x magnification (Stemi 508; Carl Zeiss, Jena, Germany). The same procedure was used to determine the WL. set 1 mm short than this measurement. Canals that did not meet these criteria were discarded and replaced with new specimens.

Randomization

The specimens were randomly allocated using the Random Allocation Software, version 1.0.0, to 6 experimental groups (n=15 each) according to the instrumentation system used (HyFlex EDM, WaveOne Gold, or Reciproc Blue) and operator experience (experienced or inexperienced). The mesiolingual canals did not undergo any instrumentation or irrigation throughout the experiment.

Instrumentation

In the EDM-E group, 15 experienced operators used the OneFile instrument (25/~, variable taper) of the HyFlex EDM system (Coltène, Altstätten, Switzerland) in rotary motion (500 rpm, 2.5 Ncm), with 3 in-andout movements (pecks) and a stroke amplitude of 3 mm in the cervical, middle, and apical thirds of the canal until the WL was reached. In the EDM-I group, 15 inexperienced operators used the same instrument and performed instrumentation in the same manner as described for the EDM-E group. In the WOG-E group, 15 experienced operators used the Primary file (25.07) of the WaveOne Gold system (Dentsply Maillefer, Ballaigues, Switzerland) in reciprocating motion, with 3 in-and-out movements (pecks) and a stroke amplitude of 3 mm in the cervical, middle, and apical thirds of the canal until the WL was reached. In the WOG-I group, 15 inexperienced operators used the same instrument and performed instrumentation in the same manner as described for the WOG-E group.

In both the RCB-E (15 experienced operators) and RCB-I (15 inexperienced operators) groups, the R25 instrument (25.08) of the Reciproc Blue system (VDW GmbH, Munich, Germany) was used in the same manner as previously described for the WOG-E group.

In all experimental groups, the instruments were driven by an X-Smart Plus motor (Dentsply Maillefer, Ballaigues, Switzerland) adjusted for each system. Regardless of the system used, each instrument was used to prepare 1 root canal only and then discarded.

Inexperienced operators received a brief training using simulated root canals in clear resin blocks (three blocks per operator), with root curvature similar to that of the human teeth used in the study. This prior contact helped to establish a standardized technique protocol.

Throughout instrumentation, the specimens were irrigated with 3 mL of double-distilled water using a side-vented needle (30G NaviTip; Ultradent Products Inc, South Jordan, UT) at each three inand-out movements or one-third of root instrumented. After each movement and irrigation cycle, foramen patency was confirmed with a #15 K-file extending 1 mm beyond the foramen in all groups. After completion of instrumentation, final irrigation was performed with 1 mL of double-distilled water, not exceeding the total amount of 10 mL of irrigant standardized for all specimens. Canals were aspirated with a capillary tip (Ultradent, South Jordan, UT) and then dried with paper points provided by the respective system's manufacturer. The canals were considered prepared when the working length was reached with the instrument. and to confirm the instrumentation's quality, the master gutta-percha point compatible with the instrument used was selected. For this, a periapical radiograph was taken.





Figure 1

Root canal instrumentation apparatus inside an opaque container, preventing the visualization of the inside of the Eppendorf tube. Manufacture of the apparatus for collecting and weighing extruded debris

The amount of apically extruded debris after instrumentation was quantified according to the method proposed by Myers & Montgomery (11) and modified by other authors (12, 13) (Figure 1).

In all experimental groups, each Eppendorf tube was weighed 3 times on the same precision balance initially used, and the average of three measurements was recorded as the final weight of the Eppendorf tube containing extruded debris. The dry weight of extruded debris (in grams) was calculated by subtracting the initial weight (empty tube) from the final weight (tube containing debris).

Evaluation of actual instrumentation time The instrumentation procedure was timed for each specimen with a digital stopwatch (Seiko, Japan). For each instrument, the timer started when the instrument was set in motion within the root canal and stopped when the instrument was removed, resulting in the actual instrumentation time.

Statistical analysis

The results obtained for debris weight and instrumentation time were statistically analyzed using BioEstat 5.0. The Shapiro-Wilk test rejected the assumption of normality of data for both the amount of extruded debris and actual instrumentation time.

First, descriptive analyses were performed, and the Kruskal-Wallis test followed by Dunn's post hoc test was used, with the level of significance at 5%. A second analysis was performed using Spearman's correlation to determine the relationships between the operator's experience with the amount of extruded debris and the instrumentation time. The correlation of the amount of extruded debris with the instrumentation time was analyzed too. For the second analysis, all 90 samples were used, divided into two groups based only on the operator's experience (Table 1 and Figure 2).

Results

There was no significant difference in the

Descriptive analysis of all samples grouped based only on the operator experience									
Operator experience Extruded Debris Instrumentation Time									
	Median±IQD Mean±SD		Median±IQD	Mean±SD					
Inexperienced (45)	0.0018100±0.0016	0.00231840.0020755	26.130±10.3100	29.103±9.5855					
Experienced (45)	0.0019000±0.0016	0.0018760± 0.0010776	25.550±10.0000	25.624±6.1516					

Table 1

IQD: interquartile deviation; SD: standard deviation.



Table 2

Differences between instrumentation systems regarding apical debris extrusion and time required for root canal instrumentation

Group	Extruded	Debris	Instrumenta	ation Time
	Median ± IQD	Mean ± SD	Median ± IQD	Mean ± SD
EDM-E (15)	0.0019±0.0015 ^{AB}	0.0020±0.0009	28.5600±6.1900AB	29.1547±4.4305
EDM-I (15)	0.0021±0.0012 ^{AB}	0.0025±0.0012	30.2800±20.2550 ^A	35.3267±11.3072
WOG-E (15)	0.0020±0.0010 ^{AB}	0.0017±0.0007	20.3500±7.5150 ^B	22.5600±5.1632
WOG-I (15)	0.0019±0.0014 ^{AB}	0.0020±0.0008	22.5600±8.0150AB	24.9227±7.0142
RCB-E (15)	0.0011±0.0020 ^{AB}	0.0019±0.0015	23.5700±9.4700 ^{AB}	25.1567±6.9997
RCB-I (15)	0.0013±0.0004 ^{AB}	0.0025±0.0034	26.1300±10.9900AB	27.0587±6.8567
P-value*	0.223	36	<0.0	05

EDM: HyFlex EDM system; WOG: WaveOne Gold system; RCB: Reciproc Blue system; E: experienced operator; I: inexperienced operator; IQD: interquartile deviation; SD: standard deviation.

Same superscript letters indicate no statistical difference between the groups, whereas different superscript letters indicate statistical difference. *Kruskal-Wallis test.

> amount of apically extruded debris between experienced and inexperienced operators or between the instrumentation systems used (Table 2) (p>0.05). Regarding instrumentation time, a significant difference was observed only between the EDM-I and WOG-E groups (p<0.05), with the latter requiring shorter instrumentation time (Table 2).

> Regarding Spearman's correlation (Table 3), no significant correlation was found between the operator experience and extruded debris (p=0.545) neither between the operator experience and instrumenta-

tion time (p=0.173). The instrumentation time and the amount of extruded debris showed no correlation too (p=0.081).

Discussion

The null hypothesis was accepted regarding the amount of extruded debris but rejected regarding instrumentation time. Extracted mandibular first molars with moderately curved roots were used in the present study, which approximates the study conditions to the difficulties routinely encountered in clinical practice



Figure 2

A) Box-plot distribution of the amount of extruded debris regarding the operator's experience. B) Box-plot distribution of the instrumentation time regarding the operator's experience.



		Operator experience	Extruded Debris	Instrumentation Time
Operator experience	Spearman's rho			
	p-value			
Extruded Debris	Spearman's rho	-0.06459		
	p-value	0.545		
Time	Spearman's rho	-0.14499	0.18494	
	p-value	0.173	0.081	

Table 3 Spearman's correlation of all samples grouped based only on the operator experience

compared to similar studies that used clear resin blocks (14-16). The crowns were sectioned, and all root lengths were standardized at 13 mm to avoid canal length interference with the results. Despite standardization efforts, it is known that mandibular molars' mesial roots have anatomic variations (17, 18). For this reason, the initial foramen diameters were also standardized by introducing a #15 K-file into the canal, thus avoiding the use of calcified or very wide canals.

We decided to recruit 15 inexperienced operators to avoid the fact that when only one operator performs all procedures, he/ she can begin to develop skills in the activity that will lead to improved performance (15). It is essential to highlight that final-year dental undergraduate, who had already performed endodontic treatments on patients but were skilled only in the use of manual files, participated in the study – this differs from previous studies in which operators were considered inexperienced if they had never had any contact with endodontics (15).

As shown in similar studies, double distilled water was used to irrigate the canals because it would not influence the final extruded debris weighing results after evaporation. The use of sodium hypochlorite could lead to the deposition of salts or the formation of crystals after the drying process, thereby increasing the mass of the extruded material (2, 6, 19). We chose the modified Myers & Montgomery weighing method (11-13) because it has been widely used in many studies (2, 13, 20).

In the present study, regardless of operator experience, there was no difference or

correlation in the amount of apically extruded debris with the instrumentation system, whether reciprocating or rotary. However, the literature is controversial in this respect. While some studies report more significant debris extrusion with reciprocating instruments (10, 21), other studies show more significant extrusion with rotary instruments (12, 22). We believe that the absence of such difference in our study results from the fact that the HyFlex EDM system, despite having different kinematics than that of the WaveOne Gold and Reciproc Blue systems, also uses only one instrument to prepare the root canal, thus reducing the possibility of debris extrusion compared with multiple-file systems (12). Despite using instruments with different kinematics, instrumentation was set to be performed by root thirds, alternating with glide path maneuvers, which have been shown to produce less debris (7). Thus, all systems followed the instrumentation protocol using sequential files in the cervical, middle, and apical thirds, with a sequence of three in-and-out movements and a stroke amplitude of 3 mm under abundant irrigation until the WL was reached.

Regarding operator experience, there was also no correlation with the amount of apically extruded debris. Given the lack of *ex vivo* studies for comparison, clinically, our results are consistent with those of previous studies that demonstrated, as a secondary endpoint, no difference in postoperative pain (a factor that may be related to the amount of extruded debris) concerning operator experience (23, 24). However, it is essential to note that such



studies did not follow the same methodological standards used in the present study since their inexperienced operators were graduated dental surgeons.

The actual instrumentation time of root canals was statistically longer in the EDM-I group than in the WOG-E group. Although this may reflect inexperienced operators' difficulty in reaching the WL with continuous rotation kinematics, we believe that this finding is clinically insignificant because there were only 13 seconds of difference between the two groups. All other instrumentation time comparisons showed no difference between the groups, and there was no correlation between the instrumentation time with the operator's experience, which can be explained by previous training in three clear resin blocks, since it has been demonstrated that, regarding instrumentation time, little practice is required for inexperienced operators to reach the WL (15). In the present study, although inexperienced operators attended a very brief training session, the use of single-file systems allowed them to safely prepare the canals, without loss of length, deviation, or any other iatrogenic factors, in a time similar to that of experienced operators.

Since the crown was removed, direct access to the canal is a limitation of this study, as it prevented us from fully simulating the difficulties encountered in clinical practice. This has already been reported as a determinant of iatrogenic events in endodontic treatment performed by inexperienced operators (25).

Siqueira (26) has pointed out that endodontic treatment is technically demanding, and general dentists are not prepared to provide adequate endodontic care, explaining that a possible solution to the problem would be a paradigm shift in education in dental schools. It is known that a more significant amount of debris extruded apically during endodontic treatment can lead to flare-ups, pain, and even treatment failure (27). Since there was no correlation of the amount of extruded debris with the operator's experience in this study and that mechanized instrumentation is easy to learn (15), the introduction of mechanized instrumentation can be a safe way in an attempt to improve the endodontic treatment success and reduction of postoperative pain incidence (28).

Conclusions

Within this study's limitations, it can be concluded that all instrumentation systems produced debris extrusion, with no difference between them or between experienced and inexperienced operators. Also, in most comparisons, the actual instrumentation time of root canals did not differ between the groups – only the EDM-I group required longer instrumentation time than the WOG-E group. No significant correlation was found between extruded debris, instrumentation time and operator's experience. All instrumentation systems tested can be safely used even by inexperienced operators regarding the amount of apically extruded debris, thus paving the way for further research to replicate these findings in clinical settings.

Clinical Relevance

The knowledge about the influence of the operator's experience on extruded debris is an important factor considering the importance of teaching mechanical instrumentation to dental undergraduates without generating risk for patients.

Conflict of Interest

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

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ORIGINAL ARTICLE

Residual dentin thickness at the apical third of mandibular first molar mesial root instrumented by nickel-titanium rotary and manual files with different tapers: an *ex vivo* study

ABSTRACT

Aim: The residual root dentin thickness (RDT) in endodontically treated teeth is a major factor affecting their longevity. This study aimed to assess and compare the RDT in teeth instrumented with hand files and also with Dia-PT, Edge Taper Platinum (ETP), and Mani-Silk rotary instruments with different tapers.

Materials and Methods: Sixty-eight extracted human mandibular first molars with separate mesial and distal roots were evaluated. The teeth were mounted in Speedex putty and underwent cone-beam computed tomography (CBCT). The primary thickness of the mesial and distal root canal walls was measured at 1, 2, and 3 mm from the apex. The teeth were divided into four groups (n=17) for instrumentation of the mesiobuccal canal with Dia-PT with 0.09 taper, ETP with 0.08 taper, Mani-Silk with 0.04 taper, and stainless steel K files with 0.02 taper. They were then placed back in the putty in their previous position and underwent CBCT again. The RDT was measured at 1, 2, and 3 mm from the apex in the mesial and distal surfaces, and compared with the baseline values. Data were analyzed by the Kruskal-Wallis and Mann-Whitney tests using SPSS version 20 at P<0.05 level of significance.

Results: In the mesial surface, hand files yielded the maximum RDT. Mani-Silk and Dia-PT showed comparable RDT at all levels from the apex lower than the values yielded by hand files, except at 3 mm from the apex, where Mani-Silk yielded RDT comparable to hand files. In the distal surface, hand files and Mani-Silk yielded maximum RDT at all levels from the apex, except at 1 mm, where Mani-Silk showed significantly lower RDT. RDT in ETP and Dia-PT groups was almost similar, and significantly lower than other groups at all levels, except at 1 mm where RDT in ETP group was similar to that in Mani-Silk and hand file groups. **Conclusions:** Use of hand files resulted in maximum RDT at all three levels from the apex. Files with greater taper removed greater amount of dentin from the distal surface.

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Introduction

echanical preparation of the root canal system is an important step in endodontic treatment. According to the qualitative guidelines of the European Endodontics Society, elimination of the residual pulpal tissue and debris, and preservation of the original shape of the canal curvature during the cleaning and shaping process are the main goals of root canal instrumentation (1). The amount of dentin removed in the process of instrumentation is an important parameter in prevention of procedural errors such as strip perforation (1). The residual root dentin thickness (RDT) after instrumentation should not be lower than 0.3 mm in order to be able to resist condensing forces during obturation (2).

Morfis et al. (3) found a direct association between the amount of lost tooth structure and risk of crown or root fracture. Cheung et al. (4) evaluated the mesial root of mandibular first molars and demonstrated that nickel-titanium (NiTi) files left a thicker layer of RDT than stainless steel files after use. In another study, Portenier et al. (5) showed that the RDT was higher after using the Light Speed system (Lightspeed Technology Inc., San Antonio, TX, USA) compared with manual filing by the stepback technique.

Dia-PT rotary file (Dia-Dent, Cheongwon, Korea) is a commercial subtype of the ProTaper rotary system, and is similar to the ProTaper system in terms of shape and transverse cross-section. It has a triangular convex cross-section and properties similar to those of ProTaper in root canal preparation (6).

Edge Taper Platinum (ETP) rotary system (EdgeEndo, Albuquerqu, NM, USA) was recently introduced on the market and has the properties of heated NiTi files. Its characteristics are similar to those of ProTaper Gold files (PTG; Dentsply Maillefer, Ballaigues, Switzerland). Its manufacturer claims that ETP files have a fatigue resistance twice that of ProTaper Gold and 6 times that of ProTaper Universal files (PTU, Dentsply Maillefer) under optimal speed and torque conditions (7).

The Mani-Silk (Mani, Japan) file has a tearshaped cross-section, which results in unique cutting of dentin, and confers fracture resistance to the file. It eliminates the screwing effect and decreases the amount of debris, and the stress applied to the file as such. On the other hand, decreasing the number of instruments in this system decreases the instrumentation time and allows more efficient treatment by the clinician (8).

Considering the significance of adequate RDT, this study aimed to compare the RDT following instrumentation of root canals with Dia-PT, ETP, and Mani-Silk NiTi rotary instruments with different tapers in comparison with hand files using conebeam computed tomography (CBCT).

Materials and Methods

Sixty-eight human mandibular first molars of patients between 20-30 years that had been extracted due to periodontal reasons were collected. The study was approved by the ethics committee of Zahedan University of Medical Sciences (IR.ZAUMS. REC.1397.358). The teeth were first immersed in 2.5% sodium hypochlorite (Cerkamed, Stalowa Wola, Poland) for 3 h, and calculus and debris were removed by an ultrasonic scaler.

The inclusion criteria were teeth with separate mesial and distal roots, Vertucci's type II (9) mesial canals such that the canals merged at over 3 mm from the apex, 20-22 mm root length, 20-35° canal curvature, and closed apices.

The exclusion criteria were teeth with cracks, fracture, caries, calcifications, internal and/or external root resorption, and open apex (Figure 1).

Standard access cavity was prepared by a diamond fissure bur (Diatech, Coltene Whaledent, Altstetten, Switzerland) under air and water coolant. A #10 K-file was introduced into the canal to ensure canal patency. The apical diameter of the canal was evaluated by introducing a #15 K-file into the canal (Mani, Tochigi, Japan) not passing through the apex. A #15 K-file was



Figure 1 Flowchart of the study.

then introduced into the mesiobuccal canal and a digital radiograph (EZ Sensor; Vatech, Hwaseong, Republic of Korea) was obtained by the parallel technique from different directions. The degree of curvature of the mesiobuccal canal was then determined according to the Schneider's method (10). Teeth with 20-35° curvature were included in this study. The teeth were stored in saline until the experiment. They were then coded and mounted in Speedex putty blocks measuring 15x15 mm. Next, they underwent CBCT (New-Tom Giano; QR, Verona, Italy) with the exposure settings of 8x5 field of view, 9 s exposure time, 90 kV voltage, and 59-30 mA amperage. Images of different sections with 0.16 mm slice thickness were reconstructed. After determining the center of the mesiobuccal canal, the primary thickness of the mesial and distal root canal surfaces was measured by drawing lines perpendicular to the center of the canal. For each surface, the distance between the inner and outer walls of the root canal was considered as the thickness of the respective surface. The primary thickness of mesial and distal surfaces at 1, 2 and 3 mm from the apex of the mesiobuccal canal was measured independently by an endodontist and an oral and maxillofacial radiologist. The measurements were repeated in cases where different values were reported by the endodontist and oral and maxillofacial radiologis. The measurement accuracy of the device software was 0.01 mm. The teeth were then randomly divided into four groups (n=17). A



 Table 1

 Residual dentin thickness of the mesial wall before and after instrumentation (mm)

P value	After Mean (SD)	Before Mean (SD)	Distance from apex							
	Dia-PT	ETP	Mani silk	Hand file	Dia-PT	ETP	Mani silk	Hand file		
0.005	0.67±0.13⁵	0.80±0.14ª	0.64±0.20 ^b	0.79±0.17ª	0.87±0.15	1.05±0.25	0.86±0.21	1.16±0.23	1 mm	
0.000	0.85±0.15⁵	0.65±0.24°	0.94±0.16 ^b	1.24±0.23ª	0.91±0.26	0.85±0.21	1.32±0.18	1.38±0.20	2 mm	
0.000	0.89±0.12 ^b	0.78±0.20 ^b	1.25±0.22ª	1.38±0.25ª	1.06±0.15	1.01±0.16	1.75±0.21	1.61±0.24	3 mm	

SD: Standard deviation.

Different superscripted letters indicate a significant difference between groups (P<0.05).

Table 2 Residual dentin thickness of the distal wall before and after instrumentation (mm)

P value	After Mean (SD)	Before Mean (SD)	Distance from apex							
	Dia-PT	ETP	Mani silk	Hand file	Dia-PT	ETP	Mani silk	Hand file		
0.008	0.67±0.14 ^b	0.86±0.17ª	0.70±0.18 ^b	0.75±0.18ª	0.90±0.20	1.16±0.29	0.89±0.19	1.10±0.27	1 mm	
0.000	0.88±0.19 ^b	0.70±0.17⁵	1.11±0.23ª	1.18±0.23ª	0.99±0.21	0.92±0.21	1.34±0.21	1.33±0.23	2 mm	
0.000	0.92±0.19 ^b	0.81±0.19 ^b	1.45±0.43ª	1.38±0.28ª	1.01±0.28	1.10±0.17	1.76±0.27	1.59±0.24	3 mm	

SD: Standard deviation.

Different superscripted letters indicate a significant difference between groups (P<0.05).

#10 K-file was inserted into the mesiobuccal canal until its tip was visible at the apex; 1 mm was subtracted from this length to determine the working length.

Root canal cleaning and shaping

The process of cleaning and shaping of the canals was performed by an experienced operator. Apical preparation size was #25 in all canals, and all preparations were performed by a rotary motor (VDW Silver; VDW, Munich, Germany). During instrumentation, each root canal was rinsed with 5 mL of double-distilled water using a syringe with a side-vented needle (0.3x25 mm; Endo-Top; Cerkamed, Stalowa, Wola, Poland). Next, 2 mL of 17% ethylenediaminetetraacetic acid (Meta BioMed, Korea) was used for smear layer removal

followed by 2 mL of 5% sodium hypochlorite (Cerkamed, Stalowa Wola, Poland), and a final rinse with 2 mL of saline. The canals were then dried and divided into 4 groups (group 1: hand files, group 2: Mani-Silk, group 3: ETP and group 4: Dia-PT).

Root canal Instrumentation

In group 1, which was prepared manually, #2 and #3 Gates-Glidden drills were used in the crown-down technique for coronal flaring. The apical area was prepared up to file #25. The rest of the canal was prepared by the step-back technique up to file #40 by subtracting 1 mm from the file length.

In group 2, Mani-Silk (Mani, Japan) pack was used at a speed of 500 rpm and 3 N/ cm torque according to the manufacturer's

Figure 2 Residual dentin thickness of

the mesial wall before and after instrumentation (mm).





Figure 3

Residual dentin thickness of the distal wall before and after instrumentation (mm).

instructions as follows: First #25/0.08 file was used for coronal preparation and then files #20/0.04 and #25/0.04 were used to the working length.

In group 3, Edge Taper Platinum (ETP) rotary system (EdgeEndo, Albuquerqu, NM, USA) was used at 300 rpm speed and 3 N/cm torque according to the manufacturer's instructions: SX file was first used for coronal preparation followed by S2, F1, and F2 (#25/0.08) to the working length. In group 4, Dia-PT (Dia-Dent, Cheongwon,

Korea) was used at 300 rpm speed and 2 N/cm torque according to the manufacturer's instructions: DX file was first used for coronal flaring followed by D1 to half the working length, D2 to two-thirds of the working length, and D3 and D4 (#25/0.09) to the working length.

After cleaning and shaping, the teeth were placed back in the putty in their previous position and underwent CBCT with the same parameters. The preoperative and postoperative CBCT scans of each tooth



were compared, and the RDT was measured at 1, 2 and 3 mm from the apex in the mesial and distal surfaces, and compared with the primary thickness.

Statistical analysis

Data were analyzed by the Kruskal-Wallis and Mann-Whitney tests using SPSS version 20 (SPSS Inc., IL, USA) at P<0.05 level of significance.

Results

Tables 1 and 2 and Figures 2 and 3 present the RDT of the mesial and distal walls before and after instrumentation with hand files with 0.02 taper, Mani-Silk files with 0.04 taper, ETP files with 0.08 taper, and Dia-PT files with 0.09 taper at 1, 2 and 3 mm from the apex.

In both the mesial and distal walls, the RDT was maximum at 3 mm and minimum at 1 mm from the apex (P=0.000).

In the mesial surface, hand files yielded the maximum RDT. Mani-Silk and Dia-PT showed comparable RDT at all levels from the apex lower than the values yielded by hand files, except at 3 mm from the apex, where Mani-Silk yielded RDT comparable to hand files. In the distal surface, hand files and Mani-Silk yielded maximum RDT at all levels from the apex, except at 1 mm, where Mani-Silk showed significantly lower RDT. RDT in ETP and Dia-PT groups was almost similar, and significantly lower than other groups at all levels, except at 1 mm where RDT in ETP group was similar to that in Mani-Silk and hand file groups.

Discussion

This study assessed the RDT in the apical third of the mesiobuccal canal of mandibular first molars following instrumentation with three rotary files in comparison with hand files. During root canal preparation, high amounts of tooth structure are removed, which increases the risk of mechanical fracture of endodontically treated teeth compared with vital teeth (11). Evidence shows that a minimum of 0.3 mm root dentin thickness should remain after root canal instrumentation as the minimum RDT (2). In the present study, over 0.3 mm of RDT remained after using all four types of files; thus, all tested systems left adequate thickness of residual dentin. However, Sathorn et al. (12) discussed that dentin removal does not necessarily increase the risk of tooth fracture and some other factors are also involved in this matter.

At present, CBCT enables precise assessment of the function of rotary files and evaluation of canal transportation. In this study, measurements were made on preoperative and postoperative CBCT scans, and the values were compared to determine the changes. This technique is highly accurate and provides several images without damaging the specimens. Also, evidence shows that CBCT measurements made in the apical third are comparable with the standard references with no significant difference (13).

The present study, similar to some other investigations (14, 15), was conducted on the mesiobuccal canal of the mesial root of mandibular first molars because accessing its canals is difficult and these canals have high frequency of procedural errors due to the presence of concavity at the furcation area and having a curvature. Also, maximum curvature is often at the apical third. Thus, the amount of dentin removed from the mesial and distal walls at 1, 2 and 3 mm from the apex was quantified in this study.

In both the mesial and distal canal walls, the RDT was maximum at 3 mm and minimum at 1 mm from the apex; this difference was statistically significant. In line with the present results, Garala et al. (16) reported that the primary root dentin thickness appears to be the most important factor in determining the RDT after root canal instrumentation.

The RDT in both the mesial and distal walls was significantly higher in the hand file group at 1, 2 and 3 mm from the apex. Shahriari et al. (17) assessed the RDT of the mesiobuccal canals of mandibular molars following instrumentation with Profile and hand files. They concluded that dentin removal by hand files at all sections



was greater than that by rotary files. Difference between their results and ours may be due to the manual preparation technique since they first prepared the apical third and then the coronal third while we first performed coronal flaring by the Gates Glidden drills and then prepared the apical third.

This sequence can significantly affect the involvement of the file with the root canal walls in the apical third and consequently the RDT. The same technique was used by Rama Roa et al (18). They compared the RDT in use of hand files and four rotary file systems. They found that the RDT was greater in use of hand files than rotary files. In the present study, Mani-Silk and Dia-PT showed comparable mesial RDT at all levels from the apex lower than the values yielded by hand files, except at 3 mm from the apex, where Mani-Silk yielded RDT comparable to hand files. This finding is due to a constant increase in taper of Mani-Silk file, which results in an increase in the material volume in the entire body of the file, resulting in greater stiffness and subsequently greater dentin removal (19). On the other hand, although teeth with severe root curvature were excluded from the study, root canal irregularities can increase the risk of procedural errors such as root canal transportation and excessive dentin removal (20).

This may explain comparable dentin removal by Mani-Silk and Dia-PT although Dia-PT has greater taper (0.09.). Also, the operation speed was higher for Mani-Silk than other files, which may also explain greater dentin removal from the apical third. In the distal surface, hand files and Mani-Silk vielded maximum RDT at all levels from the apex, except at 1 mm, where Mani-Silk showed significantly lower RDT. RDT in ETP and Dia-PT groups was almost similar, and significantly lower than other groups at all levels, except at 1 mm where RDT in ETP group was similar to that in Mani-Silk and hand file groups. It appears that by an increase in file taper,

RDT deceases in the distal surface, which may be attributed to the rotational movement of high-taper rotary files, which results in greater dentin removal compared with manual filing. Also, Kunert et al, (21) and Gundappa et al. (22) discussed that file taper is an important factor that plays a pivotal role in canal transportation and dentin removal from the root canal walls. It should be noted that several factors such as the age and dentin properties (23) can affect the results. Although teeth with severe root curvature were excluded from this study, different degrees of curvature significantly affect the treatment results. Thus, use of precise measurement techniques such as micro computed tomography and evaluation of higher number of sections are recommended.

Conclusions

This *ex vivo* study indicated that all root canal cleaning and shaping techniques decreased the dentin thickness. However, increasing the file taper is not directly correlated with greater removal of dentin or RDT.

Clinical Relevance

The result of this study showed that increasing the file taper does not necessarily lead to greater dentin removal.

Conflict of Interest

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

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ORIGINAL ARTICLE

Interfacial characterization of hydraulic calcium silicate-based sealers by Scanning Electron **Microscopy**

ABSTRACT

Aim: To characterize the sealer-dentin interface of hydraulic calcium silicate-based sealers TotalFill and Bioroot and compare it to an epoxy-resin sealer AH Plus.

Methodology: An experimental ex vivo study was conducted were 15 single-root extracted premolars were divided into three experimental groups. The teeth were prepared and filled using a single tapered gutta-percha cone. Samples were cut and analyzed using scanning electron microscopy. The images were analyzed using ImageJ software.

Results: The median interface value for the three sealers was between 0.6 and 2.5 µm. The largest interface was observed in the TotalFill group in the middle third, and this difference was significant compared to AH Plus (p<0.05). The largest sealer area was for Bioroot in the apical third, but the difference was not significant.

Conclusions: Within the limitations of this study, AH Plus had better marginal adaptation in the middle third. The interfacial gaps were similar in the apical third for all sealers.

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KEYWORDS Hydraulic calcium silicate-based sealers, interface characterization, sealer interface, BioRoot, TotalFill RCS

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Introduction

oot canal filling materials are necessary to avoid root canal reinfection and to entomb remaining bacteria (1), and to fulfil these purposes, root canal sealers must provide excellent sealing ability, adequate dimensional stability, a reasonable setting time to ensure proper handling, insolubility in tissue fluids and ideally, high cytocompatibility (1, 2). However, most materials fail to provide an effective seal (3) and moisture inside the canal before obturation appears to be critical (4). Conventional resin-based sealers are hydrophobic, and their properties are altered by moisture (4), but this is not the case for Bioceramic (BC) or hydraulic sealers because they are hydrophilic, and root canal wall and tubule moisture is necessary for setting (2).

BC sealers were created to overcome several disadvantages of traditional resin-based sealers. They present properties such as a non-existent setting contraction (5) or slight expansion and an alkaline pH, which gives them antibacterial capacity before setting and chemical stability (6). Other properties include biocompatibility and the ability to create a link between dentine and the obturation material (7). All of these qualities make them, in theory, excellent obturation materials.

Among the most frequently used BC sealers is TotalFill BC SealerTM (FKG Dentaire, La Chaux-de-Fonds, Switzerland), a premixed tricalcium silicate-based sealer available in a syringe presentation. Its composition includes monoclinic zirconia, calcium silicate, monobasic calcium phosphate, calcium hydroxide, and tantalum pentoxide (8). Its working time is over four hours at room temperature, and the setting time is more than four hours; however, in an environment with low humidity, the final setting time may be up to 23 hours (8).

Other properties reported are the ability to produce an appropriate seal, adequate radiopacity, flow and bioactivity produced by ion release (8).

BioRoot RCS[™] (Septodont, Saint-Maurdes-Fosses, France) is a sealer based on tricalcium silicate and zirconium oxide (9). Its presentation is powder-liquid. According to the manufacturer, the powder is based on tricalcium silicate, zirconium oxide and iodine, and the liquid is composed of calcium chloride and polycarboxylate. It has ten minutes of working time and a final setting time of five hours (10). Other reported properties include adequate radiopacity, bioactivity and an alkaline pH (10).

Considering the lack of contraction upon setting and the possibility of obtaining a chemical bond to the root canal wall, obturation with hydraulic calcium silicate-based sealers can work differently than obturation with resin sealers. Its qualities make it unnecessary to maintain the sealers' thickness to its minimum (6). It is used in a hydraulic technique based on a tapered 4 or 6% gutta-percha point of the calibre of the last used instrument that acts as a piston allowing the sealer to flow into the canals' irregularities leaving a higher percentage of sealer (6). However, to allow a higher percentage of sealer, it should be first determined whether the interface between dentine and sealer is minimum or ideally non-existent.

This study aims to characterise the sealer-dentine interface in teeth obturated with hydraulic calcium silicate-based sealers: TotalFill BC SealerTM and BioRoot RCSTM analysed under a scanning electron microscope (SEM).

Materials and Methods

An experimental ex-vivo study was conducted prior to the approval of the ethics committee of San Sebastian University (Resolution Number 2018-14).

Sample Selection

The sample consisted of fifteen one-rooted mandibular premolars extracted for orthodontic reasons, with a type I Vertucci configuration (11), complete apical formation and a radicular curvature of no more than 10° , of patients between the age of 15 and 30.

Teeth that presented canal obliteration, internal or external radicular resorption, or endodontic treatment, were discarded.



Sample Preparation

Teeth were cleaned of organic residues with an ultrasonic scaler and then stored in distilled water for no longer than 30 days. The roots were sectioned to a length of 12mm from the apex with a diamond bur under constant refrigeration. Working length was determined with a K10 file observed under a 3.5x loupe when it came out of the apical foramen; 1mm of this length was subtracted. Root canals were then prepared with the Reciproc system to R40 file (VDW, München, Germany), using 10 mL of 5.25% sodium hypochlorite (NaClO), activated by the Endoactivator (Dentsply Maillefer, Ballaigues, Switzerland) for one minute. Final irrigation was performed with 3mL ethylendiaminetetracetic acid (EDTA) 17%, 3 mL of NaClO for 60 seconds, and 5 mL of saline solution for 60 seconds. The canals were dried with aspiration and paper points.

Experimental Groups Preparation

Teeth were divided into three groups of five premolars each. Group 1: TotalFill BC SealerTM Group 2: BioRoot RCSTM Group 3: AH PlusTM (Dentsply DeTrey, Konstanz, Germany) All groups were obturated using the hydraulic technique with an R40 gutta-percha point. The sealers were manipulated according to the manufacturers' indications. The samples were coronally sealed using Vitremer ionomer (3M Espe, St Paul, USA) and stored at 37 °C and 100% relative humidity for seven days. After this period, the teeth were mounted in translucid epoxy resin (Fibratec, Santiago, Chile), to be sectioned at 3 and 6mm from the apex using a precision sectioning saw (ISOMET) 1000, Buehler Ltd., Lake Bluff, IL, USA).

Sample Observation and Analysis

The samples were sent to the Advanced Microscopy Unit of the Catholic University of Chile for processing (golden shadowing on the coronal surface) and observation under SEM (Hitachi, TM3000, Tokyo, Japan). Images were obtained at 60 and 100x to completely visualise the obturation material and dentine, and two quadrants were chosen because they presented the lesser distortion of gutta-percha produced by the saw's cut. Close-ups at 500x 1,000x and 2,000x were taken. The interfacial distance was measured from the edge of the sealer to the edge of the dentine.

Before the observation, criteria for analysis was defined, and measuring methods were considered between the examiner and expert observer. To measure the interface horizontally and vertically, the examiner observed a total of twelve images of 2,000x randomly chosen, in the same conditions of daytime, light and screen resolution, and ten days later repeated the entire process. With the acquired data, an intra-class correlation coefficient was obtained using the statistical program EPIDAT 4.2 (Xunta de Galicia, Universidad CES, 2016), which showed an intra-examiner agreement of 0,97 on the horizontal plane and 0.91 on the vertical plane, which indicates a high concordance. For the sealer area percentage, the examiner observed a total of eight images at 60x that were randomly chosen, obtaining an intra-examiner correlation coefficient of 0.99 in the canal area and 0,95 for the gutta-percha point, which indicates a high concordance.

The digitalised images were observed by one operator on the same computer, with a screen resolution of 1,366x768 in a 14-inch screen with the ImageJ program (ImageJ, Wayne Rasband, National Institutes of Health, USA). Twenty different images of each group at 2000x were selected to measure the sealer-dentine interface. A printed scale of an image at 2,000x representing 30 µm was used to measure the interface. A grid was traced with 20 vertical and horizontal lines that guided the location during measurement. The images where the interface did not coincide with the vertical line were rotated (Figure 1A).

The horizontal measurement was obtained by tracing 20 segments perpendicular to the interface selected to reference the horizontal lines on the grid. After that, for the vertical measurement, segments were traced parallel to the interface taken as





Figure 1 A) Horizontal measuring and B) vertical measuring of the sealer-dentin interfase through the tool provided by ImageJ Software (2,000x). reference for the horizontal lines (Figure 1B). Ten images of each group were selected to calculate the sealer percentage inside the canal. The surface occupied by the canal was first measured, and then the surface occupied by the gutta-percha point; the difference between these determined the sealer area (Figure 2). All measurements were registered in μ m and adjusted to three digits after the decimal using a measuring tool provided by the software.

Results

A total of 240 images were obtained: 60 at 2,000x, 60 at 1,000x, 60 at 500x, 30 at 100x and 30 at 60x. For the interfacial measurement, 60 images at 2,000x were analysed, founding the largest horizontal interface

in the TotalFill BC SealerTM group in the apical zone (1,091 μ m on average), and the smallest vertical interface was in the Bio-Root RCSTM group in the middle third (57,1%) (Table 1).

Sealer area was measured in relation to the canal area; the images taken were analysed at 60x, and the most substantial proportion of sealer was found on the BioRoot RCS[™] group in the middle third (Table 1).

To verify that the differences observed were statistically significant, the Shapiro Wilk test was applied, which showed that data had a non-normal distribution (p<0.05). Secondly, Kruskal Wallis non-parametric test was applied and showed significant differences in the horizontal interface of AH Plus[™] middle third



Figure 2 Sealer area measurement through the tool provided by the ImageJ software (60x).



Table 1

Sealer-Dentin Interfase and Sealer Area Vertical Interfase Measurements in µm Horizontal Interfase Measurements in µm **Sealer Area** Interfase Total Sealer Median DS Minimum Maximum % Interfase % Sealer Length Interfase 1.091 0.020 79.7% 0,720 2,609 52,711 66,298 23.2% Apical TotalFill Middle 0.393 0.330 0.000 67.593 71.1% 32.7% 1.161 48.100 Bioroot 0,681 0,544 0,000 58.6% Apical 1,898 39,317 67,260 29.4% Middle 0.801 0.615 0.000 2,003 40,703 70,977 57.1% 35.6% 0,640 0.519 0.042 66.654 Topseal Apical 1.756 41,580 62.8% 28.1% Middle 0,357 0.281 0,044 0,983 66,344 48.8% 32,472 35.4%

Measurements of central tendency of the sealer-dentin interfase in the horizontal plane, in the vertical plane and sealer area inside the root canal

vs BioRoot RCS^{TM} middle third (p=0.001). No significant differences were observed for the vertical interface and sealer area (p<0.05).

Discussion

This study aimed to determine the sealer-dentine interface in teeth filled with BC sealers TotalFill BC SealerTM and BioRoot RCS[™] under SEM. There were no differences in the sealers' vertical and horizontal interfacial adaptation in the apical third. These results agree with what was observed by Mohammadian (2016), who also found no differences in the apical third when comparing hydraulic calcium silicate-based sealers to AH PlusTM (12). AH PlusTM presented significantly better adaptation in the middle third when compared to BioRoot RCS[™]. The results obtained in this study agree with Al-Haddad and Aziz (2015) when they compared hydraulic calcium silicate-based sealers to AH Plus and found that the interface was slightly smaller for AH PlusTM, but the differences were not significant. (13) In a study conducted by Arikatla (2018), they also observed better adaptation for AH PlusTM when compared to hydraulic calcium silicate-based sealers (BioRoot RCS™ and MTA FillapexTM sealer) (14). One of the reasons why AH PlusTM might have presented better adaptation in the middle third could be because of its chemical bonding to dentine (13) and higher flow (15); premolars with one canal tend to have irregular shapes in the middle third, and this ability would prove helpful to fill this area.

