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The value of Endodontic Sciences experts

Endodontics is a specialty taught with great rigor, criteria and scientific base in most schools of Dentistry. This fact stimulates dental surgeons to seek a postgraduate course in order to complement their formal education.

Among the wide variety of postgraduate courses in Endodontics available in Brazil, the pursuit of technical-scientific improvement has been one of the main targets. The great majority comprises scientific-based courses, but our practice reveals an avalanche of discussions that focus on a few technological resources. Perfect scientific-based endodontic knowledge is essential to establish the basis for the application of new technology. In many cases, the professional lacks knowledge on diagnosis, which will certainly affect other areas, starting with planning and therapeutic management. Professionals with psychomotor skills, who master the new technologies and scientific knowledge available, will certainly stand out from the crowd.

Scientific knowledge has been increasingly required, given that Endodontics establishes a strong relationship with other specialties, namely: Cosmetic Dentistry, Periodontology, Prosthesis, Surgery, Pathology, Radiology, Orthodontics, Pediatric Dentistry, among others. Seen in these terms, the specialist must cross the borders of his own space, which creates a need for broadening his knowledge.

Nevertheless, any information revolution brings change. The success of this specialty is of great value for health and is responsible for conducting changes in the professional's daily behavior with regard to technological innovations. In this context, the specialization courses must be urgently reviewed, better planned, discussed, structured and implemented.

Good endodontists are essential to Dentistry, since they occupy a privileged position of healthcare professionals who are highly respected by professionals of other areas. The professional of Endodontics is expected not only to have good formal education, but also to implement and correctly as well as coherently disclose the Endodontic Science.

Carlos Estrela
Editor-in-chief

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The presence of pus indicates bacteria at the site!

Alberto **CONSOLARO**¹

ABSTRACT

The presence of pus necessarily suggests bacterial contamination caused by staphylococcus and streptococcus. Interaction of neutrophils with these bacteria represents the mechanism of formation of pus in the human body. The presence of these bacteria can be analyzed and questioned as follows:

1. Were the bacteria already present prior to the surgical procedure? 2. Was the material previously contaminated by bacteria? 3. Was there any failure in the aseptic procedure? 4. Was there lack of oral hygiene in the postoperative phase? If the pus is formed, staphylococcus and streptococcus are present.

Keywords: Abscess. Bacteria. Pus. Contamination.

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The human body has ten trillion cells, but it also has ten times more bacteria (a hundred trillion). This impressive datum allows us to highlight the importance of the microbiota with which we have a close relationship. The presence of bacteria in our lives is essential to stimulate our defense mechanism.^{3,4,21,22} Initially, they are known as animalcules and share the environment with fungi, viruses and parasite.

The skin and mucosae, natural integument, act in our defense mechanism as a physical, chemical and biological barrier that prevents microorganisms from entering the internal environment. Connective tissue represents the internal environment and comprises the oral submucosa, the dermis and bone tissue.

When the skin is cut, lacerated, subject to surgery, puncture or other methods that cause loss of skin or mucosa continuity, the integuments are transposed, overcome or ignored and the bacteria found in the microbiota enter the underlying connective tissue. In the event of loss of skin or mucosa continuity, bacteria will inevitably enter the internal environment due to the large amount of opportunities that are offered.

After all, we have a close relationship, we share and we biologically balance ourselves with a great amount of bacteria present in our inner and outer surfaces. The human body is prepared for the bacteria that occasionally enter its internal environment, which occurs practically every day.

When bacteria enter the connective tissues, inflammation is immediately triggered, and the blood vessels allow many plasmatic substances, the exudate, as well as many leukocyte cells, known as the infiltrate, to go to the affected area (Fig 1).

Blood vessels exude plasmatic components

Antibodies or immunoglobulins are among the substances that immediately flow out the blood vessels after bacteria enter the organism. These products, along with other plasmatic components such as proteins and enzymes that altogether are known as the complement system, react in a specific manner and provoke mechanisms that destroy the bacteria comprising human microbiota.

How does the organism previously know that these specific bacteria will enter the internal environment? Every day, these bacteria enter or try to enter our organism. Every day, our immune system is stimulated to produce antibodies and other products specifically aimed at destroying them. This is the reason why the blood plasma has high levels of specific antibodies that fight against the bacteria present in the microbiota, of which the most predominant are the staphylococcus and streptococcus.

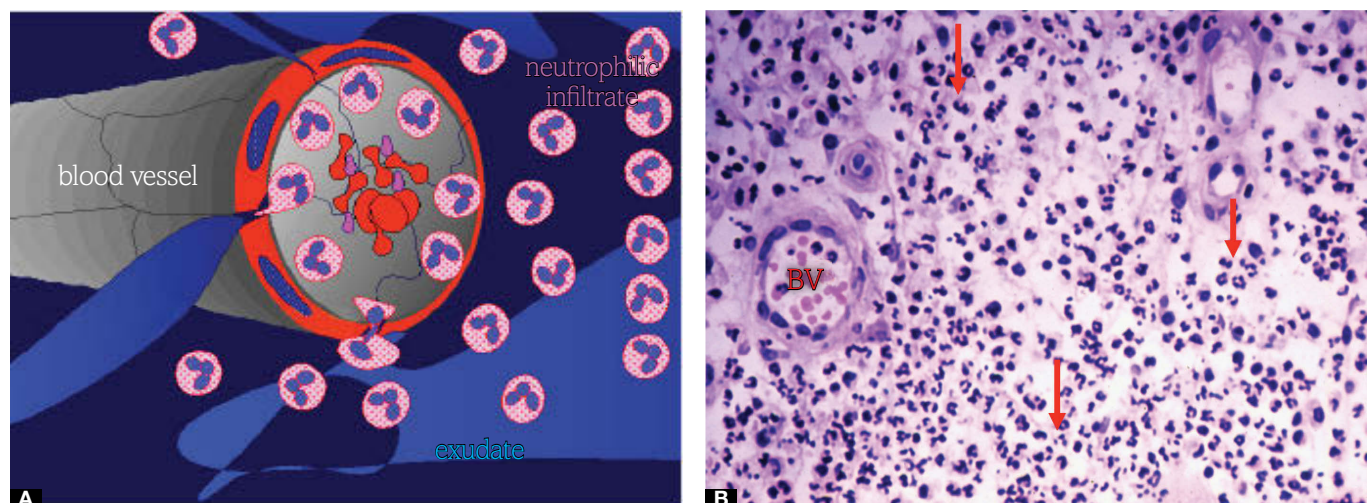


Figure 1. The neutrophils reach the attacked site through the vascular walls by means of leukodiapedesis (bent arrow in **A**) and predominate for 48-72h (smaller arrows in **B**). After 90 minutes, they interact with the bacteria and destroy them while releasing their lysosomal components. Should there be no bacteria at site, the neutrophils migrate or disappear by apoptosis and the site moves on to repair (**B** = H.E.; 160X).

The arrival of neutrophils and the formation of pus

Ninety minutes after any attack is caused to the connective tissues, for instance, when bacteria enter it, the first inflammatory cells that arrive in a considerable large number — almost exclusively within the next 48-72 h — are the neutrophils. The inflammatory, immune or defense cells are the leukocytes that continuously circulate in the blood (Fig 1).

The neutrophils are the most numerous leukocytes present in the blood (50-60%) and are specialized in phagocytizing bacteria, especially staphylococcus and streptococcus. Their function of interacting, phagocytizing and destroying these types of bacteria is related to the presence of many granules full of enzymes and other cytoplasmic powerful products (Fig 2). Other types of bacteria, such as tuberculosis and leprosy bacilli, do not interact with neutrophils. In short, neutrophils interact almost exclusively with staphylococcus and streptococcus.

The neutrophils are programmed to interact with staphylococcus and streptococcus, given that the latter are the most predominant microorganisms of our microbiota, in inner and outer surfaces, including the mouth.

When microbiota bacteria are able to cross or enter through open skin and mucosa barriers, antibodies, proteins of the complement system and other enzymes and products are immediately ready to inhibit,

destroy and neutralize them. The neutrophils arrive ninety minutes later to phagocytize and completely eliminate them.

When neutrophils interact with bacteria, before closing the “clasp” they build around the microorganism to phagocytize it, the cytoplasm releases the content of its granules, the proteolytic degrading enzyme-based lysosomes as well as bactericidal substances such as hydrogen peroxide and chlorine solutions (Fig 2).

Neutrophil products also degrade collagen and other components of the extracellular matrix of the connective tissue. Degraded bacteria, dissolved components of connective tissue as well as dead neutrophils join the inflammatory exudate which, at this point, is no longer a serous fluid, but a yellow and viscous fluid (Fig 4), thus originating purulent exudate, also known as pus.

Neutrophils that cannot destroy the bacteria they have phagocytized burst and release a massive amount of degrading enzymes, a phenomenon known as cytolysis (Fig 3).

Neutrophils are also known as pyocytes or pus cells, whereas staphylococcus and streptococcus are also known as pyogenic bacteria or pus producers. The cluster of pus and neutrophils is generally known as abscess.