BioRoot RCSTM presented the highest sealer percentage in the apical third, but the difference was not significant. Theoretically, the sealer percentage should be minimal, considering that most sealers contract upon setting (13). However, this is not the case for Hydraulic calcium silicate-based sealers, in which a slight expansion is observed (6); a large percentage of sealer in the apical third would not be detrimental to the sealing ability (16).

The three sealers studied presented gaps in the apical and middle third; this agrees with Eltair (2018) in that both TotalFill BC SealerTM and AH PlusTM showed visible gaps in all root thirds (3). Marginal adaptation is critical in root canal treatment. Root canal obturation aims to produce a bacteria-tight seal and entomb possible remaining bacteria (1). Root canal biofilm has a variable thickness between a few and hundreds of cells, and the microorganisms



that regularly take part in biofilm foundation, such as *Enterococcus faecalis*, have a diameter between 0,6-2,5 μ m (17). The interface in this study had a median value of 0,357 to 1,091 μ m, which is minimal. Even though all sealers present an interface, it is unlikely that a biofilm could form in such a confined space, indicating that all the sealers studied would be effective obturation materials.

Conclusions

This study compared the sealer-dentine interface of three root canal sealers: Total-Fill BC SealerTM, BioRoot RCSTM, and AH PlusTM. All sealers presented a minimal interface in the horizontal and vertical planes. Within the limitations of this study, AH PlusTM had better marginal adaptation in the middle third. The interfacial gaps were similar in the apical third for all sealers. The sealer percentage was slightly higher for BioRoot RCSTM in the apical third, but the difference was not significant.

Clinical Relevance

Endodontic sealers are an essential part of canal obturation since they effectively seal against bacteria. Under the conditions of this study, it can be inferred that BC sealers TotalFill BC SealerTM and BioRoot RCSTM provide a proper marginal adaptation that could lead to successful obturations.

Conflict of Interest

None.

Acknowledgments

Nothing to declare.

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ORIGINAL ARTICLE

Evaluation of the push out of bond strength of different bioceramic root canal sealers with different obturation techniques

ABSTRACT

Aim: This study aims to evaluate the effect of different obturation techniques [single cone (SC) and warm vertical compaction (WVC)] on the push-out bond strength (PBS) of gutta-percha (GP) with three calcium silicate-based sealers (CSBSs).

Methodology: Sixty single-rooted mandibular premolars were selected and decoronated to obtain a standardized root length of 16 mm. The canals were instrumented using VDW. Rotate system and were assigned into six equal Groups based upon the CSBS and obturation technique used following; Group 1 and Group 2; BC Sealer HiFlow (HiFlow; Brasseler USA) with SC and WVC, Group 3 and Group 4; CeraSeal (Meta Biomed Co., Cheongju, Korea) with SC and WVC, Group 5 and Group 6; BioRoot RCS (Septodont, Saint-Maur-des-Fosses Cedex, France) with SC and WVC. Roots were sectioned transversally at the thickness of 1±0.1 mm in thickness at 5 and 10 mm from the apex. The specimens were subjected to PBS test using a universal test machine at a loading speed of 0.5 mm/ min examined for their failure modes. Statistical analysis was performed using Kruskal-Wallis and multiple comparison tests.

Results: BioRoot-WVC Group was a significantly lower PBS value than other Groups at 5 and 10 mm from the apex (p<0.05). At 5 and 10 mm from the apex, no significant difference in PBS was found among the BC Sealer HiFlow and CeraSeal sealers obturated using two different techniques (P>0.05). The most common failure mode found in all Groups was a mixed failure.

Conclusions: WVC significantly reduced the PBS of the BioRoot RCS sealer compared to SC technique, but this was not significant with the BC Sealer HiFlow and CeraSeal.

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Introduction

oot canal filling is one of the crucial factors for successful endodontic treatment. Using a root canal sealer with gutta-percha (GP) is the most widely accepted obturation technique. The root canal sealer establishes a good connection between the root canal wall and the GP, preventing microleakage causing re-infection (1, 2). In recent years, calcium silicate-based sealers (CSBSs) have been widely used in endodontics. CSBSs are biocompatible and bioactive, thanks to their calcium silicate formulation. CSBSs form an apatite layer in contact with tissue and chemically bond to dentin. Micromechanical interlocking between CSBS and root dentin helps maintain the integrity of the sealer-dentin interface during a function (3. 4).

The EndoSequence BC Sealer HiFlow (BCH; HiFlow; Brasseler USA) exhibits a lower viscosity when heated and is more radiopaque, making it optimized for warm obturation techniques compared to standard BC Sealer (5). BioRoot RCS (BR; Septodont, Saint-Maur-des-Fosses Cedex, France) sealer induces hard tissue barrier formation in the presence of a physiologic solution (6). CeraSeal (CS; Meta Biomed Co., Cheongju, Korea) has an antibacterial effect, high volumetric stability, and high flowability according to manufacturer (Meta Biomed. CeraSeal Brochure [Internet]. 2022. Available from: https://www. meta-europe.com/en/produkt/ceraseal/) (7). The composition of the three CSBSs is represented in Table 1.

The single-cone technique (SC) has become very popular because of is easy and fast. CSBSs should be used with the SC technique, as recommended in the manufacturer's manual (8). The warm vertical compaction technique (WVC) might be the best to fill the pulp space in three dimensions (9). Because, in WVC technique, plasticized GP with heat can adapt well to the root canal geometry and achieve a good seal at all root canals (10). However, in WVC, using heat leads to changing the physical properties of CBCSs and this may affect the quality of the root canal filling (11, 12). The adhesion of endodontic sealer to root canal dentine has been reported to be affected by the obturation technique (12, 13).

The push-out of bond strength (PBS) tests are used as a measure of the bond strength of root canal filling materials to the root dentin. PBS results in shear stress at the dentin-cement interface, comparable with the stress in clinical conditions (14). The ability of the PBS test to evaluate adhesion is superior to other tests, as it creates parallel fractures in the interfacial area of the dentin bond (15). There are a few studies about the PBS of BCH, BR and CS (3,5,16,17). However, no data is available for comparing these CSBSs with each other in terms of PBS with SC and WVC techniques. Therefore, this study aimed to evaluate the PBS of BCH, BR, and CS with different obturation techniques. The null hypothesis was that there are no differences between these CSBSs regarding PBS and failure mode with varying obturation techniques.

Materials and Methods

The sample size calculation was performed with G Power software (Heinrich Heine University, Dusseldorf, Germany) with α =0.05 and β =0.95, and each Group must be a minimum of nine samples (13). Sixty human mandibular premolars with only one straight root canal (curvature <5°) and a single apical foramen were included. The teeth were decoronated to achieve a standardize length of 16 mm. After determining the working length (WL), 1 mm short of the apex, the root canals were instrumented with a series of VDW.Rotate rotary file system (VDW GmbH, Munich, Germany) to #40/04. 10 ml of 2.5% NaOCl was used with a 30-G IrriFlex needle (Produits Dentaires SA, Switzerland) inserted to 1 mm short of the WL during instrumentation. Following mechanical instrumentation, each root canal was irrigated using 2 ml of 17% ethylenediaminetetraacetic acid (EDTA) solution for 1 min and 5 ml distilled water. The canals were dried using paper points.

Composition of the sealers used								
Material	Manufacturer	Туре	Composition					
EndoSequence BC Sealer HiFlow	HiFlow; Brasseler USA	Premixed	Zirconium oxide, calcium silicates, calcium phosphate, calcium hydroxide, filler, and thickening agents					
BioRoot RCS	Septodont; Saint-Maur-des-Fosses Cedex, France	Powder-Liquid	Powder: tricalcium silicate, zirconium oxide, and povidone Liquid: aqueous solution of calcium chloride and polycarboxylate					
CeraSeal	Meta Biomed Co.; Cheongju, Korea	Premixed	Calcium silicates, zirconium oxide, and thickening agent.					

Toble 1

Prepared roots were randomly assigned to six Groups (n=10) for testing the obturation technique with different CBCSs. Group 1 (BCH-SC)

According to the manufacturer's instruction, the canals were obturated with a size 40/0.04 BC point (Brasseler USA) sealed with a HiFlow sealer using the SC obturation technique. Before the sealer was placed into the coronal one-third of the canal with an intracanal tip, the tug back was evaluated with a master gutta-percha cone.

The master cone was coated with a thin layer of sealer and slowly inserted to the full WL, cut at the orifice level, and plugged.

Group 2 (BCH-WVC)

Same with the previous method, the master cone 40/0.04 BC point was inserted into the canal. The down pack (EQ-V Endodontic Obturation System, MetaBiomed, Cheongju, Korea) was 5 mm from the WL. Then, the hot GP was condensed with a hand plugger to achieve complete obturation of the root canal.

Group 3 (CS-SC)

Root canal obturation was used using the SC technique with a 40/0.04 GP cone (VDW GmbH, Munich, Germany) and the CeraSeal described in Group 1.

Group 4 (CS-WVC)

Root canal obturation was used using the WVC technique with a 40/0.04 GP cone and the CeraSeal described in Group 2. Group 5 (BR-SC)

Root canal obturation was used using the

SC technique with a 40/0.04 GP cone (VDW GmbH, Munich, Germany) and the BioRoot described in Group 1. Group 6 (BR-WVC)

Root canal obturation was used using the WVC technique with a 40/0.04 GP cone and the BioRoot described in Group 2. The radiographs were taken in the buccolingual, mesiodistal directions to ensure that the canals were without voids, the temporary filling material (Cavit G; 3 M ESPE, Seefeld, Germany) was placed. Then, the roots were kept in normal saline at 37 °C and 100% humidity for two weeks to set the fillings.

The roots were embedded into acrylic resin vertically and sectioned horizontally to obtain two slices 1±0.1 mm in thickness at 5 and 10 mm from the apex using a rotating diamond disk under water-cooling. Each slice was subjected to PBS test in a universal testing machine (Shimadzu, Kyoto, Japan) using a metallic indentor with a round cross-section and diameter of 0.5 mm and a crosshead speed of 0.5 mm/min was used. The load applied at the time of displacement was recorded in Newton. The bond strength was calculated in MPa according to the formula: Load/Adhesion surface area. The adhesion (bonding) surface area of each section was calculated as: $[(r^1+r^2)/2]\times\pi\times h$, where π is the constant 3.14. r^1 and r^2 are the smaller and larger radii, respectively, and h is the thickness of the section in mm. After the test, samples were examined under a stereomicroscope at ×40 magnification.



The failure mode was determined into three categories; adhesive, cohesive, and mixed.

-Adhesive: the failure is between the sealer and root canal dentin;

-cohesive: the failure is between the GP cone and the sealer,

-mixed: the failure is between both interfaces (12).

The PBS from samples of the six Groups was statistically analyzed. Shapiro Wilk test indicated that the data showed non-normal distribution (p<0.05) and compared using Kruskal–Wallis and multiple comparison tests using SPSS 17.0 (SPSS software, SPSS Inc., Chicago, IL, USA).

Results

The descriptive statistics of all specimens had median PBS values in Table 2. BR-WVC Group was a significantly lower PBS value than other Groups at 5 and 10 mm from the apex (p<0.05). At 5 and 10 mm from the apex, no significant difference in PBS was found among the BCH and CS sealers obturated using two different techniques (P>0.05). The results of the mode of failure analysis are shown in Table 3 and the most common failure mode found in all Groups was mixed failure.

Discussion

Adhesive root canal sealers enhance resistance to fracture and minimize microleakage of canal obturation (18). CBCSs are hydrophilic materials with a low contact angle and good flowability, helping them bond to the root canal wall (19). The penetration of endodontic sealers into the dentinal tubules reduces the core material and dentin interface. Therefore, mechanical locking may improve the retention of the core material. The PBS test provides valuable information about the retention of sealers on root canal walls (20). The present study used PBS test to evaluate the adhesion of BR, BCH, and CS are reportedly efficient, practical, and reliable (20). To the best of our knowledge, no other study assessing the PBS of BR, BCH, and CS with different obturation techniques is

vet available. A measurable adhesive property was seen in all the Groups in this study. The lowest PBS value was observed in the BR-WVC Group. The null hypothesis was rejected. The reason for that could be the high temperature helps push GP to the root canal dentin wall; there have been concerns about the potential adverse effects of the heat on sealer characteristics (21-24). Chen et al. (11) reported that the GP and root canal are likely to modify the effects of heat on the physicochemical properties of the sealer and the interaction between the viscosity and the shear rates. Also, heat may accelerate hydration and hydroxyapatite formation in CBCSs (25). However, the WVC technique demonstrated the same bond strength values in BCH, and CS Groups. Atmeh et al. (26) reported that in the WVC technique, the amount of heat transmitted to the root canal filling may not cause a crucial change to the properties of CBCSs and does not affect the setting reaction of the CBCSs. Also, Dewi et al. (5) reported that WVC method did not affect the PBS of the BCH. It is consistent with the data obtained in our study about BCH. According to Abdelwahed et al. (16), CS showed higher PBS values when used in the WVC technique compared to SC and lateral compaction techniques. This contradictory result could be attributed to different methodology according to our study.

In our opinion, the PBS results of sealers with the WVC technique may be due to the physicochemical properties.

Kharouf et al. (27) compared the physicochemical properties, filling ability of CS and BR sealers. They reported that CS might have the superior filling ability and lower solubility than the BR sealer due to its specific chemical composition and mixing method. These physical properties of the sealer used with the warm technique may affect the quality of the root canal obturation (12).

Additionally, López-García et al. (28) reported that CS and EndoSequence BC Sealer displayed high cell viability, cell attachment, cell migration rates, and ion release rates. BCH has similar physico-chemical and biological properties with



Table 2

Mean of the push-out bond strength (MPa) of specimens obtained from different distances from the working length

Sealers and Obturation Technique								
	BC HiFlow CeraSeal BioRoot							
	SC	WVC	SC	WVC	SC	wvc		
5 mm	5.28 ^A	5.43	5.24	5.17	5.18	3.32 [₿]		
10 mm	2.34ª	2.25ª	2.17ª	2.24ª	2.26ª	1.28 ^b		

Kruskal Wallis H-test. Different superscript letters indicate a statistically significant difference between the mean value of PBS of specimens obtained from the same distance from the WL (P<0.05). SC, single-cone obturation technique; WVC, warm vertical compaction obturation technique.

	I	Table 3 Failure pattern dist	ribution (n)			
	B	C HiFlow	Cera	Seal	Bio	Root
	SC	WVC	SC	WVC	SC	WVC
5 mm						
Adhesive	0	0	0	0	0	0
Cohesive	2	1	3	2	3	2
Mixed	8	9	7	8	7	8
Total	10	10	10	10	10	10
10 mm						
Adhesive	0	0	0	0	0	0
Cohesive	4	2	4	3	5	4
Mixed	6	8	6	7	5	6
Total	10	10	10	10	10	10

SC, single-cone obturation technique; WVC, warm vertical compaction obturation technique.

Different superscript letters indicate statistically significant differences - Kruskall-Wallis (P<.05).

EndoSequence BC Sealer (29). In the present study, the CS-WVC Group showed higher PBS values than the BR-WVC values and had no significant difference with the BCH-WVC Group. CBCSs are used with the SC technique to the manufacturer's instructions (8).

Our study found no differences between the three CBCSs with the SC technique. Previous studies reported that use of the SC to obturate root canals with an irregular shape results in formation of bubbles (30,31) and an increase in the volume of sealer in the root canal obturation (32). Retana-Lobo et al. (33) showed that the different CSBSs values for PBS observed in their study, were higher when the root canal was obturated with just the sealer. We think the difference between BR Groups might be because the amount of sealer increases in SC, and the interaction of BR with WVC is not fully known.

In a previous study comparing the physical properties of different premixed formulation CBCSs and BR, the physical properties of BR were affected by thermal treatment. They explained that different behavior of formulations (premixed and powder-liquid) could be due to the momentum of water uptake and the beginning of the setting reaction (34).

Also, the thermoplastic GP shrinks on



cooling; shrinkage may lead to stress concentration on the sealer, affecting its bond to the root dentine surface (35). Furthermore, when using a coated GP cone with CBCS, a chemical bond could help reduce leakage and reinforce the monoblock obturation concept (5). However, Eltair et al. (36) reported that an interfacial gap could be between BC sealer and coated GP. Therefore, although standard GP cone with BR and CS, there was no significant difference between all SC Groups.

Our study's most common failure mode is a mixed failure, followed by cohesive failure. These results were consistent with studies about CBCSs that also showed the dominance of mixed or cohesive failure (12,35,37). That may be, CBCSs have more potent chemical, physical bonds to radicular dentin than to the GP (33). Also, the plastic deformation of the GP may negatively affect the PBS (14).

One of the limitations of this study was to investigate the PBS of three CBCSs after two weeks following obturation, and saline was used to give the humidity to the samples. However, the phosphate-buffered saline (PBS) or Hank's balanced salt solution (HBSS) may enhance the bond strength of CBCSs (38,39). For this reason, comparing different filling techniques with CBCSs in PBS or HBSS should be considered in future research.

Conclusions

Within the limitation of this in vitro study, the following was concluded the WVC method did not affect the PBS of the premixed CS and BCH. However, the heatbased root canal obturation method negatively impacted the BR's PBS. Concerning this study's results, CS can be used with standard GP since the PBS of the SC technique was equal to the WVC. Therefore, further clinical investigations are required to evaluate the PBS of BCH, BR, and CS with different obturation techniques.

Clinical Relevance

The present study results suggest that BC Sealer HiFlow and CeraSeal are suitable

calcium silicate-based sealers with warm vertical compaction for root canal treatment.

Conflict of Interest

The authors declare that they have no confict of interest.

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Ethical approval

Ethical approval was obtained from the research ethics committee of İstanbul Medipol University (Ref. Number: 137).

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ORIGINAL ARTICLE

Comparative evaluation of the effectiveness of different rotary systems in removal of root canal filling materials

ABSTRACT

Aim: The aim of this study was to compare the remaining root canal filling materials after instrumentation using ProTaper Universal Retreatment (PTUR) system alone or combined with Neoniti, One Curve, and using hand Hedstrom files and Gates Glidden drills using cone beam computed tomography (CBCT) images.

Methodology: Fifty-two mandibular premolars with single and straight canals were used. The canals were instrumented with ProTaper rotary instruments up to F3 and filled with gutta-percha and AH26 sealer. All the samples were placed into silicone models. Samples were scanned with CBCT and assigned into four groups (n=13): the PTUR system group, the PTUR system plus Neoniti group, the PTUR system plus One Curve group, and the hand Hedstrom files plus Gates Glidden group. The specimens were scanned once again after retreatment procedures, and the volume of the remaining filling materials was determined. Data were analyzed using Kruskal-Wallis and Dunn tests (a=0.05).

Results: None of the retreatment procedures provided complete removal of the filling materials. Hedstrom files plus Gates Glidden removed more residual obturation materials than the other groups. The additional use of the Neoniti or One Curve systems significantly improved the removal of filling materials when compared with the PTUR system alone (P<0.05). The differences between the PTUR plus Neoniti group and the PTUR plus One Curve group were not statistically significant (P>0.05).

Conclusions: Using Gates Glidden and Hedstrom files was the most effective way for retrieval of endodontic material from the root canals, while PTUR alone was the least effective method. Re-instrumenting with rotary files significantly improved the removal of root filling materials. Neda Shekarchizade¹ Masoume Shahpouri² Mohammadreza Avalin Charsooghi² Gianrico Spagnuolo³ Parisa Soltani^{4*}

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Introduction

he main reason for failure of endodontic treatment is remaining infection in the root canal (1). Nonsurgical endodontic retreatment aims to eliminate the infection from the root canal and thus resolve the inflammatory response (2). One of the crucial components of endodontic retreatment is effective removal of root filling material in order to allow access for better debridement and disinfection of the root canal system. Remnant necrotic tissues and bacteria within the gutta percha and sealer may lead to persistence of the infection and therefore may compromise the outcome of endodontic retreatments (3, 4). Success rate of appropriate endodontic retreatments is reported to be as high as 81% (5).

Several techniques are available for removal of endodontic materials, including manual or rotary nickel titanium (NiTi) instruments, endodontic solvents, and ultrasonic activation. A combination of these techniques can be used for removing the root canal filling materials (6, 7). One of the most common techniques, involve the use of Hedstrom files combined with Gates Glidden drills with or without solvents such as chloroform. This technique can be particularly difficult and time-consuming when the root filling materials are dense and compact (8). Therefore, rotary endodontic systems are preferred due to their safety and speed. Several studies have reported the effectiveness of these rotary instruments to be comparable with manual files (9-11). In addition, rotary files specifically designed for retreatment purposes have been developed. One of these systems is ProTaper Universal Retreatment (PTUR) with continuous rotation. This system contains three rotary instruments: D1(30, 0.09), D2 (25, 0.08), and D3 (20, 0.07). D1 file has a cutting tip which can penetrate the root filling material and is used to remove the material from the coronal third of the root canal. D2 and D3 files have non-cutting tips and are used to remove the endodontic materials from middle and apical thirds of the root canal, respectively (12-14).

One of the newly introduced rotary systems for root canal preparation is Neoniti which uses a continuous rotation movement. This system contains C1 (25, 0.12) and A1 (25, 0.06) files. C1 is used as an orifice shaper and A1 is used to the working length for preparation of the root canal. This system uses CM-wire alloy with heat treatment technology. Therefore, it possesses high flexibility and shape memory, allowing these files to be pre-curved. In addition, modern wire cut electrical discharge machining technique used for surface preparation increases surface roughness of these endodontic files, which can potentially increase the effectiveness of root canal shaping (15-17).

One Curve rotary system is another endodontic rotary system with a single file (25, 0.06) used to the working length. This file is made from heat-treated C-wire alloy and has a high flexibility and shape memory. Therefore, it can be pre-curved to preserve the curve and shape of the root canal (18, 19).

Removal of all endodontic materials during retreatment is virtually impossible and remnants of gutta percha and endodontic sealer remain attached to the root canal walls (20). Different methods can be used in order to measure the amount of these remnant materials. One method is sectioning the teeth and examining the samples under optical microscope (21, 22). This technique disrupts the integrity of the tooth and can distort and scatter the remnant endodontic materials (14). A more conservative approach is using imaging techniques such as cone beam computed tomography (CBCT) which allows three-dimensional (3D) observation of the root canal system and remnant endodontic material. CBCT is a reliable and non-invasive technique for detecting the configuration of the root canal system (23, 24). No study has been previously performed to compare the effectiveness of PTUR, Neoniti, and One Curve rotary systems for endodontic retreatments. Therefore, the aim of the present study was to compare the effectiveness of these files in removal of root canal filling materials during endodontic retreatments.



Materials and Methods

This study was approved by the Ethics Committee of Isfahan University of Medical Sciences (approval date: 25.02.2020, #IR.MUI.RESEARCH.REC.1398.698). Informed consent is not applicable.

In this experimental study, fifty-two human mandibular premolar teeth which were extracted due to periodontal diseases or orthodontic reasons were selected. All teeth had a single straight root with a single canal and completely developed apex. The selected teeth were free from root caries, root fracture, root canal calcification, and external or internal resorption. Debris and attached soft tissue were removed from the teeth and the samples were kept in normal saline 0.9% solution (Samen, Mashhad, Iran) in a temperature of 4 °C. The crowns of the teeth were cut and a root with a length of 16 mm was prepared. Then, a hand K file #10 (Dentsply Maillefer, Ballaigues, Switzerland) was inserted into each root canal until the tip of the file was visible from the apical foramen using a microscope (×12.5). The working length was determined 1 mm short of this length.

Root canal treatment

Root canals were prepared using ProTaper rotary system (Dentsply Maillefer, Ballaigues, Switzerland) using the crown-down technique. At first, the cervical and middle thirds of the root canals were prepared using SX file. Thereafter, S1, S2, F1, F2, and F3 files were inserted to the working length. Apical patency was achieved using hand K file #15 (Dentsply Maillefer, Ballaigues, Switzerland) before insertion of the next rotary file. Irrigation was performed by 2 mL of sodium hypochlorite 2.0% (Cerkamed, Stalowa Wola, Poland). In order to remove the smear layer, the final irrigation was carried out by 2 mL of EDTA 17% (Cerkamed, Stalowa Wola, Poland), 2 mL sodium hypochlorite, and 5 mL sterile water. Then, the root canals were dried by paper cones (Dentsply Maillefer, Ballaigues, Switzerland) and filled by gutta percha and AH-26 sealer (Dentsply DeTrey, Konstanz, Germany) using lateral condensation technique. In order to assess the quality of root canal treatments, radiographs were obtained from the samples in buccolingual and mesiodistal directions using an intraoral size 2 film (Kodak, NY, USA). Samples which had voids in the root filling material were excluded and replaced by other teeth. Thereafter, the coronal portion of the roots was sealed using temporary restorations (Cavit, 3M ESPE, Seefeld, Germany). The samples were kept for two weeks in a temperature of 37 $^\circ C$ and humidity of 100% to allow complete setting of the endodontic sealer. Then, in order to create repeatable position of the samples for scanning, the roots were mounted on a putty model.

CBCT imaging

The samples were scanned using ProMax 3D Max scanner (Planmeca, Helsinki, Finland) with exposure parameters of 10 mA and 90 kVp, with a voxel size of 76 μ m and 16 mm ×16 mm field of view. The imaging dataset was then exported in digital imaging and communication in medicine (DICOM) format and transferred into Mimics innovation/research software (v.21, Materialise, Leuven, Belgium) for calculating the volume of root canal filling material. Threshold gray value limits were defined between 2604-3095 which corresponds to gutta percha. A separate mask was created for each sample containing the endodontic material within the root canal using the split mask tool. Then, using the edit mask tool, each mask was meticulously edited in coronal, sagittal, and axial planes (Figure 1A). The volume of the root canal filling material was then measured.

Endodontic retreatment

The roots were removed from the putty model and were randomly assigned into four groups based on endodontic retreatment technique. Removal of gutta percha from the root canals was performed by an endodontist each lasting for 9 minutes.

PTUR group: D1, D2, and D3 files were used to remove the root filling material from the coronal, middle, and apical thirds



Figure 1 Three-dimensional visualization of remaining root filling material (A) before and (B) after endodontic retreatment.





of the root canal, respectively, using slight pulsed apical pressure. The files were used to the working length until no further filling material was retrieved from the canal. For all rotary files, an endo-motor (NSK, Tochigi, Japan) with 500 rpm and 3 N.cm torque was used according to the manufacturer's instructions. PTUR with Neoniti re-instrumentation group: After application of PTUR system, Neoniti A1 file was passively used to the working length with slight pulsed apical pressure with 300 rpm and 1.5 N.cm torque. PTUR with One Curve re-instrumentation group: After application of PTUR system, One Curve rotary file was used to the working length with 300 rpm and 2.5 N.cm torque.

Gates Glidden and Hedstrom hand file group: In this group, Gates Glidden size 3 and size 2 (Dentsply Maillefer, Ballaigues, Switzerland) with 2000 rpm for removal of root canal filling material from the coronal portion of the canal. Then, 0.1 mL of chloroform was used as solvent for each root canal. Hedstrom hand files #35, 30, 25 were inserted in the root canal with filing, pushpull, quarter-turn, and circumferential motion in order to reach the working length. During the retreatment procedure, irrigation was performed by 2 mL sodium hypochlorite 2% solution. Final irrigation was carried out by 2 mL of EDTA 17% solution, 2 mL sodium hypochlorite 2% solution, and 5 mL of sterile water. The samples were mounted back on the putty model and were scanned by CBCT using the previous setting. The volume of the remaining root canal filling material after retreatment was calculated by Mimics software as described (Figure 1B). Then, the volume of remaining root canal filling material was divided by the volume of endodontic material after root canal treatment, in order to provide the percentage of remaining endodontic material in each sample.

Statistical analysis

Kruskal-Wallis nonparametric test and Dunn's test were used in order to compare the effectiveness of four systems in removal of endodontic material from the root canal. Statistical analysis was performed by Statistical Package for the Social Sciences (SPSS, v. 22, IBM, NY, USA, α =0.05). Data was presented as median due to non-normal distribution.


Table 1 Ratio of remaining material after endodontic retreatment using different file systems									
	Before (mm ³)		After (mm ³)		After/Before				
	Median	Q1-Q2	Median	Q1-Q2	Median	Q1-Q2			
PTUR	15.88	14.79-16.19	2.59	2.05-5.31	0.17	0.13-0.24			
PTUR+ Neoniti	14.64	12.55-20.13	1.74	0.15-3.03	0.10	0.01-0.17			
PTUR+ One Curve	19.12	16.27-21.77	1.72	0.40-3.47	0.11	0.02-0.19			
Gates Glidden + H file	18.08	15.74-20.79	1.32	0.12-2.31	0.08	0.01-0.12			

PTUR: ProTaper universal retreatment

Results

None of the tested systems were able to remove all the root canal filling material. The most effective system for removal of root canal filling material was Gates Glidden drills and Hedstrom files (table 1). Re-instrumentation with Neoniti and One Curve rotary systems were significantly more effective in retrieving endodontic material compared to PTUR file (P=0.024 and P=0.041, respectively). Re-instrumenting with Neoniti files led to better removal of root filling material from the root canal. However, this difference was not statistically significant (table 2, P=0.826).

Discussion

In the present study, the volumes of root canal filling material before retreatment were not significantly different among the experimental groups. Therefore, the comparison of root canal filling material volume after retreatment was possible. Based

on our findings, using Gates Glidden drills and Hedstrom files was the most effective way for retrieval of endodontic material from the root canals, while PTUR alone was the least effective system. Re-instrumenting with rotary files significantly improved the removal of root canal filling material.

Removal of root canal filling materials from an inadequately treated root canal increases the effectiveness of instruments and irrigants on debris and microorganisms responsible for apical periodontitis (25, 26). Although, it has not been proven that remaining gutta percha causes failure of endodontic retreatments, adequate retrieval of material from the root canal system is an important factor for eliminating the necrotic tissues and bacteria from the canal (2, 27). However, none of the systems were able to completely remove the endodontic material. This finding has been shown in other studies as well (16, 24, 28-30).

In this study, PTUR was used initially

Table 2			
Comparison of different retreatment techniques in remova	al of root canal filling	material	

Groups	Mean difference	SE	p-value
PTUR vs. PTUR+ Neoniti	0.129	0.046	0.024
PTUR vs. PTUR+ one curve	0.114	0.046	0.041
PTUR vs. Gates Glidden + H file	0.145	0.046	0.004
PTUR+ Neoniti vs. PTUR+ One Curve	-0.014	0.046	0.826
PTUR+ Neoniti vs. Gates Glidden + H file	0.016	0.046	0.518
PTUR+ One Curve vs. Gates Glidden + H file	0.031	0.046	0.386

PTUR: ProTaper universal retreatment



followed by Neoniti or One Curve rotary files for additional removal and shaping of the canals. Chloroform was used only in the Gates Glidden and H file group, where manual removal of the gutta percha was performed. Retrieving the endodontic material from the root canals by manual techniques alone can be time-consuming, especially when the endodontic materials are thoroughly condensed (8). Applying a small amount of solvent during endodontic retreatment can facilitate the retreatment process. Using chloroform with rotary instruments can leave a thin layer of sealer and gutta percha attached to the root canal which is difficult to remove. Therefore, chloroform was not used with rotary files (31).

In this study, PTUR was the least effective system for retrieval of endodontic material. All root canals were prepared with F3 ProTaper file with a tip size of 30, while the tip size of D3 ProTaper retreatment file is 20, meaning that the tip of D3 is not completely engaging with the canal walls in the apical portion of the root during the retreatment procedure (24).

Recently, it has been recommended that using a combination of endodontic instruments leads to more effective removal of root canal filling materials (32). Bueno et al. showed that Hedstrom files are more effective compared with PTUR in retrieving gutta percha from the root canals. The flute design and circumferential filing techniques used with Hedstrom files can facilitate removal of endodontic material. The authors recommended initiation of endodontic retreatment with rotary files followed by application of Hedstrom manual files as a complementary technique (33).

Yürüker et al. evaluated the effectiveness of different techniques in removal of endodontic materials from the root canal. They reported that combined use of PTUR and Hedstrom files or Reciproc rotary instrument significantly enhanced the retrieval of root canal filling material compared with use of PTUR alone or combined with self-adjusting files (24). These findings are consistent with our results, showing that complementing PTUR with rotary instruments improves the effectiveness of endodontic material removal.

Similarly, Aksel et al. reported that re-instrumenting with XP-Endo finisher system after PTUR enhances the removal of root canal filling material regardless of sealer type (34).

However, Ealla et al. conducted a study comparing PTUR and D-RaCe retreatment systems with hand Hedstrom files. They found that D-RaCe rotary system is more effective in removing endodontic material from the root canal (35).

n their study, for the Hedstrom file group they have only used hand H-files and K-files, while we used H-files combined with Gates Glidden drills, which performed better than the other groups in retrieving endodontic material from the root canals.

One limitation of the present study was not using micro-CT for evaluation of the samples (36). However, application of high resolution CBCT with artifact reducing algorithms and accurate thresholding techniques in third party software enabled for determination of the volume of gutta percha before and after endodontic retreatment.

Conclusions

Using Gates Glidden drills and Hedstrom files was the most effective way for retrieval of endodontic material from the root canals, while PTUR alone was the least effective method. Re-instrumenting with rotary files significantly improved the removal of root filling material.

Clinical Relevance

Based on the findings of this study, combined use of Gates Glidden and Hedstrom files is recommended as the most effective way for removal of endodontic material from the root canals for nonsurgical endodontic retreatment. However, in case of application of rotary instruments for retrieval of root filling material, re-instrumenting with a second rotary instrument improved the effectiveness of PTUR system.



Conflict of Interest

All authors declare that they have no conflict of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the Helsinki Declaration as revised in 2013.

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Informed consent

Not applicable.

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Effect of fentanyl on the success of inferior alveolar nerve block for teeth with symptomatic irreversible pulpitis: a randomized clinical trial

ABSTRACT

Aim: The purpose of this prospective, randomized, double-blind study was to evaluate the effect of adding fentanyl to lidocaine 2% with epinephrine 1:80,000 on the success of the inferior alveolar nerve block in mandibular molar teeth with symptomatic irreversible pulpitis.

Methodology: 100 healthy adult patients with diagnosis of symptomatic irreversible pulpitis in one of the mandibular molar tooth were selected and randomly divided in two groups of 50 patients each. In the first group (fentanyl group), 0.25 ml of a cartridge of 1.8 ml of 2% lidocaine with 1:80,000 epinephrine solution was drained and the same amount from $50\mu g/ml$ fentanyl solution was added to the cartridge. In the second group (non-fentanyl group) 0.25 ml of a cartridge of 1.8 ml of 2% lidocaine with 1:80,000 epinephrine solution was drained and the same amount from $50\mu g/ml$ fentanyl solution was drained and the same amount from solution was added to the cartridge. In the second group (non-fentanyl group) 0.25 ml of a cartridge of 1.8 ml of 2% lidocaine with 1:80,000 epinephrine solution was drained and the same amount from saline solution was added to the cartridge. Each group received two cartridges of prepared soloution with inferior alveolar nerve block injection technique. Access cavity preparation started 15 minautes after injection and after confirming the lip numbness. Success defined as no pain or mild pain on the basis of Heft-Parker visual analog scale during access cavity preparation or initial instrumentation. Data were analyzed by T-test and Chi-square

Results: The success rate of inferior alveolar nerve block injection was 58% for Fentanyl group and 46% for Non-Fentanyl group. There was no significant difference between the two groups (P=0.23).

Conclusions: The addition of fentanyl to lidocaine 2% with epinephrine 1:80,000 did not increase the success rate of the inferior alveolar nerve block in mandibular molar teeth with symptomatic irreversible pulpitis

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Introduction

eep anesthesia is an important factor during root canal treatment procedures. However, some factors during endodontic treatments can make obtaining appropriate anesthesia challenging (1). Inferior alveolar nerve block is the standard technique for anesthesia of posterior mandibular teeth. Failure in this technique is a considerable clinical problem during root canal treatment particularly in teeth with irreversible pulpitis (1). The failure rate for inferior alveolar nerve block in asymptomatic molar teeth is 10-39% (2-5). The success rate of this technique is reduced to 24% for molar teeth with irreversible pulpitis (4, 6). Several hypotheses have been developed to explain the reduced effectiveness of local anesthesia in inflamed teeth. These hypotheses include the reduction in pain threshold of inflamed tissues, hyperalgesia, the reduction in penetration of the basic form of the anesthetic molecules from the neural membrane as a result of reduced pH in tissues, the increase in anesthetic-resistant TTX-R sodium channels, and the reduction in pain threshold of patients as a result of anxiety (7-10). Different studies have proposed different methods for increasing the chance of success in inferior alveolar nerve blocks, such as increasing the volume of the local anesthetic solution, increasing the concentration of epinephrine, alternative local anesthetic solutions, alternative injection sites, supplemental injection techniques, increasing injection time, buffered anesthetic solutions, and premedication with non-steroid anti-inflammatory drugs (NSAIDs) or sedatives (11-19). Several studies have evaluated the effects of adding different therapeutic agents to local anesthetic solutions. These drugs include NSAIDs, opioids, anti-anxiety drugs and anesthetics (20-23).

Fentanyl is a potent synthetic opioid which is used as an analgesic agent and also an additive drug for local and general anesthesia (24). Fentanyl is 50-100 times more potent than morphine and is thus more effective in lower serum concentrations (25). The effectiveness of fentanyl combined with its low rate of side effects has garnered attention to this opioid agent (26). The onset of fentanyl is quick and duration of action is short (24).

To the authors' knowledge, no previous study has been performed on the effects of addition of fentanyl to 2% lidocaine solutions in success rate of inferior alveolar nerve blocks in molar teeth with symptomatic irreversible pulpitis. Therefore, the aim of this study was to evaluate the success rate of local anesthesia by inferior alveolar nerve block using lidocaine 2%+fentanyl solution for teeth with symptomatic irreversible pulpitis.

Materials and Methods

100 healthy adult individuals who have attended for emergency treatment to the Endodontics Department of Isfahan Dental School have participated in this study. Individuals under 18 years, those with a history of systemic diseases, pregnant women, patients with allergy to local anesthetic drugs or sulphites, patients taking drugs affecting the evaluation of anesthetics, those with active pathoses in the injection site, and those unable for informed consent were excluded from the study. The Research Ethics Committee in Isfahan University of Medical Sciences approved the protocol for this study (IR.MUI.RE-SEARCH.REC.1398.430). This clinical trial has been registered in the Iranian Registry for Clinical Trials (IRCT20191114045441N1). Informed consent was obtained from all participants. Inclusion criteria was presence of one vital mandibular first molar with moderate to severe pain and elongated response to cold test with Endo-Frost cold spray (Coltene-Whaledent, Langenau, Germany). Teeth without a response to cold test, or with necrotic coronal pulp tissues during access cavity preparation, or with periapical lesions were not included. Therefore, each patient had one mandibular first molar with clinical diagnosis of symptomatic irreversible pulpitis.

The permitted injection dose for fentanyl for a 50 kg adult human was calculated



according to the Pain Assessment and Management Initiative as 0.5 to 1 mL. In this study, the lower threshold (0.5 mL) was used. In both study groups, 0.25 mL of the solution in 1.8 mL anesthetic solutions cartridges containing 2% lidocaine+1:80,000 epinephrine (Darupakhsh, Iran) was removed using an aspirating syringe. In the fentanyl group, 0.25 mL of 50 µg/mL fentanyl solution (Abureihan, Tehran, Iran) was then added to the anesthetic cartridge. In order for proper mixing, the anesthetic cartridge was reversed for 5 times. In the control group, 0.25 mL of normal saline was added to the anesthetic cartridge and the cartridge was reversed for 5 times for proper mixing. At first, the patients marked their preoperative pain levels on the Heft-Parker Visual Analogue Scale (HP-VAS) (27). This visual scale for measurement of pain levels is a 10-cm scale divided into four segments: no pain (0 cm), mild pain (1-3 cm), moderate pain (4-6 cm), and severe pain (7-10 cm) (27). Patients with moderate to severe pain were included in this study.

A trained dental assistant prepared the anesthetic solutions immediately before injection and coded them based on randomized blocks. In each group, two cartridges of the prepared solutions were injected after aspiration using the inferior alveolar nerve block technique. All of the anesthetic injections were performed by one operator. The operator and the patient were blinded to the components of each anesthetic cartridge. All of the injections were made using standard aspirating dental syringes with 31 mm gauge 27 needles. Anesthesia of the lower lip was considered as the indicator for success of the inferior alveolar nerve block. 15 min after injection, if anesthesia was obtained in the lower lip and chin, the therapeutic treatment was performed. Otherwise, the patient was excluded from the study and required treatment was performed after supplementary injection techniques. In case of presence of pain during access cavity preparation and initial file insertion, the treatment procedure was suspended and the patients were asked to mark their pain levels on HP-VAS. This marked the end of the study for that particular patient. Success was defined as no pain or mild pain during access cavity preparation and initial file insertion and failure was defined as presence of moderate or severe pain in each of these stages.

Statistical Analysis

The data pertaining to preoperative pain and success rate of inferior alveolar nerve block were statistically analyzed using SPSS (version 20, IBM Corporation, Armonk, NY, USA). Comparison between fentanyl and control groups for success of inferior alveolar nerve block was analyzed

Table 1 Age distribution									
Group	Number	Minimum	Maximum	Mean	standard deviatior				
Fentanyl	50	18	52	33.14	11.68				
Control	50	18	55	31.06	9.89				
	Group	Sex	Table 2 distribution	Number	Percentage				
Fant		Man		27	54				
Fenta		Woman		23	46				
Control				00					
Cont	wal	Man		23	46				



using chi-square test, while preoperative and intraoperative pain were compared using t-test. Level of significance was set at 0.05.

Results

100 adult individuals (50 men and 50 women) with an age range of 18-55 were enrolled in this study. variables for fentanyl and control groups are shown in tables 1 and 2.

Mean age and sex distribution were not significantly different between the two groups (p=0.339 and p=0.424, respectively). Mean preoperative pain was 6.28 in the fentanyl group and 6.74 in the control group. T-test revealed that no statistically significant difference was observed between preoperative pain in the fentanyl and control groups (p=0.375).

Mean intraoperative pain in the fentanyl group and control group were 3.48 and 4.08, respectively. The mean recorded intraoperative pain in the fentanyl group was lower than that of the control group. Based on t-test, however, the difference was not statistically significant (p=0.417) (table 3). Success rate of the inferior alveolar nerve block was 58% in the fentanyl group and 46% in the control group. Although the success rate in the fentanyl group was higher than in the control group, this difference was not statistically significant according to chi-square test (p=0.23) (table 4).

Discussion

Based on the findings of this study, addition of fentanyl to lidocaine cartridges increased the effectiveness of inferior alveolar nerve block in mandibular molars with symptomatic irreversible pulpitis. The basic parameters of the participants (age, sex, and preoperative pain) were not significantly different between the study groups and thus these variables did not alter the results.

The standard anesthetic technique for mandibular posterior teeth is inferior alveolar nerve block. However, this technique has a high failure rate particularly

for teeth with symptomatic irreversible pulpitis. The present findings confirmed the results of previous studies reporting low success rate of inferior alveolar nerve block for teeth with symptomatic irreversible pulpitis (4, 6). In the present study, the success rate of the anesthetic injection for teeth with symptomatic irreversible pulpitis was 46%. A number of studies have assessed the effects of addition of therapeutic agents to the original local anesthetic. These additives include NSAIDs, opioids, anti-anxiety drugs, and anesthetics (20-23). Addition of opioids to local anesthetic agents can provide several benefits. Among different opioids, fentanyl with its high analgesic effects 50-100 times more potent than morphine), its anesthetic and sedative properties, and its lower rate of side effects compared to its effectiveness, is an appropriate choice. Fentanyl is a potent synthetic opioid used as analgesic medication and also in combination with other drugs for local and general anesthesia (24). Fentanyl is 50 to 100 times more potent than morphine and will therefore provide acceptable effects in lower serum concentrations (25). The effectiveness of fentanyl in combination with its low rate of side effects has garnered attention to this opioid agent (26). The onset of fentanyl is quick and duration of action is short (24). Intravascular injection of fentanyl is usually applied for general anesthesia, local anesthesia, and pain control (28).Intrathecal application of fentanyl is used as a component of spinal anesthesia and its epidural injection is used for epidural anesthesia and analgesia. Due to its high fat solubility, fentanyl's effects are more localized compared with morphine (29). Fentanyl is used for emergency pain control (usually in the form of nasal spray), control of chronic pain (e.g., in patients with cancer, in the form of skin patches) and quick pain control (in the form of sublingual tablets) (30-31). Other major applications of fentanyl include pre-anesthetic sedation in operative rooms and sedation for intubated patients (25).

The action mechanism of fentanyl is similar to other opioid drugs. Fentanyl affects



Table 3 Pain distribution in patient (according to HP-VAS)								
Group		Number	Minimum	Standard deviation				
Duranting	fentanyl	50	0	10	6.28	2.95		
Preoperative pain	Control	50	3	10	6.74	2.16		
Intro on orativo noin	fentanyl	50	0	10	3.48	3.7		
Intraoperative pain	Control	50	0	10	4.08	3.66		

Table 4 Success rate								
Gro	up	Success	Unsuccessful	Total				
Fontonyl	Number	29	21	50				
Fentanyl	Percentage	58	42	100				
0 1 1	Number	23	27	50				
Control	Percentage	46	54	100				
Total	Number	52	48	100				
Total	Percentage	52	48	100				

a subgroup of opioid receptors which are primarily in the brain (in the neuroanatomic structures for feelings, pain, and speech). Fentanyl is a mu-selective opioid agonist which can also activate other opioid receptors such as delta and kappa receptors, leading to its analgesic effects (21). The most common side effects of fentanyl include sleepiness, confusion, malaise, xerostomia, diarrhea, nausea, constipation, and sweating (25). Although fentanyl is more potent that morphine, nausea occurs less frequently after fentanyl administration compared with morphine (32). Injection of fentanyl provides a quick action onset within the first minutes and peaking at the second minutes (33). Additionally, by using the low dosage of fentanyl in this study, the probability of side effects was extremely low. However, any experienced side effect will resolve quickly and the patients will be able to be dismissed without the need for a companion.

De Pedro-Muñoz and Mena-Álvarez evaluated the effect of submucosal injection of tramadol as an adjunct injection technique on success rate of inferior alveolar nerve block with 4% articaine+1:100,000 epinephrine solution in teeth with irreversible pulpitis. Their findings indicated that submucosal injection of tramadol prior to the inferior alveolar nerve block injection significantly increased its success rate (34). Aksoy and Ege assessed the effectiveness of submucosal injection of tramadol as a complementary injection technique for inferior alveolar nerve block in teeth with symptomatic irreversible pulpitis. They concluded that submucosal administration of tramadol did not significantly influence the success rate of inferior alveolar nerve block in teeth with symptomatic irreversible pulpitis (35).

Rodríguez-Wong et al. analyzed the effect of adding tramadol to mepivacaine 2%+1:100,000 epinephrine solution on success rate of inferior alveolar nerve block in molars with symptomatic irreversible pulpitis. Based on their findings, although the success rate of inferior alveolar nerve block was improved by addition of fentanyl, no significant difference was observed between the two study groups (36). Bigby et al. evaluated the effect of addition of meperidine to 2% lidocaine+1:100,000 epinephrine solution on success rate of



inferior alveolar nerve block for molars with symptomatic irreversible pulpitis and reported that adding meperidine does not significantly enhance the success rate of inferior alveolar nerve block (21). The findings of these two studies were consistent with the present findings indicating that addition of opioids to local anesthetic solutions does not significantly improve the success of inferior alveolar nerve block.

Conclusions

Based on the findings of the present study, addition of fentanyl to lidocaine anesthetic cartridges improved the effectiveness of inferior alveolar nerve block. However, this increase was not statistically significant.

Clinical Relevance

Failure in IANB technique is a considerable clinical problem during root canal treatment particularly in teeth with irreversible pulpitis. Addition of fentanyl to lidocaine anesthetic cartridges improved the effectiveness of IANB.

Conflict of Interest

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

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Microbiological analysis of root canals of primary teeth with pulp necrosis caused by caries or trauma

ABSTRACT

Aim: This study aimed to investigate the microorganisms detected from root canals of primary teeth with pulp necrosis caused by dental caries or trauma.

Methodology: Microbial samples were taken from 44 cases in primary teeth with pulp necrosis either due to dental caries or trauma. DNA was extracted from the samples, which were analysed for the presence of fifteen endodontic pathogens by using PCR species-specific primers.

Results: The bacteria most detected in necrotic primary teeth due to caries (37/44) were P. micra (76.3%), P. nigrescens (76.3%), A. naeslundii (47.4%), and F. nucleatum (42.1%). On the other hand, A. naeslundii (83.3%), T. forsythia (83.3%), P. nigrescens (66.7%), and F. nucleatum (66.7%) were most frequently recovered from a root canal with pulp necrosis due to trauma (7/44). Significant associations were found between the presence of P. micra and the existence of caries (p=0.023) and sinus tract (p=0.044). The presence of T. forsythia was associated with the existence of trauma (p=0.033).

Conclusions: The microbiota recovered from root canals of primary teeth with pulp necrosis caused by dental caries or trauma is similar, with the predominance of anaerobic microorganisms

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Introduction

rimary teeth are subject to physical, chemical and/or biological injuries. Extensive dental caries and some traumatic injuries can result in pulp necrosis, which can lead to their premature loss, causing functional and aesthetic damage, as well as injuring the underlying permanent tooth germ, affecting its development (1). Although pulp necrosis has been reported as a common sequel after dental caries and trauma in the primary dentition (2), its mechanisms vary according to the causal condition. After pulpal exposure by caries, pulp surface is colonized and covered by bacteria present in the caries biofilm, which will adhere to the dentinal walls colonizing these surfaces and forming root canal biofilms (1). This microbial load exerts a direct and by-product pulpal reaction characterized by severe inflammation (3). Lactobacillus acidophilus, Strep-

Figura 1 STROBE diagram of the patients' enrolment process.



tococcus mutans, and *Streptococcus sobrinus* have been related to the initial stages of dental caries and the pulpal surface colonization (4).

In some traumatic injuries, coagulation necrosis can happen as a result of a permanent break of the blood supply connected to the pulp tissues (5). The pulp and the periodontium communicate between themselves through the apical foramen and lateral canals, which is an acceptable pathway for bacterial entrance into the root canals (6). After this, the microenvironment of the pulp space becomes propitious to factors that influence the microbial colonization and multiplication (7). Anaerobic bacteria, such as black-pigmented rods (BPRs) and Fusobacterium nucleatum, have been reported as the most predominant microorganisms in primary teeth with endodontic infection after traumatic injury (8).

The success of root canal therapy depends directly on reducing or eliminating the endodontic microbiota. Several studies have evaluated the microbiota of permanent teeth (9-11), however few of them have investigated the primary dentition infection and also the effect of dental caries and trauma, on the microbial profile (8, 12, 13). Thus, this study aimed to investigate the microorganisms present in root canals of primary teeth with pulp necrosis caused by dental caries or trauma.

Material and Methods

Patient selection

A cross-sectional study was conducted, following the *strengthening the reporting of observational studies in epidemiology* (STROBE) guidelines. Eligible 44 children, who had been referred for endodontic treatment and who presented at least 1 primary tooth with pulp necrosis due to carious lesions or dental trauma, were enrolled in this study (Figure 1).

The qualified participants had tooth with evidence of primary endodontic infection (necrotic pulp tissue, sinus tract, periapical lesion, no previous endodontic treatment), intact root or less than two thirds of root resorption, enough coronal structure to

			Groups	
Variable	Category	Total	Caries	Trauma
Condor	Female	21	17	4
Gender	Male	23	20	3
٨٢٥	≥6 years	30	30	0
Age	<6 years	14	7	7
	Single-rooted	7	0	7
Range of teeth	Bi-rooted	19	19	0
5	Multi-rooted	18	18	0
Teeth localization	Upper	25	18	7
	Lower	19	19	0
Discoloration	Yes	7	0	7
Discoloration	No	37	37	0
Dain on polyotion	Yes	6	6	0
Pain on palpation	No	38	31	7
Tandarnaaa ta narayaajan	Yes	6	6	0
Tenderness to percussion	No	38	31	7
Sinus tract	Yes	21	17	4
Sinus tract	No	23	20	3
Mobility	Yes	1	1	0
WODIIIty	No	43	36	7
Periapical lesion ≤2mm	Yes	44	37	7
renapical lesion szimm	No	0	0	0

Table 1

allow a full isolation with a rubber dam, and absence of deep periodontal pockets in the involved tooth. Patients could not present systemic alterations or have used antibiotics or antifungals within the previous 3 months. Patients, who had used antibiotics or antimicrobial mouthwashes during the course of the trial and had an uncooperative behaviour, not allowing the microbial sample collection, were excluded from this study.

Clinical and radiographic features were observed and recorded for all patients, including presence or absence of teeth mobility, pain on palpation, tenderness to percussion, discoloration, sinus tract, and periapical lesion (Table 1).

This study was carried out in accordance with the International Code of Medical Ethics, and the research protocol was approved by the Institutional Research Ethics Committee (process n°. 224/10), including the description of the sample collection for this investigation. The purposes were fully explained to the guardians, who signed a written informed consent form authorizing their children's enrolment in the study, and the privacy rights of subjects were observed. An experienced operator performed the endodontic procedures and sample collection in all cases included in this investigation.

Clinical procedures

All clinical procedures have been previously described (10, 12). Firstly, an infiltrative local anaesthetic was applied followed by tooth isolation with a rubber dam. The rubber dam and the tooth were disinfected using 30% hydrogen peroxide and then 2.5% sodium hypochlorite (NaO-Cl). The later was neutralized with 5% sodium thiosulfate to avoid carry-over the antimicrobial effect of NaOCl during the bacteriological sampling.

The disinfection of the tooth surface was monitored by taking a swab sample from both external and internal surfaces of the crown and from its surrounding structure area. Next, a swab sample was streaked on a plate containing 5% defibrinated sheep blood and fastidious anaerobe agar (FAA

- LAB M, Heywood, Lancashire, UK) before being incubated anaerobically and aerobically, respectively, for up to 14 days followed by DNA extraction from the swab and PCR run by using universal bacterial primers. If any positive culture or presence of bands on the agarose gel was detected, then the patient was excluded from the study.

Root canals were accessed through the crown with high-speed round diamond burs (KG Sorensen Industria e Comercio, São Paulo, SP, Brazil), which was made without the use of water spray but under manual irrigation with sterile saline solution. The pulp chamber was irrigated with sterile saline to remove the contents from the pulp space. The microbial sample was obtained from a single root canal. If the



PCR primers sequences, with expected amplicon size and thermocycling parameters								
Target bacteria	Primers pairs (5' to 3')	Amplicon size (bp)	Cycles					
Actinomyces naeslundii	Forward: GCG CCT TTT TTG GTG TTT TTG G	274	Initial denaturation at 94 °C for 1 min and 35 cycles of: 94 °C for 1 min, 60 °C for 1 min, 72 °C for 90s and final					
	Reverse: CAC CCA CAA ACG AGG CAG GCC TG Foward: TTC TAA GCA TCG CAT GGT GC		step continued at 72 °C for 10 min Initial denaturation at 95 °C for 2min and 36 cycles of:					
Dialister pneumosintes	Reverse: GAT TTC GCT TCT CTT TGT TG	1105	94 °C for 30s, 55 °C for 1min, 72 °C for 2 min and a final step 72 °C for 2 min					
Enterococcus faecalis	Forward: CCG AGT GCT TGC ACT CAA TTG G Reverse: CTC TTA TGC CAT GCG GCA TAA AC	138	Initial denaturation at 95 °C for 2 min and 36 cycles of: 95 °C for 1 min, 57 °C for 1 min, 72 °C for 1 min and a final step 72 °C for 7 min					
Filifactor alocis	Forward: CAG GTG GTT TAA CAA GTT AGT GG	594	Initial denaturation at 95 °C for 2 min and 26 cycles of: 95 °C for 30 s, 58 °C for 1 min, 72 °C for 1 min and a					
	Reverse: CTA AGT TGT CCT TAG CTG TCT CG		final step 72 °C for 2 min					
Fusobacterium nucleatum	Foward: AGT AGC ACA AGG GAG ATG TAT G Reverse: CAA GAA CTA CAA TAG AAC CTG A	1000	Initial denaturation at 95 °C for 5min and 30 cycles of: 94 °C for 30 s, 40 °C for 1 min, 72 °C for 2 min and a final step 72 °C for 10 min					
Parvimonas	Forward: AGA GTT TGA TCC TGG CTC AG	207	Initial denaturation at 95 °C for 2 min and 36 cycles of: 94 °C for 30s , 60 °C for 1 min, 72 °C for 1 min and a					
micra	Reverse: ATA TCA TGC GAT TCT GTG GTC TC	-	final step 72 °C for 10 min					
Porphyromonas endodontalis	Forward: GCT GCA GCT CAA CTG TAG TC Reverse: CCG CTT CAT GTC ACC ATG TC	672	Initial denaturation at 95 °C for 2 min and 36 cycles of: 94 °C for 30 s, 58 °C for 1 min, 72 °C for 2 min and a					
Porphyromonas	Forward: AGG CAG CTT GCC ATA CTG CG	404	final step 72 °C for 10 min Initial denaturation at 95 °C for 2 min and 36 cycles of: 94 °C for 30 s, 60 °C for 1 min, 72 °C for 2 min and a					
gingivalis	Reverse: ACT GTT AGC AAC TAC CGA TGT	-0-	final step 72 °C for 2 min					
Prevotella intermedia	Forward: TTT GTT GGG GAG TAA AGC GGG Reverse: TCA ACA TCT CTG TAT CCT GCG T	575	Initial denaturation at 95 °C for 2 min and 36 cycles of: 94 °C for 30 s, 58 °C for 1 min, 72 °C for 2 min and a					
Prevotella	Forward: ATG AAA CAA AGG TTT TCC GGT AAG	804	final step at 72 °C for 10 min Initial denaturation at 95 °C for 2 min and 36 cycles of: 94 °C for 30 s, 58 °C for 1 min, 72 °C for 2 min and a					
nigrescens	Reverse: CCC ACG TCT CTG TGG GCT GCG A		final step at 72 °C for 10 min					
Streptococcus mitis	Forward: GTC GAA GGT GAT GAT ATG AC Reverse: GAC AGT ACG CAG TCT TAC GTC	372	Initial denaturation at 94 °C for 2 min and 30 cycles of: 94 °C for 1 min, 54 °C for 1 min, 72 °C for 1min and a final step 72 °C for 10 min					
	Forward: ATT GAA GGC GAG CCT TTA GAA AG		Initial denaturation at 94 °C for 2 min and 30 cycles of:					
Streptococcus mutans	Reverse: CTA GGA CAA TAG CAA C	351	94 °C for 1 min, 54 °C for 1 min, 72 °C for 1 min and a final step 72 °C for 10 min					
Streptococcus sanguis	Forward: GTC GAT GGC GAG GAT CTA GAG C	208	Initial denaturation at 94 °C for 2 min and 30 cycles of: 94 °C for 1 min, 54 °C for 1 min, 72 °C for 1 min and a					
30118013	Reverse: TGC CGA GCG CTC TAA CTC CA		final step 72 °C for 10 min					
Tannerella forsythia	Forward: GCG TAT GTA ACC TGC CCG CA Reverse: TGC TTC AGT GTC AGT TAT ACC T	641	Initial denaturation at 95°C for 1 min and 36 cycles of: 95 °C for 30 s, 60 °C for 1 min, 72 °C for 1 min and a final step at 72 °C for 2 min					
Treponema	Forward: TAA TAC CGA ATG TGC TCA TTT ACA T		Initial denaturation at 95 °C for 2 min and 36 cycles of:					
denticola	Reverse: TCA AAG AAG CAT TCC CTC TTC TTA	316	94 °C for 30 s, 60 °C for 1 min, 72 °C for 2 min, and a final step at 72 °C for10 min					
Universal 16S rDNA	Forward: TCC TAC GGG AGG CAG CAG T Reverse: GGA CTA CCA GGG TAT CTA ATC CTG TT	466	Initial denaturation at 95 °C for 10 min and 40 cycles of: 95 °C for 10 s, 60 °C for 10 s and a final extension step at 72 °C for 25 s					
	Reverse: GGA CTA CCA GGG TAT CTA ATC CTG TT		at 72 °C for 25 s					

Table 2

tooth was multi-rooted, the largest canal or the canal with periapical lesion was chosen. Three sterile absorbent paper points of a diameter compatible with the root canals were successively introduced and maintained into the canals for 60 seconds each, up to 1 mm shorter of the root apex, established by the initial radiograph. In the case of a dry canal, 0.85% sterile saline was used to moisten the root



canals to allow better sample collection. In case of a wet canal (or those that have been previously irrigated with saline) numerous paper points were used to absorb all the fluid inside the canal.

The paper points were then transferred to sterile Eppendorf tubes containing VMGA III transport medium and were frozen immediately at -80 °C and stored until assayed by Polymerase Chain Reaction (PCR).

DNA extraction and PCR assay

DNA from clinical samples was extracted and purified using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The DNA concentration was recorded by a DNA quantification machine (NanoDrop 2000; Thermo Scientific, Wilmington, DE, USA).

The PCR reactions were performed as previously described (14) (MyCycler; Bio-Rad, Hercules, CA, USA) with modification to consist of a final volume of 25 μ L of reaction mixture. The primer sequences and PCR cycling parameters are listed in Table 2 (27, 28). Once the PCR reactions were concluded they were loaded into 1% agarose gel electrophoresis, stained with ethidium bromide, and analysed under ultraviolet transillumination. The presence of determined bacteria was confirmed by a positive band of positive control in the expected molecular weight and a negative band of negative control.

Data analysis

Data were tabulated in Excel spread sheet (Microsoft, Redmond, WA, USA) and statistically analysed by using SPSS 21 (IBM, Chicago, IL, USA). A descriptive analysis was performed on all data. Fisher's exact or Pearson Chi-square tests, when appropriate, were used to test the null hypothesis that there is no relation between clinical and radiographic features and presence of specific bacteria. Significance level was set at 5% (P<0.05).

A Venn's diagram was drawn to easily visualize both common and different species found in necrosis after caries and trauma (15).

Results

Clinical and radiographic features

Forty-four patients were enrolled in this study, 37 were due to dental caries, while 7 were due to trauma. Eligible children were selected from 2 to 9-year-old, mean of 6 years old (±1), being 21 (47.72%) females and 23 (52.28%) males.

Traumatised teeth corresponded to the anterior single-rooted, while necrotic teeth due to caries were posterior and bi-rooted or multi-rooted. All teeth presented periapical lesion equal or lower than 2 mm.

Regarding the patients' clinical features, none of them reported acute pain (acute abscess). However, 21 patients presented sinus tract (chronic abscess). A detailed explanation of the clinical features according to each group (necrosis caused by dental caries or trauma) is shown in Table 1.

Overall microbial findings

Samples collected from the operatory field, including external and internal surfaces of the crown and its surrounding structures using sterile swabs presented no positive cultures and no bacterial DNA after performing culture and molecular methods, respectively.

The mean number of species detected was 4.16 (\pm 1.88), ranging from 1 to 9 species per sample. In samples from pulp necrosis caused by caries, the mean number of species detected was 3.97 (\pm 1.64), ranging from 1 to 7, while in samples from pulp necrosis caused by trauma the mean was 5.33 (\pm 2.94), ranging from 2 to 9 species. No statistically significant differences were observed among specific bacteria from pulp necrosis caused by caries or trauma (p>0.05).

Prevotella nigrescens (75%), Parvimonas micra (70.5%), Actinomyces naeslundii (52.3%), and Fusobacterium nucleatum (45.5%) were the most prevalent species in primary teeth with primary endodontic infection (n=44).

The bacteria most detected in necrotic primary teeth due to caries (37/44) were *P. micra* (76.3%), *P. nigrescens* (76.3%), *A. naeslundii* (47.4%), and *F. nucleatum* (42.1%). On the other hand, *A. naeslundii* (83.3%), *Tannerella forsythia* (83.3%), *P.*





Figura 2

Venn's diagram showing the overlap of bacterial species detected by PCR in pulp necrosis due to trauma or caries.

nigrescens (66.7%), and *F. nucleatum* (66.7%) were most frequently recovered from a root canal with pulp necrosis due to trauma (7/44).

Treponema denticola and Filifactor alocis were detected only from a root canal with pulp necrosis due to caries. *S. mutans, S. mitis* and *P. intermedia* were detected only from a root canal with pulp necrosis due to trauma. *Enterococcus faecalis* was not observed in any of the root canals (Figure 2). Significant associations were found between the presence of *P. micra* and the existence of caries (p=0.023) and sinus tract (p=0.044). The presence of *T. forsythia* was associated with the existence of trauma (p=0.035), and the presence of *F.nucleatum* was associated with positive pain on palpation (p=0.033).

Discussion

There is a well-established consensus that microorganisms are the main cause of endodontic infections. Of all microorganisms inhabiting the oral cavity, only a few of them can invade the pulp and compromise its function. Studies have well-documented these species in endodontic infection in permanent teeth either by culture method (10) or molecular techniques (9, 12). However, few studies have been carried out to identify microorganisms in root canals of primary teeth (8, 11-13, 16-18). The present study sought to investigate 15 bacterial species frequently detected from root canals of primary teeth with pulp necrosis after caries or trauma using PCR.

The microbiota of primary teeth with pulp necrosis, identified in this study, was similar to permanent teeth regarding the bacteria respiration metabolism, being the anaerobes frequently detected (10, 12). This result suggests that, despite the different causes of pulp necrosis and different mechanisms of microbial invasion, the conditions inside the root canal probably favours the colonization and multiplication of a restricted group of species with a predominance of anaerobic species.

Actinomyces naeslundii was found commonly in necrotic root canals either after caries (47.4%) or after trauma (83.3%). Actinomyces naeslundii, a facultative Gram-positive bacterium, has been associated with secondary endodontic infections. A low frequency of this microorganism has been reported previously in primary infections of permanent teeth using culture techniques (10).

Prevotella nigrescens was found often in necrotic root canals either after caries (76.3%) or after trauma (66.7%). *Fusobacterium nucleatum* was also regularly detect-



ed in necrotic root canals either after caries (42.1%) or trauma (66.7%), and it was associated with positive pain on palpation (p=0.033). Prevotella and Fusobacterium, which are Gram-negative anaerobic bacilli, have been previously associated with the presence of acute symptoms of pain, history of previous pain, tenderness to percussion and swelling in permanent teeth (10). In our study, Fusobacterium was associated with pain, and this may be explained by the presence of lipopolysaccharide, an outer membrane component of Gram-negative bacteria that induces, among other issues, up-regulation of bradykinin, a potent pain mediator (10).

Parvimonas micra were the species most commonly detected in root canals of primary teeth with pulp necrosis after caries (76.3%). This species was not generally detected in pulp necrosis after trauma and had an association with the existence of caries (p=0.023) and sinus tract (p=0.044). *P. micra*, Gram-positive anaerobic cocci, have been previously associated with a history of pain, tenderness to percussion and wet canals in permanent teeth (10).

Treponema denticola (Gram-negative anaerobic bacilli) and *Filifactor alocis* (Gram-positive anaerobic bacilli) were detected only from samples of root canals with pulp necrosis after caries. These bacteria have been previously observed in great prevalence in primary endodontic infections in permanent teeth, implying they may be implicated in the pathogenesis of periapical diseases (19, 20).

Tannerella forsythia was the species most frequently detected in root canals of primary teeth with pulp necrosis after trauma (83.3%). This bacterium had an association with the presence of trauma (p=0.035), and it was not frequently detected in pulp necrosis after caries.

Our results have similarities with other findings (18), which showed a low frequency of *T. forsythia* in primary teeth with pulp necrosis caused after caries. *T. forsythia*, a Gram-negative anaerobic bacillus, has been previously associated with tenderness to percussion, mobility, wet canals, and purulent exudate in permanent teeth (21). *S. mutans, S. mitis* (both facultative Gram-positive cocci) and *P. intermedia* (black-pigmented anaerobic Gram-negative bacilli) were detected only from a root canal with pulp necrosis after trauma. According to the literature, *P. intermedia* have been most frequently detected in permanent teeth with necrotic pulp, and are related to the appearance of signs and symptoms of periapical disease in permanent dentition (22).

Enterococcus faecalis was not detected in any root canal in this study. This result is in disagreement with a previous study (18) which observed a high frequency of Enterococcus spp. (50%) in the necrotic pulp of children using PCR. E. faecalis was also previously detected in 63% in primary teeth using culture technique (23). E. faecalis was frequently detected in cases of primary and secondary infections in permanent teeth using PCR (19), and in the root canals and their combined periodontal pockets in cases of endodontic-periodontal lesions (24). Our results are consistent with other studies, which say the microbial composition of unexposed and exposed pulp tissues do not present impressive distinction (25, 26). This evidence suggests pulpal exposure may not play an important role in establishing the selection of bacteria present in infected root canals (26).

Conclusions

The microbiota recovered from root canals of primary teeth with pulp necrosis caused by dental caries or trauma is very similar, with the predominance of anaerobic microorganisms.

Clinical Relevance

Despite the different causes of pulp necrosis and different mechanisms of microbial invasion, the conditions inside the root canal probably favors the colonization and multiplication of a restricted group of species with a predominance of strict anaerobic species.

Conflicts of Interest

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



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ORIGINAL ARTICLE

Application of artificial intelligence in a visual-based fluid motion estimator surrounding a vibrating EDDY[®] tip

ABSTRACT

Aim: To improve our initial understanding of the vibrational behavior and fluid flow of an EDDY® tip when irrigation is activated using artificial intelligence (AI).

Methodology: A straight glass model was filled with a solution containing 3% NaOCI. A-28 mm polymer noncutting #20 0.04 taper file was driven by an air sonic handpiece at 6,200 Hz for five seconds. The fluid flow behavior was visualized using a Miro 320S high-speed imaging system (Phantom, Wayne, NJ, USA). The recordings of the hydrodynamic response were then analyzed using motion estimation program, supported by LiteFlowNet.

Results: Rapid fluid flow was visualized clearly in the model when activated by an air sonic driven EDDY[®] tip. The distal end of the EDDY[®] tip generated a near-wall high-gradient velocity apically in all directions of the oscillation.

Conclusions: The proposed motion estimation program, supported by LiteFlowNet, could perform flow estimation of a non-PIV experiment in detail.

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Introduction

new polyamide tip, referred to as an EDDY® tip (VDW, Munich, Germany), has recently been developed for activating root canal irrigants and released onto the market. An EDDY® tip is a smooth polymer tip driven by an air sonic scaler at 5,100-6,200 Hz. Numerous studies have found sonically and ultrasonically driven electrical devices to be effective in cleaning root canals (1-5). Several researchers have found that sonically activated irrigation is less effective than its ultrasonically activated counterpart, while others have suggested that the two methods produce comparable results (6-8). However, the physical mechanism relating to the efficiency of EDDY® tip has vet to be assessed.

Several studies on dentistry have employed powerful particle image velocimetry (PIV) technology to observe the natural flow behavior of hydrodynamic response during irrigation activation (9-12). This conventional PIV method uses microscopic tracer particles intended to be a non-intrusive means of obtaining a deeper insight into complex flow phenomena used in the measuring of a velocity field. The tracer particles previously seeded into the flow medium do not affect the natural flow motion. The displacement of the seeding particles is recorded by means of a highspeed digital imaging system and subsequently analyzed using a specific program such as cross-correlation with a window deformation iterative method to identify the velocity distribution in the investigated location (13-15).

At the present time, in order to further improve the PIV image-processing program, the implementation of a state-of-theart innovation constitutes a potential solution. A deep learning approach for motion estimation has shown promising results with higher accuracy and enhanced computational performance (16). Currently, the LiteFlowNet network, a state-of-the-art deep learning model used for motion estimation, has been developed by Hui and colleagues (17). The program utilizes a relatively smaller model size than the other solutions, thus enabling faster inference speed. Furthermore, reproducing a deep learning model from estimator programs is not significantly different to undertaking measured investigations using PIV image processing method. The differences occur only in cases of different randomization incorporating floating number error (18). In dentistry, an artificial intelligence application model has been employed to support the clinical decision-making process in certain clinical disciplines (19) such as radiology (20-22), endodontics (23), and surgery (24).

The main challenge of this study is to develop a deep learning estimator for non-PIV (absence of seeding particles) based on LiteFlowNet (17) which can be applied to a relatively limited area such as a root canal space as a means of producing high-resolution images. Furthermore, the program can process a non-particle image pair input to produce a velocity field output with displacement vectors at every pixel.

To the best of our knowledge, no previous studies of velocity field estimation of hydrodynamic response in a small area relating to air sonic driven EDDY® tips during activation of an irrigation solution using a deep learning program have been conducted. In the present non-PIV study, the occurring phenomena were investigated by acquiring real-time data using a transparent glass block model of a root canal in order to visualize its behavior when in the solution.

Materials and Methods

Basic visualization

The study was designed to observe the hydrodynamic response to EDDY® tip solution activation in the root canal model. The process was recorded using a Phantom Miro 320S high-speed digital imaging system (Wayne, NJ, USA) which incorporated a macro lens capable of producing 25,000 frames per second with 320×240 pixels per image (60 mm, f/2.8; Nikon, Tokyo, Japan). Each sample was illuminated by means of a Fibre-Lite LMI-6000 LED continuous light source (Dolan-Jenner In-





Figure 1

 A) The deep learning motion estimator process in an optically accessible root canal model.
 B) The workflow featured in each validation test.

dustries, Boxborough, MA, USA). The study method implemented was designed in such a manner as to be non-destructive. A single glass tooth model was used during each treatment to ensure uniformity of root canal width and size. After each test, the model was carefully cleaned for re-use in the subsequent one. Experiments were conducted three times on each sample. In order to obtain estimation quantitative data images, the recordings of the hydrodynamic response were analyzed using an in-house PIV software developed by the Aerodynamics Laboratory at ITB (Institut Teknologi Bandung, West Java, Indonesia). This software utilizes optical flow with a convolutional neural network for velocity field estimation rather than measured experimental PIV settings with tracer particles.

Sonic parameters and procedure

A-28 mm polymer noncutting # 20/0.04 (VDW) tip was screwed onto a Ti-Max S970 air scaler (NSK, Tochigi, Japan) set at level 3 (5,800–6,200 Hz) in accordance with the manufacturer's instructions and employed to activate the irrigant. The sample was subjected to active sonic irrigation without use of a water spray, while the tip was activated passively without any filing motion.

Experiment: visualization of an EDDY® tip during irrigant activation

The conditions within a straight root canal were simulated and visualized using a glass block model with an artificial canal and pulp cavity acting as a reservoir (Kimia Farma, Bandung, West Java, Indonesia). The crown height was one of 7 mm, while the root length was 18 mm with an inner diameter of 0.4 mm at the apex and 0.06 taper. Each model was filled with a solution containing 3% NaOCl. The apex of the model was sealed with composite to simulate the internal conditions of a root canal. The sonic instrument tip was inserted to a depth of 3 mm below the orifice and activated passively for five seconds without any filing motion. Maintenance of the desired position, fluid activation and pre-



vention of the tip binding vibrating against the canal wall were ensured by fixing the tip in a holder.

Image processing

Data from non-PIV experiments was extracted by means of a deep learning estimator incorporating a deep learning estimator incorporating PIV software developed by the Aerodynamics Laboratory at ITB (Institut Teknologi Bandung, West Java, Indonesia). Since the images obtained during the conduct of this experiment were not of PIV particles, the non-enhanced LiteFlowNet version (17) proved more suitable. This was due to the LiteFlowNet model being trained using images with general objects (and RGB colored), unlike the PIV-LiteFlowNet-en that is trained specifically with grayscale particle images. It is important to note that the same program was employed in this case by reproducing the PIV-LiteFlowNet-en in a Python program with PyTorch, thereby allowing the user to control the modification of each layer. Therefore, in order to generate a LiteFlowNet version, the user should disregard the last NetE level and impose the LiteFlowNet trained weights. The flowchart outlining the stages of the data execution process is contained in Figure 1A.

Program validation

It is necessary to prove the validity of the motion program in handling the images resulting from the root canal experiment with LiteFlowNet. Thus, a validation test was performed by transforming several

Table 1 Average end-point error evaluation (AEE) for both shifting and rotational transformation. The mean value and the 99th percentile errors are presented for both tests

Method	AEE
MPI Sintel - final	5.38
KITTI12	1.60
Shifting test - mean	0.10
Shifting test - 99th	0.26
Rotation test - mean	0.22
Rotation test - 99th	1.31

images in order to estimate the velocity field produced by the corresponding transformation. Figure 1B presents the test procedure relating to a single sample image. To validate the images generated during the experiment, they were either shifted or rotated according to their respective randomized input parameters. Therefore, it also proved possible to extrapolate the ground-truth velocity field from the parameters. The LiteFlowNet estimated the velocity field occurring between the original and transformed images. The average end-point error (AEE) was calculated on the basis of the estimated and ground-truth velocity fields. The experiment images were shifted randomly between -11 to 11 pixels in both vertical and horizontal directions. These parameters were selected on the basis of a study conducted by Butler et al. (25) that a displacement above 12 ppf is considered to be significant. This resulted in various shifting combinations, ranging from displacement of 0.5 pixel per frame (ppf) to one of 14.7 ppf. Evaluation based on different velocity gradients and rotational transformation was also included. The images were then rotated randomly from -18 to 18 degree direction,

In order to avoid potential confusion due to the blank pixel, error evaluation was performed only around the center region of the images, as depicted by the red-bordered boxes contained in Figure 2. While avoiding the blank pixels, the evaluation region relating to the shifting transformation, Figure 2B captured the most crucial area located around the irrigation solution. On the other hand, the smaller bordered box for the rotational test shown in Figure 2E was intended to allow larger image rotation of up to 18 degrees while maintaining reasonable pixel displacement. Hence, error evaluation of high-velocity gradient cases is facilitated by the larger image rotation. The greatest recorded pixel displacement during the rotational test was one of 14.1 ppf. The evaluation results for both the shifting and rotational transformation tests are presented in Table 1.

The 99th percentile AEE value is also provided to enable analysis of the critical condition. The rotational test indicated that Figure 2

Illustration of the original and transformed. A) Sample



С F A D





Figure 3

A) The velocity scale for each image is indicated by the color bar on the left. Direction of the flow caused by an EDDY® oscilating tip (indicated by solid arrows). B) Upward direction: C) downward direction; **D**, **E**) the broken circles indicate the flow pattern occasionally occuring perpendicular to the axial of the tip. F-K) Indicating instantaneous dense velocity fields within small-scale flow structures near the wall were visualized. The orange to red color indicates the location where a near-wall high-velocity gradient occurs.

the highest 1% AEE results were all produced by those images rotated by >16.6 degree. It was suspected that the higher velocity gradient (due to greater image rotation) constituted the main causal factor. This was due to there being a strong positive correlation between the image rotation angle that was proportional to the velocity gradient and the AEE with a Pearson's correlation coefficient (PCC) score of 0.64. On the other hand, the shifting test proved that there was almost no correlation between the pixel displacement and the AEE score of -0.06 PCC. This is reason for the highest 1% AEE results of the shifting test being produced by images transformed with varying displacement ranging from 0.55 ppf to 14.7 ppf. Therefore, it can be concluded that the effect of larger pixel displacement was insignificant to the high-velocity gradient in terms of reducing

the program performance. Furthermore, the tests showed that the LiteFlowNet-based estimator program was valid for processing the root canal experiment images. It is because all of the AEE results were relatively lower than those of the LiteFlowNet benchmark (17) in the popular dataset (MPI Sintel and KITTI) (25, 26), even for the 99th percentile value.

Results

Data extraction

The study results indicate that the modified non-enhanced LiteFlowNet program successfully extracted non-particle image pair input, producing velocity field output with displacement vectors in every pixel. Furthermore, the program provided detailed high-resolution images enabling smaller scale motion detection, while also gener-







Figure 4 A) Column level of air depression and B) Average flow magnitude.

> ating a dense motion field for all images, and rapidly completing computer processing.

Experiment

The flow motion was highly unsteady. In order to highlight the observation, the study

focused on only a limited portion of the canal to enable velocity field visualization. The portion was located around the distal end of the tip. The instantaneous images contained in Figure 3 illustrate the flow pattern of the solution near the distal end of the tip where the highest amplitude



occurs. The flow pattern of strong axial components was both upwards and downwards (figure 3B and C). A flow pattern perpendicular to the axial flow along the canal was periodically observed (figure 3D and E). A periodic flow pattern within which velocity gradients occurred near the wall during activation, such as shown in Figure 3F-K, was observed. The velocity fields within this region (shown in red) were approximately three times higher than those in the surrounding area. The length of the arrows also indicates the magnitude of the velocity.