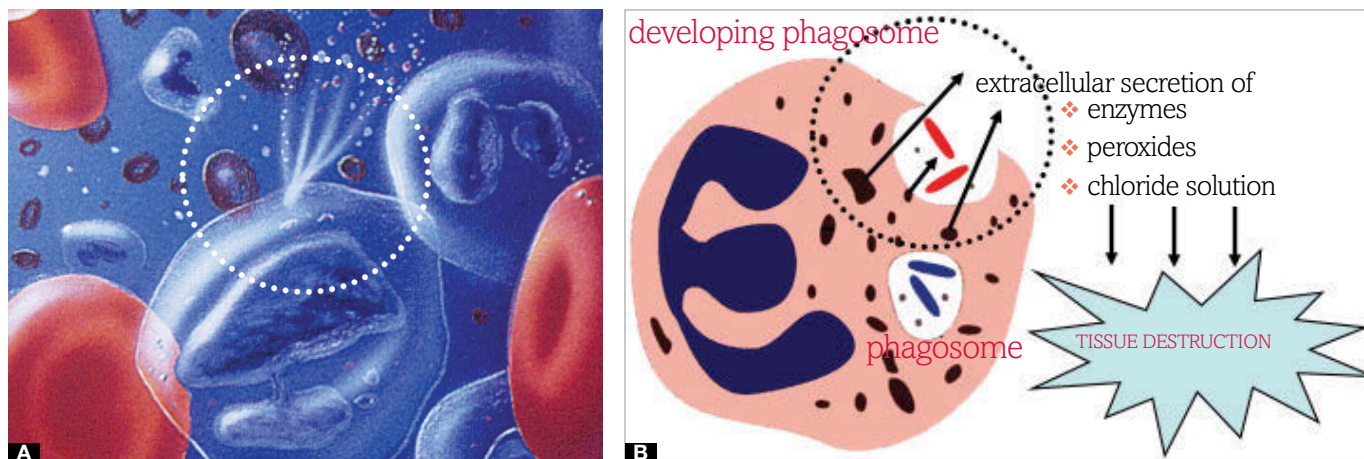
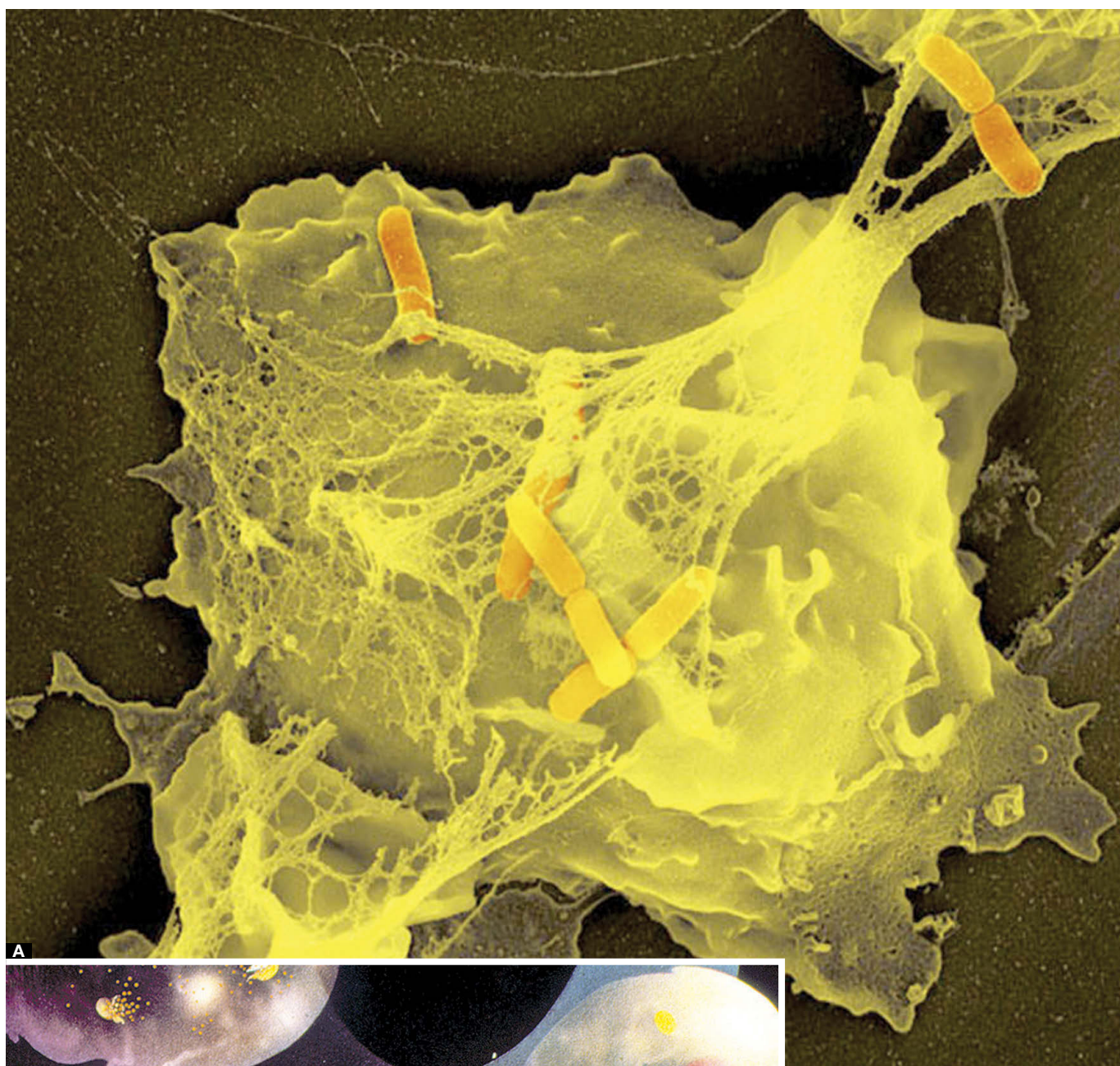


Figure 2. Phagocytosis of neutrophils is characterized by enzyme regurgitation (circles). Lysosomes release their powerful content into the phagocytic vacuole (arrows) before the latter is closed and, as a consequence, into the tissues. Depending on the amount of bacteria, tissue destruction occurs at different levels where the neutrophils are phagocytizing.



A



B

Figure 3. During phagocytosis of neutrophils (A), cytolysis may occur when the neutrophils cannot destroy the bacteria they have phagocytized (arrows). This causes the lysosomal products to be released into the tissues, increasing tissue destruction where phagocytosis of neutrophils occur. Enzyme regurgitation and cytolysis are mechanisms of pus formation. (A = Max Planck Institute for Infection Biology)

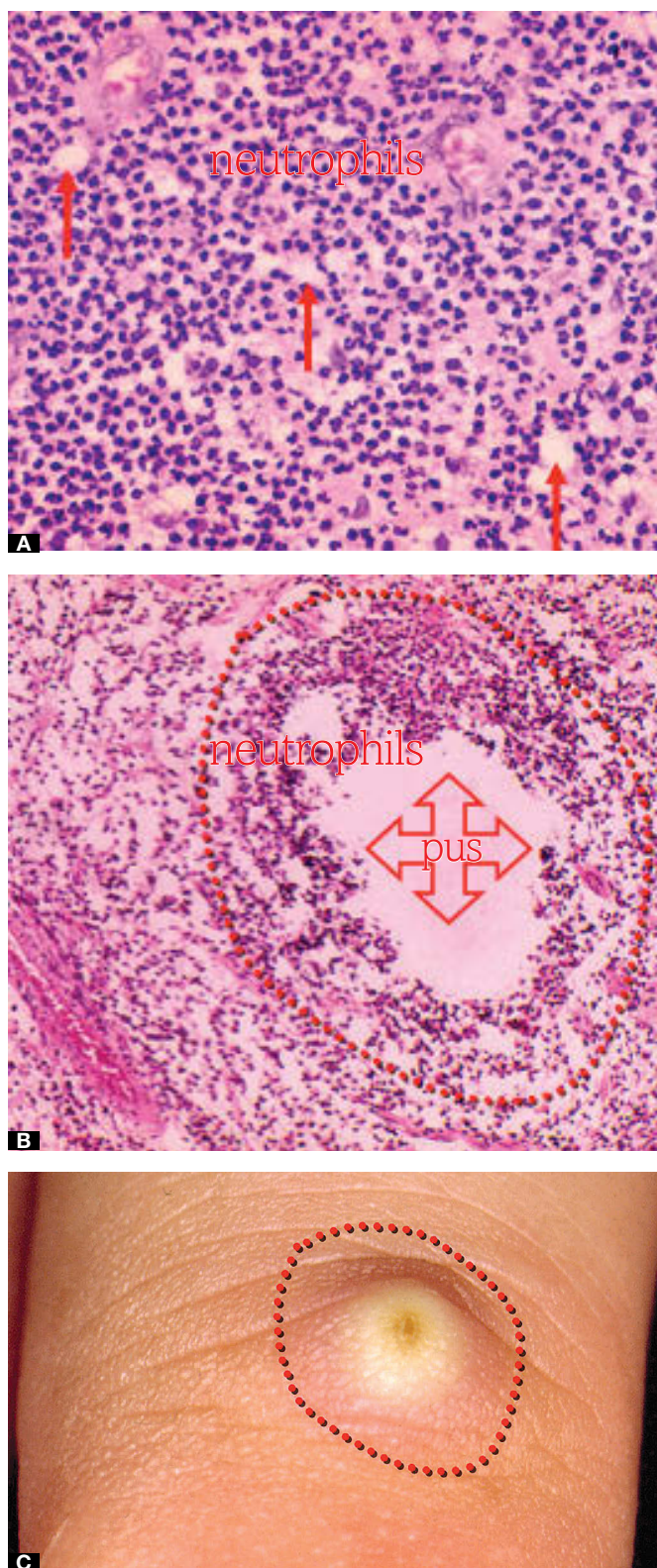


Figure 4. The extracellular matrix is filled with inflammatory exudate in the affected area and as enzyme regurgitation and cytolysis occur, the exudate ceases to be a serous fluid to become the yellow and viscous fluid that characterizes the formation of pus in microabscess (arrows in **A**). The microabscess gradually coalesces, forming clusters that characterize the abscesses (longer arrow and circles in **B** and **C**).

Little amount of bacteria forms no pus!

The destructive phenomena of bacteria-neutrophils interaction will occur regardless of the number of bacteria that enter a certain area of the connective tissue. However, should there only be a small amount of bacteria, the formation of pus will not be seen, i.e., the initially serous fluid does not become a yellow and viscous fluid, even though the phenomena occur likewise.

In other words: the amount of bacteria that enter the tissues is also important to clinically determine whether or not pus will be formed.

In general, a small amount of bacteria enters the surgical site, particularly in cases of parodontic, periodontal and implant placement surgeries as well as in direct pulp procedures. However, because they are in small amounts, the antibodies, proteins and neutrophils are able to quickly destroy them, thus avoiding the formation of pus. Likewise, in endodontic procedures, bacteria can reach the stump and the apical periodontal ligament, but if in small amounts, they will not cause the formation of pus.

Asepsis represents a set of procedures aimed at preventing bacteria from entering sites where they had not existed before. To this end, sterilization, disinfection and antisepsis procedures are carried out. Nevertheless, it is quite impossible to prevent bacteria from entering the oral environment, especially into connective tissue exposed by endodontic or surgical procedures. No problems occur in the majority of cases because, when proper aseptic care is taken, only a small number of bacteria reach the surgical wound, even in cases of staphylococcus and streptococcus.

Should there be formation of pus, bacteria are present!

Regardless of where, the formation of pus has a well-established meaning: presence of staphylococcus and streptococcus. It may occur around a suture wire, in the alveolus, after extraction, in the gingiva after surgery or around a dental implant. Some rare cases involving chemical products, as it is the case of turpentine, may lead to the formation of pus when the

products are inserted in large amounts in the connective tissue. Nevertheless, this practice only happens in experimental procedures.

The main and almost exclusive cause of pus formation is the interaction between bacteria and neutrophils. The presence of pus does not suggest rejection, immune rejection, bad material quality, improper composition of filling cement, bad suture wire, occlusal overload or trauma.

Solid/particulate material usually placed within tissues does not induce the formation of pus. Metals and alloys, surgical and filling cements, resin, polymers and other solid material alone do not lead to the formation of pus, unless they have been contaminated with bacteria. Staphylococcus and streptococcus contamination is what induces the formation of pus. The presence of these bacteria can be analyzed and questioned as follows:

- ❖ Were the bacteria already present prior to the surgical procedure?
- ❖ Was the material previously contaminated by bacteria?
- ❖ Was there any failure in the aseptic procedure?
- ❖ Was there lack of oral hygiene in the postoperative phase?

No matter the explanation, if pus is formed, staphylococcus and streptococcus are present.

Final considerations

The presence of pus necessarily suggests bacterial contamination caused by staphylococcus and streptococcus. The neutrophil-bacteria interaction is the main and most important mechanism of pus formation in the human body. Additionally, it may be an important point for clinical thinking: how, when and why these bacteria reach the spot.

I have been questioned and induced to answer the following question:⁷ What does the presence of pus around osseointegrated implants mean? This question is naturally extended to other clinical conditions, especially with regard to Endodontics: in clinical practice, the formation of pus only and necessarily occurs when bacteria are present!

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Comparison of the flexibility and torsional resistance of nickel-titanium rotary instruments

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ABSTRACT

Introduction: This study compared the flexibility and torsional resistance of two types of instruments manufactured with special NiTi alloys, and one with conventional NiTi.

Methods: Twisted File (TF) instruments manufactured with the R-phase of NiTi (SybronEndo, Orange, CA), and ProFile Vortex instruments (Dentsply Tulsa Dental, Tulsa, OK, USA) made of M-Wire NiTi were compared with RaCe (FKG Dentaire, La Chaux-de-Fonds, Switzerland) instruments made of conventional NiTi. Flexibility and torsion assays were carried out using twenty 25/0.06 instruments from each manufacturer. Statistical analysis was performed by ANOVA.

Results: The mechanical resistance of the instruments tested was significantly different. TF were the most flexible

instruments, followed by RaCe and ProFile Vortex ($P < 0.01$). In the torsion assay, ProFile Vortex instruments endured the greatest maximum load and maximum torque values prior to fracture, followed by RaCe and TF ($P < 0.01$). The torsional resistance values of RaCe and TF were not significantly different ($P = 0.061$). **Conclusion:** We observed a relationship between flexibility and torsional resistance (maximum torque and maximum angular deflection in torsion). The most flexible instrument (TF) was the least resistant to torsion, while the least flexible (ProFile Vortex) was the most resistant to torsion. RaCe presented intermediate results for both flexibility and torsional resistance.

Keywords: Mechanical torsion. Nickel. Dental instruments. Titanium.

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Introduction

Since the introduction of NiTi in Endodontics by Walia et al,¹ the technological evolution for fabricating NiTi instruments has allowed the production of more flexible and resistant instruments,^{2,3} revolutionizing the process of root canal shaping. The ability to widen the apical diameter of a curved canal, the availability of instruments with different tapers and cross-sections, the achievement of more centered preparations, and the reduction in the learning curve of endodontic systems are among the evidences of this paradigm shift.^{4,5} The super elasticity and shape memory effect of NiTi alloys are recognized as properties that allowed this revolution to take place.⁶

More recently, advances in the development of endodontic instruments reflect improvements in the thermal treatment of NiTi, culminating in the emergence of two special Nitinol alloys: R-phase and M-Wire. Twisted Files (TF - SybronEndo, Orange, CA, USA) instruments are manufactured by twisting a super elastic R-phase NiTi wire, as opposed to grinding or machining.⁷⁻⁹ ProFile Vortex instruments (Dentsply Tulsa Dental, Tulsa, OK, USA) represent the new generation of ProFile and are made of machined M-Wire, a NiTi alloy obtained by a proprietary process. In this manufacturing process, the alloy is subjected to a special thermomechanical treatment during the cooling and heating cycles.⁸⁻¹¹

In previous studies, Rodrigues et al¹² and Lopes et al¹³ assessed some mechanical properties of TF, RaCe and Vortex instruments. The purpose of the present study was to extend these findings by assessing the mechanical behavior, more specifically the bending and torsional resistance of two types of endodontic instrument fabricated with special NiTi alloys (TF and ProFile Vortex) and one instrument manufactured with conventional alloy (RaCe, FKG Dentaire, La Chaux-de-Fonds, Switzerland).

Material and Methods

Sixty rotary NiTi endodontic files were used in this study: Twenty 25/0.06 RaCe files (FKG Dentaire, La Chaux-de-Fonds, Switzerland), measuring 25 mm in nominal length; twenty 25/0.06 Twisted Files (TF) (SybronEndo, Orange, CA, USA), measuring 27 mm in nominal length; and twenty 25/0.06 ProFile Vortex (Dentsply Tulsa Dental, Tulsa, OK, USA), with nominal length of 25 mm.

Geometric characterization of instruments

Ten instruments of each brand were analyzed according to the following parameters: taper; length of the working portion; diameter at D0, D3, and D13; total number of flutes; and number of flutes per millimeter. These data were obtained with the aid of a Zeiss® optical microscope (Carl Zeiss do Brasil Ltda., Cambuci, SP, Brazil) to which a PixeLINK model PL-A662 camera (PixeLINK, Ottawa, Canada) was attached. All dimensions were obtained under 6.5X magnification except for the taper which was calculated according to the methodology described by Stenman & Spangberg.¹⁴ The AxioVision 4.4® imaging software (Carl Zeiss MicroImaging, Thornwood, NY, USA) was also used to aid the measurements.

Flexibility assay

The bending resistance was assessed by the cantilever bending test using a universal testing machine (EMIC, DL10000) as described in previous studies,^{15,16} with a downward incline of 45° in relation to the horizontal plane. A 20 N load was applied by means of a stainless steel wire measuring 30 cm in length and 0.3 mm in diameter, with one end attached to the cross head and the other end 3 mm from the instrument tip (load application point). Testing was conducted at a speed of 15 mm/min.

Torsional assay

The instruments were subjected to clockwise rotation with no axial load by using an apparatus attached to the universal testing machine, as described in a previous study.¹⁷ The apparatus monitored the rotation and the load applied to the instrument. The file was held by a vise placed at 3 mm from the instrument's tip, and the other end of the file was attached to a mandrel connected to the rotating shaft of the apparatus.

Torsion was achieved by twisting a braided nylon string measuring 0.3 in diameter around the rotating shaft which measured 8 mm in diameter. This nylon thread connected the rotating shaft to a 20 N load attached to the testing machine cross head, causing the shaft to rotate at 2 rpm. The load applied and the displacement of the nylon string until the instrument fractured were continuously monitored by a computer attached to the testing machine. The maximum angular deflection and maximum torque were

assessed with the aid of the M Test 1.01 software (EMIC DL 10000).

The fractured surfaces were analyzed under SEM to determine the type of fracture and the presence of plastic deformation on the instrument shafts. The values obtained in the bending and torsional assays were subjected to ANOVA.

Results

Geometric characterization of instruments

The mean diameters at D0, D3, and D13, the taper, the length of the working portion, the total number of flutes, and the number of flutes per millimeter are shown in Table 1.

Bending assay

The mean and the standard deviation for the maximum load to bend each instrument are presented on Table 2. Statistically significant difference was observed between the values of the maximum load necessary to bend the instruments. TF were the most flexible among the instruments tested, followed by RaCe and ProFile Vortex ($P < 0.01$).

Table 1. Mean values for diameters at D0, D3, and D13; taper; length of the working portion; number of flutes; and number of flutes per millimeter.

Instruments	n	Diameter (mm)			T	WL	NF	F/mm
		D0	D3	D13				
RaCe	10	0.28	0.47	1.10	0.06	17.56	7	0.4
TF	10	0.23	0.41	0.97	0.06	15.53	11	0.7
ProFile Vortex	10	0.24	0.42	1.00	0.06	16.75	10	0.6

Table 2. Means \pm standard deviation of the maximum loads (gf) necessary to bend the instruments tested.

Instrument	Number of instruments	Maximum load (gf)
RaCe	10	333.4 \pm 16.5
TF	10	228.4 \pm 15.18
ProFile Vortex	10	603.7 \pm 29.3

Torsional assay

The means and standard deviations for the maximum load and maximum torque necessary to fracture the instrument are shown in Table 3. Significant difference was observed between the three types of instruments. ProFile Vortex withstood greater values of maximum load and maximum torque, followed by RaCe and TF ($P < 0.01$).

Table 4 shows the means and standard deviations for the maximum angular deflection before torsional failure as well as the number of turns that are necessary to fracture the instrument. TF and RaCe instruments did not show significant differences among each other ($P = 0.061$), but both presented greater angular deflection values and number of turns than ProFile Vortex ($P < 0.01$).

In order to confirm the association between flexibility and maximum torsional torque, a graph presenting the relationship between these parameters was constructed (Fig 1). Another graph shows the relationship between the maximum angular deflection in torsion and flexibility (Fig 2). Finally, a third graph was constructed to show the association between the mean maximum angular deflection and the maximum torsional torque (Fig 4).

Table 3. Means \pm standard deviation for the maximum loads and maximum torque at fracture of the instruments tested.

Instrument	Number of instruments	Maximum load (gf)	Maximum torque (gf-mm)
RaCe	10	184.5 \pm 7.61	765.71 \pm 31.59
TF	10	107.27 \pm 8.50	445.19 \pm 35.28
ProFile Vortex	10	250.93 \pm 31.15	1041.39 \pm 129.26

Table 4. Means \pm standard deviation for the maximum angular deflection at torsional fracture and number of turns necessary to fracture the instrument in the torsional assay for the instruments tested

Instrument	Number of instruments	Maximum deflection (°)	Number of turns
RaCe	10	578.88 \pm 50.96	1.61 \pm 0.14
TF	10	688 \pm 154.92	1.91 \pm 0.43
ProFile Vortex	10	394.56 \pm 72.0	1.10 \pm 0.20

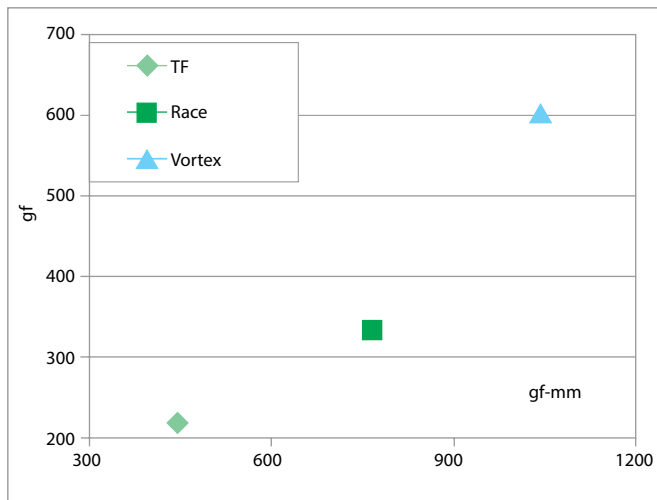


Figure 1. Graphic representation of the relationship between flexibility (gf) and maximum torque (gf-mm).