Figure 4 depicts the duration of energy accumulation and the average fluid magnitude during activation. The correlation between air column depression and bubbles formation during activation could be observed.

Discussion

The experiment results revealed that the process of EDDY® tip activation in the root canal model occurred in three stages.

Stage 1: the vibrating EDDY® tip transferred its energy to the solution and created waves which, in turn, caused surface waves to form at the interfaces. In the air-solution interface system, the surface of the solution moved downwards on one side and upwards on the other. The duration of this energy accumulation period was in the range of the initial 0-0.03 seconds (figure 4Aa).

Stage 2: this stage lasted between approximately 0.03 and 0.34 seconds (figure 4Aa-b). When the amplitude reached or approached its peak, kinetic energy increased to the point that it was sufficiently high to disrupt the interface with an average fluid magnitude close to 300 mm/sec. This disruption, in turn, forced part of the solution upwards into the coronal portion where droplets formed on the wall. Their accumulation caused mass displacement of the solution, while downward pressure waves subsequently forced the air to displace it. The solution and the air moved spontaneously and simultaneously. The pressure of the air on the solution forced it far beyond the interface which created an air depression column. The possible reasons for this may be that the specific natural frequency of the EDDY® tip was achieved, while the amplitude concomitantly approached or reached its peak. It means that, at the setting concerned, one of the natural frequencies of the tip matched the frequency of excitation.

By way of confirmation, the results of the pilot study conducted demonstrated that when an Eddy tip was being forced to vibrate in the air at its natural frequency for less than a second the distal end of the tip might separate. This means that the EDDY® tip achieved its resonant frequency which caused damage due to excessive vibration (27). The velocity of the moving air depression column was approximately 20 mm/ sec. During this period, accumulation of energy occurred in the form of flow magnitude generating velocity field throughout the canal. Significant data at this stage indicates that the natural frequency of the tip generates intense solution flow. It was observed that the flow pattern periodically moved up and down throughout the canal (figure 3B, 3C). This action may develop a push-pull mechanism by which removal of smear layer and debris will be enhanced. Furthermore, the natural frequency of the tip produces a near-wall high-velocity gradient which is proportional to wallshear stresses. The areas of significant changes in velocity over a short distance are indicated in red (figure 3 F-K). From a fluid dynamic perspective, a near-wall high-velocity gradient and the bulk transports of solution are, potentially, the most important variables of the cleaning process within the root canal during activation (11). Finally, it was observed that high-velocity gradient on the wall largely occurred more apically (around the lower end of the tip) and periodically in the same area as indicated in red (figure 3 F-K). The shear stress coverage area was approximately 3-4 mm which enhances the efficacy of the cleaning effect around the apex.

Based on the data relating to this stage, it is recommended that the tip should be moved up and down in order to distribute the shear stresses evenly along the canal, while the pumping action of the tip induc-



es additional shear stresses on the wall. Its cleaning efficacy has been attributed to the combination of either mechanical forces or fluid dynamics with chemical irrigants (28-30). Indeed, this stage is a crucial one within which the near-wall high-velocity gradients are of approximately 0.30 seconds in duration. Within clinical settings, it would be possible for irrigant activation with the cleaning process relating to the entire single root canal to be completed within 30 seconds, thereby reducing treatment time. Additional studies are required to produce further confirmatory evidence in support of this assumption.

Stage 3: during the first 350,000-430,000 µs, the oscillating free end of the EDDY® tip (antinode) did not generate bubbles. After approximately 450,000 µs (figure 4Ab-c), the air depression column reached the distal end of the tip (antinode) where the maximum flow velocity reached approximately 1,500 mm/sec, as indicated by the red section of the color bar (figure 3A). Intense bubble fragmentation occurred when the bubbles reached the oscillating distal end of the tip. The vibrations of the free end of the tip generated new bubbles, a continuous process (340,000-450,000 µs) occurring during activation which culminated in the entire canal being filled largely with micro-bubbles (i.e., clouds of bubbles), while simultaneously producing circular solution flow along the root canal wall and creating a chaotic pattern at 450,000 µs. The results showed that the bubble cloud at the distal end of the tip was formed at around 450,000 µs post-EDDY® tip activation. The life cycle of an observed bubble 500 µm in diameter ranges from 300,000 to 500,000 µs. This intense bubble formation can cause an expansion in volume of up to 1,600 times the original, thereby enabling easier access of the irrigant to the apical region of the canal which might assist in the cleaning ability of various shapes (31). This bubble formation was visualized by means of high-speed imaging. However, during intense bubble formation, when the velocity fields in the solution could not be observed simultaneously, the velocity decreased to its lowest level reaching a constant speed of approximately 250 mm/sec (figure 4B, C) where

near-wall velocity gradients did not occur. As a consequence of this phenomenon, continuous replenishment of irrigation solution during activation is recommended as this may reduce the prolonged bubble regime.

Interestingly, the modified estimator program appears to offer the considerable advantage of high efficiency through a reduction in time image processing compared to the original PIV program (32). The whole image extracting process (approximately 13,000 images) employed in this study was of approximately an hour in duration. Furthermore, the study did not require a complicated experimental set-up but one which was, ultimately, extremely cost effective.

Conclusions

The results indicate that the proposed LiteFlowNet-supported motion estimation program was able to conduct detailed flow estimation of a non-PIV experiment and extract dense velocity fields. Moreover, it was capable of capturing transient flow behavior, a near-wall high-velocity gradient and intense bubble formation occurring in a restricted area when the solution was activated by an air sonic driven EDDY® tip. More importantly, there is enormous potential for the development of related topics and this model facilitates further similar research in dentistry.

Clinical Relevance

Detailed flow pattern during irrigant activation is a fundamental information. Clinicians may use this information to modify treatment procedures to achieve better expected results.

Conflict of Interest

The authors declare that no conflicts of interests exist regarding the contents of this article.

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The impact of composition, core metal mass and phase transformation behaviour on the dynamic cyclic fatigue of Ni-Ti files at different temperatures

ABSTRACT

Aim: To assess impact of elemental composition, core metal mass and phase transformation behaviour on the dynamic cyclic fatigue resistance of three Ni-Ti rotary files at room and body temperatures.

Methods: Twenty instruments of each system were tested for dynamic cyclic fatigue resistance in a simulated root canal with a 90° angle of curvature and a 5-mm radius of curvature at room and body temperature. The core metal mass at the fractured surface of each instrument was calculated by Image J software analysis of SEM images. The energy dispersive X-ray analysis was used to assess file composition. Scanning calorimetry was used to assess the structural phase state and the transformation temperature. One-way analysis of variance (ANOVA) was performed to determine any statistical difference amongst groups. For inter-group comparison, the unpaired t-test was used.

Results: HEDM showed significantly higher TtF and NCF values than AFBS and ZB-F6 instruments, at both temperatures tested. The mean core metal mass was smallest in HEDM followed by AFBS with no statistical difference between them, while ZB-F6 had the significantly largest metal core. EDX analysis showed that all the instruments were mainly composed by nickel and titanium. DSC analysis revealed that HEDM and AFBS exhibited a martensitic phase at body (37 °C) and room temperature (25 °C), whereas ZB-F6 revealed an austenitic phase at body temperature.

Conclusions: Dynamic cyclic fatigue resistance increased when the instruments had less cross-sectional metal mass, less Ni (wt%), a thermally treated surface, and a martensite phase at body temperature.

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Introduction

ickel-titanium (NiTi) instruments can shape root canals faster, with better-centered preparations and fewer procedural errors than stainless steel ones (1). Yet, unexpected instrument fracture still does occur. Two modes of fracture were identified by Sattapan et al. (1), torsional and fatigue failures. Fracture due to torsion occurs upon reaching the ultimate shear strength of the file, while cyclic fatigue is attributed to metal fatigue when it rotates freely in a curved canal at the point of maximum flexure (2, 3). Several factors contribute to the cyclic fatigue resistance of NiTi instruments, including operational settings, alloy composition, metallurgical properties, and the thermomechanical history of the instrument (1-5). Recent studies have shown that environmental temperature during testing also influences the cyclic fatigue failure of NiTi instruments (6, 7). As instrument fracture could jeopardize the outcome of endodontic treatment, it is essential to understand the impact of elemental composition and structural phase state on the fatigue resistance of NiTi endodontic files, especially in a dynamic model and a simulated body temperature.

The present study aimed to study the impact of elemental composition, core metal mass and phase transformation behaviour on the dynamic cyclic fatigue resistance of Hyflex EDM One file (COLTENE/Whaledent, Altstatten, Switzerland), (HEDM), AF blue S one (Fanta Dental Materials Co. Ltd., Shanghai, China) (AFBS) and ZB-F6 Ni-Ti rotary files (Foshan Qiyang Medical Equipment Limited. Guangdong, Guangdong, China) at room and simulated body temperatures. The null hypotheses tested were that there would not be significant differences among the instruments regarding their cyclic fatigue resistance and that cyclic fatigue is not influenced by the testing temperature.

Materials and Methods

Sample size calculation

A statistical sample size calculation was performed using the G-power program based on a previous study. An alpha-type error of 0.05, a beta power of 0.95, and an N2/N1 ratio of 1.

Dynamic cyclic fatigue test

Twenty new instruments with tip sizes (#25) from three NiTi rotary systems, Hy-Flex EDM OneFile (HEDM), AF Blue S One (AFBS), and ZB-F6 were tested at room



Figure 1

Cyclic fatigue assembly: A) custom-made dynamic cyclic fatigue device; B) custom-made water bath; C) endodontic motor; D) screen display of the code responsible for dynamic movement; E) Arduino/ CNC shield complex.

El-wakeel M, Hashem A, Fahmy S et al.





Figure 2

SEM photomicrographs of the fractured surface after cyclic fatigue testing. General view of HEDM (A), AFBS (D) and ZB-F6 (G); instruments showing area of elevation (black arrows), and high-magnification view of HEDM (B), AFBS (E) and ZB-F6 (H); instruments showing microstructure voids (white arrows), both typical signs of ductile failure. And (x500) used to determine the amount of metal mass (in pixels) at the level of the instrument fracture for of HEDM (C), AFBS (F) and ZB-F6 (I).

> temperature (25 °C, n=10) and body temperature (37 °C, n=10). Instruments were operated according to the manufacturer's instructions in a simulated stainless-steel canal (tip size #25/0.08 taper +0.2 mm offset) using a 16:1 reduction handpiece (Saeshin Precision Co., Ltd, Korea). The simulated root canal was 16 mm long with a 90° angle of curvature and a 5mm radius of curvature according to Pruett's method (8). The straight segment of the canal was 11 mm and the center of curvature was 5 mm away from the instrument tip. A new custom-made device was designed (Egyptian patent number 265/2021) and constructed to be used for dynamic cyclic fatigue testing in the present study (Figure 1). The device is composed of a custom-made linear actuator attached to a custom-made frame holding the handpiece

and the artificial canal. The linear actuator is connected to a CNC shield/Arduino Uno complex (Arduino. cc, USA).

A custom code written by the Arduino software (version 1.8.13, Arduino.cc, USA) controlled the magnitude of the vertical distance and time (1.5 mm/0.5 s upwards and 1.5 mm/0.5 s downwards) of the rotating file. The code allowed repeatable cycles with a 1 second relay. Both the device and the endodontic motor were started simultaneously. The auto-reverse mode of the endodontic motor was turned off. The cyclic fatigue apparatus was inserted in a custom-made water bath with a heat controller to regulate the desired testing temperature. The test was performed two times for each group, one at room temperature 25 °C ±0.5 °C and the other at body temperature 37 °C ±0.5 °C. A video was cap-







Figure 3

DSC analysis, showing heating and cooling curve with phase transformation temperatures for each file. tured and time to fracture (Ttf) was evaluated frame by frame using a special video editing program (Adobe Premiere Pro 2020, Adobe Inc, USA), recorded and tabulated for all groups. The number of cycles to fracture (NCF) was then calculated and the fractured fragment length (FL) was measured in mm unit for each instrument tested.

Fractographic and metal mass examination by scanning electron microscope (SEM)



Table 1

Time to fracture (seconds), number of cycles to failure, fragment length (mm), metal mass (pixels), the Predicted cycles for 99% survival and correlation coefficient for the different instruments at the different tested temperatures

	T	:F N		CF	Fragment length						lation cient
	25 °C	37 °C	25 °C	37 °C	25 °C	37 °C	pixels	25 °C	37 °C	25 °C	37 °C
HEDM	40.0160 ±0.0389ª	39.9170 ±0.2068ª	266.773 ±0.260ª	266.113 ±1.378ª	3.18 ±0.092ª	3.18 ±0.123ª	68727 ±2006ª	269.072	268.329	0.944	0.829
AFBS	38.8290 ±0.0706⁵	38.7760 ±0.0517⁵	258.860 ±0.471 ^b	258.505 ±0.345⁵	3.15 ±0.108ª	3.17 ±0.095ª	78617 ±2568ª	261.091	260.650	0.957	0.874
ZB-F6	8.0360 ±0.0331°	5.8460 ±0.1166°	52.5733 ±0.220°	38.974 ±0.778°	3.26 ±0.108ª	3.25 ±0.151ª	97828 ±3190 ^b	54.0347	39.1740	0.984	0.957

Different superscript letters in the same column were statistically significant (p<0.05).

The values are means \pm standard deviations.

TtF: time to fracture, NCF: number of cycles to fracture, metal mass and fractured fragment length.

All fractured instruments were examined by SEM (Philips SEM 515, Eindhoven, Netherlands). The broken fragments were ultrasonically cleaned in absolute ethyl alcohol for approximately ten minutes before analysis at 500x and 2000x magnification at 25 kV and room temperature to evaluate the type of fracture. The 500x SEM images were analyzed using Image J software (Image J, USA) to determine the amount of metal mass of each file in pixels at the fracture level (Figure 2).

Elemental analysis by energy-dispersive X-ray (EDX) analysis

The surfaces of three new instruments from each brand were analyzed to evaluate their chemical composition by EDX analysis using a field emission SEM (FEI Company, Hillsboro, Oregon-USA). Samples were mounted onto SEM stubs and analyzed at a 10.1 mm working distance, with an inlens detector and an excitation voltage of 20 kV.

Phase transformation behaviour by differential scanning calorimetry (DSC)

Four new instruments from each file system were evaluated using DSC (Diamond Dsc, PerkinElmer, Waltham, USA) with scans ranging from approximately 65 $^{\circ}$ C to -5 $^{\circ}$ C to assess the metallurgic phase of the

file at different temperatures (4 $^{\circ}$ C, 25 $^{\circ}$ C, 37 $^{\circ}$ C) and transformation temperatures. Two different tests on two new instruments from each system were conducted, aiming the second to confirm the result of the first one.

Statistical analysis

The Shapiro-Wilk and Levene test was used to evaluate the assumption of normality and the equality of variance of the data sets. Considering that the dynamic cyclic fatigue resistance results were normally distributed (P>0.05), they were presented as mean and standard deviation values of TtF, NCF, FL, and metal mass. These results were analyzed using one-way analysis of variance (ANOVA) and Tukey Honestly Significant Difference (HSD) tests using Minitab 19 program (version 19, Minitab, LLC, USA) to determine any statistical difference amongst groups. For inter-group comparison, the unpaired *t*-test was used. In the present study, P≤0.05 was considered as the level of significance. Pearson correlation test was conducted with confidence level 95 to correlate metal mass, Ni (Wt%), and Af temperature with TtF.

Using Weibull reliability analysis, the probability for survival was calculated and charted in a reliability plot the steepness of the slope evaluated which represents the



Element	HEDM	AFBS	ZB-F6
С	19.328±1.294	8.874±1.247	10.815±1.449
0	18.995±0.516	12.474±0.392	4.160±0.913
AL	3.447±0.379	3.440±0.403	4.308±1.205
Si	0.5600±0.0283	-	-
Ti	32.047±0.495	32.998±1.251	35.730±0.939
Ni	41.297±1.889	41.138±1.519	44.710±1.568

 Table 2

 EDX analysis showing the mean Wt% ± standard deviations of each element of the three tested files

C: carbon, O: oxygen, AL: aluminium, Si: silicon, Ti: titanium, Ni: nickel.

Beta value. The correlation coefficient was calculated. The predicted maximum cycles for 99% probability of survival were calculated.

Results

Mean TtF and NCF after dynamic cyclic fatigue testing for all the instruments tested are displayed in Table 1. One-way ANOVA revealed a significant difference among the three tested instruments (P<0.05). HEDM showed the highest resistance to cyclic fatigue followed by AFBS and ZB-F6 files at both room and body temperatures (P<0.05). A significant difference was observed between body temperature and room temperature for the ZB-F6 file only (P>0.05). The length of all fractured segments showed no significant difference among all tested groups denoting accurate device trajectory.

Weibull probability plots (reliability Vs time) per group revealed that the ZB-F6 file has the lowest Beta value among the tested groups at body temperature. The Predicted cycles for 99% survival, the correlation coefficient is represented in (Table 1). HEDM at room temperature showed the highest predicted number of cycles to fracture for 99% survival by 269.072 cycles while ZB-F6 at body temperature showed the lowest predicted number of cycles to fracture for 99% survival by 39.174 cycles. All groups showed a correlation coefficient higher than 0.75.

The fractographic examination confirmed a predominantly ductile mode of fracture for all tested instruments, typical for fractures due to accumulation of metal fatigue (Figure 2). This was evident by areas of elevations at the peripheries at high magnification and dimpled surfaces at low magnification. The dimpled surfaces revealed different characteristics for each instrument type: for HEDM and AFBS instruments, the dimples were fewer and relatively smaller, while for ZB-F6 instruments the dimples were larger and more numerous. The metal mass calculation (Table 1) revealed that the mean core metal mass was smallest in HEDM (68727±2006) followed by AFBS (78617±2568) with no statistical difference between them (p>0.05), while ZB-F6 had the significantly largest metal core (9782± 3190) (p<0.05).

EDX analysis (Table 2) showed that all the instruments were mainly composed of nickel, titanium with slightly different percentages depending on the instrument tested.

Regarding the phase transformation behaviour, all tested brands showed a DSC curve with a single exothermic peak at cooling and a single endothermic peak at heating at the examined temperature range (Figure 3). The exothermic peaks represented the martensitic phase changes for AFBS



and ZB-F6 and the R-phase change for HEDM instruments, while the endothermic peaks represented the austenitic phase changes for all instruments. Martensitic start and finish temperatures and austenitic start and finish temperatures are listed in (Table 3). HEDM and AFBS files displayed a higher Af $(53.505\pm0.375,$ 45.62 ± 2.376) than the body temperature. In comparison, ZB-F6 showed a lower Af (26.44 ± 0.523) than the body temperature. Pearson's correlation test showed a negative correlation between TtF and metal mass (-0.953) and Ni Wt% (-0.996) and a positive correlation with Af temperature (0.953).

Discussion

The present study evaluated the impact of elemental composition, core metal mass and phase transformation behaviour on of the dynamic cyclic fatigue resistance of Hyflex EDM One file (HEDM), AF blue S one (AFBS), and ZB-F6 Ni-Ti rotary files at room and simulated body temperatures. Recent studies advocated the simulation of clinical conditions during cyclic fatigue testing (6-11). The present study used a computer-guided novel dynamic model that allowed a pecking motion of 1.5 mm in both directions and can be operated in a water bath with simulated body temperature. It has an advantage over former dynamic models operated by universal testing machines or custom-made devices that

cannot be used in a water bath (12, 13). Results of the present study showed that HEDM files reported the highest cyclic fatigue resistance followed by AFBS and ZB-F6 files respectively, at both room and body temperatures. ZB-F6 showed the lowest cyclic fatigue at both temperatures (P<0.05). This can be attributed to the thermomechanical processing treatment of the instruments during manufacturing. HEDM undergoes electric discharge machining which is a noncontact thermal erosion process that partially melts and evaporates the NiTi wire by high-frequency spark discharges. This technique was proved to enhance the mechanical properties greatly through reducing the surface defects and decreasing stresses on the instrument (13-15). The manufacturer of AFBS instruments claims instead a flowless surface finishing with a titanium oxide surface treatment that contributes to a better fatigue resistance (http://www.fanta-dental.com/static/ upload/file/ 20211101/1635745409400497. pdf). This agrees to former studies highlighting the positive impact of heat treatment to reduce and correct machining defects (15, 16). The low cyclic fatigue resistance of ZB files can be attributed to the absence of such treatments.

The Weibull cumulative distribution function, which appears as a straight-line where the beta parameter is the slope of the line, indicated that ZB-F6 file showed the lowest predictability among the tested groups. The

	Phase transformation temperatures and enthalpy changes (ΔH)									
	М _s (°С)	М _f (°С)	ΔΗ (j ⁻¹)	R _s (°C)	R, (°Ć)	ΔH (j⁻¹)	A (°Ĉ)	A, (°Ć)	ΔH (j⁻¹)	
				44.41	35.875	1.1862	43.03	53.505	2.549	
HEDM				±0.509	±0.332	±0.568	±0.537	±0.375	±0.438	
	38.33	33.825	41.17				41.17	45.62	2.265	
AFBS	±1.004	±0.757	±1.853				±1.853	±2.376	±0.529	
ZB-F6	19.265 ±0.177	15.28 ±0.057	1.913 ±0.446				21.305 ±0.516	26.44 ±0.523	2.265 ±0.529	

Table 3Phase transformation temperatures and enthalpy changes (ΔH

The values are means ± standard deviations.

A; austenitic start, A; austenitic finish, M; martensitic start, M; martensitic finish, R; R-phase start, R; R-phase finish.



steeper the slope (beta), the smaller the variation in the time to failure and the more predictable the results will be. The correlation coefficient, which is the degree of the relation between the probability of survival and the predicted maximum cycles for each instrument, was higher than 0.75 for all files, which indicates a strong correlation.

Examination of the fractured instruments showed that they all fractured at the level of D3 to D4. At this level, HEDM has a quadratic cross-section and an 8% taper, the AFBS has an almost oval cross-section and 4% taper, while the ZB-F6 has a triangular cross-section and a 4% taper. Using Image J software (Image J, USA), the metal mass was calculated at the fracture level revealing that ZB-F6 files showed the largest metal mass that would explain the lower cyclic fatigue resistance obtained by the results of the study. Although the significant influence of cross-sectional design on the fatigue resistance had been reported by some studies (17, 18), other studies found that the cross-sectional design and the core size did not influence the fatigue resistance of Ni-Ti instruments (19, 20).

EDX analysis showed that the principal element in all files was nickel, 41.2 wt% for the HEDM instruments, 41.1 wt% for the AFBS instruments, and 44.7 wt% for the ZB-F6 instruments. It has been reported that the Ni content of a NiTi alloy influences the mechanical properties of endodontic instruments (20)which exhibit a lower percent in weight of nickel (52 Ni %wt. With the reduction of the Ni content, there is an increased tendency to obtain stable martensite, which is more flexible than austenite, at the working temperature (21). This finding is consistent with the results obtained in the present study by the mechanical dynamic cyclic fatigue test. It has been postulated that the fatigue life

of NiTi files was sensitive to the test environment (6, 22). Therefore, this study sought to compare the dynamic cyclic fatigue test at both room and simulated body temperatures. Theoretically, heat treatment is done during file manufacturing to increase the austenitic transformation finish temperature above the intracanal temperature. This

would help increase the fatigue life of the file (23). DSC analysis showed that the austenitic transformation of both HEDM and AFBS was above body temperature, while for ZB-F6 it was below body temperature. Moreover, HEDM showed an R-phase transformation around 37 °C, consistent with the previous studies (24-26) Cuyahoga Falls, OH [#25/.08, manufactured by electrical discharge machining]. This explains the superior fatigue resistance of HEDM and AFBS over ZB-F6 instruments. Transformation of ZB-F6 to the austenitic phase below room temperature might also explain the difference observed in its cyclic fatigue resistance between the simulated room and body temperatures.

According to the results of the present study, both null hypotheses are rejected. HEDM and AFBS had significantly better fatigue resistance than ZB-F6 instruments and the fatigue life of ZB-F6 instruments was influenced by the testing temperature. Recently, the scientific value of studies on the fatigue resistance of rotary and reciprocating NiTi instruments has been questioned because of the large variability of the tested protocols, in absence of standardized specifications, that would make a comparison among studies difficult and problematic (27). It was also pointed out that this type of research does not resemble the reality because pure rotation inside an artificial canal without any torque on the instrument, such as in cyclic fatigue tests, was unlikely to happen in a clinical scenario. Although these statements are true, it is important to emphasize that cyclic fatigue and torsional resistance tests allow the variables to be isolated and tested individually, increasing the internal validity and reproducibility of the study, which agrees with basic concepts of the scientific method (28). Well-designed studies are warranted to evaluate the claims of the manufacturers and rank different generations of NiTi instruments.

Conclusions

Dynamic cyclic fatigue resistance of the rotary NiTi instruments tested increased when the instruments had less cross-sec-
tional metal mass, less Ni (wt%), a thermally treated surface, and a martensite phase at body temperature.

Clinical Relevance

The present study demonstrated clinical relevance for testing the resistance to cyclic fatigue of three instruments and thus making the root canal exploration with rotary instruments safer.

Conflict of Interest

Authors deny any conflict of interest.

Acknowledgments

None

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CASE REPORT

Maintenance of vitality following horizontal root fracture of a lower central incisor

ABSTRACT

Aim: To maintain vitality in a lower central incisor with a horizontal root fracture. The patient was a seventeen years old male in optimal health conditions. He did not want lose his damage tooth at all costs.

Summary: Through of a three-layer glass fiber splinting, the tooth was immobilized by joining it to the adjacent teeth and leaving it in light occlusion. The clinical examination to date confirms the positive vitality of the tooth, with no change in color or presence of fistulas. At the fourteenth month control, the intraoral radiographs show how the lumen of the root canal reduces increasingly into a very thin layer.

Key Learning Points

- Horizontal root fracture: if the tooth is vital, you can try to save by a splint of fiberglass.
- Maintenance pulp vitality: monitored with vitality test, no change in color or presence of fistulas.
- Glass fiber splinting: it's a method to preserve the tooth from possible trauma by joining it to the adjacent teeth.

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Introduction

mong children and adolescents, the teeth that suffer dental trauma more frequently are the maxillary central incisors (1). Root fracture is a consequence of these dental traumas and represents on average between 0.5% and 7% of all dental traumas occurring in individuals aged 11 to 20 years.

Horizontal root fractures are usually caused by direct physical impact on the tooth, sport activities, traffic accidents and falls (2).

The predisposing factors are childhood obesity, increased overlap, protrusion of the central incisors and labial incompetence; these aspects which are considered as individual predisposing factors.

Root fractures can be divided according to direction; they can be either horizontal or vertical (3).

Radiography is needed to confirm a definitive diagnosis (4). In another study conducted by Barayan et al. (5), it was highlighted that the various filters present in the digital intraoral radiograph program did not improve the identification of the fractures.

Many authors, such as Lo Giudice et al. (6) and Salineiro et al. (7), have stated that endodontic fractures or lesions are better highlighted with CBCT, but in our case, the lesion was already clearly visible with on a periapical radiograph. Furthermore, the first author in his study concluded that CBCT is considered level II exam and could be used to address diagnostic questions, essential to a proper management of the endodontic problems (6, 7).

The (American Academy of Pediatric Dentistry (AAPD) recommends performing the X-ray examinations using different projections and angles (8).

If a fracture is not seen on intraoral radiography, CBCT is the diagnostic tool of choice (in suspected cases) (9).

The most frequent horizontal fractures, occur in the middle third (57%) and in the apical third (34%) and are caused by a frontal impact that involves enamel, dentin, pulp and cement; these fractures are

associated with injuries of the periodontal ligament and the alveolar bone. Usually, such fractures are observed on the permanent incisors that have erupted completely with closed apices and fully formed roots. These fractures have longer survival if compared than those in the apical third (10, 11).

After the impact, the pulp and damaged tissues begin the healing process.

The prognosis depends on a range of factors, such as the patient's age, tooth mobility, the state of the pulp and position of the line of fracture, degree of root formation, location of the radicular fracture, diastasis of fragments and time between trauma and treatment (12-14).

According to DiAngelis et al. (8), the treatment of this type of fracture consists of the correct repositioning of the fragment and the stabilization of the fragment through a fixed splinting to the adjacent teeth. These actions can favour the preservation of the pulp vitality and the healing of the tissue around the fracture line. According to IADT guidelines, if the pulp necrosis does not occur, the root canal treatment is not necessary (8).

The tooth may have increased mobility and bleeding from the periodontal ligament. Pulp necrosis of the displaced coronal segment occurs in approximately 25% of patients, and the necrosis of the apical segment is rare (3, 15-17).

This case report highlights the possibility of maintaining pulp viability after a horizontal fracture of the middle third using a glass fiber splint.

Report

On July 14th, 2016, a fifteen-year-old boy appeared at our clinic after a minor motorcycle accident. As a result of falling from his vehicle, he slammed his face and afterwards complained of pain and mobility of one tooth in the lower arch. His general medical history revealed neither loss of consciousness, nor major trauma. During the clinical check, tooth 31 (international FDA numbering) was noted, which presented an abnormal mobility and a vestibular position compared to the





A) Position of the tooth 31 vestibularized with respect to the normal dental axis.
B) The intraoral radiograph shows the presence of a horizontal root fracture in the middle third. C, D) Splinting of the tooth to the adjacent teeth by glass fibre (vestibular and lingual vision).



Figure 2

The intraoral radiograph shows that the fracture zone was slightly calcified and that the lumen of the pulp canal had decreased compared to that shown in Figure 1B.

characteristics of the adjacent teeth (figure 1A). The internal mucosa of the lower lip was superficially lacerated and not require sutures. An intraoral X-ray (Planmeca Asentajankatu 6, 00880 Helsinki, Finlandia) scan processed with an intraoral phosphor plate (VistaScan Dürr Dental Höpfigheimer Str. 17 74321 Bietigheim-Bissingen Germany) was performed to check the condition of the tooth. The results showed the presence of a horizontal root fracture in the level of the middle third (figure 1B). The vitality of the tooth in question was checked through a cold vitality test with ice spray Crio Spray (Sz 114 Carl Sanremo). We performed this vitality test because we did not have the means to perform an electrical test. The vitality of the tooth was positive. Infiltrative anesthesia was carried out in the vestibular area of the tooth with 4% articaine hydrochloride with 1:100,000 adrenaline (Alfacaina Dentsply France S.A.S. 4 rue Michael Faraday Montigny le Bretonneux). We proceeded with the preparation of the fibreglass splint (Fibre-Splint Polydentia SA, via Cantonale 47, CH 6805 Mezzovico-Vira Swiss). We measured the distance between teeth 32 and 41 and we cut three pieces of fibreglass tape.

Based on our clinical experience, it is clear that the splinting using fibreglass and composites is provides a more stable result than the splinting with metal wires and composites. First, a thirty-seven percent orthophosphoric acid (Total Etch, Ivoclar Vivadent Via del Lavoro 47, 40033 Casalecchio di Reno - BO) in gel was used to etch the teeth, and then the primer and bonding (Prime & Bond active Dentsply DeTrey Gmbh, De Trey str. 1 78467 Konstanz Germany) were applied; finally, after polymerizing (B-Cure Carlo De Giorgi S.R. L - Via Tonale N. 1-20021 Baranzate, Milano, Italy) the teeth, glass fibre tapes were applied, one at a time, with flow A3 (Ivoclar Vivadent AG 9494 Schaan/Liechtenstein). Each layer was light-cured for 40 seconds, and finally, the occlusion was checked using an articulation map (Bausch Artikulationspapier 200 μ , 50769 Köln, Germany) to verify that the splinting did not cause pre-contacts (figure figure 1C and and D).

First control

On September 29th, 2016 (75 days later), the col*d vitality* test was performed again with Crio Spray ice and the results were positive.

We proceeded by taking an intraoral X-ray and noted that the fracture zone was slightly calcified and the lumen of the pulp canal was reduced (figure 2).

Second control

Another check was performed on September 13th, 2017, fifteen months after the accident. A pulp viability test was performed, which had a positive outcome, along with an intraoral radiograph. The results showed a further decrease in the lumen of the root canal compared to a normal condition, and the recalcification was complete except in areas that were 0.5 mm both distal and mesial (figure 3).

Third control

On November 14th, 2018, the assessment during the visit showed positive vitality. The intraoral X-ray results appeared very similar to those of the previous assessments regarding the root canal lumen. Furthermore, a better calcification of the fracture was noted (figure 4A). The splinting was renewed because it was worn out (figure 4B and C).





Complete calcification except in areas that were 0.5 mm distal and mesial of the fracture zone and reduction of the canal lumen compared to that shown in Figure 1B.

Figure 4

A) Better calcification of the fracture line and canal lumen similar to that shown in Figure 3. B, C) Lingual and vestibular vision splintings were renewed.

Fourth control

On September 3rd, 2019, the vitality was positive again. As a result of an intraoral radiography, the lumen and the calcification of the fracture were considered superimposable over the previous control (figure 5). The thirty-eight-months point of follow up was reached.

Fifth control

On January 25th, 2021, we again made a control radiograph and conducted a vitality test, which had positive results. The colour did not change, and periodontal probing remained within physiological limits.

It is possible to appreciate the preservation of the interdental bone peaks and the invagination of the alveolar bone at the level of the most proximal portion of the fracture gap. The lumen of the root canal remained very narrow but allowed the flow of a solution of continuity between the coronal and apical parts of the endodontum (figure 6).

Discussion

The distance between fragments greatly influences the possibility of healing by hard tissue fusion. In our case, the radiolucent area of the fracture was significant, and from a diagnostic point of view, the area was immediately noticeable by the intraoral radiographs without having to resort to a CBCT. By comparing the patients' radiographs taken on during the first visit to the last follow-up, the way in which spontaneous calcification of the root united the two parts could be observed. Cvek in 2001 and Andreasen in 2004 in their studies stated that the healing rate for root fractures is was between 77% and 80%. There are 4 types of possible ways of healing for root fractures; Andreasen and Hjorting-Hansen, described these possibilities in their study.

1) Healing with hard tissue across the fracture.

2) Healing with the interposition of hard and soft tissue between fragments.

3) Healing with interposition of soft tissue only.

4) No healing.

The ideal outcome is healing with hard tissue (18).

Andreasen et al (3) stated that the clinical and radiographic findings showed that 120 teeth out of 400 teeth (30%) had healed by hard tissue fusion of the fragments. Interposition of the periodontal ligament (PDL) and bone between fragments was found in 22 teeth (5%), whereas interposition of the PDL alone was found in 170 teeth (43%). Finally, nonhealing, with pulp necrosis and inflammatory changes between fragments, was seen in 88 teeth (22%) (14).

The splinting procedure of the tooth made the recalcification manoeuvre safer, ensuring that fragments remained immobilized. As stated by Chala et al. (19), teeth with horizontal fracture have a greater pulp preservation than teeth with dislocation without root fracture.

Therefore, our patient's outcome demonstrates that it is actually worth trying to use a splint it if the tooth has vitality. The procedure should follow the one illustrated by DiAngelis et al. (8): a splint held in place from 4 four weeks up to 4





 A) Calcification of the fracture and canal lumen similar to that observed during the previous visit. B,
 C) Lingual and vestibular vision. four months, waiting for the fracture to heal by welding the two fragments. In contrast, we decided to keep the splints in place longer than the indicated time, since this tended to have a positive influence on the tooth stability, as Westphalen et al. (20) said reported in their study.

Many studies have shown a good prognosis for the horizontal fracture of a tooth with vitality even in follow-up evaluations occurring ten or more years later. However, this is not always the case. Evaluating the lumen of the root canal after two years, it was stated that there was a decrease in size. It is important to emphasize that between the first and second controls, the reduction is greater than the others (figures 2 and 3). Instead, between the third and fourth control, the reduction of the lumen appears minimal: this suggests the beginning of calcification of the canal from the outside (figures 3 and 4).

Our case report confirms that if a tooth

is subjected to a horizontal root fracture

but maintains maintenance of the vital pulps, splinting is an excellent option for safeguarding the vitality of the tooth and the tooth itself.

Surely, a constant monitoring is necessary to check the state of vitality, the position of the coronal fragment and the periodontal situation as well as any colour changes of the crown.

The timely treatment of intra-alveolar horizontal root fractures is important for the good prognosis and long-term success. In our patient after having applied a rigorous therapeutic and control protocol, after approximately sixty-six months of follow-up, we can say that the situation was stable and the prognosis was good with an excellent aesthetic result.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Clinical Relevance

Fiberglass splinting is an effective method that allows the fractured tooth to be im-



Figure 6 A) Intraoral radiograph. B) Lingual vision. C) Vitality test

results.

Conclusions

mobilized to the adjacent ones, to ensure that the horizontal fracture fixes spontaneously, maintaining vitality.

Acknowlegments

None.

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CASE REPORT

Apicectomy and simultaneous obturation of root canals: a clinical case report

ABSTRACT

Aim: This case report presents a maxillary lateral incisor that had already being treated by endodontic therapy and apicectomy surgery, but still had a persistent lesion. To maintain the tooth in the oral cavity, a third intervention was necessary in which parendodontic surgery followed by retro-obturation was performed. The aim of this report is to demonstrate the effectiveness of the treatment by means of clinical and radiographic follow-ups (6 and 12 months).

Summary: Considering the patient's complaint and the clinical and radiographic aspects, it was decided to perform the parendodontic surgery. The surgery procedures were osteotomy, curettage, apicectomy, root canal retreatment and retrofilling with Mineral Trioxide Aggregate, graft with lyophilized bone and, finally, use of collagen membrane. Through clinical and radiographic examinations were possible to observe that there was good healing of the soft and hard tissues, suggesting total regression of the lesion, and absence of signs and symptoms. Even if periapical lesions persist for a long time after interventions, the association of multiple procedures should be considered to enable the maintenance of the tooth in the oral cavity.

Key learning points

- The parendodontic surgery associating osteotomy, curettage, apicectomy, root canal retreatment and retrofilling can be effective to treat persistent periapical lesion.
- The use of graft with lyophilized bone and collagen membrane helps in bone repair.

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Introduction

ndodontic therapy is the first choice to treat periapicopathies that originate in the pulp and root canal, however, this treatment presents risk of failure. Failures in endodontic treatment may be related to persistent cystic lesion, inflammatory foreign-body-type reaction (caused by leakage of endodontic material and others), endogenous accumulation of cholesterol crystals in apical tissues and extraradicular infections, such as periapical actinomycosis, for example (1).

Non-surgical endodontic retreatment is often the first option to treat failure of conventional endodontic treatment (2). However, there are clinical situations in which this approach becomes unfeasible or has an unfavorable prognosis. Thus, the surgical approach in the periapex region is indicated (3, 4).

Apicectomy or root resection is also a surgical procedure that involves exposing the periapical lesion, through an osteotomy, removal of the lesion, removal of part of the root apex and disinfection. Additionally, retro-obturation or apical sealing is applied (5). In addition, root canal retreatment can be used simultaneously after the parendodontic surgery (6).

The use of guided tissue regeneration (GTR) techniques has been proposed as complementary method to endodontic surgery in order to promote bone tissue healing (7). Regarding the topic, many techniques and material, such as bone replacement grafts from numerous sources, nonresorbable and bioabsorbable membranes (8), have been developed that show good clinical and histologic outcomes.

An important step in apical surgery is to identify possible areas that have not been filled in the root apex and, subsequently, to perform adequate filling of the root tip. Only an adequate apical filling will allow satisfactory long-term results. Among the materials used for this filling, the Mineral Trioxide Aggregate (MTA) has been widely used (9). Studies have shown that MTA has a high sealing capacity, good stability and excellent biocompatibility (10). Prospective randomized clinical studies have reported high level of success rates in teeth treated with MTA in apical surgery (10, 11).

This case report shows a maxillary lateral incisor that had already being treated by endodontic therapy and apicectomy surgery, but still had a persistent lesion. To maintain the tooth in the oral cavity, a third intervention was necessary in which parendodontic surgery followed by retro-obturation was performed. Therefore, the aim of this report is to demonstrate the effectiveness of the treatment by means of clinical and radiographic follow-ups (6 and 12 months), showing complete lesion regression, bone formation and absence of symptoms.