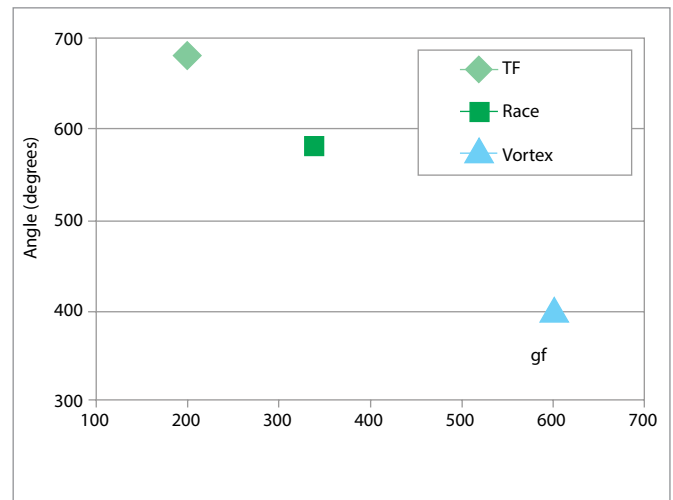


Figure 2. Graphic representation of the relationship between maximum angular deflection (degrees) and flexibility (gf).

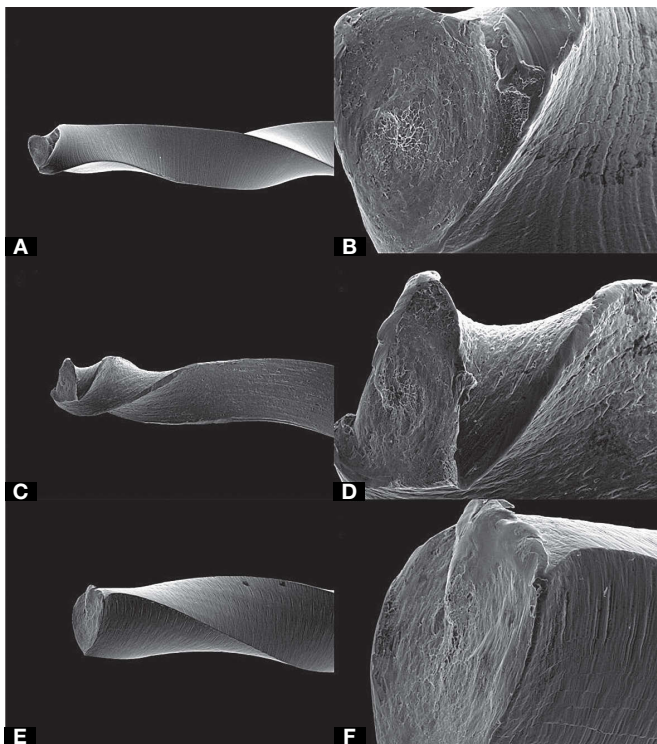


Figure 3. Appearance of the fractured surfaces, showing reversed flutes near the tip, where the instruments were held by the vise. Instruments: RaCe (**A** and **B**), TF (**C** and **D**), and ProFile Vortex (**E** and **F**). Presence of plastic deformation, grooves, and cracks of varying depths (**A**, **C**, and **E** under 100x magnification; **B**, **D**, and **F** under 500x magnification).

SEM showed that all instruments tested displayed features of ductile mode fracture. Plastic deformation was observed in the helical shaft of all instruments (Fig 3).

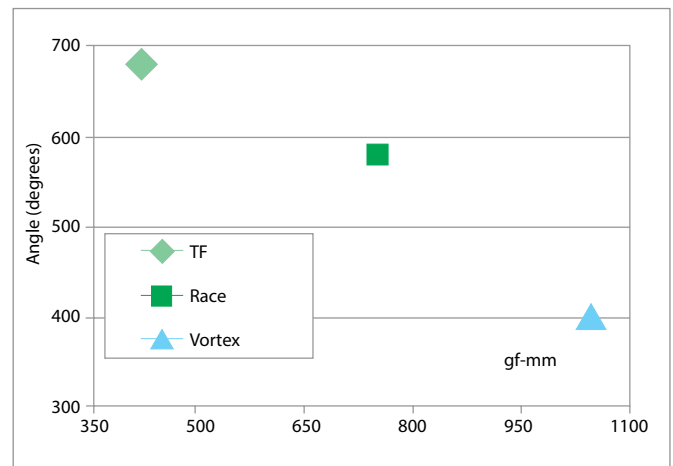


Figure 4. Graphic representation of the relationship between maximum angular deflection (degrees) and maximum torque (gf-mm).

Discussion

Although the instruments selected for the present study were made of different types of NiTi, all of them had similar cross-sectional designs (triangular), since this variable is known to influence the performance of instruments in mechanical assays.¹⁸

In the present study, TF instruments required the smallest load to deflect 45° below the horizontal plane. Clinically, the greater an instrument's flexibility, the less likely it is to produce apical deviation during root canal instrumentation.¹⁹ Based on our results, we expect TF to present more satisfactory clinical performance.

The greater flexibility of TF may be due to the fact that these files are made of R-phase NiTi, which provides greater flexibility, lower elasticity modulus, and less rigidity in comparison with conventional austenite NiTi instruments.^{20,21} Thus, instruments fabricated with this special alloy are able to withstand greater elastic and plastic deformation than conventional alloys, when subjected to similar torque. Our results corroborate previous studies.^{7,22-24} Moreover, TF instruments are manufactured by twisting NiTi wires, which distinguishes these instruments from the two other groups manufactured by grinding. Twisted instruments present significantly less surface flaws than ground files.^{7,9,23-26}

The results from the torsional assay demonstrated that ProFile Vortex instruments are able to withstand significantly greater maximum torque than RaCe and TF. Some factors may explain this difference:

- » The cross-sections of TF and RaCe are equilateral triangles, while the cross-section of ProFile Vortex is a convex triangle.
- » The resistance to torsional fracture of engine-driven NiTi instruments increases with the cross-sectional area and the moment of inertia. Profile Vortex files has larger cross-sectional areas.
- » Previous studies have demonstrated a direct correlation between the diameter and the cross-sectional area.^{24,25,27-32} However, these studies do not analyze the cross-sectional area, which depends on both instrument diameter and shape.
- » The manufacturing process/thermomechanical treatment of the alloy may also have influenced the maximum torque values. Variations in the final thermomechanical state of the alloy (austenite, martensite, or R-phase) lead to different mechanical properties.

With regard to the maximum angular deflection before torsional failure, our results show significantly higher values for TF in comparison with ProFile Vortex. Conversely, no statistically significant difference was observed between TF and RaCe. Several authors suggest that resistance to torsion be assessed by angular deflection, not by the maximum torque.^{28,33,34} This is justified by the fact that control of the torsional deflection (measured either in degrees or number of turns) may represent a safety factor when hand-operated instruments are used in clinical settings. In the event in which a hand-operated instrument may become lodged inside the

canal, the clinician can apply torque within the torsional deflection limits, thus preventing instrument fracture caused by torsion. In engine-driven rotary instruments, however, it is not possible to control the angular deflection in torsion. Instead, these engines prevent instrument failure by controlling the maximum torque.^{20,24,32,35}

Another important parameter that should be taken into account in order to explain the higher maximum angular deflection values of TF is related to the manufacturing process and the resulting surface finish of these instruments. Although TF instruments display the worst surface finishing, these manufacturing imperfections are longitudinal and perpendicular to the fracture plane. The nucleated cracks develop along the longitudinal imperfections, and do not contribute to form the fracture plane. After the torsion test, several cracks were observed on the surface of TF. In the remaining instruments, which present circumferential manufacturing imperfections, the cracks tend to develop more easily along these grooves, leading to instrument failure under smaller angular deflection (Fig 3).

The results obtained in the present study revealed a relationship between maximum torque, bending resistance, and maximum angular deflection until torsional failure. This may be explained by differences in instrument geometry, cross-sectional area, and moment of inertia. The cross-section shape plays an important role in the process of instrument fracture, since the maximum load (L_{max}) is directly proportional to the radius (R) and to the maximum torque (M_t), and inversely proportional to the moment of inertia (I), as demonstrated by the following equation: $L_{max} = M_t R / I$.

The differences in torque resistance verified in the present study cannot be associated with the initial diameter at the instrument tips (standardized at 0.25 mm) or to the diameter at D3, (approximately the same for all instruments), nor to the taper (standardized at 0.06 mm). ProFile Vortex presented the greatest torsional resistance among the instruments tested. On the other hand, TF, the instrument with the lowest resistance to torsion, presented the greatest flexibility and the highest angular deflection before torsional failure. This result corroborates observations of other authors who reported that the cross-sectional area is inversely proportional to the flexibility of endodontic files.^{6,18,20,30,36-39} It is important to mention that instruments with the same cross-sectional area may present different moments of inertia.

Based on the findings of the present study, it was possible to establish a relationship between flexibility and maximum torque, as well as between flexibility and maximum angular deflection for the instruments tested. The most flexible instrument (TF) was the least resistant to torsion, while the most resistant to

torsion (ProFile Vortex) was the least flexible. TF, the most flexible, was also able to withstand the highest angular deflection until torsional failure, and ProFile Vortex, the least flexible, had the lowest angular deflection values. RaCe had intermediate results for both flexibility and angular deflection.

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Root apical third and canal morphology of teeth with hypercementosis

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ABSTRACT

Objective: This study aims at studying the influence of hypercementosis over root and root canal morphology using different methods of observation (clearing technique, radiography, stereomicroscopy and optical microscopy).

Methods: 130 teeth were selected for morphological comparative evaluation; all teeth were previously radiographed and stereomicroscopically evaluated. Out of these, 60 teeth with hypercementosis and 30 without it were selected for clearing technique evaluation. The analysis was based on aspects such as: type of hypercementosis; root canal number and configuration; root surface and presence of apical foramen and apical deltas. The remaining 20 teeth with hypercementosis were microscopically compared to 20 teeth with normal root formation by means of the Hematoxylin and Eosin (H.E.) staining technique, so as to study the cementum deposition pattern and morphological aspects of the root canal. The evaluation was performed by two ex-

aminers and submitted to Kappa agreement test. The data obtained was compared through non-parametric Kruskal-Wallis one-way analysis of variance test, and the Dunn test was applied for individual comparisons. **Results:** The root clearing examination showed higher frequency of club shaped hypercementosis (65%) followed by focal hypercementosis (35%). Teeth with hypercementosis showed significant increase in the presence of apical deltas (53.3%). A higher frequency of root canal constrictions (55%), and changes in the original root canal path (46.6%) were also observed. Microscopic evaluation supports the influence of hypercementosis over the morphological characteristics of root apical third formation. **Conclusions:** These findings show the existence of a complex root canal anatomy at the apical third of teeth with hypercementosis, which may hinder root canal treatment.