Case Report

This study was approved by the Research Ethics Committee, protocol CAAE: 29178120.7.0000.5220. The patient agreed to participate and signed the consent form. A 36-year-old female patient, who reported a history of endodontic treatment with subsequent apicectomy performed in 2007, sought dental care for period evaluation in 2014. Through radiographic examinations, a slight bone rarefaction was observed at the apex of tooth 12, which presented a root canal filling. Thus, radiographic monitoring and proper follow-up were chosen as treatment. In 2016, increase in the radiolucent area was observed. Clinically, the patient was asymptomatic, and the treatment decision was to follow-up the case.

In 2019, the patient sought dental care complaining of tooth proclination. The clinical examination showed no signs of inflammation or infection. Regarding radiographic evaluation, a radiolucent and well-defined area was observed associated with the periapex of tooth 12. Due to the radiographic aspect of the lesion in which progression was detected (Figure 1), a refractory periapical lesion was suspected. As part of the clinical examination, diagnostic tests were performed. The palpation test was positive in the periapex area.





Radiographic evaluation: A) Baseline (2014); B) 12-months follow-up; C) 24-months follow-up - increase in the radiolucent area; D) 36-months follow-up - lesion progression showing a refractory periapical lesion. Regarding percussion tests, vertical test was positive and the horizontal one was negative. Thus, considering the patient's complaining and the clinical and radiographic aspects, it was decided to perform the parendodontic surgery. The surgery procedures were osteotomy, curettage, apicectomy, root canal retreatment and retrofilling with MTA. Additionally, the GTR was applied using graft with lyophilized bone and bioabsorbable membrane.

Considering the clinical steps, firstly, intraoral antisepsis was performed using chlorhexidine digluconate 0.12% (Rioquímica, São José do Rio Preto, Brazil) and extraoral antisepsis using iodine-polyvinylpyrrolidone (PVPI - Rioquímica, São José do Rio Preto, Brazil).

The pulp chamber was accessed to remove gutta-percha. After that, the surgery started with anesthesia by regional block with lidocaine solution (2%) with epinephrine 1:100.000 (Alphacaíne - DFL, Rio de Janeiro, Brazil).

Partsch incision was performed with a scalpel blade no. 15 (Solidor, Barueri, Brazil) and flap divulsion with Molt 2-4 detacher (Trinity, São Paulo, Brazil). Osteotomy was performed using surgical drills 702 (KG – Sorensen, São Paulo, Brazil) and Zecrya drill (Microdont, São Paulo, Brazil) under irrigation with saline solution. The lesion was detected and curettage and smoothing (adjacent structures and the root apex) was performed using curette by Lucas no. 85 (Quinelato, Rio Claro, Brazil). Macroscopically, gran-

ulomatous inflammatory tissue was observed.

Apicectomy was performed with a Zecrya drill (Microdont, São Paulo, Brazil) approximately 3 mm from the apex, seeking a 45° angle with a bezel to the vestibular face due to facilitate the subsequent condensation of the retrofilling material. The cavity was cleaned using sterile gauze soaked with chlorhexidine 2% (Rioquímica, São José do Rio Preto, Brazil). After, gutta-percha was removed from the root canal with largo II drills (Dentisply Malleifer, Ballaigues, Switzerland), Reciproc primmary file (VDW GmBG, Munich, Germany) and, for root canal preparation a rotary file size F5 (Universal Protaper -Dentisply Malleifer, Ballaigues, Switzerland) was used.

After the gutta-percha removal, the apical portion was prepared with the aid of ultrasonic device, obtaining approximately 2 mm for the MTA filling cement (Angelus, Londrina, Brazil). Gutta-percha cone size F5 (Universal Protaper - Dentisply, Malleifer, Balliagues, Switzerland) was used to fill the root canal. For this, the cone was adapted at the apical third of the root and was cut at the level of the dental apex with a scalpel no. 15 (Solidor, São Paulo, Brazil). Thus, the cone was removed and recalibrated on a sterile glass plate, in order to keep it 2 mm above the root apex, providing enough space for a correct condensation of the retrofilling material – MTA (Figure 2).

The MTA Repair HP was mixed according to the manufacturer's instructions. Then,





Parendodontic surgery: A) Curettage, B) Gutta-percha removal and root canal preparation; C) Apical preparation with ultrasonic device; D) Apical gutta-percha cone adaptation.

> the material was condensed into the cavity. The surgical area was filled with lyophilized bone (GenOx Org - Baumer, Pacaembu, Brazil). Then, collagenous membrane (Lumina Coat - Critéria, São Carlos, Brazil) was used to accelerate and improve tissue healing (Figure 3). Regarding suture, 3-0 silk thread (Shalon, São Luís de Montes Belos, Brazil) was used. Seven days after surgery, the stitches were removed. Radiographic assessment was performed at 15 days, six and twelve months (Figure 4). Through clinical and radiographic examinations were possible to observe that there was good healing of the soft tissues, suggesting total regression of the lesion, and absence of signs and symptoms.

Discussion

Periapical lesions can be treated through conventional endodontic treatment, associated or not with surgical endodontic therapy, and even by tooth extraction (12). However, more conservative practices should be advocated, whenever its possible. Even periapical lesions with large extension can be treated by non-surgical endodontic therapies (13). Success in conventional endodontic treatment usually occurs when the lesion presents direct communication with the root canal. In these situations, to obtain the best possible clinical result, pus drainage should occur by the access cavity. On the other hand, when the lesion is separated from the apical foramen due to the presence of an intact epithelium, it may not heal after non-surgical therapies (12).

Parendodontic surgery is a well-known procedure and a meta-analysis reported that it can present success and failure rates about 91.6% and 4.7%, respectively (7). In the present clinical case, the authors decided to perform parendodontic surgery that involved osteotomy, curettage, apicectomy, retreatment of the root canal associated with retrofilling, GTR, in the same surgical session, due to a persistent and refractory periapical lesion.

According to Fehlberg and Bittencourt (14), the aim of the apicectomy is to eliminate bacteria and areas of imperfection in the



Parendodontic surgery: A) MTA condensation, B) filling the surgical area with lyophilized bone; C) using the collagenous membrane. root canal with persistent apical lesion. Thus, the apicectomy provides airtight seal and facilitates access to the root canal. It is recommended to remove three or more millimeters of depth from the root apex to obtain a safe and effective closure of the region and to have enough space for adequate curettage of the root surface and bone cavity (14). In the present study, the patient had already undergone previous apicectomy surgery. Therefore, it was decided to remove 3 mm of the root apex in order to avoid further wear and loss of root structure.

Although it is an invasive procedure, during parendodontic surgery, necrotic cells, tissue debris and bacteria from periapical area are removed (15). If the case selection is adequate, compared to non-surgical endodontic therapy, healing of the periapical lesion is much more effective and faster when parendodontic surgery is performed (15). However, if endodontic treatment is unsatisfactory, surgical intervention will not work. In the present case, the patient reported a previous history of surgical intervention, but had a persistent lesion. This long-term failure can probably be associated with unsatisfactory root canal filling. Other factors could be considered as the partial removal of the cystic lesion, and even individual factors such as the organism response associated to the tissue repair process (16).

Although uncommon, as in the present case, chronic periapical cysts with persistent exudate may occur. The indicated clinical intervention is to perform the root canal retreatment simultaneous to the surgical procedures. This approach is mainly indicated for cases in which it is not possible to eliminate persistent exudate through systemic and intracanal medication (14). In this clinical case, although there was no exudate, the lesion was persistent even after clinical interventions already performed.

Endodontic retreatment is an excellent



Figura 4 Radiographic follow-up after surgery: A) 15 days B) 6-months follow-up C) 12-months follow-up.



option, as it promotes good root sealing and restores favorable conditions for bone neoformation (4, 14). Endodontic retreatment with simultaneous parendodontic surgery has a significantly higher healing rates compared to endodontic treatment only (6). Considering the present clinical case, previously, the patient had undergone an apicectomy, however, without retreatment. For this reason, it was decided to associate endodontic retreatment with the surgical procedure in order to optimize the clinical results.

Regarding the endodontic retreatment, different techniques can be used to remove gutta-percha from the root canals. Therefore, the clinician needs to choose a technique that is both effective and safe. The use of ultrasonic devices has shown good results in relation to gutta-percha removal (17). Therefore, it was decided to use this technique in the present clinical case. Different materials can be used in the retrofilling procedure, such as amalgam, composite resin and glass ionomer. However, the MTA seems to be the gold standard as a root filling material, presenting higher success rates (9, 18). In the case reported by Garlapati et al. (15), MTA has been used as a root filling material producing favorable results in terms of absence of inflammation and hard tissue and cementum formation (15, 4). Additionally, considering periapical lesions larger than 5mm, lyophilized bone grafts could be used and represent a good resource for better healing of hard tissues (19). In the present clinical case, several procedures were performed in a single session. It is paramount to consider the skill and clinical experience of the professional to perform all these steps associated. The intervention was proposed as a last attempt to intervene in the maxillary lateral incisor.

Conclusion

Even if periapical lesions persist for a long time after interventions, the association of multiple procedures, including conventional and surgical ones, should be considered to enable the maintenance of the tooth in the oral cavity.

Clinical Relevance

The association of multiple procedures can be a valid resource to treat persistent periapical lesions, allowing the maintenance of tooth in the oral cavity.

Conflict of Interest

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

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CASE REPORT

One-year follow-up of endodontic retreatment in a patient with severe Hemophilia A

ABSTRACT

Aim: To describe the clinical management of a patient diagnosed with severe Hemophilia A presenting an endodontically treated tooth (ETT) with a persistent periapical radiolucent lesion.

Summary: This case report has been written according to Preferred Reporting Items for Case reports in Endodontics (PRICE) 2020 guidelines. Inherited bleeding disorders (IBDs) are a heterogeneous group of genetic conditions in which missing or defective clotting factors prevent normal blood clotting from occurring. Among those conditions, Hemophilia A (deficiency of factor VIII) is the most prevalent, representing about 85% of all inherited bleeding disorders. A 21-years-old male was referred to our service to retreat a left mandibular first molar. At clinical examination, the ETT showed signs of persistent symptomatic apical periodontitis, confirmed by the radiographic exam. After reviewing the risks and benefits, written informed consent was obtained from the patient, and then, non-surgical root canal retreatment was performed on the tooth under dental operating microscopy. Reciproc R25 NiTi file was used to remove the previous fillings and reprepare the root canal system, and Bio-C Sealer was the chosen sealer to obturate the root canals. The entire retreatment procedure was performed in a single-visit. At the subsequent follow-up visits one year later, the periapical radiograph suggests a successful outcome with periapical repair, a substantial improvement in bone density, and the patient reported that he was free of symptoms and using the tooth as usual.

Key learning points

- Although no complications or excessive bleeding was observed, proper multidisciplinary
 planning must be carried when treating patients with inherited bleeding disorders.
- All efforts should be made to avoid intraoperative complications or multiple sessions that also require multiple clotting factor infusions, and
- further clinical investigations should focus on the need for prophylactic protocols in order to safely perform endodontic treatment on patients diagnosed with different severity levels of IBDs.

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Introduction

nherited bleeding disorders (IBDs) are a heterogeneous group of genetic conditions in which missing or defective clotting factors prevent normal blood clotting from occurring (1). Among those conditions, Hemophilia A (deficiency of factor VIII) is the most prevalent, representing about 85% of all inherited bleeding disorders (2). Accordingly to the severity, this condition can be divided into mild (up to 5% of FVIII in the blood), moderate (1-5%), or severe (<1%). In this way, a proper diagnose and severity classification is important since such a condition is directly related to an increased number of spontaneous or traumatic bleeding episodes (3).

Endodontic treatment is considered a non-invasive dental procedure (4). Although the risk of excessive bleeding during treatment is not common, it can sometimes occur during apical enlargement, removal of reminiscent of vital pulp, or during apical patency establishment. When treating hemophilic patients, a higher risk of bleeding is expected, with also an increased risk of postoperative complications (5). Therefore, root canal treatment should be performed with caution and under previous medical advice in order to avoid any further complications. In this perspective, a previously published Australian hospital-based dentists consensus reported that root canal procedures in mild and moderate hemophilic patients could be performed under regular dental office settings (4); however, this very consensus also suggested that adults with severe hemophilia must have endodontic treatments performed at hospital settings (6). Such recommendation is based on the assumption that excessive bleeding in severe hemophilic patients might impose the necessity of additional replacement therapies. Also, some studies reported complications as minor discomforts to acute swelling (7, 8)

When planning an endodontic intervention in a hemophilic patient, the dentist should consider some important challenges: (1) to reduce or eliminate the risk of bleeding during the procedure and post-treatment, and (2) to achieve hemostasis and root canal dryness in order to properly fill the root canal (9). Likewise, the scientific literature about root canal procedures in hemophilic patients is still scarce. Therefore, the aim of the present case report was to describe the planning and treatment of a patient with severe Hemophilia A at a dental office setting, with a multi-professional approach to, including hematological advice and clotting factor replacement therapy, and to report specific endodontic considerations.

Report

This case report has been written according to *Preferred Reporting Items for Case reports in Endodontics* (PRICE) 2020 guidelines (10).

A 21-years-old caucasian male patient diagnosed with severe congenital hemophilia A was referred by the Regional Blood Center of Pelotas (HEMOPEL) to the School of Dentistry of the Federal University of Pelotas. The patient's chief complaint was pain and tenderness on the left mandibular first molar. The patient also reported that he remembers undergoing an endodontic procedure in the referred region a few years ago.

The visual inspection of the patient identified restorations in multiple teeth and the clinical examination of the tooth with referred pain presented moderate sensitivity to vertical percussion, with no discomfort at palpation or extra-oral abnormalities as described in Table 1. The intraoral periapical digital radiographic (CDR Elite size 2, Dentsply Sirona, Charlotte, NC, USA) image showed a previous endodontic treatment with insufficient root canal fillings, persistency of periapical radiolucency, and an apical widening of the periodontal ligament space characterizing a periradicular pathological condition due to the failure of the previous root canal treatment (Figure 1). Based on these pieces of information, the pulpal and periapical diagnosis of a previously treated tooth with symptomatic apical periodontitis was made to the left mandibular first molar,



Table 1 Diagnostic assessment results							
Tooth number	Cold	Heat	Percussion	Palpation	Mobility	Extra-oral	
#36	No response	No response	Moderate	No abnormalities	Within normal limit	No abnormalities	

Table 2 **Hematology tests** Result **Blood** assay **Reference values** Activated partial thromboplastin time 103.3 seconds 34.3 seconds Prothrombin time 13.5 seconds 13.4 seconds At least 0.25 IU/ml Factor VIII activity (FVIII:C) Less than 0.01 IU/ml von Willebrand factor activity (FvW:Ag) 1.19 IU/ml 0.90-1.20 IU/ml Anti-factor VIII inhibitor antibody 1.3 UB/ml At least 0.6 UB/ml

and root canal retreatment was indicated. The periapical index (PAI) scoring system (11) was used to register the intervention outcome, and the initial PAI score was '4'. After reviewing the risks, benefits, and treatment options, written informed consent from the patient was obtained for the proposed treatment and followed the ethical principles of the World Medical Association's Declaration of Helsinki. The patient was referred back to the Blood Center for medical assessment and a prophylactic treatment plan. The results of the blood tests are presented in Table 2. The prophylactic protocol consisted of infusion of 3000 UI/dL recombinant factor VIII concentrate (Hemo-8R, Hemobrás, Federal District, Brazil) one hour before the root canal treatment due to the necessity of alveolar nerve block anesthesia and to reduce the risk of intraoperative bleeding or complications.

The alveolar nerve block was performed using 1.8 mL of 2% lidocaine with 1:100.000 epinephrine (Alphacaine 2%, Nova DFL, Rio de Janeiro, Brazil), and supplementary anesthesia was carried out with a buccal infiltration of 1.8 mL of 4%



Figure 2 Access opening and canal location.

Figure 1 Initial radiographic assessment of the left mandibular first molar. validation test.





Figure 3 Root canal fillings removal with the Reciproc R25 file.



Figure 4 Reprepared tooth prior to filling procedures.

articaine with 1:100.000 epinephrine (Nova DFL). After that, a rubber dam was placed to isolate the operating field. The access cavity was opened with high-speed diamond burs (Figure 2) and refined under magnification provided by a dental operating microscope (OPMI Pico, Zeiss, Oberkochen, Germany) using an E6D pear diamond ultrasonic tip (Helse Ultrasonics, Sao Paulo, Brazil) to carefully remove interferences and previous restorative materials. Initial root fillings removal (Figure 3) was performed using a Reciproc R25 file (VDW GmbH, München, Germany), and apical patency was achieved in all root canals with small size C-Pilot files (VDW GmbH) and was maintained at a size 15

K-file (VDW GmbH). Chlorhexidine gluconate gel at 2% (LenzaFarm, Minas Gerais, Brazil) was used as a chemical auxiliary substance associated with 0.9% sterile saline solution as an irrigation substance. The root canals length measures were determined electronically by a RomiApex A-15 apex locator (Romidan LTD, Kirvat Ono, Israel) and were as follows: mesiolingual 21mm; mesiobuccal 21mm; and distal 22mm. Working length was established at the "0.0" reading of the electronic apex locator, and the glide path was conducted in all canals with an R-Pilot reciprocating file (VDW GmbH) prior to the apical enlargement using a Reciproc R25 file. At the end of the root canal repreparation (Figure



Figure 5 Fitting assessment of the gutta-percha cone and Bio-C Sealer.



Figure 6 Quality radiographic assessment of the root canal filling.





One-year follow-up periapical radiograph of the root canal retreatment.



Immediate postoperative periapical radiograph.

4), three cycles of passive ultrasonic irrigation were performed in each canal using an E1 Irrisonic ultrasonic tip (Helse Ultrasonic) associated with 1mL of EDTA 17% (Biodinamica, Parana, Brazil), renewing this substance at the end of each cycle. A final flush of 0.9% sterile saline solution was carried out to remove any trace of the previous substances, and the canals were dried with sterile paper points. The root canals were filled with the single-cone obturation technique using a calibrated gutta-percha point in each canal, associated with Bio-C Sealer (Angelus, Parana, Brazil), a tricalcium silicate-based root canal sealer (Figure 5). Before cutting and condensing the gutta-percha points, a quality radiographic assessment was performed to evaluate the root filling apical sealability (Figure 6). The tooth received an immediate coronal restoration with Opus A2 (FGM, Santa Catarina. Brazil) bulk-fill resin-based composite. Afterward, a final radiograph was taken to assess the quality of the root canal retreatment (Figure 7).

No complication occurred during or after the procedure, and a telephone inquiry was made with the patient after 48 hours and three months as an initial follow-up method to evaluate the patient's comfort and symptom conditions.

The patient was then recalled after twelve months for periapical healing assessment, and the one-year clinical and radiographic follow-up showed periapical healing with a reduction of the PAI score to '2' (Figure 8). Also, no sensitivity on percussion or palpation was observed, and the patient reported adequate function of the tooth with no pain. Figure 9 is a schematic flowchart that summarizes the steps involved in this case report and its clinical outcome, and Table 3 represents the treatment timeline.

Discussion

The present study reports a case of persistent apical periodontitis resolved with root canal retreatment on a severe hemophilia A patient. There is still a lack of studies reporting endodontic therapy in IBDs patients. In this way, the present case report provides important information regarding root canal retreatment and the radiographic assessment of one year of follow-up with adequate periapical bone repair without the presence of any signs or symptoms.

According to a case-control study, hemophilic patients present a 2.2 higher odds ratio of presenting teeth with apical periodontitis but about 70% less prevalence of endodontic treated teeth when compared to patients without IBDs (12). In this perspective, another survey on IBDs patients in hemophilia treatment centers across the United States identified that financial barriers, lack of skilled providers, and patient anxiety contribute directly to less



Timeline of the patient plaining, treatment, and follow-up							
Time	Event	Symptons					
0	Patient visited the clinic, history, clinical and radiographic examination.	Moderate pain at vertical percussion in the left mandibular first molar					
+24 hours	Factor XIII prophylactic treatment	-					
+1 hour	Endodontic retreatment of tooth #36	-					
101	1st Follow-up	No pain, no swelling (sympton free)					
+12 hours	(Phone inquiry)						
	2nd Follow-up	No pain, no swelling					
+12 hours	(Phone inquiry)	(sympton free)					
.041	3rd Follow-up	No pain, no swelling					
+24 hours	(Phone inquiry)	(sympton free)					
2	4th Follow-up	No pain, no swelling					
+3 months	(Phone inquiry)	(sympton free)					
+9 months	5th Follow-up (clinical and radiographic assessment)	No pain, no swelling (sympton free) Radiographic evidence of periapical repair					

 Table 3

 Timeline of the patient planning, treatment, and follow-up

access to oral health care (13). On the other hand, it is well-known that root canal treatment is a conservative technique that reduces the need for tooth extractions, providing high long-term survival rates (14). The maintenance of teeth in IBDs patients is extremely important once it reduces the need for more invasive procedures such as tooth extraction and dental implant placement, which are more prone to intra and postoperative complications. Importance should be given to the adequate management of IBDs subjects, especially those with high severities due to the increased risk of bleeding episodes during their lifetime (2). Considering the possible implications of IBDs, dental professionals might be afraid of providing invasive procedures in IBDs patients, such as anesthetic nerve block and other specific endodontic procedures. A consensus statement by hospital-based dentists suggests that endodontic therapy in patients with IBDs could be considered a low-risk procedure and can be performed routinely (4). On the same perspective, a recent scoping review on invasive oral procedures (e.g. tooth extraction and anesthesia) highlighted the importance of a multidisciplinary team when treating patients with IBDs to reduce

complications and provide a safe treatment (15). In this sense, dentists willing to perform endodontic therapy on IBD patients should be aware of some particularities in order to reduce the risk of bleeding-related complications. In this way, it can be suggested that infiltrative anesthesia and the maintenance of the working length within the root canal region should be preferred in IBDs subjects, unless clotting factor replacement therapy has been previously administrated.

It has been suggested that a single dose of 0.1 mg desmopressin acetate tablet associated with 250 mg tranexamic acid could be used as a prophylactic protocol to perform inferior alveolar nerve block in IBDs patients (16). However, considering the severity of our patient and the increased risk of complications, our multi-professional team decided to prescribe the infusion of clotting factor VIII one hour prior to the dental appointment; Also, 2% chlorhexidine gluconate gel was chosen as the preferred irrigation substance instead of sodium hypochlorite due to the necessity of avoiding intraoperative accidents in patients with this special healthcare need, since there is no difference regarding clinical and radiographic outcomes when



Figura 9 Schematic PRICE 2020 flowchart.



comparing those two irrigation substances (17).

The root canal retreatment was conducted following the gold standard endodontic procedures with apical patency achievement in all root canals. The reciprocating file used was an *M-Wire* nickel-titanium alloy that has been widely investigated in the literature (18-21), presenting adequate performance on root canal filling removal due to the file design allied with the kinematic motion, allowing greater resistance to cyclic and torsion fatigue with excellent shaping efficacy. Moreover, the chosen root canal sealer may present a bioactivity and biomineralization potential (22-24), and although no significant difference has been observed in recent clinical trials that evaluated the outcomes of this sealer (25. 26), its use in teeth with apical periodontitis is supported by the current literature. In the present case report, apically extrusion of the sealer happened during the endodontic treatment; however, a cohort study showed that although calcium silicate-based sealers have a high extrusion rate, this unintentional overfilling has no significant effect on the treatment outcome (27), and it is expected that such material extrusion would resorb in the future follow-up assessments since this sealer has a considerable solubility in the periapical tissues (28).

Considering that adequate hemostasis and dryness in the root canal were obtained, the treatment was performed in a single session to avoid the need for a second appointment and, consequently, the need for another clotting factor infusion. A cost-analysis study revealed that the treatment of hemophilia patients is very expensive and may also affect public policies adopted by health systems (29). For this reason, it is important to correctly administrate the clotting factor replacement therapy in order to reduce the occurrence of complications during and after the procedures, impacting the patient's health positively and reducing the costs to the public health systems.

Although IBDs are considered rare, it is important to highlight that dental professionals must consider inquiring all patients



about their health condition and signs of abnormal or excessive bleeding, including family background assessment and, whenever treating patients with diagnosed or suggestive signs and symptoms of IBDs, these patients must be referred to hematologists or blood centers with a multi-professional team prior to any dental therapy in order to assess the specific patient's conditions as well as to plan the need of prophylactic measures and the performance of the treatment in a dental office setting.

Conclusions

The present study reports a non-surgical endodontic retreatment of a left mandibular first molar of a patient with severe hemophilia A with one year of follow-up and radiographic images suggesting a successful periapical repair and no report of symptoms. Although no complications or excessive bleeding was observed, proper multidisciplinary planning must be carried when treating patients with inherited bleeding disorders. Further clinical investigations should focus on the need for prophylactic protocols in order to safely perform endodontic treatment on patients diagnosed with different severity levels of IBDs.

Clinical Relevance

Clinical and radiographic outcomes after 1-year of endodontic treatment in a patient with Hemophilia A were adequate and compatible with those expected when treating healthy patients. However, when treating patients with such condition a multidisciplinary assessment and planning must be made in order to avoid intraoperative complications.

Conflict of Interest

None.

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CASE SERIES

Management of teeth with open apex and apical periodontitis using MTA and OrthoMTA

ABSTRACT

Aim: To describe successful managements of teeth with open apex using ProRoot MTA and OrthoMTA.

Summary: Teeth with open apex and apical periodontitis present multiple challenges specially in disinfecting the root canal space and providing an artificial apical barrier to achieve an optimal filling of the root canal. In the past decade, Mineral Trioxide Aggregate (MTA) has steadily gain popularity among clinicians to be used as a biocompatible apical stop for apexification of teeth with open apex. With strict adherent to the standard root canal protocol, teeth treated with ProRoot MTA and Ortho MTA in this case series showed satisfactory apical healing radiographically and improvement of signs and symptoms. Studies featuring OrthoMTA in the last ten years showing satisfactory clinical successes in various clinical scenarios such as pulpotomy in primary molars, partial pulpotomy in permanent teeth and apexification in immature apex of permanent teeth have also been listed.

Key Learning Points

 Clinicians need to be extra cautious with the aggressive use of endodontic files and have thorough knowledge of standard root canal protocols in establishing the correct working length, advancements in the disinfection procedures, materials, and techniques to be used to create an apical barrier.

• The obturation of the root canal space shall focus on the prevention of the extrusion of the root filling material into the periapical tissues and reinforcement of the weakened root against fracture during and after an apical stop is provided.

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Introduction

he open apex in permanent teeth is mainly resulted when the pulp undergoes necrosis before the competition of root development, often caused by trauma (1), carious exposure (2, 3) and presence of an existing dental anomaly such as dens evaginatus and dens invaginatus that renders the developing tooth susceptible to the pulpal necrosis (4, 5). Endodontic treatment of a permanent tooth with an open apex poses a different set of challenges compared to the tooth with a mature root apex. Incomplete root and thin dentinal walls make the tooth more susceptible to fracture (6) and compromised crown to root ratio may cause increased mobility (7). An absence of an apical stop can lead to extrusion of irrigating solution and/or sealer into periradicular tissues leading to a negative effect on the apical healing process (8). A wide root apex may also create difficultly in achieving the apical seal during the conventional root canal filling, resulting in apical leakage. To overcome these problems, a hard tissue barrier that allows the optimal filling of the root canal and the strengthening of the weakened root walls against secondary fracture must be formed.

Apexification has traditionally been the treatment of choice to manage teeth with necrotic pulps and open apices. Apexification is a process in which a non-vital, immature, permanent tooth that has lost the capacity for further root development, root end closure is induced to form a calcified root barrier at the root terminus (9).

Calcium hydroxide as an intracanal medicament has been historically used to induce the formation of a hard tissue barrier at the root apex with great success (10-14). The mechanism of action of calcium hydroxide on apical tissue is not clearly understood however, its antimicrobial activity, high pH or its direct effect on the apical and periapical soft tissues have been discussed in the literature (15). The effect of optimal timing and frequency to change the calcium hydroxide dressing on the rate and quality of apical barrier formation is debatable that may vary from changing it every month, every 3 month, every 6-8 months or not at all (16-20).

However, despite its many advantages, there are several limitations relating to the use of calcium hydroxide for apexification such as long time required for hard tissue barrier formation (21), possibility of root canal contamination during the apexification process (10, 13), multiple and repeated "dressings" required to achieve the root apex closure (16, 17, 19, 20), reduced fracture resistance of teeth after the longterm application of calcium hydroxide (22-24), and patient's compliance (25). Mineral trioxide aggregate (MTA) has steadily gained popularity amongst clinicians in the past three decades as it offers multiple benefits compared to calcium hydroxide in the apexification procedure. The principal components of MTA include tricalcium silicate, tricalcium aluminate, tricalcium oxide and silicate oxide. MTA has been successfully used as root-end filling during apical surgery (26) and repair of root perforations (27, 28). Use of MTA in vital pulp therapy even for the primary dentition has been supported by a systemic review done by Sanz et al. (2020), that shows that hydraulic calcium silicate cements (HCSC) which includes ProRoot MTA when cultured with human exfoliated deciduous teeth (SHEDs) shows cytocompatibility and bioactivity (29). Apexification done with MTA can be completed in one to two visits, saving time, relying less on patient compliance, and greatly reducing the risk of fracture and reinfection of the tooth. MTA is mixed and packed into the apical 3-4mm of the tooth. It reaches a high pH of 10.2 to 12.5 in three hours, possibly inducing hard tissue formation of the apical tissue, similar to that of calcium hydroxide (30). ProRoot MTA (Dentsply



Tulsa Dental, Tulsa, OK, USA) was launched in the United States in 1999, and was the first commercial product made available. With its wide array of usage and benefits, ProRoot MTA also has some limitations: high material cost, long setting time (which requires additional visits), difficult handling characteristics and tooth discoloration (31-33). As the demand increases and companies attempt to improve and upgrade this Portland cement, many brands of MTA were created. Few of the many are Biodentine (Septodont, Saint-Maur-des-Fosses, France), and OrthoMTA (BioMTA, Seoul, Korea). Biodentine is a calcium silicate-based material used for crown and root dentin repair treatment, repair of perforations or resorptions, apexification and root-end fillings. The material can also be used in class II fillings as a temporary enamel substitute and as permanent dentine substitute in large carious lesions. Its manufacturer claims that Biodentine has a faster setting time compared to ProRoot MTA, due to calcium chloride as a setting accelerator in the liquid component (34).

Having the same indications as MTA, Bio-C is a ready-to-use bioactive reparative putty. Besides having a setting time of less than 120 minutes, its bioceramic formulation and high alkalinity induces tissue regeneration, setting expansion, chemical adhesion to dentine and inhibits bacterial infiltration. An in vitro study by Rodríguez-Lozano et al. (2020) (35) showed the cytocompatibility and high bioactive potential of Bio-C Repair and TotalFill BC RRM putty that can promote the osteo- and cementogenic differentiation of human periodontal ligament stem cells. Similarly, Ghilotti et al. (2020) (36) showed adequate attachment of hDPCS to the vital pulp materials, indicating excellent biocompatibility of Bio-C Repair similar to BioDentine and ProRoot MTA.

Recently, a newly developed MTA, OrthoMTA claims to be as biocompatible as ProRoot MTA, but without hexavalent chromium, a carcinogenic heavy metal. In addition, due to its bioactive characteristic: releasing calcium ions through the apical foramen, OrthoMTA neutralizes the apical tissue. This results in the formation of an interfacing layer of hydroxyapatite (Hap) between the OrthoMTA and the canal wall. Hap prevents microleakage and induces regeneration of the apical periodontium (BioMTA http://www. biomta.com/shop/eng/product_1. php?).

However, in apexification the longterm survival of the teeth is questionable due to its thin and prone-to-fracture dentinal walls (23). Regenerative endodontics procedure (REP) presents a viable treatment alternative for these teeth. By utilizing the key elements of tissue engineering: growth factors, stem cells and scaffold, REP allows healing of the teeth by complete restoration of pulpal function and subsequent completion of root development (38). REP presents a promising future in the world of endodontics based on largely reported successful clinical cases and studies (39-43). However, the generally recognized current protocol of REP suggested by American Association of Endodontics (44) too presents with its own sets of limitation, such as inability to invoke bleeding, sub-optimal barrier placement (e.g. MTA), and coronal staining (45). Therefore, careful case selection is imperative in determining case success, and further research is warranted to explore alternative methods and materials to further improve clinical outcomes.

Therefore, this case series describes the successful management of teeth with open apex using MTA and OrthoMTA and lists various studies done on the properties of OrthoMTA.

Report

#Case1

A healthy 57-year-old patient was referred to the dental clinic for the treat-





A) radiological working length determination, B) collagen sponge placement, C) apex view after the placement of the collagen sponge beyond the apex to create a barrier (tooth 11), D) 4 mm MTA cement placement control, (E) The rest of the canal was filled with injectable thermoplasticized gutta-percha (tooth 12).



Figure 1

Preoperative radiograph showing teeth 12,1 1, 21 and 22 with previous endodontic treatments and radiolucent periapical lesions adjacent to the wide apices of teeth.

> ment of the upper incisors. The patient had a history of orthodontic treatment and orthognathic surgery for maxilla in the year 1991. The teeth 12,11, 21 and 22 had previous endodontic treatments performed 15 years back and tooth 21 had a history of Endodontic surgery in 2008. Percussion and palpation tests were negative for teeth 12, 11, 21 and 22, with physiologic mobility.

> Radiographic examination showed radiolucent periapical lesions adjacent to the wide apices of teeth 12, 11, and 22 (Figure 1). A common diagnosis of asymptomatic apical periodontitis was made for the latter teeth. After explaining all treatment options (retreatment and apexification with MTA, Endodontics surgery), risks and benefits, an informed consent was obtained from the patient and the decision was to attempt a non-surgical endodontic treatment with MTA apexification on teeth 12, 11 and 22.

First Appointment

The root canal system of the three teeth was accessed after local anesthetic injection with 4% articaine hydrochloride with epinephrine 1:200,000 (Septanest, Septodont, France) under rubber dam isolation. Each root canal was desobturated using D2 files (Dentsply Maillefer, Ballaigues, Switzerland) in the coronal/middle third of the canal then gently irrigated with 5 mL of 3% sodium hypochlorite (NaOCl) (Coltene/Whaledent, GnbH, USA). The rest of the canal was desobturated using manual K-files Dentsply Maillefer, Ballaigues, Switzerland) then the working length was established with both electronic apex locator (Root ZX (J. Morita, Tokyo, Japan) and reconfirmed using the intraoral periapical radiograph (Figure 2A).

10 mL of NaOCl was delivered using EndoVac system and activated with EndoActivator (Dentsply Tulsa Dental, Tulsa, OK, USA) for 1 min. Calcium hydroxide paste (mixture of Ca(OH)₂ powder (Merck, Darmstadt, Germany) with sterile water) was placed in the canal and gently packed with paper points. The access cavity was sealed with Cavit (3M Espe, St. Paul, MN, USA).

Second Appointment

After two weeks, the patient came for treatment completion. Teeth were similarly anesthetized and isolated with a rubber dam. Calcium hydroxide paste was removed with copious irrigation with 5 mL of NaOCl using Endovac system. Apical gauging was done using K-files (Dentsply Maillefer, Ballaigues, Switzerland)

Tooth 22 had an apex of 90/100, tooth 11 an apex superior to 140/100, and tooth 12 an apex of 120/100. The final



irrigation protocol was performed by a continuous delivery of solutions as follows: 5 mL of 17% ethylenediaminetetraacetic acid (EDTA) (produits dentaires, Switzerland), 5mL of 3% NaOCl (Coltene/Whaledent, GnbH, USA) followed by a 5mL of distilled water followed by an activation with endoactivator (Dentsply Tulsa Dental, Tulsa, OK. USA) for 1 min for each solution. Canals were dried and collagen sponge (Etik Collagene Aceton, Merignac, France) was placed beyond the apex and compressed with a #70 K-file (Figure 2B and 2C). This procedure was performed under microscope (CJ-Optik GmbH & Co. KG, Aßlar, Germany) to control the sponge placement. Immediately after, the mineral trioxide aggregate (MTA) cement was inserted into the apical third of the canal. The teeth were filled with 4 mm of white ProRoot MTA® (Dentsply International) mixed with sterile water in a 0.26 WP ratio using MTA gun system (Dentsply Maillefer, Ballaigues, Switzerland) (Figure 2D). After the placement of the first millimetre, an X-ray control was taken to control the placement of the MTA cement. The MTA plug placement was also performed under microscope. A wet paper point was placed inside each canal then the teeth were closed with Cavit (3M Espe, St. Paul, MN, USA) and temporary crowns.

Third Appointment

The patient was recalled 48 hours after the appointment to finish the treatment. Rubber dam isolation was placed, and the paper points were removed from the canals. The MTA setting was assessed using the hand plugger. Following, the rest of the canal was filled using injectable thermoplasticized gutta-percha (Kerr Sybron Endo, Orange, CA, USA) (Figure 2E). Access cavity was sealed with Cavit and temporary crowns were placed. Patient was referred to her general dentist to complete the prosthetic treatment. *Follow Up*

One-month post-operative radiograph showed well obturated root canals of teeth 12, 11, 21 and 22 with reduction in the size of periapical radiolucency (Figure 3B). In the18 month follow-up, the teeth were asymptomatic. Clinical examination showed physiological mobility and absence of sensitivity to percussion and palpation. The follow-up radiographs revealed a satisfactory periapical healing (Figure 3C).

#Case2

A 38-year-old male reported to the clinic with a concern of pain on biting in his lower left back tooth for past few days. Clinical examination showed a temporary restoration on tooth 35 with a history of initiation of root canal



Figure 3

A) Pre-operative radiographs of maxillary incisors, B) post-operative radiographs, C) eighteen months follow-up radiographs showing a satisfactory periapical healing of teeth 12, 11, 21 and 22.





A) In intraoral periapical radiograph showing tooth 35 with large peri-apical lesion with an open apex.
 B) Completion on tooth 35 root canal filling using OrthoMTA.
 C) Three months follow up radiograph showing tooth 35 with complete resolution of the periapical lesion.
 D) Thirty months follow up radiograph tooth 35 showing satisfactory healing.

titis was made and root canal therapy with the management of open apex using OrthoMTA was advised. *First Appointment*

After obtaining the written consent, the treatment was carried out. The area was anesthetised using 2% lignocaine with

anesthetised using 2% lignocaine with epinephrine 1:200,000 (inibsa, Barcelona, Spain) and tooth was isolated using the rubber dam. Old temporary restoration was removed, and an access cavity was modified using endo access burs (Dentsply Maillefer, Ballagues, Switzerland)), pulp chamber roof was completely removed. The working length was confirmed using the intraoral periapical radiograph and circum-

Figure 5 Biomaterial intracanal grafting kit including OrthoMTA, gun, compactors

and plugger.



tion of the entire root canal with OrthoMTA was planned. In this appointment, non-setting calcium hydroxide (Voco GmbH, Germany), an intracanal medicament was placed into the root canal and the cavity was temporarily restored with Cavit (3M Espe, St. Paul, MN, USA).

Second Appointment

The patient was recalled after oneweek, old temporary dressing was removed, canal was irrigated with 5 ml of 3% NaOCl (Coltene/Whaledent, GnbH, USA), saline and 17% ethylenediaminetetraacetic acid (EDTA) (PULPDENT, Watertown, MA, USA) each for 1 minute ensuring no extrusion of any irrigating solution. The mastercone radiograph was taken to reconfirm the extension of root canal filling material. OrthoMTA (BioMTA, Seoul, Republic of Korea, Figure 5) was mixed according to the manufacturer's instruction and placed in the canal using the MTA gun. The compactor with the established working length was used to place OrthoMTA (BioM-TA, Seoul, Republic of Korea) upto the working length of the root canal following the manufacturer's instruction. The hand plugger was used to compact OrthoMTA (BioMTA, Seoul, Republic of Korea) in the canal with the controlled working length. The excess OrthoMTA (BioMTA, Seoul, Republic of Korea) was removed from the canal orifice area and a damped cotton pellet was placed covering the OrthoMTA



(BioMTA, Seoul, Republic of Korea) in the pulp chamber area (Figure 4B). A temporary restoration using Cavit Cavit (3M Espe, St. Paul, MN, USA) was placed to close the access cavity and recalled after a day for further treatment.