Keywords: Hypercementosis. Dental pulp cavity. Endodontics.

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Introduction

Hypercementosis is characterized by excessive deposit of cementum beyond the physiologic limits of the teeth, promoting an abnormal thickness of the apex that becomes round-shaped and/or with a macroscopically altered appearance.

The incidence of hypercementosis by race or population group has not yet been established. Gardner and Goldstein¹ studied the frequency of the phenomenon in 137 individuals, with 529 teeth being compromised by the process. Their study reported an average of 3.8 teeth with hypercementosis per individual, with a mean age of 47.3 years old and whose most affected teeth were the premolars. Another study demonstrated tooth root hypercementosis in 84% of a collection comprising 104 skeletons of Barbadian slaves from XVII to XIX centuries.² Additionally, root hypercementosis was found in 10 out of 54 specimens gathered from prehistoric coastal populations of Texas, USA.³

The etiology of hypercementosis is attributed to several conditions such as functional stress due to occlusion forces; continuous dental eruption; incorporation of periodontal cementicle during physiologic cementum deposition; inflammatory reactional deposition; as well as systemic factors such as atherosclerosis, acromegaly, arthritis, thyroid diseases and Paget's disease.¹⁻¹³

Alterations in root morphology due to hypercementosis comprise club shape hypercementosis, which results from cementum deposition in all root surfaces; focal hypercementosis, globular cementum deposition in one of the root surfaces; and circular cementum hyperplasia (CCH), cementum lateral deposition on the root surface without affecting the apex.¹⁴

Hypercementosis does not radiographically alter the biologic space relationship between the root surface, periodontal ligament and the alveolar bone. Although hypercementosis can be identified through common radiographic techniques, the latter do not allow one to estimate the amount of extra cementum in the affected root since dentin and cementum have the same radiodensity.^{1,15}

Microscopic studies of hypercementosis report thick layers of cementum characterized by deposition of symmetric, highly basophilic lines parallel to the dentinal surface. Occasionally, atypical cementum depositions are identified in focal areas as external cementum projections. The presence of blood vessels and nervous filaments associated with irregular apical cementum

deposition can contribute to the formation of multiple foramina during cementum deposition resulting from hypercementosis, thus originating the apical deltas.^{1,9}

The lack of recent studies related to this subject, added to potential endodontic implications, inspire the assessment of morphological differences in the apical third of the root and the root canal of teeth with hypercementosis. The present study applied different methods to perform these morphological observations and a comparative study was established among them in order to judge their diagnostic value.

Material and Methods

The 130 teeth examined in this study were selected from the teeth bank of the routine archives of one of the author's laboratory of Oral Pathology. The specimens were kept in 10% formalin solution, placed into small containers and labeled for identification. A consent for using human tissue was obtained from an Institutional Review Board.

In selecting the sample, the following diagnostic criteria were applied: cementum formation beyond the physiologic limits of the teeth, with changes in root morphology that could be macroscopically detectable. The sample comprised 80 teeth with hypercementosis and 50 without it. Out of these specimens, 60 teeth with hypercementosis and 30 without it were selected for analysis with the clearing technique. Out of the 40 remaining teeth, 20 with hypercementosis and 20 without it were selected for microscopic analysis.

The specimens with hypercementosis were additionally classified by means of stereomicroscopy according to their morphological characteristics, including the type of hypercementosis, the surface, the longitudinal root axis and the visualization of the apical foramen.

After this classification, the teeth were radiographed with a 70Kvp and 7,5mA x-ray device (Dabi-Atlante, SP, Brazil), with a 16-cm cone, during an exposition period of 0.5 seconds. The radiographic films used were Kodak Ultraspeed DF-58 (Eastman Kodak, NY, USA) and the radiographic developing process used was the temperature-time one during a 3.5-minute developing period.

The radiographs were examined by two independently trained observers using an X-ray viewer and magnifying lens (2X) in a dark room. The radiographic aspects of the alterations in root radiodensity between the cementum and the dentin at the apical third were considered.

Additionally, the morphological aspects of the root such as number, distribution and surface, as well as the direction, acquired due to hypercementosis, and root canal breadth at the apical third were also considered. The 50 teeth without hypercementosis were used as comparative basis.

The 90 teeth chosen for this part of the study (60 with and 30 without hypercementosis) were divided into groups of maxillary molars, mandibular molars, maxillary premolars, mandibular premolars, maxillary anterior teeth and mandibular anterior teeth. Each group comprised 10 teeth with hypercementosis and 5 teeth without it.

Access cavities were prepared and the teeth were immersed in 5% sodium hypochlorite solution for 24 hours, followed by ultrasonication (Ultrasonic bath, EM Scope lab Ltd, London, UK) to dissolve the pulp tissue. The teeth were dried and the root canal systems coronally injected with Indian ink (Windsor & Newton Indian Ink, London, UK) with a hypodermic needle (Sherwood Medical Company, St. Louis, MO, USA) apically assisted by vacuum suction. After that, self-cured resin was placed in the coronal access.

After another 12-hour period of drying, the teeth were decalcified in 5% hydrochloric acid for 48–72 hours, and the process was monitored by periodic radiography. The decalcified teeth were washed in running tap water for 4 hours and dehydrated in ascending concentrations of ethanol (60%, 70%, 80%, 90%, 95%) (MJ Patterson, Dunstable, UK) for 2 days, then rendered transparent by immersion in xylene and stored in methyl salicylate for observation (Pharmacos Ltd, Southend-on-Sea, UK).

After transparency was achieved, the specimens were observed by two independent investigators, other than those who evaluated the radiographs, using a light stereomicroscope with magnification set at 5X. They were asked to collect the data by means of a technique similar to that used for the radiographic analysis. The data collected by the observers working with the clearing technique were compared to those obtained from the radiographs.

All data obtained from the 90 teeth mentioned above were entered into a spreadsheet (Excel, Microsoft Corporation, WA, USA). The Kappa agreement test values between evaluators, with regard to the characteristics of root and root canal obtained by the radiographic method and the clearing technique, were computed.

Root canal clearing was established as a standard for all morphological aspects studied.

Statistical analysis was carried out by means of the non-parametric Kruskal-Wallis one-way analysis of variance test which was used to determine if there were significant differences between the hypercementosis and no hypercementosis group. The Dunn test was applied for individual comparisons. Differences were considered significant at $P < 0.05$.

The 40 remaining specimens were divided into two groups: A = 20 teeth with hypercementosis; B = 20 teeth without hypercementosis, with 5 maxillary molars, 5 mandibular molars, 5 maxillary premolars and 5 mandibular premolars in each group. The root apical third of the specimens were sectioned for histochemical procedures using a carborundum sectioning disk. Demineralization was performed by means of 5% EDTA solution (pH 7.0). After being embedded in paraffin, the specimens were horizontally sectioned at 5 μm and stained with the Hematoxylin and Eosin (H.E.) technique.

The morphological comparative observation aimed at dentin and cementum root canals, the cementum deposition pattern, presence of lateral canal or apical deltas, presence of periodontal cementicles, incremental lines of cement as well as presence of internal or external root resorption.

Results

The data obtained from the interaction between morphological and radiographic findings; from stereomicroscopy and the tooth clearing technique applied to the different types of hypercementosis; as well as the root canal morphology in each dental group of affected teeth are shown in Tables 1 and 2. Tables 3 and 4 display the morphological findings obtained with the referred methods in teeth with and without hypercementosis as well as the root canal configuration of each dental group.

As for root morphology, out of the 60 teeth with hypercementosis studied by means of the clearing technique, 39 (65%) presented club shape hypercementosis, whereas 21 (35%) presented focal hypercementosis and none of them presented CCH. Kappa test results, comparing the radiographic and root clearing techniques, was substantial ($\kappa = 0.6$) with regard to the type of hypercementosis.

The root surface was found irregular in 86.6% of the teeth with hypercementosis and in 90% of the teeth

without it. However, these irregularities were more easily detected through root clearing than through radiographic examination ($\kappa = 0.2$ and 0.1 respectively). Deformation of the root longitudinal axis was observed in 66.6% of teeth with hypercementosis and in 60% of teeth without it. This characteristic showed substantial concordance when the radiographic and root clearing techniques were compared ($\kappa = 0.9$ and 0.9 respectively).

The most distinguishable alteration during the comparison between teeth with and without hypercementosis was the high frequency of apical deltas in those with hypercementosis: 53.3% ($n = 32$) against 20% ($n = 6$) of teeth without hypercementosis. This morphological alteration was the only one that showed significant values ($p = 0.01$) between groups and individual comparison. These apical deltas were only observed by means of the root clearing technique $\kappa = 0$ (Fig 1).

Table 1. Morphologic findings of the different types of hypercementosis in each dental group detected through radiography, stereomicroscopy and tooth clearing technique analyses.

		Maxillary molar	Mandibular molar	Maxillary premolar	Mandibular premolar	Anterior maxillary teeth	Anterior mandibular teeth	
Type	Club	9	4	8	9	2	7	
	CCH	0	0	0	0	0	0	
	Focal	1	6	2	1	8	3	
Surface	Regular	1	3	1	0	1	1	
	Irregular	9	7	9	10	9	9	
Longitudinal axis	Normal	7	4	1	1	4	3	
	Deformed	3	6	9	9	6	7	
Apical foramen	Visible	10	10	10	10	10	10	
	Non-visible	0	0	0	0	0	0	
Dental resorption	Internal	0	6	3	3	1	4	
	External	Apical	5	7	3	6	7	10
		Lateral	3	0	5	7	8	10

Table 2. Morphologic findings of root canal configuration in each dental group with hypercementosis detected through radiography, stereomicroscopy and tooth clearing technique analyses.

		Maxillary molar	Mandibular molar	Maxillary premolar	Mandibular premolar	Anterior maxillary teeth	Anterior mandibular teeth		
Root canal	Number and distribution	Single root canal	10	6	9	9	10	10	
		Two root canals	0	4	1	1	0	0	
		Lateral root canal	3	3	2	2	0	2	
		Collateral root canal	5	3	5	6	5	4	
		Apical delta	6	7	8	4	5	2	
	Surface	Regular	3	1	2	4	4	3	
		Irregular	7	9	8	6	6	7	
	Root canal breadth	Continuous	5	2	5	6	6	4	
		Constriction	Progressive	1	1	2	3	0	2
			Abrupt	4	7	3	2	4	4
Direction	Same	4	7	6	7	5	5		
	Modified	Mild	3	2	3	0	3	5	
		Moderate	3	3	0	4	0	0	
		Severe	0	1	0	0	0	0	

Table 3. Morphologic findings of each dental group without hypercementosis detected through radiography, stereomicroscopy and tooth clearing technique analyses.