Third Appointment

The patient was recalled 24 hours after the second appointment, old temporary dressing was removed, canal was irrigated with 5 ml of 3% NaOCl (Coltene/Whaledent, GnbH, USA), saline and 17% ethylenediaminetetraacetic acid (EDTA) (Pulpdent, Watertown, MA, USA) each for 1 minute ensuring no extrusion of any irrigating solution. old temporary restoration was removed, and the hardness of OrthoMTA (BioMTA, Seoul, Republic of Korea) was assessed. The root canal filling was solidified and thereafter, the access cavity was restored suing composite resin (3M ESPE, St.Paul, MN, USA). the patient was recalled for periodic follow ups.

Follow up visit

The patient was recalled for follow up after one month to assess the clinical signs and symptoms such as absence of tenderness to percussion or palpation, swelling, locally deep periodontal probing defect, tooth mobility and condition of coronal seal. An intraoral radiograph was taken to assess the reduction/absence of periapical lesion. The clinical and radiographic findings of the follow up visit suggested satis-

factory healing process (Figure 4C). The follow up was done at regular interval up to 30 months to ensure the positive outcome of the treatment (Figure 4D).

#Case3

A 20-year-old male reported to the clinic with a concern of caries and mild pain on biting in his upper front teeth. Clinical examination showed a restoration on tooth 11 and deep caries on tooth 12. The tooth 11 was tender on percussion with no mobility. Periodontal examination showed healthy gingival tissue around the tooth. Radiographic examination showed tooth 11 with open apex and large peri-radicular radiolucency and coronal radiolucency on tooth 12 suggesting dentinal caries (Figure 6A). After considering the clinical and radiographical findings the diagnosis of pulpal necrosis with symptomatic apical periodontitis was made and root canal therapy with the management of open apex using OrthoMTA was advised for tooth 11 and composite resin restoration for tooth 12.

First Appointment

After obtaining the written consent, the treatment was carried out. The area was anesthetised using 2% lignocaine with epinephrine 1:200,000 (inibsa, Barcelona, Spain) and an access cavity was prepared under rubber dam isolation. The working length was established using the intraoral



Figure 6

A) Preoperative intra-oral radiogrpah showing open apex on tooth 11 with large peri-apical radiolucency and coronal radiolucency on tooth 12. B) Establishing the working length. C) Obturation done using OrthoMTA. D) Three months follow up showing satisfactory periapical healing and closure of root apex, (E) Thirty months follow up radiograph tooth 12 showing complete resolution of peri-apical lesion.



periapical radiograph (Figure 6B) and the size of the apical end of the canal was gauged to size 140. The canal was circumferentially filed using Hand K files (Dentsply Maillefer, Ballagues, Switzerland), disinfected with 5 ml of 3% NaOCl (Coltene/Whaledent, GnbH, USA) and saline each for 1 minute ensuring no extrusion of any irrigating solution. Thereafter, obturation of the entire root canal with OrthoMTA was planned. In this appointment, non-setting calcium hydroxide (Voco GmbH, Germany), an intracanal medicament was placed into the root canal and the cavity was temporarily restored with Intermediate Restorative Material (IRM, Dentsply Sirona, Konstanz, Germany).

Second Appointment

The patient was recalled after oneweek, old temporary dressing was removed, canal was irrigated with 5 ml of 3% NaOCl (Coltene/Whaledent, GnbH, USA), saline and 17% EDTA (Pulpdent, Watertown, MA, USA) each for 1 minute ensuring no extrusion of any irrigating solution. The mastercone radiograph was taken to reconfirm the extension of root canal filling material. OrthoMTA (BioMTA, Seoul, Republic of Korea) was mixed according to the manufacturer's instruction and placed in the canal using the MTA gun as described in case 1 (Figure 6C) and the patient was recalled after a day for further treatment. Third Appointment

The patient was recalled after 24 hours, old temporary restoration was removed, and the hardness of OrthoM-TA (BioMTA, Seoul, Republic of Korea) was assessed. The root canal filling was solidified and thereafter, the access cavity was restored suing composite resin (3M ESPE, St.Paul, MN, USA). the patient was recalled for periodic follow ups to assess the clinical signs and symptoms such as absence of tenderness to percussion or palpation, swelling, locally deep periodontal probing defect, tooth mobility and condition of coronal seal. An intraoral

radiograph was taken to assess the reduction/absence of periapical lesion. The clinical and radiographic findings of the follow up visit suggested satisfactory healing process (Figure 6D). A thirty month follow up radiograph showed complete healing of the periapical lesion (Figure 6E).

Discussion

Appropriate management of open apex largely depends on the vitality of the tooth. Through detailed history taking, pulp sensibility tests, supported by radiographical evidence, an accurate diagnosis can be obtained. For teeth with viable pulpal status, vital pulp therapy is the primary treatment option (46). This will allow continued physiological development and formation of the root. For irreversibly inflamed or necrotic pulp, current evidence shows two treatment options such as root-end closure by apexification or pulpal regeneration (46). To achieve root-end closure, large interest has been expressed in the use of mineral trioxide aggregate and MTA like materials such as OrthoMTA.

The literature has shown satisfactory clinical successes of OrthoMTA in various clinical scenarios such as pulpotomy in primary molars, partial pulpotomy in permanent teeth and apexification in immature apex of permanent teeth (Table 1). Despite it being newly developed, OrthoMTA is very much comparable to ProRoot MTA in terms of biocompatibility, mineralization -inducing potential, fracture resistance, and marginal adaptation. In an in vitro research by Kum et al. (2013) (47) using RNA isolation and revers transcription-polymerase chain reaction, osteopontin levels were increased in incubated cells in both ProRoot MTA and Ortho MTA groups. However, Kim et al. (2014) (89) reported increased cytotocixity with OrthoMTA compared to ProRoot MTA and Endocem MTA. This finding was supported by Lee et



Table 1

Studies showing various parameters and outcomes using OrthoMTA in last ten years

					_		
First author	Year	Country	Objective	Sample type	Sample size	Experimental groups	Outcome
Song et al. (50)	2011	Korea	To evaluate the influence of root canal filling material composed of MTA on tubular penetration	Human teeth	50	 Angelus Fillapex MTA sealer Gutta-percha Portland cement OrthoMTA ProRoot MTA 	OrthoMTA group showed deepest pe- netration of particles in the dentinal tubule with no significant difference with other groups (p>0.05)
Chang et al. (51)	2011	Korea	To investigate and compare the levels of arsenic(As), chromium (Cr), hexavalent chromium (Cr6+), and lead (Pb) in Ortho MTA and ProRoot MTA	-	-	1. OrthoMTA 2. ProRoot MTA	OrthoMTA and ProRoot MTA meet the ISO specification 9917-1 regarding the safety limits of As and Pb and are safe biomaterials when the purity of As, Cr6+, and Pb is considered
Lee et al. (101)	2012	Korea	To compare the cyto-toxicity of four root-end filling materials	MG-63 cells derived from a human osteo- sarcoma	-	 Fuji II GIC (Glass Ionomer Cement) IRM (Intermediate Restorati- ve Material) OrthoMTA ProRoot MTA 	ProRoot MTA and GIC showed good biocompatibility. However, OrthoMTA showed lower biocompatibility compa- red with ProRoot MTA and GIC
Kum et al. (52)	2013	Korea	To investigate the levels of cad- mium (Cd), copper (Cu), iron (Fe),manganese (Mn), nickel (Ni) and zinc (Zn) in Ortho MTA and ProRoot MTA.	-	-	1. Ortho MTA 2. ProRoot MTA	Ortho MTA had lower levels of Cd, Cu,Fe, Mn and Ni than ProRoot MTA
Kum et al. (47)		Korea	To compare the elemental con- stitution, morphological cha- racteristics, particle size distri- bution, biocompatibility, and mineralization potential of Or- tho MTA and ProRoot MTA	•	-	1. Ortho MTA 2. ProRoot MTA	The morphology of OrthoMTA powders was similar to that of ProRoot MTA. The constituent elements of both MTAs were calcium, silicon, and alu- minum. The mean particle sizes of OrthoMTA and ProRoot MTA were 4.60 and 3.34 mm, respectively. Both MTAs had equally favorable in vitro biocom- patibility
Chang et al. (48)	2014	Korea	To evaluate the biocompatibility, inflammatory response, and odontoblastic potential of Bio- dentine, Ortho-MTA, Angelus- MTA, and IRM on human dental pulp cell (HDPCs)	-	-	 Biodentine Ortho-MTA Angelus-MTA IRM 	The biocompatibility, inflammatory response, and odontoblastic differen- tiation of OrthoMTA were similar to Bio-dentine
Ghorbanza- deh et al. (103)	2014	Iran	To compare the marginal adap- tation of MTA and MTA-like ma- terials as root-end fillings after incubation in phosphate buffer saline (PBS), a synthetic tissue fluid, for either 1 week or 2 months	Human single- rooted teeth	72	 ProRoot MTA Ortho- MTA Retro MTA 	There was no difference between the marginal adaptation of ProRoot MTA, OrthoMTA, and RetroMTA as root-end filling materials
Kang et al. (100)	2015	Korea	To determine the clinical effica- cy of the newly developed Or- thoMTA and RetroMTA, compa- red to Pro-Root MTA for pulpotomy in primary teeth	Human primary teeth	143	OrthoMTA RetroMTA ProRoot MTA	The success rates of RetroMTA, Or- thoMTA and ProRoot MTA were almost similar, indicating that pulpotomy with these material can be carried out success-fully in primary molars
Kim et al. (89)	2014	Korea	To compare the biological pro- perties of OrthoMTA and Endo- cem MTA with those of ProRoot MTA	Preosteoblast like cell line MC3T3-E1	-	 OrthoMTA Endocem MTA ProRoot MTA 	Pro-Root MTA appeared to be superior to OrthoMTA and Endocem MTA in terms of biological properties althou- gh Endocem MTA exhibited the shor- test setting time and presented lower cytotoxicity.
Soram et al. (104)	2016	Korea	To evaluate the effects of three acids on the microhardness of set OrthoMTA and root dentin, and cytotoxicity on murine ma- crophage	Human teeth	40	 10% citric acid (CA) 5% glycolic acid (GA) 17% ethylenediaminetetra- acetic acid (EDTA) Saline 	Tested acidic solutions reduced microhardness of root dentin. Five minutes application of 10% CA and 5% GA significantly reduced the microhardness of set OrthoMTA (p<0.05) with lower cellular cytotoxicity compared to 17% EDTA
Kang et al. (105)	2017	Korea	To evaluate and compare the clinical applicability of various MTA materials as partial pulpo- tomy materials in permanent teeth	Human teeth	104	 ProRoot MTA Ortho-MTA RetroMTA 	ProRoot MTA, OrthoMTA and RetroM- TA had favorable clinical and radio- graphic results after one year.



Table 1

Studies showing various parameters and outcomes using OrthoMTA in last ten years

Rahoma et al. (106)	2018	Saudi Arabia	To measure the push-out bond strength of three types of mine- ral trioxide aggregate (MTA) materials in root dentin	Human maxilla- ry central inci- sors	30	1. 2. 3.	OrthoMTA MTA Angelus ProRoot MTA	OrthoMTA, MTA Angelus, and ProRoot MTA materials showed similar push- out bond strength values in root den- tin
Mousavi et al. (107)	2018	Iran	To evaluate the sealing ability of ProRoot MTA, Biodentine, and OrthoMTA as the root canal obturation materials using the fluid infiltration method	Human mandi- bular premo- lars	66	1. 2. 3. 4. 5.	Negative group Positive group (gutta- percha) ProRoot MTA Biodentine OrthoMTA	OrthoMTA, ProRoot MTA and Bioden- tine showed similar sealing ability
Khedmat et al. (111)	2018	Iran	To assess the antibacterial ac- tivities of OrthoMTA, RetroMTA, and ProRoot MTA	Fusobacterium nucleatum (Fn), Porphyromonas gingivalis (Pg), and Prevotella intermedia (Pi)	-	1. 2. 3.	Ortho-MTA RetroMTA ProRoot	OrthoMTA had the highest antibacte- rial activity against Pi. The mean num- ber of CFU/ml of Fn in the presence of ProRoot MTA and RetroMTA was significantly lower than that in positi- ve controls (p<0.05). ProRoot MTA and OrthoMTA both had equal signif- cant antibacterial effect against Pg compared to positive controls
Aslan et al. (108)	2018	Turkey	To evaluate the forces required to fracture roots obturated with different calcium silicate based materials, after applying a frac- tured instrument removal simu- lation	Human mandi- bular premo- lars	75	1. 2. 3. 4.	ProRoot MTA Ortho- MTA Biodentine Endocem MTA	Any of the tested materials could be chosen to reinforce the root after the removal of a fractured instrument
Kang et al. (109)	2018	Korea	To compare the inflammatory response and mineralization- inducing potential of three cal- cium silicate cements	Dog's teeth	44	1. 2. 3.	ProRoot MTA OrthoMTA Endocem MTA	ProRoot MTA and OrthoMTA resulted in reduced pulpal inflammation and more complete calcific barrier forma- tion, whereas Endocem MTA caused a lower level of calcific barrier conti- nuity with tunnel defects
Kim et al. (102)	2019	Korea	To evaluate the initial cytotoxi- city of four different commer- cially available MTA materials	Bone- marrowderived human mesenchymal stem cells (hMSCs)	-	1. 2. 3. 4.	Endocem MTA Ortho MTA ProRoot MTA MTA Angelus	100% extracts from completely set MTAs showed similar cell viability with the control group without cytotoxicity. However, all four MTA products tested during setting showed severe cytoto- xicity at original and 50% extracts
Ballal et al. (53)	2020	Switzer- land	To evaluate the influence of ProRoot MTA and OrthoMTA as an obturating material on the fracture resistance of endodon- tically treated teeth	Human maxilla- ry central inci- sors	30	1. 2. 3.	Ortho- MTA ProRoot MTA Positive control	OrthoMTA showed the highest fractu- re resistance and showed better tu- bular biomineralization when compa- red to ProRoot MTA
Bolbolian et al. (110)	2020	Iraq	To compare microleakage of resin modified glass ionomer and OrthoMTA used as an intra- orifice barrier in non-vital blea- ching	Human mandi- bular premo- lars	36	1. 2. 3. 4.	OrthoMTA RMGIC (Resin Modifies Glass lonomer Cement) Positive group Negative group	OrthoMTA had less leakage than RMGIC but both materials can be used as suitable barriers for internal tooth bleaching

al. (2012) (101) study, stating ProRoot MTA has higher biocompatibility compared to OrthoMTA. Nonetheless, Kim et al. (102), Chang et al. (48), Kum et al. (47) and Kang et al. (109) reported that Ortho MTA is equally favorable in cell biocompatibility when compa-red to other calcium silicate cements (CSCs). In addition to biocompatibility and sealing ability, Khedmat et al. (37) demonstrated that OrthoMTA has antibacterial effects against bacteria involved in endodontic periodontal infections. Arising concerns regarding heavy metal content in MTA has led to few studies investigating the issue. The manufacturer of OrthoMTA claims that it has similar components as ProRoot MTA but less heavy metal contents than ProRoot MTA. Chang et al. (2011) (51) and Kum et al. (2013) (52) concluded that both OrthoMTA and ProRoot MTA meet the ISO specification 9917-1 regarding the safety limits of As and Pb, and the latter stating that OrthoMTA has lower levels of Cd, Cu, Fe, Mn and Ni than ProRoot MTA.

In case reports two and three use of OrthoMTA has shown positive outcomes



with successful healing of the periapical lesion. This could be because, in addition to the properties explained earlier, OrthoMTA or BioMTA as an orthograde root canal grafting material forms an interfacial hydroxyapatite layer between Ortho MTA and the root canal wall and prevents microleakage and entombs the remaining bacteria by intratubular mineralization.

Endodontically treated teeth are usually weak because of loss of tooth structure due to caries, access cavity preparation, and instrumentation of the root canal and with the right obturating material and techniques, fracture resistance of the tooth can be greatly enhanced. Ballal et al. (2020) (53) stated that among OrthoMTA, ProRoot MTA and the control group, Ortho-MTA demonstrated the highest fracture resistance and better tubular biomineralization.

Due to the presence of fracture-prone thin dentinal wall, disinfection of the root canal system relies solely on chemical disinfection using irrigants and intracanal medicament. Therefore, careful selection of irrigants/intracanal medicaments is imperative to ensure successful outcomes. Non-setting calcium hydroxide was placed as an inter-appointment intracanal medicament as it is showed to effectively disinfect the canal system against common endodontic pathogens (54). This highly alkaline medicament with a pH of around 12.5, releases hydroxyl ions in an aqueous environment potentially causes damage to the bacterial cytoplasmic membrane, protein denaturation, and damages DNA. Although the mechanism of action is not clearly understood, the antimicrobial action of calcium hydroxide has been documented in many studies (55-58). However, it remains controversial as some authors reported ineffectiveness of calcium hydroxide (49, 59, 60).

3% Sodium hypochlorite was used as an irrigating solution to disinfect the root canal and rinse off the intracanal medicament placed in the previous

appointment. NaOCl used in various concentration (0.5-5.25%) has shown to be an excellent non-specific proteolytic and antimicrobial agent to disinfect the root canal system (61-63). A systemic review by Arruda et al. (2009) (64) showed that there is insufficient research done on the time of irrigation, concentration or volume of NaOCl solution that is optimal in endodontic treatment without causing significant changes in the mechanical properties of dentin. Moreover, extreme caution must be exercised when using this cytotoxic irrigant, especially in cases of open apex. Many reports describe clinical complications arising from accidents such as injection of Na-OCl into periapical tissue, maxillary sinus, splashing into eyes, leakage of NaOCl through the rubber dam causing severe mucosa/cutaneous chemical burns (65-71). In addition, rare case reports of hypersensitivity and allergy towards NaOCl has also been reported (72-74). To prevent extrusion of NaOCl into periapical tissue, EndoVac system was utilized in this case report. This system applies suction to pull irrigant down the root canal, instead of excessive digital pressure applied through conventional irrigating syringe. Careful working length determination through an apex locator and reconfirming it with the introoral periapical radiograph is recommended in open apex cases. Standard operating procedure such as rubber dam isolation, straight line access, maintaining the working length 2 mm short of root end, loose placement of irrigating needles in the canal and constant in and out movements of irrigating needle must be strictly adhered to avoid potential tissue damage (75).

Smear layer which consists of dentine, pulp remnants, odontoblastic processes and bacteria is formed on canal walls after instrumentation. It is said to inhibit tubular penetration of sealant material during canal obturation (76). Many researchers support the usage of EDTA 17% as a chelating agent to remove this



inorganic layer which cannot be removed by NaOCl alone (76-79) .Using scanning electron microscope, Niu et al. (2012) (80) showed that final irrigation with 3% NaOCl following treatment with 17% EDTA produces clean and exposed dentinal tubule orifices, as compared to EDTA or NaOCl alone.

Although MTA has been the material of choice for apexification due to its biocompatibility, over extrusion of the material can impede periapical healing and prolong patient discomfort. A controlled condensation pressure of MTA that normally produces a uniform apical plug and good seal might cause the material to be pushed through the apex in an immature open apex tooth (81, 82). Acting as an apical barrier, a collagen sponge was pushed through the apex and compacted in place prior to the placement of MTA in case report 1. The extra radicular membrane improved the adaptation of the MTA in the open apex tooth, achieving a good seal. However, Zou et al. (2008) (83) reported no significant difference in either leakage or overfilling of MTA when internal matrix is used for the repair of furcation perforations. Therefore, it is a matter of the dentist's personal preference as there are few successful case reports showing favorable prognosis with the usage of a collagen sponge apical barrier (84-86). The use of magnification and illumination play an essential role in managing these kinds of cases to ensure the adequate placement of collagen sponge and MTA.

A waiting period of 24 hours was given after the placement of MTA in the canal as MTA has a long setting time (20, 31, 87-89). Manufacturer's instructions have stated that the ProRoot MTA and OrthoMTA sets in 4-6 hours and 3-5 hours respectively. However, Kim et al. (2014) reported that ProRoot MTA and OrthoMTA has a setting time of 318.0±56.0 and 324.3±2.1 minutes respectively in an in vitro study (89), significantly different from the said instructions. Therefore, to ensure that the MTA has completely solidified

prior to obturation or coronal restoration, a two-visit endodontic visit was performed in all three case reports. A damp cotton pellet was placed on top of the newly placed MTA as it provides the moisture MTA needed for a proper set. This was demonstrated by an in vitro study by Craig D. Johnson (2010) (90) in which MTA with the placement of a moist cotton pellet showed significantly lesser penetration resistance for 6 hours and set significantly (P<0.05)slower than MTA without the moist pellet. However, the MTA without the moist pellet was significantly (P<0.05) softer than the MTA with the moist pellet at day 1 and 3.

In all three case reports treated with ProRoot MTA and OrthoMTA, the follow up was done up to thirty months to ensure the successful outcome of these treatment modalities. This time-period is an accordance to Travassos et al. (91) who recommended a period of two to five years to observe complete repair. By providing follow-ups, clinicians can deduce and solve the post-operative problem which can adversely affect a patient's quality of life in the long run (92).

Future Direction

Apexification with calcium hydroxide and MTA barrier technique fails to induce continued root maturation which makes the tooth susceptible to root fracture. Hence, an ideal outcome for such a tooth should be regeneration of pulp like tissue into the root canal capable of continuing normal root maturation. Regenerative endodontic therapy has been defined as "biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex" (41).

A very specific environment must be created for revascularization of the pulp to take place. The absence of intracanal infection and presence of a scaffold (e.g., blood clot or platelet-rich plasma) conducive to tissue ingrowth, and a permanent coronal seal



(e.g., MTA or resin-modified glass-ionomer) are very crucial in REP. Similar to conventional retrograde endodontic therapy, disinfection of the root canal system is paramount to the success of REP. Due to its thin weakened dentinal wall, disinfection relies less on mechanical instrumentation but rather relies heavily on the use of chemicals. consisting of irrigating solutions and intracanal antibiotic dressings. A combination of antibiotics, specifically ciprofloxacin, metronidazole and minocycline has been shown to properly kill common endodontic pathogens in the infected root canal (93). The blood clot can be created by instrumenting the tooth beyond apex to approximately 1-2 mm to permit bleeding into root canal system. Multiple theories for the mechanism of revascularization have been proposed and discussed.

Remaining vital pulp cells (94), stem cells from the dental pulp (95), periodontal ligament (96), or mesenchymal stem cell from the bones (97) could be the ones responsible for the differentiation into odontoblast. The newly formed odontoblast lay down atubular dentin at the apical end, causing elongation of root and thickening of the dentinal walls, reinforcing and strengthening the root. Not only that, platelet-derived growth factor, vascular endothelial growth factor (VEGF), platelet-derived epithelial growth factor, and tissue growth factor found in the blood clot, could play an important role in regeneration. These growth factors causes the fibroblasts, odontoblasts, cementoblasts etc from the immature, undifferentiated mesenchymal cells in the newly formed tissue matrix to be stimulated to differentiate, grow, and mature (98).

Tatullo et al. (2019) (99) showed cell proliferation, cells viability, and gene expression for osteogenic and odontogenic differentiation when Human periapical cyst mesenchymal stem cells (hPCy-MSCs) derived from inflammatory periapical cysts were seeded on mineral doped bioactive scaffold. This study is one among the many research making new discoveries and advancement in the evolving world of regenerative dentistry.

Conclusions

This case series showed the successful management of teeth with open apex with periapical lesions using Pro-RootMTA and OrthoMTA. In all three cases, the tooth restored showed complete resolution of pain and satisfactory healing of periapical lesion radiographically. Clinicians need to have a thorough knowledge of standard root canal protocols to be followed in the managing open apex cases and understanding of ProRoot MTA and OrthoM-TA materials to achieve clinical and radiographical success.

Clinical Relevance

Clinicians need to know how to handle teeth with open apex and apical periodontitis as it may present multiple challenges specially in disinfecting the root canal space and providing an artificial apical barrier using Mineral Trioxide Aggregate (MTA) and OrthoMTA.

Conflict of Interest

The authors declare that they have no conflict of interests.

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All authors conceived the original idea, designed, treated these cases, and wrote the manuscript.

All authors have read, reviewed, and approved the manuscript.

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Intentional replantation of severely compromised teeth

ABSTRACT

Aim: The present case series aims to show the applicability of intentional replantation to successfully solved cases with root perforations, external cervical resorption, and chronic pain due to material extrusion to the periapical tissues.

Summary: After medical and dental history review, the signs, symptoms and imaging examinations were analysed in order to perform a correct diagnosis. All cases had indication for intentional replantation procedures which were addressed according to the best practices. The teeth were extracted in the most atraumatic way possible while avoiding the handling interaction with the periodontal ligament. Hard tissues repair was conducted by using ultrasonic tips and mineral trioxide aggregate (MTA) application before getting the teeth positioned back in their original socket. Extra-oral time was less than 15 minutes and the operating microscope was used in all cases. After a period of two years all teeth were asymptomatic and functional.

Key learning points

- Root perforations, external cervical resorption, and chronic pain due to material extrusion may be solved using intentional replantation techniques
- Reduced extra-oral time and careful periodontal ligament handling is mandatory
- Intentional replantation techniques appears to be a reliable clinical procedure

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Introduction

btaining proper root canal disinfection, preventing reinfection, achieving a healthy periapical tissue, and keeping a tooth in function, thus contributing to its longevity, are among the objectives of a root canal treatment. However, when the treatment is performed according to a high standard of care and there is still persistence of symptoms and apical pathology, derived by either persistent bacteria, extrusion of endodontic sealer (1) or even by the presence of a root perforation, the root canal treatment should be re-evaluated and alternative treatments considered.

When initial root canal treatment fails. root canal retreatment and endodontic microsurgery are the subsequent treatment options to be considered (2). Root canal retreatments have been reported to present an overall success rate of approximately 78% (3, 4) while for the surgical option it may be as high as 95% (5). Nonetheless, when a root canal retreatment cannot be performed, for instances due to tooth inner anatomical complexity leading to inherent limitations of chemomechanical preparations (6), or when endodontic microsurgery is contraindicated, for instances due to proximity to anatomic landmarks such as the mental foramen, mandibular canal or thick mandibular bone, the intentional replantation may be considered (7).

Originally described by Grossman (8), intentional replantation consists of the deliberate extraction of a tooth in order to directly evaluate the root surfaces, conduct an endodontic manipulation and repair of the problematic area and placement of the tooth back into its original socket. This procedure has a reported survival rate of approximately 89% (2) and can be applied for treatment of vertical root and crownroot fractures, external root resorptions, persistent chronic pain, or previous failure of root canal retreatment and endodontic microsurgery (2, 9, 10). Yet, intentional replantation should not be considered an option when there is periodontal and furcation involvement, an extensive carious lesion, very long and curved roots, or in case of septal bone loss (8, 9).

Despite the growing reports of favourable outcomes (2), intentional replantation as a treatment option is not widespread in the clinical community (11). Thus, the objective of this case series is to illustrate the intentional replantation technique by presenting four surgical cases in which this modality was successfully chosen to treat previous root canal treatment failures.

Report

General clinical procedures

All patients were referred for an endodontic appointment and had their medical histories reviewed and considered as non-contributory. Signs and symptoms were analysed, and complementary imaging examinations requested. Periodontal pockets and tooth mobility were considered within normal limits in all cases. Periapical radiolucencies were detected with periapical radiographs and confirmed through cone-beam computed tomography (CBCT), which was used to establish a proper treatment plan. Pulpal and periapical diagnosis was made based on clinical findings, pulp tests and imaging analysis. Once verified the inaccessibility to perform endodontic microsurgery, intentional replantation was recommended and accepted as the treatment option by all patients, who signed informed consents.

All cases were performed by the same clinician (AG) under a dental operating microscope (M320, Leica Microsystems, Wetzlar, Germany) with an effort of preserving the viability and integrity of the periodontal ligament (PDL) by reducing the extra-oral time to the minimum and never exceeding 15 minutes. After proper local anesthesia with articaine (Artinibsa, Inibsa, Barcelona, Spain), teeth extractions were done in the most atraumatic way possible with nº 150 universal apical extraction forceps, and never interacting with the PDL. The perforation repair cases were performed using a microsurgical ultrasonic tip KiS nº 1 (Obtura-Spartan, Algonquin, USA) with water irrigation to clean and



Figure 1

Representative images of the surgical procedure conducted on tooth 25 presenting a preoperative root perforation: A) initial radiograph; B) photograph of atraumatic tooth extraction; C) photograph of granulation tissue on the perforation site; D) clinical photograph of the granulation tissue removal; E) photograph of ultrasonic preparation of perforation before sealing with Grey MTA; F) post-operative radiograph; G) 9 months follow-up radiograph; H) 3-year follow-up radiograph.

prepare for mineral trioxide aggregate (MTA) placement. As for the other case, after extraction, a 3 mm root end resection was performed under water irrigation, followed by the root end preparation with a microsurgical ultrasonic tip KiS nº 1 (Obtura-Spartan, Algonquin, USA) with water irrigation and subsequent retro-fill with MTA. After teeth reposition in the sockets, sutures were performed and post-surgical instructions were given. Sutures were removed a week later and the follow-up appointments showed an improvement of the clinical condition of all cases without any sign of structural failure.

Case 1

A 37-year-old male patient was referred for a disto-palatal root perforation repair on the maxillary left second premolar (tooth 25) (Figure 1A). The dental history revealed a failed attempt to repair it during a previous root canal treatment. The patient was asymptomatic and the clinical diagnosis was previous endodontically treated tooth with a large persistent radiolucency, corresponding to asymptomatic apical periodontitis. The intentional replantation was performed to access and repair the disto-palatal perforation using grey MTA (Grey ProRoot MTA, Dentsply Tulsa Dental, USA) (Figures 1B to 1F). At 9 months (Figure 1G), and 3 years follow-up appointments (Figure 1H), the tooth was functional, asymptomatic and showing signs of radiolucency healing. The patient skipped the 5 years recall, however mentioning that the tooth was still asymptomatic and functional.

Case 2

A 55-year-old female patient with a diagnosis of pulp necrosis and asymptomatic apical periodontitis on the maxillary left first molar (tooth 26) was referred for root canal treatment, which was accomplished at that time (Figures 2A and 2B). The pa-



tient returned 5 years later with a post and crown and complaining of a palatal swelling (Figure 2C). The CBCT analysis revealed a perforation on the distal aspect of the palatal root, which was inaccessible by conventional endodontic microsurgery due to the position of the disto-buccal root (Figures 2D to 2F). Intentional replantation was advised and performed (Figures 2G to 2I). The perforation sealing and retro-obturation was performed with white Pro-Root MTA (White ProRoot MTA, Dentsply Tulsa Dental, USA) (Figure 2L). Six months later, the patient was recalled for control (Figure 2M) and the tooth was asymptomatic and functional. The 2 years radiographic follow up revealed complete bone healing (Figure 2N).

Case 3

A 44-year-old male patient was referred for an endodontic appointment with a previous endodontic treatment and symptomatic apical periodontitis on the maxillary left central incisor (tooth 21) (Figure 3A). The tooth had a cast post and a ceram-



Figure 2

Documentation of the intentional replantation conducted on tooth 26 presenting a root perforation: **A**) initial radiograph; **B**) endodontic treatment final radiograph; **C**) 5 years post endodontic treatment radiograph showing root perforation; **D**) pre-operative CBCT frontal view; **E**) pre-operative CBCT sagittal view with an orange arrow pointing towards the perforation site; **F**) pre-operative CBCT axial view; **G**) photograph before atraumatic extraction; **H**) photograph of granulation tissue removal; **I**) clinical photograph of root resection; **J**) examination after root resection; **K**) photograph of the perforation handling; **L**) photograph of the MTA obturation and sealing of the perforation; **M**) 6 months follow up radiograph; **N**) 2 years follow up radiograph.





Figure 3

Clinical procedures of the surgical treatment of tooth 21: A) initial radiograph; B) post-operative radiograph of the microsurgical endodontic procedure which included root apicectomy; C) 4 months post-operative radiograph of the first surgical procedure; D) pre-operative CBCT frontal view; E) pre-operative CBCT sagittal view; 3F) pre-operative CBCT axial view; G) clinical photograph exhibiting resorption site; H) post intentional replantation photograph of the resorption treated and sealed with MTA; I) photograph of tooth splinting; J) 8 months follow up radiograph; K) 2 years follow up radiograph; L) 2 years follow up CBCT frontal view; M) 2 years follow up CBCT axial view.

ic crown. The initial treatment plan involved the orthograde root canal retreatment, however it was not possible to remove the cast post, so endodontic microsurgery was proposed and accepted (Figure 3B to 3F). Four months later, a disto-palatal cervical invasive root resorption was diagnosed (Figures 3G), and the intentional replantation proposed. A 90% aqueous trichloroacetic acid was applied as per guidelines (12), and the sealing was done with white ProRoot MTA (White ProRoot MTA, Dentsply Tulsa Dental, USA) (Figure 3H). The tooth was then splinted with flowable composite (Tetric EvoFlow, Ivoclar Vivadent, Schaan, Liechtenstein) (Figure 3I). The patient returned after 8 months for a follow-up and had the tooth asymptomatic and functional (Figure 3J). Complete bone healing was observed in the 2 years recall (Figures 3K to 3N).

Case 4

A 37-year-old female patient presented with pain on the maxillary left second molar (tooth 27). The diagnosis of the in-





Figure 4

Representative radiographs of the clinical case of tooth 27: **A**) initial radiograph exhibiting large sealer extrusion; **B**) Post intentional replantation radiograph; **C**) 3 months follow up radiograph; **D**) 2 years follow up radiograph.

> volved tooth was previous root canal treatment with symptomatic apical periodontitis. The previous root canal therapy had been concluded a few weeks before and the access cavity was still restored with a provisional restoration (Figure 4A). Since the previous root canal obturation resulted in a heavy extrusion of endodontic sealer to the periapical tissues, intentional replantation was proposed and performed (Figure 4B). White ProRoot MTA (White ProRoot MTA, Dentsply Tulsa Dental, USA) was used as a retro-filling material. The patient returned 3 months later for a follow-up appointment which revealed an asymptomatic and functional tooth (Figure 4C). The same outcome was observed at the two years recall (Figure 4D).

Discussion

As life expectancy of the population is increasing globally (13), all viable treatment options should be considered to prolong the survival of the natural dentition (14). Intentional replantation has often been regarded as an unreliable procedure in endodontics (15) and overlooked as a viable treatment option, especially given the high survival rate of tooth implants (2).

As a treatment performed since the 18th century (10, 16), the intentional replantation procedure and its indications have changed over time (17). Recent literature presenting treatment protocols based on the understanding of root resorption and splinting protocols have proven this procedure to be more predictable than previously considered and a viable treatment option to be considered in specific cases (7, 10, 11, 14). With modern treatment protocols using updated equipment, instruments and materials, this surgical procedure has been shown to have a success rate ranging from 72% to 94% (2, 17), similar to that of unitary implant rehabilitation. Intentional replantation treatment protocols advocate a minimally traumatic extraction (18, 19) and an extra oral dry time shorter than 15 minutes (7, 11, 14, 19, 20, 21). These are considered key prognostic factors for the treatment success as the preservation of viable cementum and periodontal fibers is of the utmost importance to reduce the likelihood of external replacement resorption (10). Also, the tooth



type (19), apical anatomy (22), root surface hydration (11, 22), illumination and magnification (10, 22) and meticulous instrumentation are important factors that weigh in the success of the treatment (22).

When the extra oral dry time exceeds 15 minutes, previous literature shows that the risk of complications is 1.7 times higher when compared to shorter periods which are associated with a higher survival rate and fewer complications (2, 11). A systematic review by Mainkar in 2017 compared the survival of intentionally replanted teeth with single-tooth implants stated the risks of external replacement resorption (ranging from 0% to 7%) or external root resorption (from 3 to 5%) were lower when extra oral dry time was kept to a minimum (2). Additionally, since these complications occur mostly in the first year after treatment (11, 14), it is prudent to perform regular follow-ups during this time frame, in accordance with European Society of Endodontology guidelines (23).

In presented cases 2 and 3, which were related with root canal perforations in the distal aspect of teeth 21 and 26, a CBCT scan was made to localize the exact perforation site and plan for appropriate treatment.

CBCT imaging overcomes several limitations of conventional radiographs such as the anatomical noise, radiographic two-dimensional nature, or geometric distortion that may impede the correct detection of periapical lesions in cancellous bone (24). In endodontics, a CBCT small field of view examination can be considered if the additional information obtained aids in the diagnosis and treatment planning and ultimately enhances the prognosis of clinical management (24).

In case 3, an external cervical resorption was detected 4 months after endodontic microsurgery. Patel et al. (14) point out that the treatment of this type of resorption will depend on the severity and location of the defect and its restorability (14). According to a newly proposed 3-dimensional classification, the lesion was classified as 3Ap because it extends to the mid-third of the root with a total area less than 90° with pulpal involvement (25), and the

CBCT analysis proved that the location of the defect was not accessible through endodontic microsurgery, thus the intentional replantation was considered. In this case, after curettage of the granulation tissue, the application of 90% aqueous trichloroacetic acid was made to obtain coagulation necrosis of the resorptive tissue and halt the pathologic process (26), given that incomplete removal is likely to result in the recurrence of external cervical resorption (27). After debridement, external cervical resorption defects can be restored with a glass ionomer cement or composite (14). However, the use of bioactive materials such as MTA (11, 18) as opposed to other repair materials (such as amalgam, IRM and Super EBA) has been shown to improve the outcome of intentional replantation (28). White ProRoot MTA was used to seal the defect as it presents an excellent sealing capacity (29, 30) associated not only to its mechanical seal but also to a chemical adhesion provided by by-products of the setting and hydration reaction of the material (30).

A wide range of indications were listed by Grossman (8) including natural or iatrogenic root canal obstructions, complex root canal morphology, presence of periapical irritants and/or extruded materials and perforation sealing when apical surgery was not viable (31). Extruded material in the periapical tissues has been stated previously as one of six biological factors that may lead to asymptomatic radiolucency persisting after root canal treatment (1). As microsurgery was impracticable, intentional replantation was performed in case 4 in order to remove all the remnants of root canal sealer and control the pain which led the patient to look for clinical help.

Conclusions

Intentional replantation may be considered a suitable option to successfully maintain which were once deemed untreatable through conventional endodontic procedures, especially considering the favourable prognosis and outcomes recently reported in the literature.



Clinical Relevance

Intentional replantation requires the deliberate extraction of a tooth allowing its manipulation and repair through an endodontic surgery outside the socket, followed by the tooth placement back into its original position. As emphasized by recent literature, with a well-designed treatment protocol this procedure may be considered a viable option for teeth otherwise considered lost, presenting high success and survival rates.

Conflict of Interest

None.

Acknowledgements

None.

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Intentional replantation in Endodontics: review of literature

ABSTRACT

Intentional replantation (IR) is a surgical approach consisting of a tooth's controlled extraction. The latter is repositioned in its original alveolar socket after being endodontically treated extra-orally.

The present work was conducted to assess the Intentional replantation by reviewing the literature of articles presenting case reports treated with the IR technique.

The current literature search was conducted through PubMed, Scopus, EMBASE, and Google Scholar. Articles updated from 1996 to December 1st 2021, have been included. Each reviewed article was evaluated using a ten-question data extraction form to identify the type of study, the sex and age of the patients, the teeth treated, the extra-alveolar time of the teeth, the techniques used and the time of follow up.

Modern technologies recently introduced in dentistry aided in achieving encouraging results; in particular, it has been highlighted that surgical interventions are shorter and less invasive and with a lower percentage of failures.

In conclusion, this work aimed to analyze and discuss the surgical procedures of IR described in the literature by different authors through a review of the literature. Furthermore, a clinical case using the intentional reimplantation technique was also reported. Giuseppe Sangiovanni^{1*} Giuseppe Scelza¹ Francesco Giordano² Andrea Chiacchio¹ Roberto Ferrigno¹ Carlo Rengo¹ Massimo Pisano¹

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Introduction

ntentional replantation (IR) is a surgical technique that consists of the controlled extraction of a tooth and repositioning the latter in its original alveolus. This procedure is done in order to allow, in an extraoral environment, the evaluation of root surfaces and endodontic treatment when the orthograde treatment or apical surgery cannot be made (1). The intentional re-implant is indicated: in cases of persistent symptomatic apical periodontitis after orthograde endodontic therapy for teeth in which the orthograde path is either complex or blocked (2-4). Also, IR can be applied in cases with improper endodontic therapy with overfilling material beyond the apex with the persistence of the lesion even after periapical surgery was done (3). Moreover, it can be applied when a surgical endodontic flap retraction is contraindicated due to anatomical or accessibility limitations (5). In addition, in conditions of inaccessible external root resorption (6), or in special cases of root perforations (7) and in cases of complex root or root-crown fractures (7-9), the IR can be recommended. Furthermore, IR can be beneficial for treating teeth presented with development anomalies such as fused teeth or with canal configuration type C (10-12).

In summary, the IR can be the last resort when all the possible orthograde and periapical surgery approaches have failed or are contraindicated.

The intentional re-implant can represent a therapeutic choice even when the alveolar bone level is required to be preserved for subsequent implant insertion (13-14).

The surgical phase in IR must be performed with extreme precision to improve the results and the percentage of success. The extraction tactic must be the least traumatic to avoid tooth fractures and damage to the periodontal ligament (PDL), which can comprehend a critical role in the treatment's healing and success (15). Some authors consider the extraction part the most technically sensitive phase of the procedure (16). Once extraction is done, the extracted tooth is then analyzed in order to underline the presence or absence of fractures or anatomical characteristics that require attentiveness, for example, the presence of additional or accessory canals or multiple foramina (17). Subsequently, based on the case, the decision to proceed to extra-oral endodontic treatment can be developed (18-21).

Of fundamental importance for the treatment success is the management and the conservation of the PDL and, in particular, the extra-alveolar environment conditions (22, 23). It was established that an extra-alveolar time greater than 15 minutes in a dry environment compromises the vitality of the PDL by increasing the possibility of replacement and ankylotic reabsorption (24). Therefore, the extra-alveolar time must be minimized, and the conservation of the tooth occurs in a humid environment to render the surgical procedure more predictable (25, 26).

Removing any cystic or granulomatous tissue residues in the alveolar socket (alveolar curettage) aiming to initiate alveolar healing is very debated in the literature as the main objective is to avoid removing or damaging the PDL attached to the alveolar walls (27). Some authors have advocated a healing technique of the apical portion implicating removing the lesion without affecting the pocket walls (28). Removing the cystic tissue in the latter technique involves using the laser to reduce inflammation and speed up the healing (29).

Once the alveolar socket is prepared, the treated tooth is gently inserted in an axial direction using digital pressure. In case of resistance to the replanting procedure, some authors have suggested utilizing the pressure of the patient's bite to insert the original socket (30, 31). The splinting phase is controversial; in fact, several methods and different types are reported in the literature, including a splint with orthodontic wire, with composite resin or with sutures (27). The removal times vary accordingly; some cases may require removing the splint after seven-ten days, others after three-four weeks.



Figure 1 Keywords with the number of articles identified.

Keywords	n°
Intentional Replantation	263
Intentional Replantation Procedure	179
Intentional Replantation Techniques	175
Intentional Replantation Case-Report	163

The aim of this work was to analyze and discuss, through a review of the literature, the surgical procedures of intentional replacement described in the literature by different authors, to report the success rates of the technique and to describe what are the main causes of treatment and, in addition, provide an explanatory case report.

Review

A review of articles that have reported case reports dealt with the intentional re-implant technique was performed.

Eligibility criteria

Inclusion criteria

-Type of study: case report or case series published between 1996 and 2021 about Replantation Techniques, which explained the cause of treatment and outcome.

-Type of population: studies performed in human subjects using permanent teeth as a study unit.

-Type of intervention: studies using Replantation Techniques as a treatment modality.

Studies have been excluded when the cause of treatment and outcome was not specified.

Search strategy

The research was conducted independently by three authors, G.S., A.I. and G.S. This bibliographical search has been carried out through four databases, PubMed, Scopus, EMBASE and Google Scholar for gray literature, using as "Intentional Replantation" keywords, "Intentional Replantation Techniques", "Intentional Replantation Case-Report", "Intentional Replantation Procedure" (Figure 1).

Articles updated from 1996 to December

1st 2021, were included. Each reviewed article was evaluated using a ten-question data extraction form to identify the type of study, the sex and age of the patients, the teeth treated, the extra-alveolar time of the elements, the techniques used and the time of follow up. In case of disagreement between the authors in evaluating the included studies, the majority's evaluation was considered (two authors out of three). The overlaps were removed using EndNote. The authors categorized all the cases according to age, patient sex, age, type of tooth re-implanted, cause of treatment, extra-alveolar tooth-time, outcome and healing time.

Statistical analysis

A descriptive analysis measured the relationship between studied factors, follow-up period, and outcomes of the procedure. The outcomes of the Replantation Technique "successful " or " failure " of each factor were recorded as dichotomous data. Were reported: the percentage of subjects divided by sex, the mean age of the population, the percentage of items treated and the causes of treatment, the success rate and the mean follow-up time

Results

Seven hundred eighty articles related to the keywords entered were selected from a first search. Of these, 128 were excluded as duplicates or full-text not available. Of the remaining 652 items, 130 were considered eligible, but 77 were excluded because the full-text analysis did not show clinical cases covered with the intentional re-implant technique. At the end of the screening, 43 articles were definitively included (Figure 2).

Forty-three articles were included as com-





Figure 2

Flow chart depicting the article selection process. *Exclusion criteria: studies have been excluded when the cause of treatment and outcome was not specified.

> patible with relevant inclusion criteria, with a total of 82 clinical cases covered with the technique of intentional replantation. Successively, a statistical analysis of the results was carried out.

> The sample consisted of 82 subjects, of which 35 males (42.7%) and 47 females (57.3%) (Table 1). The age varied from seven to 86 years, with an average age of 35.8 years.

The total number of teeth on which the technique of the re-implant intention was practised is 86. Most of the treated teeth were the upper right central incisors (11.7%) and the first mandibular right molars (11.7%). Following the second mandibular right molars (9.3%) and the second mandibular left molars (9.3%). Central maxillary left incisor (8.1%), first

mandibular left molars (8.1%), lateral maxillary left incisors (4.6%), first maxillary right premolars (4.6%), and first mandibular left premolars (4.6%) were processed in these percentages (Figure 3). The cause that led to the implementation, in 67 cases, is represented by the presence of periapical pathology (77.90%); 16 teeth have been re-implanted to treat crown-root/ root fractures (18.6%); 18 due to an endodontic failure (20.93%) of which four presented a perforation (22.2%) and five with the presence of an intracanal fractured instrument (27.7%). Ten elements presented a condition of periodontitis (11.6%), five teeth showed resorption (5.81%). The intentional replantation technique also treated a case of parenthesis and a development anomaly with fused teeth.



Figure 3 Distribution of the treated dental elements.



In 55 articles, the extra-alveolar time of the treated teeth was reported, which is an average of 13.44 minutes.

The treatment success was reported in 75 replanted teeth, with a successful percentage of 87.2% with an average follow-up time of 26.8 months. The following review was limited by the high number of articles in the literature that deal with reimplantation. Furthermore, another challenge was that the conservation method of the teeth was not considered in the data collection.

Authors	Gender	Age	Tooth	Cause	Extra-alveolar tooth-time	Outcome	Healing time
Tang 1996 (32)	Male	29 yr	3.6	latrogenic perforation of the furcation	-	success	17 months
Poi 1999 (33)	Male	30 yr	4.5	Instrument separation/root perforation	-	success	8 years
Aqrabawi 1999 (34) Case 1	Female	46 yr	3.7	Endodontic failure/periapical disease	15 min	success	5 years
Aqrabawi Case 2	Female	38 yr	3.7	Endodontic failure/periapical disease	20 min	success	5 years
Benenati 2003 (35)	Female	45 yr	4.7	Pain	-	success	16 years
Fariniuk 2003 (36)	Male	11 yr	1.1	Crown-root fracture	-	success	3 years
Ward 2004 (37)	Female	68 yr	3.4	Apical periodontitis	15 min	success	18 months
Shintani 2004 (38)	Male	7 yr	3.1	Periapical disease with coronal fracture	10 min	success	5 years
Peer 2004 Case 1 (5)	Male	47 yr	3.5	Periapical lesion	-	success	30 months
Peer 2004 Case 3	Male	70 yr	3.2	Periapical disease/sinus tract	-	success	4 years
Peer 2004 Case 4	Male	40 yr	3.7	Periapical disease/sinus tract	-	questionable	7 years
Cotter 2006 (39)	Female	47 yr	3.1	Periapical lesion	5 min	success	1 years
Herrera 2006 (40)	Female	56 yr	4.6	Periapical disease/endodontic failure	30 min	success	14 years

Table 1 Clinical case results included in the review

>>> To be continued on the next page



Table 1 Clinical case results included in the review

Authors	Gender	Age	Tooth	Cause	Extra-alveolar tooth-time	Outcome	Healing time
Penarrocha 2007 (41)	Female	20 yr	2.6	Odontogenic maxillary sinusitis	5 min	success	2 years
Sivolella 2008 (42)	Male	9 yr	1.2	Double tooth	20 min	success	6 years
Wang 2008 (43)	Female	8 yr	1.1	Complicated crown-root fracture	15 min	questionable	3 months
Al-Hezaimi 2009 (44)	Female	15 yr	1.2	Pulp necrosis with suppurative apical periodontitis	-	success	4 years
Ozer 2010 (45) Case 1	Male	36 yr	1.1 1.2	Vertical root fracture	(1.1) 12 min (1.2) 16 min	success	2 years
Ozer Case 2	Female	25 yr	2.2	Vertical root fracture	18 min	success	2 years
Ozer Case 3	Male	32 yr	1.3	Vertical root fracture	24 min	success	2 years
Hsiang Lu 2011 (46)	Male	50 yr	4.6	Periapical disease	13 min	success	3 months
Unver 2011 (47)	Female	41 yr	1.4	Vertical fracture	25 min	success	36 months
Kim 2011 (48) Case 1	Female	23 yr	1.1 2.1 2.2	Complicated crown-root fractures	-	failure success success	90 months
Kim 2011 Case 2	Female	27 yr	2.1	Complicated crown-root fracture	-	success	24 months
Moura 2012 (49)	Female	11 yr	1.1	Complicated crown-root fracture	-	failure	2 years
Dogan 2013 (50)	Female	9 yr	2.1	Complicated crown-root fracture	28 min	success	3 years
Shin 2013 (51)	Male	39 yr	4.6	Apical periodontitis	17 min	success	9 months
Yuan 2013 (52)	Female	11 yr	2.1	Complicated crown-root fracture	-	success	3.5 years+
Nagappa 2013 (53) Case 1	Female	18 yr	1.1	Severe periodontitis	-	questionable	3 months
Nagappa Case2	Male	24 yr	2.1	Severe periodontitis	-	success	14 months
Moradi Majd 2014 (54)	Female	44 yr	3.5	Periapical disease/necrotic	-	success	1 year
Subay 2014 (55)	Female	45 yr	4.3	Periapical disease/instrument separation	14 min	success	24 months
Asgary 2014 (56) Case 1	Male	25 yr	4.6	Periapical disease	14 min	success	23 months
Asgary Case 2	Male	45 yr	3.4	Periapical disease	10 min	success	30 months
Asgary Case 3	Male	41 yr	4.7	Periapical disease	8 min	success	24 months
Asgary Case 4	Male	23 yr	4.6	Periapical disease 12 min		success	15 months
Asgary Case 5	Female	46 yr	4.7	Periapical disease 8 min		success	27 months
Asgary Case 6	Female	31 yr	4.7	Periapical disease	9 min	success	12 months
Asgary Case 7	Female	30 yr	1.4	Periapical disease 10 min failur		failure	18 months
Asgary Case 8	Female	36 yr	3.6	Periapical disease	13 min	success	14 months

SIE

Table 1 Clinical case results included in the review

Authors	Gender	Age	Tooth	Cause	Extra-alveolar tooth-time	Outcome	Healing time
Asgary Case 9	Male	48 yr	4.7	Periapical disease	14 min	Success	16 months
Asgary Case 10	Female	24 yr	4.6	Periapical disease	14 min	Success	8 months
Asgary Case 11	Female	43 yr	2.6	Periapical disease	14 min	Success	17 months
Asgary Case 12	Male	34 yr	3.4	Periapical disease	12 min	Success	15 months
Asgary Case 13	Female	29 yr	3.6	Periapical disease	10 min	Success	11 months
Asgary Case 14	Male	63 yr	3.6	Periapical disease	14 min	Success	12 months
Asgary Case 15	Male	31 yr	1.7	Periapical disease	13 min	Success	10 months
Asgary Case 16	Female	46 yr	4.6	Periapical disease	14 min	Success	8 months
Asgary Case 17	Female	40 yr	4.6	Periapical disease	12 min	questionable	8 months
Asgary Case 18	Female	27 yr	4.7	Periapical disease	13 min	Success	20 months
Asgary Case 19	Female	41 yr	3.6	Periapical disease	10 min	Success	12 months
Asgary Case 20	Male	37 yr	4.7	Periapical disease	10 min	Success	9 months
Asgari 2014 (57)	Female	28 yr	1.4 1.5	Periapical disease	8 min	success	2 years
Penarrocha Diego 2014 (58)	Male	51 yr	1.7	Follicular cyst	30 min	success	12 months
Tsesis 2014 (59)	Female	20 yr	4.7	Paraesthesia	8 min	success	4 years
Keceli 2014 (60)	Female	20 yr	3.2	Severe periodontitis	6 min	success	15 months
Pruthi 2015 (61)	Male	28 yr	1.1	External root resorption	15 min	success	18 months
Forero-Lopez 2015 (62)	Male	25 yr	1.2	Apical periodontitis	8 min	success	3 months
Garrido 2016 (63)	Female	50 yr	1.1	Endo-parodontal disease	4 min	success	1 year
Oishi 2017 (64)	Male	7 yr	1.1	Trasverse root fracture/endo-perio disease	-	success	5 years
Grzanich 2017 (65) Case 1	Female	64 yr	3.1	Apical periodontitis/separated instrument	-	success	28 months
Grzanich Case 2	Male	35 yr	1.4	Periapical disease/endodontic failure	-	success	2 years
Grzanich Case 3	Female	86 yr	1.8	Apical periodontitis/vertical root fracture	-	success	2 years
Asgari 2018 (66)	Female	22 yr	4.6	Apical periodontitis	7 min	success	2 months
Krug 2019 (67)	Male	37 yr	1.1	External cervical resorption	12 min	success	2.5 years
Cunliffe 2020 (68) Case 1	Male	33 yr	4.1	Instrument separation/root perforation	15 min	success	6 months
Cunliffe Case 2	Female	45 yr	3.4	Periapical disease with missed anatomy	15 min	questionable	3 months



Table 1Clinical case results included in the review

Authors	Gender	Age	Tooth	Cause	Extra-alveolar tooth-time	Outcome	Healing time
Cunliffe Case 3	Female	52 yr	4.6	Periapical disease with over-filled	15 min	failure	3 months
Cunliffe Case 4	Female	57 yr	4.4	Periapical disease/pain	4 min	success	1 year
Cunliffe Case 5	Female	42 yr	3.6	Periapical disease	-	success	3 months
Cunliffe Case 6	Male	64 yr	2.1	External root resorption	15 min	success	4 months
Cunliffe Case 7	Female	76 yr	3.7	Periapical disease with sclerosed canals	-	failure	1 year
Cunliffe Case 8	Male	53 yr	3.7	Pulpar floor perforation	-	success	3 months
Cunliffe Case 9	Male	50 yr	2.1	Internal root resorption	-	success	15 months
Cunliffe Case 10	Female	64 yr	3.7	Instrument separation	15 min	success	6 months
Cunliffe Case 11	Female	45 yr	3.7	Periapical disease with over-filled	-	success	28 months
Cunliffe Case 12	Male	45 yr	4.5	Periapical lesion	-	success	9 months
Cunliffe Case 13	Female	39 yr	3.6	Periapical lesion with procedural errors	-	failure	3 months
Asgary 2019 (69)	Female	28 yr	3.7	Periapical lesion/endodontic failure	10 min	success	1 year
Fujii 2020 (70)	Female	30 yr	1.6	Instrument separation	15 min	success	1 year
Ganapathy 2020 (71)	Male	10 yr	2.1	Complicated crown-root fracture	-	success	2 years
Yang 2021 (72)	Male	20 yr	1.5	Chronic apical abscess with internal root resorption and root fracture	15 min	success	2 years

Clinical case

A 52 years old male patient was presented to our clinic with pain associated with the anterior mandibular area. No abnormality was observed upon traditional radiographic examination (Figure 4). On the contrary, the lingual side of central incisors was associated with swelling at clinical examination. A 3D cone beam computed tomography (CBCT) radiographic examination was performed, and a root fracture in the apical third of tooth 3.1 was observed (Figure 5). Tooth 3.1 did not respond to the vitality test. The diagnosis of the tooth was necrosis caused by the apical fracture.

The treatment of choice was non-surgical root canal treatment performing mechanical shaping, 3D cleaning and 3D obturation (Figure 6). Nevertheless, the swelling was persistent one week after the treatment, so the new treatment plan was decided in the form of intentional replantation.

The IR was chosen as the substitute treatment plan because an endodontic surgery could not be done due to an unfavourable crown-root ratio.

After tooth extraction, the fractured root fragment was removed, and the retro preparation was completed without removing any other mm of the root. This phase was done rapidly with the aid of magnification, light, and an ultrasonic tip. After retro preparation, the retro cavity was cleaned with Ethylenediaminetetraacetic acid (EDTA) and Sodium hypochlorite Intentional Replantation



Figure 4, 5 Pre-operative CBCT radiographic evaluation.

Figure 6, 7 Post-operative CBCT radiographic evaluation. (NaOCl). Afterwards, the retro cavity was dried and filled with white mineral trioxide aggregate (MTA), and the tooth was replanted and fixed to the other teeth with composite resin. After ten days, the composite was removed, and a one-year follow up showed the tooth was without swelling, pain or mobility (Figure 7).

Discussion

Despite the many indications reported in the literature, intentional replanting is considered a last resort to maintain natural teeth (1). The numerous operating steps provide various opportunities for mishaps, which therefore make this procedure highly dependent on the operator. These factors justify the diversity of success rates in the literature ranging from 80 to 100% (73). A recent systematic review of the literature found an 88% success rate for intentionally replanted teeth (74). The success rate concluded from our study is 87.2%. Therefore it is in accordance with that reported in the literature. This data is closely related to the extra-alveolar time of the re-implanted element, which has been seen to be a determining factor as it is directly involved in the conservation of PDL cells (22, 23). The extra alveolar time varies considerably in the different studies: from a maximum of 30 minutes (Penarrocha Diego et al., Herrera et al.) (40, 58) to a minimum of 4 minutes (Cunliffe et al., Garrido et al.) (63, 68). Jang et al. (75) reported higher success rates for those elements replanted with an extra-alveolar time of fewer than 15 minutes compared to those replanted with extra-alveolar time greater than 15 minutes. High success rates have also been reported in cases where the extra-alveolar time was greater than 15 minutes. This data should be associated with the approaches of extra-alveolar preservation of the teeth. As reported by a study by Andreasen in 1981, it is more promising in a humid environment (such as water, physiological solution, saliva) than in a humid environment (such as water, physiological solution a dry environment. (76) In all the reports we reviewed, which showed an extra-alveolar time greater than 15 minutes, the teeth were stored in a moist rather than dry environment to preserve the viability of the PDL cells.

Intentional re-implantation has a large number of clinical indications such as apical periodontitis, (2-4) in cases of improper endodontic therapy, (3) inaccessible external root resorption, (6) root perforations, (7) root/coronal-root fractures complex, (7-9) teeth with developmental anomalies such as fused teeth. (11) In the current review, a periapical lesion was associated with a fracture of the apical segment of the root that could not be treated with a surgical endodontic approach. The cone beam radiographic investigation played a fundamental role in the diagnosis as based on a conventional 2D examination, no fracture lines or discontinuities between the root tissues were observable.



CBCT has only recently been introduced to assist in surgical planning, and there are only case reports in the literature that report the implementation of the planning phase with the use of 3D reconstructions (77). Modern technologies such as microscopy, ultrasound, CBCT (78) and the latest generation biomaterials (79-81) can improve treatment and minimize extra alveolar time. Therefore, encouraging results have been reported, and in particular, it has been highlighted that surgical re-implantation can be shorter and less invasive and with a lower percentage of failures (82).

Conclusions

The choice of the line of treatment to be adopted must be built on the basis of the characteristics of each clinical case. Based on this review, a success rate of 87.2% of teeth treated with the replantation technique was found, confirming the high reproducibility of the treatment. Furthermore, this success can be guaranteed by the reduced extra-alveolar storage time of the replanted tooth, which, in the prior revision, is on average 13.44 minutes. In conclusion, if performed correctly, the replantation with standardized procedures that consider the basic biological principles represents a therapeutic choice with a high percentage of success and is highly predictable.

Clinical Relevance

Intentional re-implantation represents a valid therapeutic alternative in cases where definitive endodontic treatment is ineffective. With this review, we intend to analyze and evaluate the predictability of this method in the cases reported in the literature so as to provide the clinician with a complete view of the technique and the outcome associated with it.

Conflict of Interest

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

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REVIEW ARTICLE

Regenerative endodontic treatment in mature teeth: a systematic review and meta-analysis

ABSTRACT

Regenerative endodontic treatment (RET) is an alternative treatment for immature teeth, however, its efficacy on mature teeth is still controversial. This review was aimed to assess the level of evidence of clinical and radiographical outcomes of RET in mature teeth and run a meta-analysis to compare its success rate to conventional root canal treatment (CRCT). The electronic databases PubMed, Science Direct and Web of Science were used to search based on inclusion and exclusion criteria. The Randomized controlled clinical trials (RCTs), case series, and case reports studies of the RET in mature teeth published in the English language from January 2010 till December 2021 were selected. A meta-analysis was performed using the random-effects model on the randomized clinical trials that compare the success rate based on clinical and radiographic outcomes of RET and CRCT. From sixteen articles included in the narrative analysis, two studies were subjected to meta-analysis. Different protocol aspects of RET including disinfection, size of apical preparation, intracanal medications, types of scaffolds, barriers and follow-up periods were described. The meta-analysis showed no significant differences in success rate between CRCT (89.47%) and RET (95.45%) at 12 months (P>0.05), while it showed a significant increase in a positive response to the electrical pulp test of RET (P=0.010). With the limitations, the adopted protocols of RET are comparable to CRCT and could be a potential approach to treat mature teeth with pulp necrosis and/or apical periodontitis. However, providing more evidence is essential to ascertain these findings.

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Introduction

egenerative endodontic treatment (RET) is a biologically-based procedure aimed to replace damaged structures, including dentin and root structures along with cells of the pulp-dentin complex (1). The modern interest in the RET concept originated from the revascularization capacity of luxated or avulsed immature teeth with open apices providing ideal decontamination conditions (2). The outcome of RET in permanent immature teeth manifested successful restoring of pulp functions and stimulating normal physiological development of the root (3, 4).

Generally, conventional root canal treatment (CRCT) is the standard care for permanent mature teeth with necrotic pulp and apical periodontitis while the incidence of large periapical lesions may require surgical removal. The success rate of CRCT ranged between 68% to 85% in the last 4 to 5 decades (5). The main goal of CRCT is to eliminate clinical signs/ symptoms and resolve periapical lesions (6). Evidently, the American Association of Endodontics specified the same goal of CRCT as a primary objective for RET, while increased thickening of the root walls or root length and regained pulp vitality are secondary and tertiary goals respectively (7). Whilst the primary goal is an objective for both endodontic treatments, the secondary goal is beneficial for immature teeth to minimize potential root fractures caused by thin and/or weak instrumented root walls. The tertiary goal could be measured as a desirable goal which is possibly not essential to determine the clinical success of RET due to uncertain response of sensibility tests that may encounter false negative or false positive response (8).

Recently, RET has been investigated to treat permanent mature teeth with necrotic pulps and/or apical periodontitis (9, 10). Unlike CRCT, the apical third is commonly over instrumented and apical foramen is enlarged to remove apical ramifications and bacterial load within root canals (11). Subsequently, no obturation material is used in RET and as an alternative, the root canal is filled with biological scaffolds such as blood that is induced from the apical area manually by extending the file to the periapical area, or autologous platelet-rich/ poor plasma or collagen with/without hydroxyapatite or platelet-rich fibrin which may be combined with the stem cell.

RET is controversial in mature teeth due to the risk of recurrent infections through the non-obturated root canal and flare-up that might outweigh the benefits of regenerative treatment, counting complete root formation that is redundant in mature teeth where the root walls are thick and the apex is closed. Definite scientific evidence of the beneficial effects of this treatment in mature teeth should be provided before proposing RET as an alternative treatment. Therefore, this systematic review aimed to assess the level of evidence of clinical and radiographical outcomes of RET in mature teeth and run a meta-analysis to compare its success rate to conventional root canal treatment (CRCT).

Review

The protocol of this systematic review was registered in PROSPERO (CRD42020215802) (12) and followed the PRISMA statement (13). PubMed (National Library of Medicine), Science Direct (Elsevier), Web of Science core collection (Clarivate Analytics) were searched for relevant articles, published in the English language, from January, 2010 to December, 2021. This was supplemented by Manual searches in the reference lists of reviews and included studies to identify publications that might have been missed during the electronic database searches. The search terms (Appendix 1) used are mature permanent tooth/teeth, mature tooth/teeth, mature necrotic pulp, mature non-vital tooth/teeth, apical periodontitis, periapical lesion, regenerative endodontics, pulp regeneration, tooth/ pulp revascularization, pulp revitalization, non-obturation endodontic, root canal therapy. The Boolean operators



'AND' and 'OR' were employed to combine the keywords and generate the search strategy.

Inclusion criteria and study selection

Clinical studies that assessed the efficacy of RET in mature necrotic permanent teeth with or without apical periodontitis were included. While studies on the animal, laboratory, reviews, and clinical studies of immature permanent teeth were excluded.

The primary outcome was the success rate of the RET assessed by the absence of clinical signs and symptoms (pain, swelling, inflammation, and probing), and radiographic finding (changes in periapical lesion and root canal walls). The secondary outcome was teeth response to the sensibility/vitality test which could be an indicator of vital tissue presence (14).

Title and abstract screening followed by full-text assessment were undertaken by two independent reviewers. Any disagreement was resolved by discussion and consensus. Data were extracted in standardized tables by both reviewers. A kappa score of >0.80 was observed between them on the various domains of data extraction.

Data extraction

Relevant data were extracted following the Cochrane Handbook for Systematic Reviews of Interventions guidelines (15) which consisted of study and participant characteristics (Table 2), types of intervention and comparator (Table 3), and primary outcome measures (table 4).

Risk of bias and quality assessment

The quality assessment was assessed according to the study design. The revised Cochrane Risk of Bias Tool for Clinical Randomized Trials (RoB 2.0) and risk of bias because of the randomization process, deviations from the intended interventions, missing outcome data, measurement of the reported result, and overall bias were appraised to classify the selected studies into a low risk of bias, some concerns, and a high risk of bias (16). The Joanna Briggs Institute (JBI, University of Adelaide) tools were used to assess the quality of case reports (17) and case series (18). Evaluation parameters of the case reports were as follows; a clear description of the patient's demographic characteristics, case history, current clinical condition, assessment method, intervention, post-intervention condition, adverse effects, and lessons provided by the case report. The parameters of the case series were as follows; clear criteria for participants' inclusion, measuring the condition in reliable, standard and valid method, consecutive inclusion of participants, complete inclusion of participants, clear reporting of clinical information, outcomes, site clinic demographic and appropriate statistical analysis. For each parameter in both types of mentioned studies, the included articles could be awarded a "yes", "no", "unclear" or "not applicable". The overall quality of each case report and case series were allocated into three categories as follows: (i) low risk of bias (met at least 75% of the criteria), (ii) moderate risk of bias (met between 50%) and 74% of the criteria), (iii) high risk of bias (met less than 49% of the criteria) (19).

Data analysis

Statistical analysis was performed using Review Manager (RevMan, Version 5.4., Cochrane Collaboration, 2020). The outcome of interventions with direct comparison was analysed using proportion (%) for the primary outcome and Yes/No for the secondary outcome. The risk ratio (RR) with a 95% confidence interval (CI) was used to evaluate the association between the incidence of success and treatment type (RET and CRCT). Heterogeneity was tested using I² statistic. Fixed-effects model was used for low/moderate heterogeneity while the random-effect model was applied for significant heterogeneity ($I^2 \ge 50\%$).

Review data: study selection

Figure 1 illustrates a flow diagram on the selection, inclusion, and exclusion of studies according to PRISMA. The search yielded 1172 hits; 1152 hits without duplicates were screened; 27 were relevant



and obtained in full text. Subsequent full article screening excluded an additional 8 references (20-27). The reasons for exclusion are presented in Table 1.

Eventually, the remaining 19 studies (6, 9, 10, 14, 28-42) were included and subjected to data extraction, methodologic quality assessment, and data synthesis. From these included studies, 2 were involved in Meta-analysis.

Characteristics of the included studies

11 case reports (9, 30-38, 40), two case series (6, 10), one single armed clinical study with no control (14) and five randomized clinical trials (RCTs) (28, 29, 39, 41, 42) were involved in the current review with a total of 222 patients, 76.1% of them had RET. The age of the patient varied from 9-76 years old. Female gender was prominent with 66 patients (51.6 %) compared to 62 Male patients (48.4%). The maxillary central incisor was the most involved tooth (82.1%) of all treated teeth (single-rooted teeth and mandibular first molar). The aetiology of pulp necrosis was

Table 1 Excluded studies with reasons of exclusion Study ID Reason of exclusion

Study ID	Reason of exclusion
Chrepa, 2015 (21)	RET was not done completely it was initiated only to evaluate whether evoked bleeding from the periapical tissues elicits the influx of MSCs into the root canal system in mature teeth with apical lesions. After that, the root canal was filled through conventional Root canal therapy
Santiago, 2015 (24)	Studies were involved a young immature tooth
He, 2017 (23)	Review of previously published cases and no new case was presented
Gaviño Orduña, 2017 (22)	The trauma occurred when the tooth was immature with no history of tooth complete development earlier
Song, 2017 (25)	The studies involved immature teeth
Timmerman and Parashos, 2017 (26)	Teeth involved have open apices with no history of tooth complete development earlier
Al Khasawnah, 2018 (20)	Calcium hydroxide-iodoform-silicon oil paste (CHISP) as temporary canal filler and Pulpdent with Gutta-percha were used as permanent canals filler instead of regenerative induction
Zaky, 2020 (27)	In-vivo study involved animals

mainly trauma followed by failed previous endodontic treatment, crown fracture and caries. The cases were diagnosed as asymptomatic apical periodontitis (39%), symptomatic apical periodontitis (22%), acute apical abscess (17.1%), chronic apical abscess (14.6%), and avulsed tooth, chronic pulpitis and symptomatic irreversible pulpitis (2.4% each). Internal root resorption was diagnosed in one case (38) and root perforation in another (35). Radiographical evidence of periapical lesions was detected in approximately 166 teeth (98.8%) that have Periapical Index ≥ 2 . The avulsed tooth (32), mid-rooted fracture (35) and chronic pulpitis (37) cases were associated with no periapical lesions (Table 2).

Quality assessment and ROB

Three RCTs (28, 29, 39) were assessed as low risk whereas two had some concerns (41, 42). One clinical study and one case series were assessed as low risk (6, 14) whereas one case series (10) was presented a moderate risk of bias. Although two of 11 case reports (38, 40) have some concern regarding clear describe of the patient's history, overall bias was low risk (>75%) (9, 30-38, 40) (Figure 2).

Treatment protocol

1) Disinfection: the main irrigant in all cases was 1-6% sodium hypochlorite (NaOCl). Collectively, 139 cases (82.2 %) used 17% EDTA (6, 9, 14, 28-31, 34, 37-40, 42), whereas 18 cases (10.7%) used unspecified antimicrobial solution following the NaOCL (10). Additionally, triantibiotic solution was used before NaOCl in 3 cases (1.8%)(40). 36 cases (21.3%) used the Endoactivator system (39). The 3 cases (1.8%) used 10 ml of chlorhexidine gluconate irrigation (31) (Table 3).

2) Size of apical preparation was varied based on root canal diameter and operator judgment. Apical preparation of the maxillary central incisors was ranged from 0.30 mm up to 1mm using either hand, rotary or reciprocal files (6, 9, 14, 28-31, 33, 34, 37, 38, 41). For maxillary lateral incisor and premolars, the apical preparation was ranged from 0.30 to 0.60 mm (6, 30, 31, 33, 38, 41) whereas apical prepaGIE

	Table 2	2	
Characteristics	of the	included	studies

Study ID	Study design	Cases Number	Age	Gender	Tooth involved	Aetiology of pulp necrosis	Diagnosis	Preopera- tive periapical lesions
Shah and Logani, 2012 (10)	Case series	18	15-76	11 M & 7 F	Not specified	Not specified	Acute or chronic apical abscess	Yes or No
Paryani and	Case report	2	14	F	Incisor # 8	Uncomplicated crown fracture	Symptomatic apical periodontitis	Yes
Kim, 2013 (9)	Case report	2	11	F	Incisor #9	Uncomplicated crown fracture	Asymptomatic apical periodontitis	Yes
Saoud, 2014			23	F	Incisor #8	Trauma for 15 years ago	Acute apical abscess	Yes
(33)	Case report	2	23	F	Incisor # 7	Trauma at since years ago	Symptomatic apical periodontitis	Yes
.			48	F	Premolar 29	Previously treated pulp	Acute apical abscess	Yes
Nevins and Cymerman,	Case report	3	40	F	Incisors #8, 9	Previously treated pulp	Acute apical abscess	Yes
2015 (31)			28	F	Incisor #8	Previously treated pulp	Symptomatic apical periodontitis	Yes
Saoud, 2015	Casa raport	2	26	М	Incisor #9	Trauma 10 years ago and previously treated tooth	Acute apical abscess	Yes
(34)	Case report	2	12	М	Molar #19	Previously treated 17 months ago	Chronic apical abscess	Yes
Wang, 2015 (36)	Case report	1	39	F	Premolars #20, 29	Fractured dens evaginatus	Symptomatic apical periodontitis	Yes
Priya, 2016 (32)	Case report	1	11	М	Incisor # 9	Trauma	Avulsed tooth	NA
Saoud, 2016 (6)	Case series	4	11-21	2F & 2M	Incisors # 8, 9, 8, 25 & Molar #30	Trauma and caries	Chronic and acute abscess	Yes
Cooud 2016			15	М	Incisors #8	Trauma	Symptomatic irreversible pulpitis	Yes
Saoud, 2016 (35)	Case report	2	16	М	Incisors# 8	Trauma	Acute apical abscess and perforating root resorption	Yes
Kaval, 2017 (38)	Case report	1	14	М	Incisors #10	Not stated	Symptomatic apical periodontitis and internal resorption root	Yes
Xu and Zhou, 2018 (37)	Case report	1	15	F	Premolar #13	Caries	Chronic pulpitis	No
Nagas, 2018 (30)	Case report	1	21	F	Incisors #9, 10	Trauma 7 years ago	Symptomatic apical periodontitis	Yes
Nageh, 2018 (14)	Clinical Study	15	18-40	No gender preference (F>M)	Central incisors	Caries	Symptomatic or asymptomatic apical periodontitis	Yes or No
Jha, 2019 (42)	Randomized Clinical Trial	30 (15RET & 15 CRCT)	9-15	No gender preference	Not specified	Not stated	Periapical periodontitis	Yes
Arslan, 2019 (28)	A Preliminary Randomized Clinical Study	46 (26 RET & 20 CRCT)	18-30	CRCT (13M, 7F). RET (22M, 4F)	Anterior & premolar (single root) #7, 8, 9, 10, 11, 24, 25, 26, 27, 28	Not stated	Symptomatic or asymptomatic apical periodontitis. Acute and chronic abscess	Yes
El-Kateb, 2020 (29)	Randomized Clinical Trial	18 (Control: test is 1:1)	20-40	Control (3M & 6F) Test (4M & 5F)	Incisors #7, 8, 9	Trauma (n = 13) and Defective restoration (n =5)	Asymptomatic apical periodontitis and 4 teeth with chronic apical abscess	Yes

>>> To be continued on the next page



Table 2 Characteristics of the included studies								
Brizuela, 2020 (39)	Randomized Clinical Trial	36 (CRCT; control: RET; test is 1:1)	16-58	CRCT (13F & 5M) RET (12F & 6M)	Maxillary or mandibular incisors/canines & mandibular premolars	Not stated	Symptomatic or asymptomatic apical periodontitis. Acute and chronic abscess	Yes
Feitosa, 2021 (40)	Case report	3	18-40	No gender predilection	Premolar (single root) #35,15,25	Not stated	Irreversible pulpitis or pulp necrosis	Yes
Mittal, 2021 (41)	Randomized Clinical Trial	36	16-34	No gender predilection	Maxillary anterior, mandibular anterior and posterior teeth	Not stated	Pulp necrosis	Yes or No

Conventional root canal treatment; REI

ration of molars mesial and distal canals was reached the maximum of 0.30 mm and 0.40 mm respectively (6, 34). Moreover, the preparation was confined to the coronal pulp canal on top of the fracture line with no apical preparation in the case of horizontal root fracture (35). Controversy, massive apical preparation was done to avulsed tooth up to 2 mm (32) (Table 3). 3) Number of visits. Treatment of 137 (81.1%) cases were completed in two visits (9, 28-31, 34, 36, 37, 39, 41, 42). Whereas 20 (11.8%) cases were accomplished in three visit (6, 33, 38), other four cases (2.4%) in one visit (32, 40) and one case (0.6%) in 4 visits (34) and 7 cases (4.1%) in 2-3 visit (10, 35) (Table 3).

4) Medicament material. Ca(OH), was only used in 43 (25.4%) cases (6, 28, 29, 34, 38, 39) or combined with antibiotic in 4 cases (2.4%) (9, 35). Triple antibiotic (metronidazole, ciprofloxacin and minocycline or clindamycin) was used in 30 (17.8%) cases (30, 33, 35, 36), mixture of metrogyl, ciprofloxacin and tetracycline paste in 33 (19.5%) cases (10, 42), metronidazole with ciprofloxacin in 55 (32.5%) cases (14, 31, 33, 41), ciprofloxacin powder in 1 (0.6%) cases (9) and doxycycline solution (before replanted) in 1(0.6%) case (32) (Table 3). Scaffold used and coronal barrier materials. The scaffold used was mainly a blood clot (58.6%) (6, 10, 28-30, 33-35, 37, 38, 41, 42) followed by 2% calcium chloride with Platelet-Poor Plasma plus umbilical cord Mesenchymal stem cell (10.7%) (39). Platelet-rich fibrin (14.2%) (14, 41), collagen with or without hydroxyapatite (10.7%) (9, 31, 41), platelet-rich plasma (1.2%) (36) and auto-transplantation of the pulp (1.8 %) were also utilized. MTA or Biodentin were the main coronal barrier material to be used for the majority of the cases (Table 3).

5) The follow-up period ranged from 1 to 60 months. In four RCTs studies (28, 29, 39, 41), which represents 66.9% of the regenerated cases, the follow-up periods were accomplished within 12 months. Additionally, one RCT was pursued till 18 months (42), while in case series (6, 10) and case report (9, 30-38) it reached up to 2.5-3 years roughly. The longest follow-up period was approximately 5 years (60 months) (14) (Table 3).