		Maxillary molar	Mandibular molar	Maxillary premolar	Mandibular premolar	Anterior maxillary teeth	Anterior mandibular teeth	
Surface	Regular	1	0	0	0	2	0	
	Irregular	4	5	5	5	3	5	
Longitudinal axis	Normal	1	2	1	3	2	2	
	Deformed	4	3	4	2	2	3	
Apical foramen	Visible	5	5	5	5	5	5	
	Non visible	0	0	0	0	0	0	
Dental resorption	Internal	0	0	0	0	0	0	
	External	Apical	5	5	5	5	4	5
		Lateral	2	5	5	5	4	5

Table 4. Morphologic findings of root canal configuration in each dental group without hypercementosis detected through radiography, stereomicroscopy and tooth clearing technique analyses.

		Maxillary molar	Mandibular molar	Maxillary premolar	Mandibular premolar	Anterior maxillary teeth	Anterior mandibular teeth		
Root canal	Number and distribution	Single root canal	4	1	4	5	4	4	
		Two root canals	1	4	1	0	0	1	
		Lateral root canal	0	2	2	1	0	1	
		Collateral root canal	1	3	4	1	1	2	
		Apical delta	2	1	1	1	1	0	
	Surface	Regular	2	0	0	0	2	0	
		Irregular	3	5	5	5	2	5	
	Root canal breadth	Continuous	2	5	4	3	4	4	
		Constriction	Progressive	1	0	1	2	1	1
			Abrupt	2	0	0	0	0	0
	Direction	Same	2	1	4	4	5	5	
		Modified	Mild	3	2	0	1	0	0
			Moderate	0	2	1	0	0	0
			Severe	0	0	0	0	0	0

Alterations in root canal breadth at the apical third of teeth with hypercementosis revealed abrupt constriction in the apical third of the root canal in 40%, or progressive constriction in 15%, of the 60 specimens evaluated ($\kappa = 0.2$ comparing the radiographic and root clearing techniques). The specimens without hypercementosis showed continuous root canal breadth at the apical third in 73.3% of the 30 teeth assessed ($\kappa = 0.3$ comparing the radiographic and root clearing techniques). In spite of these values, a relative difference among teeth with and without hypercementosis was revealed by the Kruskal-Wallis test ($P = 0.5$).

Changes in the original root canal path were found in 46.6% of the specimens with hypercementosis (28

specimens). Agreement between the radiographic and root clearing techniques was $\kappa = 0.3$. Teeth without hypercementosis kept the same path in 70% of the cases (21 specimens). Agreement between the radiographic and root clearing techniques was $\kappa = 0.1$. Comparison between groups with and without hypercementosis was $P = 0.3$. Figure 2 shows changes in root canal path of teeth with and without hypercementosis.

Microscopically, except for cementum thickness and distribution, the morphological aspects of both groups, A and B, showed similar incremental patterns of cementum deposition. In teeth with hypercementosis, the root canal presented spherical or oval horizontal sections just like the ones without hypercementosis did.

Some sections of group A showed irregularities on the root canal wall. Such irregularities were composed by disorganized dentin mixed with cemental tissue. In other instances, the cement root canal was clearly identified, characterized by cementum deposition surrounding the root canal compartment. External cementum deposition on root surface of group A presented a regular, slightly undulated, basophilic and concentric pattern in the majority of cases. Both cellular and non cellular

cementum were present in groups A and B; additionally, the demarcating line between dentin and cementum did not always seem to be well defined. The presence of periodontal cementicles in teeth of group A could also be identified, given that they were adhered to the root surface or inserted into the cementum structure (Fig 3).

Numerous canaliculi were found in some of the specimens with hypercementosis. Considering the location, the presence of pulp cellular remnants and their relationship

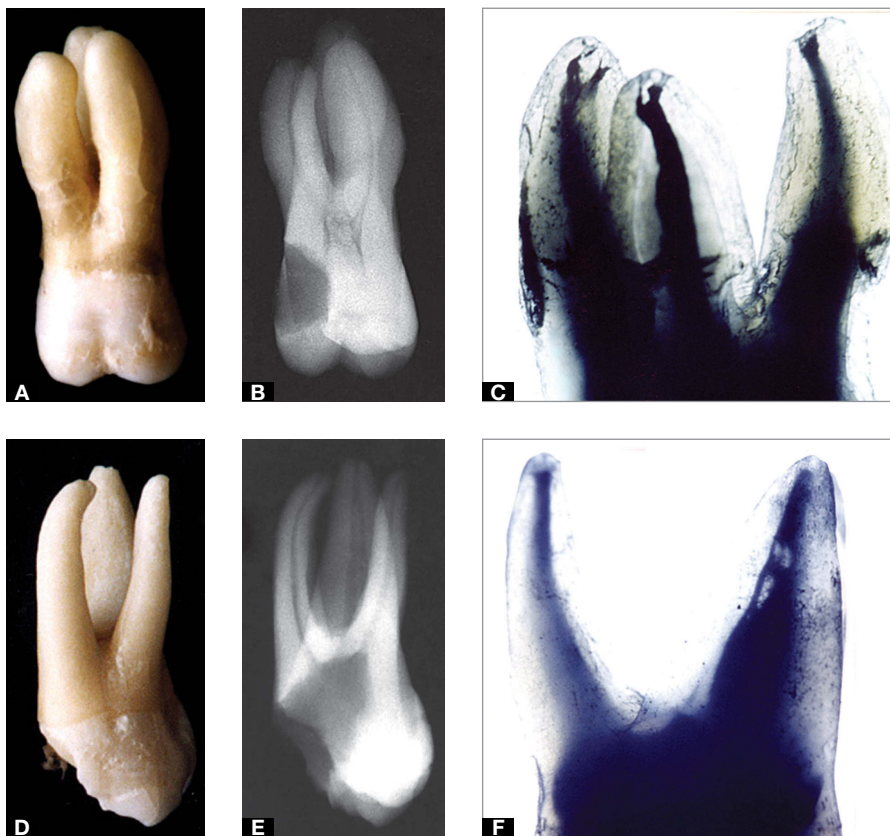


Figure 1. Comparative aspects of maxillary molars with hypercementosis (A, B and C) and without hypercementosis (D, E and F). Note the complex anatomy of the apical third of teeth with club shaped hypercementosis, only detectable through the clearing technique. Original magnification 5X.

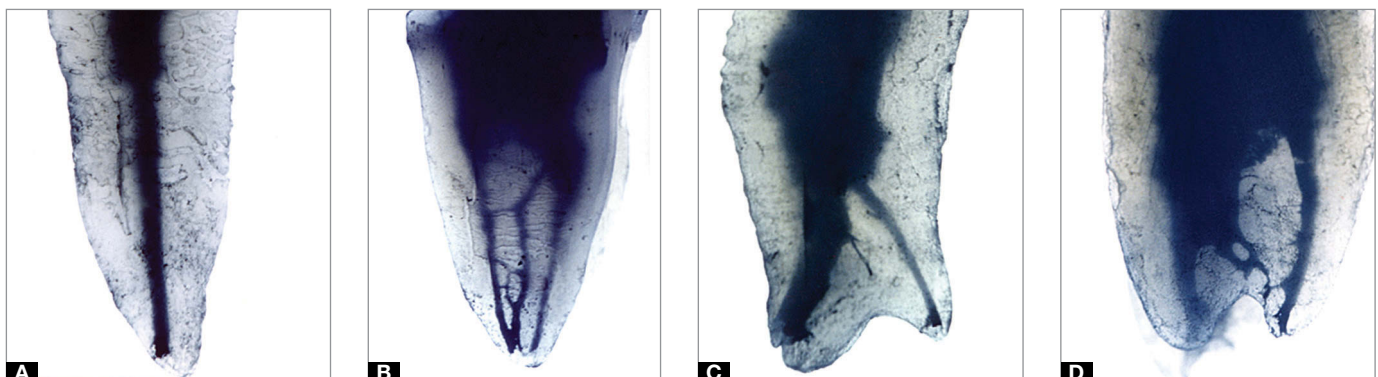


Figure 2. Differences between mandibular premolars and mandibular molars without (A and B) and with hypercementosis (C and D) observed through the clearing technique. The root canal path of the specimens without hypercementosis displays a regular trajectory. Original magnification 5X.