Clinical and radiographical outcomes of RET

Failure was reported in 4 cases (2.4%) with clinical signs and symptoms persistent though one case showed healing radiographically by reducing the size of the lesion (28). 165 (97.6%) cases were assessed as success clinically and radiographically with no signs and symptoms associated with the periapical lesion healing or completely healed at the end of follow-up time. 3.6% revealed deposition of hard



		erative Endodon		Medicaments			
Study ID	Visits	Irrigants	Apical preparation	material	Scaffold used	Barrier	Follow-up
Shah and Logani (10)	2-3	2.5% NaOCI and Antimicrobial solution	2-4 file sizes larger than the master apical file at working length	TAP (metrogyl, ciprofloxacin and tetracycline)	Blood	A calcium sulfate- based cement	6 months recall till 3 years for 5 cases, 2 ½ years for 5 cases 2 years for 5 cases and 6-months for 3 cases
Paryani and Kim (9)	2 visits with 1 week interval for tooth # 8- and 22-days intervals for tooth #9 respectively	5.25% NaOCI followed by 17% EDTA	The apical foramen was enlarged up to 0.6 mm with a # 60 K-file	Calcium hydroxide for tooth #8 Ciprofloxacin powder for tooth #9	Blood + Collacote (Absorbable Collagen)	МТА	1 month, 2 months, 1 year and 3 months, 22 months for tooth#8 1 month, 5 months and 18 months for tooth #9
Saoud (33)	3 visits with 1 week and 2 weeks intervals respectively	2.5% NaOCI followed by sterile saline solution	Instrumented to a #100 and #35 hand K- file to the WL for cases #1 and #2 respectively	TAP (metronidazole 500 mg + ciprofloxacin 200 mg + minocycline100 mg mixed with sterile saline solution)	Blood	MTA	6 months and 1 year
*Nevins and Cymerman (31)	2 visits with 1 month interval	6% NaOCI followed by 17% EDTA 2% chlorhexidine gluconate 10 MI (case 1) I & D was done on a tooth with buccal swelling	Working length was determined radiographically with #60 or #70 K-file	Ciprofloxacin and metronidazole mixed in equal amounts	Blood+ SynOss putty	MTA (case1) Bioceramic Putty (2 cases)	3-month intervals for 1 year for 2 cases and 6 months for 1 case
Saoud (34)	2 visits with 2 weeks' intervals (case #1) 4 visits and intervals of 1 week and 1 month and a half respectively (case 2)	2.5% NaOCI irrigation Saline solution and then irrigated with 17% EDTA	The canal was debrided to hand #60 K-files to the WL (case 1) Instrumentation of the canawasre done to sizes 30 in mesial and 40 in distal (case 2)	Metapaste	Blood	МТА	7 and 13 months forcases 1 and 8- and 14-months case 2
Wang (36)	2 visits with 2 weeks intervals	20 mL 2.5% NaOCI followed by 20 mL saline for each canal	Not stated	Ciprofloxacin, metronidazole, and minocycline (0.1 conc. mg/mL)	Autologous PRP	MTA	8 and 30 months
#Priya (32)	1 visit	Normal saline and 5.25% NaOCI	Root apex was enlarged to approximately 1.5-2 mm	Teeth were placed in doxycycline solution for about 15 to 20 minutes and replanted and stabilized	Autologous PRP	GIC	2 week, 2, 3, 6, 9 and 12 months
Saoud (6)	3 visits with 2 weeks interval	2.5% NaOCI irrigation	ProTaper Universal Rotary files to F5 (#40) for teeth #8 and #9, F3 (#30) for tooth #25, F2 (#25) for mesial canals and F4 (#35) for distal canals of tooth #30	Metapaste (calcium hydroxide)	Blood	MTA	ranged from 8-26 months
Saoud (35)	2 or 3 visits with 2 weeks interval	2.5% NaOCI solution followed by sterile saline solution and 17% EDTA solution	The coronal canal was debrided to #50 K-files. (case1) Gates-Glidden # 2 for the resorptive area of the canal in perforating case (case 2)	Calcium hydroxide Metapaste (case 1) TAP (case 2) (metronidazole, ciprofloxacin, and minocycline)	Blood	МТА	5,8,14 and 19 months for case 1 8, 15 and 19 months for case 2

 Table 3

 Regenerative Endodontic treatment (RET) protocol of the included studies

>>> To be continued on the next page



	Regen	erative Endodon	tic treatment (I	RET) protocol of	the included st	udies	
Kaval (38)	3 visits with 4 weeks and 3 months' intervals respectively	1% NaCL followed by 17% EDTA and distilled water	K files #80 for the root canal coronal to the resorptive area and # 45 for apical canal	Calcium hydroxide	Blood	MTA	6 months and 2 years
Xu and Zhou (37)	2 visits with 14 days interval	5.25% NaOCI followed by 17% EDTA	The apical foramen was enlarged up to 0.6 mm with a # 60 K-file	A ciprofloxacin and metronidazole	Blood	MTA	3, 6 , 12 and 30 months
Nagas (30)	2 visits with 28 days interval	20 mL of 5.25% NaOCI, followed by 10 mL of saline and then with 17% of EDTA	Not stated	TAP (ciprofloxacin, metronidazole, and clindamycin)	Blood	MTA	1-month, then every 6 months for 60 months
Nageh (14)	2 visits with 21 days interval	1.5% NaOCI. 20 mL 17% EDTA followed by saline irrigation	Apical canal preparation to K-file #60–80	metronidazole and ciprofloxacin mixed with saline	Blood+ PRF	MTA	Every 3 months up to 1 year
Jha (42)	2 visits with 1 or 2 weeks intervals	2.5% NaOCI and final rinse with 17% EDTA	Rotary protaper universal files were used and apical widening was done with K-files #25-30	ТАР	Blood	Calcium sulfate- Based cement (Cavit G)	6, 12, 18 months
Arslan (28)	2 visits with 21 days interval	5 mL of 1% NaOCI followed by 2 mL 5% EDTA and 5 mL distilled water	The root canal was enlarged using reciprocating nickel- titanium files ((#25 and #40) and stainless steel (#45- #80) hand files	CRCT group: calcium hydroxide REP Group: TAP	Blood	White MTA	12 months
El-Kateb (29)	2 visits	20 mL 1.5% NaOCI followed by a final rinse with 20 mL 17% EDTA for about 1 minute	Rotary instrumentation of the canals was performed with PTN files until sizes X3 (test group) and X5 (control group)	Calcium hydroxide	Blood	Biodentin	1, 3, 6, 9 and 12 months
Brizuela (39)	2 visits in 21 days interval	20 ml 2.5% NaOCI and Endoactivator system followed by 20 ml 17% EDTA	Selected Reciproc files	Calcium hydroxide	Blood+ PPP + UC MSCs +an absorbable gelatine sponge haemostat	Biodentin	6 and 12 months
Feitosa (40)	1 visit	TAP solution (ciprofloxacin, minocycline, and metronidazole followed by sterile saline and 17% EDTA for 5 minutes	Rotary files (WaveOne Gold)	None	pulp autotransplantation from extracted third molar	Biodentin	3, 6, 9, 12 months
Mittal (41)	2 visits with 2 weeks intervals	20 mL of 1.5% NaOCI) and 10 mL of saline	K-files #60-80 for maxillary anterior teeth, #30 for mandibular anterior and posterior teeth	Metronidazole and ciprofloxacin paste	Blood/PRF/ collagen/ hydroxyapatite- collagen (Four groups separately)	Biodentin	3, 6, 9, 12 months

 Table 3

 Regenerative Endodontic treatment (RET) protocol of the included studies

PPP (Platelet-Poor Plasma), UC MSCs (umbilical cord Mesenchymal stem cells), MTA (white mineral trioxide aggregate), SynOss (collagen hydroxyapatite scaffold), PRP (Platelet Rich Plasma), PRF (Platelet Rich Fibrin), GICs (Glass ionomer cements). *Amoxicillin 500 mg (4x1x10) was prescribed in the 1st visit, #Patient was given Doxycycline 100 mg (2x1x7) was prescribed in the 1st visit.

tissue and narrowing the root canal space (31, 33, 38). Thickening of the root canal walls was evident in 3.6% (33, 34, 37, 38). Regaining the tooth sensibility using electrical pulp test (EPT) was demonstrated in 51 (30.2%) cases (9, 14, 28, 29, 32, 37, 39, 40). Interestingly, 36 (21.3%) cases responded positively to cold test with no response to heat or EPT (41) (Table 4).

Meta-analysis

The pooled data of the two RCTs compared RET to CRCT at 12 months follow-up (28, 39) showed no significant differences in 6

Table 4Clinical and radiographical outcomes

Study ID	Signs & Symptoms	Sensibility and vitality	Periapical lesion	Root canal wall
Shah and Logani (10)	Tissue healing was excellent clinically	Not mentioned	Complete resolution or decrease in the size with increase in bone density	Increase cementum density radiographically
Paryani and Kim (9)	Asymptomatic with Probing depths ≤3 mm in one	Normal response to Endo-Ice and EPT (34 of 80) in first case and no response in the 2nd case	Complete resolution	Thinning of the apical one-third of the root canal in one of two cases
Saoud (33)	Asymptomatic	No response	The pulp cavity appeared to be obliterated by hard tissue formation in the apical portion	Thickening of the canal walls and closure of the apex
Nevins and Cymerman (31)	Asymptomatic	No response	Continuous healing	Radiopacity develops within the coronal and middle third of the root canal
Saoud (34)	Asymptomatic	No response	Complete healed	Thickening of the canal walls and the apex appeared to have closed
Wang (36)	Asymptomatic	No response	Continuous healing	No evidence of thickening in the root canal or root lengthening
Priya (32)	6 months: Symptomatic 12 months: Asymptomatic	Positive response to thermal and EPT	At 6 months: evidence of internal resorption with periapical radiolucency. At 9 and 12 months: resolution of periapical radiolucency	At 6 months: external root resorption and space were observed At 9 and 12 months: slight evidence of replacement resorption
Saoud (6)	Asymptomatic	No response	28.5% of teeth: complete healed. 71.5% of teeth: reduce in size	Not stated
Saoud (35)	Asymptomatic	No response	Not stated	Formation of hard tissue between fragments in horizontal root fracture
Kaval (38)	Asymptomatic	No response	Significant healing	Increase in root canal wall thickness with remineralization in the perforated resorptive area and between the coronal and root pulp tissue
Xu and Zhou (37)	Asymptomatic	Gradually regained pulp sensibility and responded positively to the electric pulp tester	No periapical lesion	Root wall thickening
Nagas (30)	Asymptomatic	No response to cold or EPT	Complete resolution	The dimensions of the root space had remained unchanged
Nageh (14)	Asymptomatic	60% of the patients regaining sensibility gradually to reach the highest level at 12 months	Complete healed	Not stated
Jha(42)	Asymptomatic	Not stated	13 Complete healed and 2 healing for RET	Not stated
Arslan (28)	Asymptomatic teeth are 80% in CRCT group and 92.3% in REP group	50% of REP-treated teeth responded positively	Absence and reduction of the radiolucency in 85% of CRCT and 92.4% of REP with	Not stated
El-Kateb (29)	Asymptomatic	66.7% in the X3 group and 88.9% in the X5 group had gradually regained the sensibility to reach the highest level at 12 months	Periapical healing was enhanced in all cases	The apical thirds of the canal increased from its baseline values to reach the highest values at the 3-months which became approximate to the normal contralateral tooth
Brizuela (39)	At 6 months: 5.6% of REP group had percussion pain.	Positive response to cold (56%) and heat (28%) and EPT (50%)	No Significant changes in cortical involvement and dimensions of apical lesions	Significantly median anteroposterior reduction of 0.35 mm in CRCT group and 0.94 mm in the REP group
	At 12 months: both groups had 100% efficacy			Provb
Feitosa (40)	At 3 months, slight twinges at the periapical region with no response to EPT At 6 months and 1 year, asymptomatic	Positive response to EPT at 6 months and revascularization evidence by Doppler imaging at 1 year	Complete regression of periapical lesions for patients 1 and 2 whereas the radiolucency in patient 3 was almost entirely diminished	Not stated
Mittal (41)	Asymptomatic and swelling and sinus tract had resolved completely	Positive response to cold test at 12 months with no response to heat or EPT	Periapical healing and resolution of apical periodontitis	Not stated

EPT (electrical pulp test



Figure 1

Literature search and screening according to PRISMA flow diagram on selection, inclusion, and exclusion of studies at each screening stage. clinical and radiographical outcomes (P>0.05). While it showed a significant increase in positive response to electrical pulp test (EPT) in favouring of RET (I²=9%; risk ratio; 3.97 95% CI: 1.39-11.30, P=0.010) (Figure 3).

Discussion

RET is built on the principles of regenerative medicine and tissue engineering and aimed to treat immature permanent teeth with pulpal necrosis by regenerating



*The study design is single armed clinical study with no control, authors found IJB tool for case series was suitable to th quality assessment.

Figure 2

Quality assessment results of RCTs studies according to the revised Cochrane Risk of Bias Tool for Randomized Trials (RoB 2.0), case series according to the Joanna Briggs Institute tool and case reports according to the Joanna Briggs Institute too.



Figure 3 A forest plot of the clinical and radiographical success success of RET and CRCT at 12 months.

functional pulpal tissue applying protocols (43). Hence, researchers elected to find out more the efficacy of RET on permanent mature teeth (9, 10). To our knowledge, the first study that reported regenerative endodontic treatment for mature teeth was published in 2012 (10). This systematic review aimed to search with an earlier time frame to diminish the risk of missing any study to be included.

The majority of studies in this systematic review were case reports which represented the lowest levels of causation evidence due to the inherent bias (44), Therefore, the level of evidence of RET outcomes from these groups was considered low. However, these studies are the most commonly published articles in medical journals (45). Furthermore, the existing literature lacks RCTs that compare the RET to CRCT within standard treatment protocol, follow-up and reporting methods to reduce the heterogeneity. Thus out of five RCTs, only two studies were included in the meta-analysis. The other studies lack of comparator of CRCT (29, 41) or the findings at 12 months were not reported clearly (42).

Meta-analysis showed no significant difference in clinical and radiographical success rate between the RET and CRCT. This could be attributed to the disinfection protocol of the root canal area which is a crucial step in both treatments and the key to successful outcomes. It was reported that the main cause of CRCT failure was the persistence or occurrence of intraradicular or extraradicular infections (46) and failure of coronal barrier or seal (47, 48). Likewise, failure of RET was attributed to inadequate root canal disinfection (28) besides the loss of coronal restoration that instigates reinfection (32). Disinfection of the root canal is attained through a combination of mechanical debridement and irrigation along with intracanal medicaments (if required) to disrupt biofilms on the infected canal walls (49, 50). A low concentration of NaOCl (1.5%) followed by 17% EDTA was recommended during RET of immature tooth (7) to reduce the cytotoxic effect of NaOCl on the apical papilla stem cells which is essential for RET (51, 52). According to the findings, 1-6% NaOCl was the main irrigant used since mature teeth have closed apices confining the irrigant to the canal space so a high concentration of NaOCl might lack an adverse effect on stem cells survival (53). Mechanical root dentin debridement is not recommended in immature teeth as it increases the risk


of their thin root fracture (54), while it is required in fully mature teeth with a thick root to remove infected dentin especially at the apical third of the root.

Apical preparation was followed by apical foramen widening to different sizes, based on the tooth type and the operator judgment. The successful clinical and radiographic outcomes were demonstrated in the majority of the cases which might be attributed to blood-borne and apical papilla stem cells small size (10-100 µm) that allow them to enter the canal from the periapical area through small size orifice (54). In view of that, the size of the foramen could have no significant effect on the treatment outcome. On the other hand, apical foramen enlargement is contraindicated in CRCT due to the risk of pushing necrotic debris and microorganisms into the periapical tissues and triggering periapical inflammation (55).

The root canal was filled with obturation materials and sealers in the CRCT, whereas biological active host vital tissues were obtained by inducement in the RET. Interestingly, the periapical lesion can heal without root canal fillings if the intracanal bacterial load is effectively reduced (56). This concept could explain the success rate of the regenerated cases despite different protocols applied. Prominently, leaving empty root canals is not a professional standard of care since it could allow re-infections of the root canal. Particularly with the widening procedure of the apical foramen in RET which may facilitate the apical leakage if the proposed biological tissue sealing is failed.

Blood clot alone or in combination with growth factors and/or Mesenchymal stem cells were used as filling in RET to induce the regeneration process. The blood clot was successfully leading to pulp regeneration (10, 28, 29). However, executing the bleeding technique only in RET of mature teeth might have limitations compared to those in immature teeth due to the smaller quantity of stem cells in the former, thus the implementation of PRP/MSCs in RET of the mature tooth was recommended (57, 58). A marked difference in periapical healing and dentinal wall thickening of

teeth and growth of pulp-like tissue were reported in some cases treated by revascularization with PRP and cell-based approach in different studies (32, 39). Only one study compared the success rate of RET based on the type of scaffold has been founded and it reported the efficacy of all scaffolds is comparable for clinical and radiographical outcomes however, positive response to cold was the highest with the PRF, followed by the collagen, hydroxyapatite and blood scaffolds (41). The findings of this review suggested that blood clot alone or in combination with growth factors were effective scaffolds. Furthermore, using blood scaffolds could be more practical and requires no chair-side time and effort in term of growth factors preparation. Follow-up time is a fundamental factor in clinical studies as the degree of the success rate of any treatment may change over time (59). The follow-up time in this review varied according to patients' commitment with a minimum period of 6 months (10) and a maximum of 60 months (14). It was stated that most CRCT failures occurred within 3 years of treatment (60), however, RET failure occurred at least 1-2 years from initiation of treatment (61). This is in an agreement with the recommended follow-up period for RET in immature teeth by the American association of endodontic (7).

The secondary outcome of RET assessed in the current review is regaining the pulp sensibility/vitality. In the current review, approximately 50% of the cases have a positive response to the sensibility test. This is in accordance with the percentage of a positive response in immature teeth (62). Sensibility tests are not directly related to the pulp vitality but it depends on subjective response to an external stimulus to the nervous system (63). Some histological studies reported that the vital regenerated tissues in immature teeth with apical periodontitis treated by RET were cementum-like or bone-like tissues (64, 65). Alternatively, the researchers have confirmed the presence of vascularized pulplike tissue in the mature tooth after RET by using doppler laser flowmetry (DLF) which is the best marker assessing pulp

Appendix 1

Search strategies for regenerative endodontic treatment for permanent mature teeth with pulp necrosis

permanent teeth) OR (Mature permanent tooth)) OR (mature teeth)) OR (mature tooth)) OR (mature necrotic teeth)) OR (mature necrotic tooth)) OR (mature non-vital teeth)) OR (mature non-vital tooth)) OR (apical periodontitis)) OR (periapical lesion)) OR (apical lesion)) OR (closed apex)) OR (closed apices)) AND (Endodontic regeneration)) OR (regenerative endodontics)) OR (pulp regeneration)) OR (pulp revascularization)) OR (pulp revitalization)) OR (regenerative endodontic therapies) OR (regenerative endodontics procedures) OR (tooth revascularization) OR (non-obturation endodontic treatment) AND (Endodontics) OR (Root Canal Therapy).

vitality through evaluating the vascular supply (39).

The findings of this review suggested that the positive response the to pulp sensibility test following RET could indicate the presence of a vital tissue (14) which is not necessary to be a pulp tissue (54). The negative response of pulp sensibility does not necessarily indicate a lack of vitality as it could be a sequence of false-negative and/or the deep extension of coronal barrier material into the root coronal portion (33). To the best of our knowledge, no histologic findings in mature teeth with necrotic pulp after RET have been reported yet and more evidence are needed to verify the type of tissue formed.

High heterogeneity between studies, the use of different treatment protocols, short follow-up periods, and lack of data in some included studies were among the limitations of this review. However, the findings of this review can be beneficial for guiding researchers and clinicians to explore a new approach for root canal treatment of permanent mature teeth and do more research on it. To sum up, more RCTs that have similar treatment protocol and case selection criteria with large sample size and long-term follow-ups comparing RET and CRCT had better to be established. This could increase the level of evidence that assesses both practitioners and patients to make treatment selection decisions.

Conclusions

With the limitation of this review, it appears that the adopted protocol of RET is comparable to CRCT and could be a potential approach to treat mature teeth with pulp necrosis and/or apical periodontitis. However, providing more evidence is essential to ascertain these findings.

Clinical Relevance

RET has a satisfactory clinical and radiographical outcome in necrotic pulp mature teeth with or without apical periodontitis however, the selection of the case to be treated should be based on solid evidence and agreement of the patient.

Conflict of Interest

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

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None.

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ari Soci e Amici, questa mia lettera si dividerà in due parti.
La prima rivolta alla nostra Rivista mentre la seconda al nostro primo evento in presenza del 2022, lo Spring Meeting.

Partiamo dalla rivista. L'anno scorso la mia prima lettera del GIE ha voluto fare il punto della situazione sul GIE stesso: oggi possiamo affermare che il profondo processo di cambiamento che ha interessato la rivista sta portando importanti risultati. Del resto il cambiamento è la chiave di lettura di questa crescita costante: evoluzione e adattamento alle nuove condizioni del "mercato" sono al centro di questa metamorfosi.

Dopo aver alzato l'asticella dobbiamo lavorare all'unisono affinché si possa oltrepassare l'ostacolo. In pratica dobbiamo concentrare i nostri sforzi su due aspetti principali:

1. esortare universitari, ricercatori e clinici all'invio di articoli scientifici strutturati come *review, original article e clinical trial;*

2. citare in bibliografia gli articoli del GIE su articoli di altre riviste internazionali.

Stimolate, preparate e fate sottoporre articoli. La cultura passa attraverso i testi e le riviste scientifiche. Questo mio appello è rivolto soprattutto ai giovani che troppo spesso credono di trovare nella semplice fruibilità dei social risposte esaustive a tutti i quesiti. La "cultura dei social", se non sostenuta da basi conoscitive solide, impoverisce il dibattito scientifico, portandoci inesorabilmente a una sorta di lenta schiavitù edulcorata.

Concludo questa prima parte ricordando una frase di Norman Doidge "Il cervello plasma la cultura, la cultura plasma il cervello".

Veniamo ora alla Società. Il 2022 si è aperto all'insegna della ripartenza in presenza, con il nostro Spring Meeting che ha visto la prestigiosa partnership con SIdP. L'obiettivo culturale sicuramente è stato centrato: approfondire le strette connessioni tra Endodonzia e Parodontologia in un contesto di fattiva collaborazione. Oggi, con un sempre più spiccato approccio multidisciplinare dei piani di lavoro dei pazienti, trovare delle connessioni con altre discipline è sempre più importante. Non è possibile affrontare l'Endodonzia e le altre discipline come se fossero dei compartimenti stagni. Da qui l'intento da parte nostra di stringere collaborazioni sempre più strette con le più importanti Società Scientifiche del nostro settore. Per questo motivo lavoriamo affinché l'evento di primavera arrivi, nei prossimi anni, a rappresentare un momento di incontro e condivisione imperdibile per tutti i soci.

Non mi resta che augurarvi una buona lettura!

Un caro saluto Roberto Fornara Presidente SIE

Juny

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SOCIO AGGREGATO

Per avere lo status di Socio Aggregato si dovrà presentare la documentazione descritta nel sito www.endodonzia.it che sarà valutata dalla Commissione Accettazione Soci. La documentazione che verrà presentata dovrà mostrare con rigore, attraverso casi clinici, l'interessamento del candidato alla disciplina endodontica.

Un meccanismo a punti è stato introdotto per valutare l'ammissibilità del candidato allo "status" di Socio Aggregato: i punti saranno attribuiti in base al tipo di documentazione presentata. Possono accedere alla qualifica di Socio Aggregato tutti i Soci Ordinari della SIE, in regola con le quote associative degli ultimi tre anni, che completino e forniscano la documentazione alla Segreteria Nazionale (Via Pietro Custodi 3, 20136 Milano) entro i termini che verranno indicati all'indirizzo web: www.endodonzia.it.

La domanda dovrà essere firmata da un Socio Attivo, in regola con la quota associativa per l'anno in corso, il quale è responsabile della correttezza clinica e formale della documentazione presentata.

DOCUMENTAZIONE NECESSARIA PER DIVENTARE SOCIO AGGREGATO

Qualsiasi Socio Ordinario, con i requisiti necessari, può presentare la documentazione per ottenere la qualifica di Socio Aggregato. Un meccanismo a punti è stato introdotto per valutare il candidato: un minimo di 80 punti è richiesto per divenire Socio Aggregato.

La documentazione clinica per ottenere la qualifica di Socio Aggregato dovrà presentare almeno sei casi, di cui non più di tre senza lesione visibile nella radiografia preoperatoria e non più di uno di Endodonzia Chirurgica Retrograda.

Nella domanda non potranno essere presentati casi la cui somma superi i 120 punti per la qualifica di Socio Aggregato.

L'aspirante Socio Aggregato potrà presentare la documentazione clinica in più volte, con un minimo di 40 punti per presentazione, in un arco massimo di tre anni. Il mancato rinnovo della quota associativa, anche per un solo anno, annulla l'iter di presentazione dei casi.

SOCIO ATTIVO

Per avere lo status di Socio Attivo si dovrà presentare la documentazione descritta nel sito www.endodonzia.it che sarà valutata dalla Commissione Accettazione Soci. La documentazione che verrà presentata dovrà mostrare con rigore, attraverso documentazione scientifica e casi clinici, l'interessamento del candidato alla disciplina endodontica.

Un meccanismo a punti è stato introdotto per valutare l'ammissibilità del candidato allo status di Socio Attivo: i punti saranno attribuiti in base al tipo di documentazione clinica e scientifica presentata. Possono accedere alla qualifica di Socio Attivo tutti i Soci Ordinari della SIE, in regola con le quote associative degli ultimi tre anni, che completino e forniscano la documentazione alla Segreteria Nazionale (Via Pietro Custodi 3, 20136 Milano) entro i termini che verranno indicati all'indirizzo web: www.endodonzia.it.

La domanda di ammissione allo status di Socio Attivo rivolta al Presidente della SIE dovrà essere firmata da un Socio Attivo in regola con la quota associativa per l'anno in corso, il quale dovrà aver esaminato e approvato la documentazione. Quest'ultimo è responsabile della correttezza clinica e formale della documentazione presentata.

DOCUMENTAZIONE NECESSARIA PER DIVENTARE SOCIO ATTIVO

Qualsiasi Socio Ordinario, con i requisiti necessari, può presentare la documentazione per ottenere la qualifica di Socio Attivo. Il Socio Aggregato che volesse presentare la documentazione scientifica e clinica a integrazione di quella clinica già approvata dalla CAS per lo status di socio Aggregato, potrà farlo già dall'anno successivo all'ottenimento della sua qualifica.

Un meccanismo a punti è stato introdotto per valutare il candidato a Socio Attivo. Un minimo di 200 punti è richiesto per divenire Socio Attivo.

Nella domanda non potranno essere presentati casi la cui somma superi i 240 punti per la qualifica di Socio Attivo. La documentazione scientifica potrà essere presentata, a completamento della documentazione clinica, solo per la domanda per divenire Socio Attivo e non potrà superare i 80 punti.

La documentazione clinica dovrà presentare un minimo di sei casi, di cui almeno 4 di molari pluriradicolati con delle precise tipologie: tra questi casi almeno uno deve essere un ritrattamento con lesione visibile nella radiografia preoperatoria e dei restanti tre almeno due devono avere una lesione visibile nella radiografia preoperatoria.

La documentazione clinica non deve presentare più di un caso di Endodonzia Chirurgica Retrograda con immagini e non più di uno senza immagini.

La documentazione scientifica non potrà presentare più di due articoli come coautore.

MODALITÀ DI DOCUMENTAZIONE DEI CASI CLINICI

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



CRITERI DI VALUTAZIONE

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

ADEMPIMENTI DEL CANDIDATO

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

PRESENTAZIONE DEI CASI ALLA COMMISSIONE

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

LA COMMISSIONE ACCETTAZIONE SOCI

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

MEMBRI DELLA COMMISSIONE ACCETTAZIONE SOCI BIENNIO 2021-2022

Francesco Riccitiello (Past President della Società) Maurizio Boschi Marco Colla Claudia Dettori Giuseppe Multari

The new Endo Solution: Performance Unlimited with ProTaper Ultimate



ProTaper Ultimate is the fourth generation of Dentsply Sirona's flexible endo-file system with ProTaper-Ultimate-Absorbent-Point, AH-Plus-Bioceramic, Protaper-Ultimate-HandUse File and ProTaper-Ultimate-Gutta Percha (top down).

With advances in endodontic treatment, dentists enable patients to keep their natural teeth for many years, ideally for a lifetime. This makes it even more important to have a complete set of products that can help ensure treatment success from root canal treatment to restoration. Since its first introduction 20 years ago, each generation of ProTaper from Dentsply Sirona has stood for a trusted, reliable and predictable preparation method that enables excellent clinical outcomes for patients worldwide. With ProTaper Ultimate, Dentsply Sirona is now introducing a technologically driven fourth generation system. The treatment concept is based on three pillars: shaping, 3D cleaning, and filling root canal systems. The standard sequence of the shaping pillar includes three file types; namely, slider, shaper and finishers. These feature specific files are designed to safely prepare a full range of endodontic anatomy. Novel auxiliary files are available for larger and straighter canals and all files are available to prepare canals mechanically and/or manually.

About Dentsply Sirona

Dentsply Sirona is the world's largest manufacturer of professional dental products and technologies, with over a century of innovation and service to the dental industry and patients worldwide. Dentsply Sirona develops, manufactures, and markets a comprehensive solutions offering including dental and oral health products as well as other consumable medical devices under a strong portfolio of world class brands. Dentsply Sirona's products provide innovative, high-quality and effective solutions to advance patient care and deliver better and safer dental care.

Visit www.dentsplysirona.com for more information about Dentsply Sirona and its products.

The ProTaper Ultimate Solution offers extensive options: the cleaning pillar is focused on eliminating pulp, bacteria, debris, and breaking down products, when present. To facilitate cleaning, a highly flexible, polymer canula with a bifid apical flow channel can safely deliver irrigant deep into the apical portion of the preparation. With a fluid-filled canal, a reagent of the clinician's choice can be exchanged into both the instrumentable and uninstrumentable portion of the root canal space, utilizing the novel SmartLite Pro EndoActivator, which will be launched in the coming months. The SmartLite Pro platform offers three attachments; namely, the SmartLite Pro curing light, the transilluminator, and the EndoActivator. Finally, the obturation pillar will enable dentists to successfully fill and seal the root canal system thanks to predictable shaping and cleaning. The Pro-Taper Ultimate Solution also includes dedicated gutta-percha master cones based on the unique Conform Fit concept and technology. Jointly used with a Conform Fit gutta-percha master cone, the new AH Plus Bioceramic Sealer will help ensure filling root canal systems where the sealer bonds to the cleaned dentin walls to ensure healing.

«In endodontics, the files themselves have primarily set the agenda in the past», explains Greg Sheehan, Vice President Global Endodontics Procedure at Dentsply Sirona. «With the ProTaper Ultimate Solution, we are now focusing more on the entire treatment process, in which all steps and details are precisely coordinated with each other. This simplifies the treatment itself, saves time and gives the user certainty that each procedural step will be implemented successfully».



CeraSeal

Quality and ergonomics for simple and predictable root canal fillings



Courtesy of Dott. Filippo Cardinali

Role and aims of root canal obturation

Complete filling of the endodontic space combined with an airtight seal of the foramen are essential prerequisites for a quality root canal obturation and represent the goal the clinician must aspire to when performing the obturation.

Pre-mixed bioceramic cements for root canal fillings have been in use in clinical practice for more than 10 years and their use is becoming increasingly popular due to their characteristics. The absence of shrinkage and the interaction with the canal walls during the hardening reaction allow the clinician to achieve the obturation goals using cold gutta-percha techniques, which are easier and faster to perform than hot techniques.

Ceraseal: ergonomics and safety

Ceraseal is a pre-mixed calcium-silicate bioceramic cement that can be easily applied inside the canal with disposable tips: the absence of powder-liquid mixing phases means that the cement components are in the ideal percentages, eliminating the risk of contamination during preparation and insertion of the cement into the canal.

Ceraseal has a high radiopacity that makes it clearly visible on post-operative X-rays. During the setting reaction, high pH values are reached, giving Ceraseal a powerful antibacterial action.

Hermetic Seal

Ceraseal requires moisture to start the setting reaction, which results in a chemical bond between the bioceramic cement and the dentin of the canal parts. This chemical reaction also occurs within the dentinal tubules where Ceraseal can penetrate due to its low particle size, resulting in a high-quality hermetic seal that prevents bacteria from percolating into the canal. The ability to harden in a moist environment makes Ceraseal the cement of choice when complete drying of the endodontic system is not possible for anatomical reasons.

Flowability and Stability

The high fluidity allows Ceraseal to penetrate even unshaped spaces such as isthmuses or lateral canals and fill the endodontic system three-dimensionally. Ceraseal does not contract or expand: this unique stability is the basis for its use with cold gutta-percha techniques such as single cone, not to mention that it can also be used with conventional hot root canal techniques.

Biocompatibility and Bioactivity

Biocompatibility is certainly one of the most important features of Ceraseal: in case of accidental extrusion, Ceraseal does not interfere with the health status of healthy periapical tissues, nor does it interfere with healing processes in case of periapical lesions, promoting instead peri radicular bone regeneration.





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) <u>https://www. endodonzia.it/</u>

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 <u>www.</u> 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. <u>https://onlinelibrary.</u> 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

- Aim: give a clear statement of the main aim of the study and the main hypothesis tested, if any.
 Methodology: describe the methods adopted in-
- 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 <u>www.consort-statement.org</u> A CONSORT checklist and flow diagram (as a Figure) should also be included in the submission material.

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

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

Results should present the observations with minimal reference to earlier literature or to

possible interpretations. Data should not be duplicated in Tables and Figures.

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

Conclusions should contain a summary of the findings.

Main Text of Review Articles

should be divided into Introduction, Review and Conclusions.

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

Main Text of Mini Review Articles

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

Main Text of Case Reports and Case series

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

IMPORTANT TO KNOW

Manuscript that do not conform to the general aims and scope of the Journal will be returned immediately without review. All other manuscripts will be reviewed by experts in the field (generally two referees). Giornale Italiano di Endodonzia aims to forward referees' comments and to inform the corresponding author of the result of the review process. Manuscripts will be considered for fast-track publication under special circumstances after consultation with the Editor. Giornale Italiano di Endodonzia uses **double blinded review** which menas that the names of the reviewers will thus not be disclosed to the author submitting a paper and the name(s) of the author(s) will not be disclosed to the reviewers. To allow double blinded review, please submit your main manuscript and title page as separate files.

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

References. It is the policy of the Journal to encourage reference to the original papers rather than to literature reviews. Authors should therefore keep citations of reviews to the absolute minimum. Names of products and/or companies should not be added to references. To cite a product and/or company add the same inthe text, where mentioned.

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

References in the References section must be prepared as follows:

- more than three authors cite 3 authors et al. If the paper has only 4 authors, cite all authors;
- e.g. Prati G, Lotti M, Russo F et al.
- II. title style: please use a capital letter only for the first word of the title;
- III. journal titles mentioned in the References list should be abbreviated according to the following websites:
- a. ISI Journal Abbreviations Index (<u>https://www.library.caltech.edu/journal-title-abbreviations</u>);
- Biological Journals and Abbreviations (http://home.ncifcrf.gov/research/bja);
- Medline List of Journal Titles (<u>https://www.nlm.nih.gov/bsd/serfile_addedinfo.</u> html):
- IV. put year after the journal name;
- V. never put month and day in the last part of the references;
- VI. cite only the volume (not the issue in brackets):
- VII. pages have to be abbreviated, e.g. 351-8.

We recommend the use of a tool such as End-Note or Reference Manager for reference management and formatting. EndNote reference styles can be searched for here: <u>http://www. endnote.com/support/enstyles.asp.</u> To ensure the correct citation format, please check your references in the PubMed database (<u>http://</u> www.ncbi.nlm.nih.gov/pubmed).

Examples of correct forms of reference follow. *Standard journal article*

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

Corporate author

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

Journal supplement

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

Books and other monographs

Personal author(s)

Gutmann J, Harrison JW. Surgical Endodontics, 1st edn Boston, MA, USA: Blackwell Scientific Publications, 1991.

Chapter in a book

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

Published proceedings paper

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

Agency publication

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

Dissertation or thesis

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

URLs

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

Tables, Figures and Figure Legends

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

- color (saved as CMYK): minimum 300 dpi;
- black and white/grays: minimum 600 dpi;
- one column width (8.0 cm) or 1.5 column widths (13.0 cm) or 2 columns widths (17.0 cm).

A different **caption** for each figure must be provided at the end of the manuscript, not included in the figure file. Authors must obtain **written permission** for the reproduction and adaptation of material which has already been published. A copy of the written permission has to be provided before publication (otherwise the paper cannot be published) and appropriately cited in the figure caption. The procedure for requesting the permission is the responsibility of the Authors; *PAGEPress* will not refund any costs incurred in obtaining permission. Alternatively, it is advisable to use materials from other (free) sources.

Figure legends should begin with a brief title for the whole figure and continue with a short description of each panel and the symbols used; they should not contain any details of methods.

Authorship

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

- i) conception and design, or analysis and interpretation of data;
- ii) drafting the article or revising it critically for important intellectual content;

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

Obligation to Register Clinical Trials http://www.icmje.org/#clin_trials

The ICMJE believes that it is important to foster a comprehensive, publicly available database of clinical trials.

The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or concurrent comparison or control groups to study the cause-andeffect relationship between a medical intervention and a health outcome. Medical interventions include drugs, surgical procedures,



devices, behavioral treatments, process-ofcare e changes, etc.

Our journals require, as a condition of consideration for publication, registration in a public trials registry.

The journal considers a trial for publication only if it has been registered before the enrollment of the first patient.

The journal does not advocate one particular registry, but requires authors to register their trial in a registry that meets several criteria. The registry must be accessible to the public at no charge. It must be open to all prospective registrants and managed by a non-profit organization.

There must be a mechanism to ensure the validity of the registration data, and the registry should be electronically searchable. An acceptable registry must include a minimum of data elements.

For example <u>http://www.clinicaltrials.gov</u>, sponsored by the United States National Library of Medicine, meets these requirements.

Protection of Human Subjects and Animals in Research

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

When reporting experiments on ecosystems involving non-native species, Authors are bound to ensure compliance with the institutional and national guide for the preservation of native biodiversity.

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2. A complete version of the work and all supplemental materials, including a copy of the permission as stated above, in a suitable standard electronic format is deposited immediately upon initial publication in at least one online repository that is supported by an academic institution, scholarly society, government agency, or other well-established organization that seeks to enable open access, unrestricted distribution, interoperability, and long-term archiving.

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For authors

To make a submission to an OJS journal, after registering to the website, the authors will be required to follow a procedure via the system. Once the paper has been submitted, the authors will receive a confirmation email from the Managing Editor of the Journal.

When receiving a new submission, the Managing Editor assigns it to her/himself and to the Editor-in-Chief (EiC). After a quick inhouse evaluation, if the EiC thinks that the paper is compliant with the guidelines and fits with the scope of the Journal, he/she send it out for the **peer-review phase** (=he/ she assigns reviewers). Alternatively, the EiC can assign a Section/Deputy Editor for the paper.

Once the review process is completed (*i.e.* all the assigned Reviewers have provided

their comments and recommendations on the paper), the authors will be notified via email by the editors of the editorial decision: Accepted, Rejected, Decline Submission, Minor revisions, Major revisions.

Depending on the editorial decision, and basing on the reviewers' comments, authors are required to upload their revised version (+ covering letter) within a specific deadline. At this point, they simply need to wait to hear back from the editor as to whether the revisions are acceptable.

If the editor's decision is to resubmit for review (=Major revisions or Minor revisons), the revised paper may undergo a "second round" of peer-review.

Once a paper is accepted for publication, the authors will be notified via email and their paper is moved to the "Copyediting phase", where it is improved by the work of a copyeditor. Authors can be given the opportunity to review the copyedits.

Lastly, once the copyedits are completed and approved, the submission moves to "Production stage". In Production, the copyedited files are converted to galleys (PDF). Again, the authors have the opportunity to proofread the galleys. Once everyone is satisfied, the submission is scheduled for publication in a future issue.

The online journal management system that we are using allows authors to track the progress of their manuscript through the editorial process by simply logging into the Journal website.

Peer-review policy

All manuscripts submitted to our journal are critically assessed by external and/or in-house experts in accordance with the principles of peer review (http://www.icmje.org/#peer), which is fundamental to the scientific publication process and the dissemination of sound science. Each paper is first assigned by the Editors to an appropriate Associate Editor who has knowledge of the field discussed in the manuscript. The first step of manuscript selection takes place entirely in-house and has two major objectives: i) to establish the article appropriateness for our journals readership; ii) to define the manuscript priority ranking relative to other manuscripts under consideration, since the number of papers that the journal receives is much greater than it can publish. If a manuscript does not receive a sufficiently high priority score to warrant publication, the editors will proceed to a quick rejection. The remaining articles are reviewed by at least two different external referees (second step or classical peer review). Manuscripts should be prepared according to the Uniform Requirements established by the International Committee of Medical Journal Editors (ICM-JE) (http://www.icmje.org/org/#prepare).