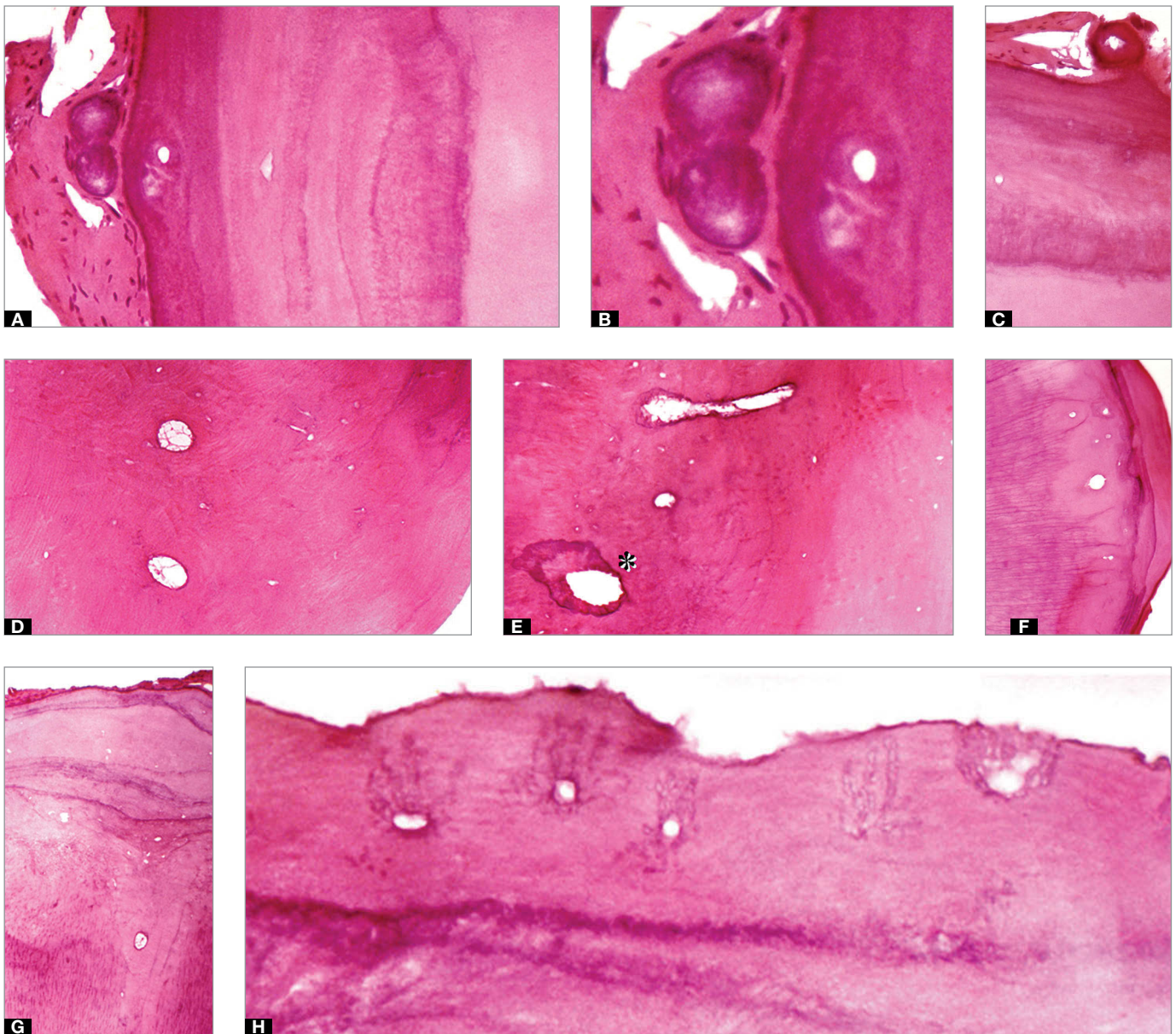


Figure 3. Microscopic aspects of hypercementosis. In **A** and **B**, the presence of cementicles closely related to cementum and included by cementum deposition, **C** shows a slight fusion between the cementum and the periodontal cementicle. Note in **D** and **E** the presence of canaliculi that might be part of apical deltas. One of the canaliculi in **E** appears partially obliterated with incremental lines of cementum deposition. The pattern of cemental deposition is highlighted in **F**, **G** (acellular cementum) and **H** (cellular cementum with innumerable cementoblasts). Original magnification: **A**= 16X, **B**= 400X, **C**= 160X, **D** and **E**=40X, **F** and **G**= 16X, **H**= 160X (H.E. stain).

with the main root canal, it is reasonable to assume that these canaliculi might be part of apical deltas. In other instances, these canaliculi would appear obliterated with incremental lines of cementum deposition (Fig 3).

Discussion

The methods applied in this study intended to assess hypercementosis and its influence over root canal morphology from different points of view. The present

radiographic data provided a perspective that is commonly found in endodontic practice; the method of canal staining and root clearing was found to be excellent for three dimensional evaluation of root canal morphology; and the microscopic evaluation enlightened the morphological aspects of cementum deposition and its relationship with root canal formation.

Radiographs alone have shown limited value when certain aspects of the root canal system are under study.^{14,16}

The interaction between the different methods applied in the present study aimed at minimizing that limitation. The only feature, strictly related to hypercementosis, that achieved substantial concordance between the radiographic and tooth clearing techniques was the one regarding the type of hypercementosis ($\kappa = 0.6$).

The presence of deformations on the longitudinal axis of the root canal ($\kappa = 0.9$ with hypercementosis and 0.9 without it) also presented substantial agreement between tooth clearing and radiography, but similarly to the findings on root surface, very similar results were present when comparing teeth with and without hypercementosis. Therefore, it is reasonable to conclude that other factors probably influence the formation of the apical third of the root canal and promote deformations of the root canal longitudinal axis.¹⁷

Although the clearing technique is useful only as a teaching/research tool, with little or no clinical applicability, it was only by means of this technique that the high frequency of apical deltas could be detected. In addition to that, other interesting morphological findings were also considered in this study: the alterations in root canal breadth at the apical third and the changes in the original root canal path due to hypercementosis. Therefore, a clinical inference can be drawn from these findings: instrumentation of teeth with hypercementosis should take into account the possibility of a complex root canal anatomy at the apical third, even if it cannot be radiographically identified.

Other radiographic methods could provide more information to this study. The methodological impact of X-ray microtomography with high quality reconstructions of the external and internal morphology of teeth with hypercementosis could be applied as a different observational technique. This method could be an alternative, a non-destructive 3D research and educational tool.¹⁸

This study draws attention to an interesting clinical fact regarding the influence of hypercementosis over electronic apex location. Previous studies have shown that as the width of the major foramen increases, the discrepancy between the electronic probe tip length induction and the actual position of the major foramen also increases.¹⁹ There is a current thinking that apex locators are only capable of detecting the major diameter of the root canal terminus, in other words,

the major foramen.²⁰ The present study found that hypercementosis usually increases the presence of apical deltas, what may reduce the presence of a major foramen. Further studies may address this issue by properly evaluating the possibility of hypercementosis affecting the accuracy of electronic apex locators.

The results observed in this study lead to future correlations established between the presence of hypercementosis and the type of root canal. It is worth noting that most classifications were made without observing the presence or absence of hypercementosis.²¹⁻²⁵

The morphological characteristics of the influence of hypercementosis over the formation of the root apical third, observed by means of stereomicroscopy, radiographic and tooth clearing techniques, were coherent with the microscopic evaluation. Continuous cementum deposition, observed in teeth with hypercementosis, was associated with the presence of numerous canaliculi, probably part of apical deltas, and so were the constrictions in the cemental canal due to incremental lines of cementum deposition surrounding the root canal compartment. These findings can be relevant when used in studies regarding the cemento-dentino-canal junction, the apical foramen and apical constriction.²⁶

The microscopic evaluation of the root and root canal morphology of teeth with hypercementosis performed in this study was similar to that reported by other studies.^{1,9} The influence of hypercementosis over root external surface constitutes the major limitation of the methods proposed herein. How and where the apical and accessory foramina are displaced due to hypercementosis, and the possible variations related to their morphotype have already been shown by means of scanning electron microscopy, by comparing teeth with and without hypercementosis.¹⁴

Conclusions

This study found that hypercementosis can cause higher frequency of apical deltas, constriction in root canal breadth, and changes in the original root canal path at the apical third of affected teeth. These findings point out the possible existence of a complex root canal anatomy at the apical third in teeth with hypercementosis, which is sometimes undetectable by common radiographic examination.

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Comparison of changes in the pH of calcium hydroxide pastes associated with different vehicles

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ABSTRACT

Introduction: The antimicrobial effect of calcium hydroxide has been assigned to its capacity to produce an alkaline shift in pH. This property is affected when calcium hydroxide is combined with other substances, such as 2% chlorhexidine gel and zinc oxide, which makes the action of the paste last longer. **Objective:** This study assessed whether calcium hydroxide paste associated with chlorhexidine gel and zinc oxide promote pH shifts at short time intervals. **Methods:** Calcium hydroxide pastes prepared with three vehicles: saline solution (paste A); propylene glycol (paste B); and 2% zinc oxide chlorhexidine gel (paste C) were placed into vials containing 15 ml of deionized water.

A pH meter was used to detect pH shifts of combinations in different vehicles at seven time intervals: 15 and 30 minutes; 1, 24 and 48 hours; as well as 7 and 14 days. **Results:** All three pastes presented a sharp increase of pH values at the first time interval and remained relatively stable at a value of about 12 from 24 hours to 7 days. After this period, the pH of pastes A and B decreased to 9.50, whereas that of paste C remained at 12. **Conclusions:** Pastes A and B produced a faster alkaline shift of the solution, whereas paste C kept an elevated pH for a longer time, however, differences were not statistically significant.

Keywords: Calcium hydroxide. Intracanal dressing. Endodontics.

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Introduction

Numerous studies in the literature report that some pathologies cannot be reversed even after endodontic treatment is performed. Such clinical condition may be associated with the persistence of microorganisms inside or outside the root system, in which case some substances are necessary not only to complement the disinfection started by cleaning and shaping procedures, but also to reduce endodontic microbiota by creating conditions that are unfavorable for the development of bacteria and favorable for the repair of periapical tissues.^{1,2}

Calcium hydroxide is among the substances recommended for intracanal dressings. It is considered an effective substance because it induces hard tissue formation, produces intratubular barriers, presents good antibacterial and anti-inflammatory properties as well as a capacity to dissolve tissues,³ act upon lipopolysaccharides and absorb CO₂.⁴

Calcium hydroxide has two fundamental properties: inhibition of enzymes that are essential for bacterial survival, which explains its antimicrobial activity; and activation of tissue enzymes, such as alkaline phosphatase, which promote mineralization.⁵

When dissolved in water, calcium hydroxide is dissociated into hydroxyl ions and calcium ions, being the former responsible for the alkaline shift of the solution. High pH values are probably responsible for the antimicrobial activity of calcium hydroxide paste, and this is the reason why calcium hydroxide paste depends on the concentration of hydroxyl ions in the solution. Therefore, the slower the dissociation of the paste, the longer the concentration of hydroxyl ions will remain constant in the solution, and the antimicrobial action inside the canal will last longer.⁶

The release of hydroxyl ions from the calcium hydroxide paste is essential for bacterial control. For this reason, the vehicle used in association with it should accelerate ion dissociation and keep pH values at high levels during long periods of activity.⁵

In certain clinical conditions, such as treatment of root resorption, trauma and incomplete root formation, calcium hydroxide paste should be kept in the root canal for a longer period of time and be replaced at regular intervals, so as to remain active.

Recent studies have found that a paste combining 2% chlorhexidine gel, calcium hydroxide and

zinc oxide does not require replacements at regular intervals. Its antimicrobial characteristics include the capacity to keep pH values at an alkaline level, rapid diffusion in root dentin and consequent inhibition of bacterial growth on external root surfaces.⁷

This study aimed at assessing whether a calcium hydroxide paste in chlorhexidine gel and zinc oxide promotes changes in pH at short time intervals, reaching and sustaining ideal pH values over time in comparison with other combinations described in the literature.

Material and Methods

Three calcium hydroxide-based pastes (Biodinâmica, Ibioporã, Brazil) were prepared with three different vehicles:

- » Paste A – calcium hydroxide with saline solution.
- » Paste B – calcium hydroxide with propylene glycol (*Fórmula Exata* Compounding Pharmacy, Campo Mourão, Brazil).
- » Paste C – calcium hydroxide with 2% chlorhexidine gel (Biodinâmica, Ibioporã, Brazil) and zinc oxide (Biodinâmica, Ibioporã, Brazil).

The pastes were prepared using a measuring spoon to standardize the amount of calcium hydroxide in each preparation. For pastes A and B, the vehicle was added so as to obtain a tooth paste consistency. As for paste C, calcium hydroxide, chlorhexidine gel and zinc oxide were combined in a 2:1:2 ratio so as to obtain a putty consistency.

After the materials had been combined, the pastes were stored in 5-mm-high plastic rings made from anesthetic cartridges. The samples were placed in 15 ml of deionized water previously stabilized at pH 7 for the analysis of ion dissociation at the following time intervals: 15 and 30 minutes; 1, 24 and 48 hours; 7 and 14 days. Measurements were taken by means of a pH meter calibrated with buffer solutions standardized at pH 7 and pH 4. The values were recorded for ANOVA statistical analysis.

Results

The pH values of the calcium hydroxide pastes in different vehicles are shown in Table 1.

The pH values of each paste under analysis were different from each other and between time intervals. All three pastes presented a sharp increase of

Table 1. Values of pH for the calcium hydroxide pastes in different vehicles.

	15 minutes	30 minutes	1 hour	24 hours	48 hours	7 day	14 days
Paste A	10.70	10.98	11.19	12.13	12.40	12.39	9.55
Paste B	9.60	10.20	11.68	12.14	12.80	11.60	9.65
Paste C	10.20	10.30	10.67	11.66	12.90	12.10	11.99

pH values at the first time interval and remained relatively stable at a value of about 12 from 24 hours to 7 days. After that, the pH values of pastes A and B decreased to 9.50, whereas the pH values of paste C remained at 12, thus demonstrating its efficacy as an intracanal dressing that lasts longer. The differences between the pH values of the calcium hydroxide pastes were not statistically significant.

Discussion

One of the etiological factors behind the need for endodontic treatment is the presence of microorganisms in the root canals. Cleaning and shaping, aided by the use of chemicals with antimicrobial properties, should remove microorganisms and their products. However, microorganisms may remain viable in inaccessible areas and may induce and perpetuate inflammation, thus hindering repair. In these cases, treatment success depends on the elimination of infection, in which case the use of intracanal dressings is essential to reduce microbial infection and ensure treatment success.^{1,8}

Calcium hydroxide pastes have several advantages including antimicrobial properties, pH values close to 13;^{9,10,11} formation of a physical and chemical barrier;¹⁰ inactivation of endotoxins present on the cell walls of gram-negative bacteria associated with pain and resorption;¹² and diffusion through dentin tubules.¹³

In some clinical cases, calcium hydroxide should be kept in the canal for longer periods of time, as it is the case of endodontic treatment for teeth with incomplete root formation and pulp necrosis, root resorption and dental trauma. A paste in which the vehicle promotes slow dissociation should be used in such cases, so that an elevated pH value is maintained. However, this may be considered as a disadvantage, given that several visits are necessary for the

dentist to replace the intracanal dressing. According to Frank,¹⁴ the time that calcium hydroxide remains in the canal for the treatment of those cases may range from 5 to 20 months. It should be noted that during this period, the tooth has a temporary restoration and, for this reason, the risks of infiltration are imminent. Additionally, the cost of this type of treatment is also high, and so it is the risk of patients dropping out.¹⁴ For this reason, studies have investigated different vehicles that can be used with calcium hydroxide at more extended intervals between visits without affecting its efficacy.

This study found that the combinations with saline solution and propylene glycol had increasing pH values up to 7 days, and decreasing values up to 14 days. The combination with 2% chlorhexidine gel and zinc oxide had a constant increase over all time intervals, in agreement with findings reported by Montagner,¹⁵ Bretas,¹⁶ Nerwich³ and Maniglia-Ferreira.¹⁷

In spite of the apparent advantage of the combination of calcium hydroxide and chlorhexidine gel in maintaining alkaline pH values, some limitations of the use of this paste should be noted. According to Kuga,¹⁸ chlorhexidine at high concentrations generates oxidative radicals that damage the cell wall and, consequently, reduce the biological compatibility of the paste.

Conclusion

Based on the results of this study it is reasonable to conclude that

» All pastes under study affected the pH of the solution and made it alkaline.

» The pastes with saline solution and propylene glycol had a faster alkaline shift, and the paste of calcium hydroxide in 2% chlorhexidine gel and zinc oxide kept an elevated pH value for a longer period, but differences were not statistically significant.

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Chlorhexidine and its applications in Endodontics: A literature review

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ABSTRACT

This study aims at presenting the properties of chlorhexidine used as an auxiliary chemical substance for endodontic instrumentation: structure and mechanism of action, substantivity, tissue solvent effect, chlorhexidine x sodium hypochlorite interaction, cytotoxicity, action over biofilm, antibacterial activity, antifungal activity, intracanal dressing, rheological action and allergic reactions. In Dentistry, chlorhexidine has been proved effective and safe against bacterial plaque since 1959. In Endodontics, it has been recommended in liquid or gel form, at different concentrations (usually 2%), as root canal irrigant and dressing (alone or associated with other substances). Additionally, it may be applied as an antimicrobial agent at all stages of root canal preparation, including disinfection of the operative field, removal of

necrotic tissues before determining the root length, chemical-mechanical preparation before foraminal clearance and enlargement, disinfection of obturation cones; to shape the main cone with gutta-percha, to remove gutta-percha during retreatment, to disinfect the prosthetic space; etc. It is reasonable to conclude that chlorhexidine, at different concentrations, has an antimicrobial activity against Gram-positive as well as gram-negative bacteria and fungus. Its antimicrobial activity, increased by the substantivity effect, does not have the ability of solving tissues, which is overcome by the rheological action of its gel form that lubricates the endodontic instrumentation used. Its biocompatibility is acceptable with relative absence of cytotoxicity.

Keywords: Chlorhexidine. Microorganism control agent. Endodontics.

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Introduction

Most bacteria found in infected root canals can be removed by the simple mechanical action of endodontic instrumentation. Nevertheless, despite thorough mechanical instrumentation, organic residues and bacteria located deeply inside the dentin tubules cannot be reached due to the anatomic complexity of root canals.^{1,2} Irrigation solutions are indicated to aid mechanical preparation and pulp space disinfection. Thus, several substances have been used not only to remove debris and necrotic pulp tissue during and immediately after root canal preparation, but also to help eliminate the microorganisms that could not be reached by mechanical instrumentation.³ The search for an ideal substance for root canal irrigation has motivated researchers since the beginning of Dentistry. Chemical agents chosen to function as endodontic irrigants have four major properties: antimicrobial activity; organic tissue dissolution that favors debridement of the root canal system; and absence of toxicity against periapical tissues.^{1,2,4} Most substances used to irrigate the root canal are liquid: sodium hypochlorite (NaOCl), chlorhexidine gluconate — also known as chlorhexidine digluconate or simply chlorhexidine (Chlorhexidine) —, 17% EDTA, citric acid, MTDA and 37% phosphoric acid solution.⁵

Sodium hypochlorite is the most popular irrigation solution due to its antimicrobial and physicochemical properties.^{6,7} The antimicrobial efficacy of NaOCl is due to its high pH (the action of hydroxyl ions) similar to the mechanism of action of calcium hydroxide.⁸ The high pH of NaOCl interferes in the integrity of the cytoplasmic membrane with an irreversible enzymatic inhibition that causes biosynthetic alterations in cellular metabolism and destruction of phospholipids, observed during lipid peroxidation. The antimicrobial activity of NaOCl leads to an irreversible enzymatic inhibition of bacteria, which originates hydroxyl ions, as well as to chloramination action.² Despite being an effective antimicrobial agent and an excellent organic solvent,⁹ NaOCl is known for being highly irritant to periapical tissues,¹⁰ especially at high concentrations.¹¹ For this reason, the search for another irrigation solution, with lower potential in inducing adverse effects, proves feasible.^{2,12}

Thus, irrigation solutions with antibacterial activity and biocompatibility, as it is the case of chlorhexidine, have been recommended to treat infected root canals. The antibacterial effect and long-term action of 2% chlorhexidine digluconate¹³⁻¹⁷ led researchers to indicate its use for endodontic treatment.^{15,16,18}

Chlorhexidine is a cationic biguanide that acts by adsorption in the bacterial wall of a microorganism, causing leakage of the intracellular components. Due to being a strong base, low-concentration chlorhexidine has a bacteriostatic effect; however, at higher concentrations, it produces a bactericidal effect. Chlorhexidine digluconate has a slightly acidic pH that varies from 5.5 to 6.0, with the ability to donate protons.¹⁹

Chlorhexidine was first introduced in the late 40s, when scientists, in the search for new agents against malaria, formulated a group of compounds with a broad antibacterial spectrum, known as polibiguanides.^{20,21} Chlorhexidine was registered in 1954 by the Imperial Chemical Co. Ltd. (Macclesfield, United Kingdom), under the trademark Hibitane. Due to its biocompatibility and broad antibacterial activity, it was the first antiseptic internationally accepted for skin, wound and mucosa cleansing.²² Since then, chlorhexidine has been used for several medical purposes, namely: gynecology, urology and ophthalmology, as well as for the treatment of skin burns and disinfection.²³

In Dentistry, chlorhexidine has been proved effective and safe against bacterial plaque since 1959. In the 70s, it was commercialized in Europe as a 0.2% mouthwash solution and in 1% gel.^{21,23}

Chlorhexidine may be applied as an antimicrobial agent at all stages of root canal preparation, including disinfection of the operative field, during root canal instrumentation, removal of necrotic tissues before determining the root length, chemical-mechanical preparation before foraminal clearance and enlargement, as an intracanal dressing (alone or in association with other substances), disinfection of obturation cones; to shape the main cone with gutta-percha, to remove gutta-percha during retreatment, to disinfect the prosthetic space; etc.⁵

Viscous irrigants, such as glycerin-based ones, have low solubility. As a result, they leave residues at the dentin walls, which hinders the final obturation

of the root canal system.^{12,24} However, Natrosol is a highly efficient non-ionic inert gel that is hydro-soluble and broadly used in cosmetic products based on cationic substances.²

Chlorhexidine gel has been widely used in Dentistry. It yields satisfactory results for cavity control, reducing *Streptococcus mutans* and *Lactobacillus*, acting as auxiliary in periodontal therapy, and controlling the growth of Gram-positive and Gram-negative bacteria.²⁵

Ferraz et al¹² demonstrated that 2% chlorhexidine gel is highly advantageous in comparison to 2% chlorhexidine solution, even though both of them have similar antimicrobial, substantivity and biocompatibility properties. Chlorhexidine gel lubricates the root canal walls, which reduces friction between the endodontic file and the dentin surface. As a result, it favors instrumentation, improves file performance and reduces the risk of file fracture inside the root canal. Additionally, chlorhexidine gel allows better debridement and, as a consequence, compensates its inability in organic tissue dissolution.^{2,26} Chlorhexidine gel leaves the majority of dentin tubules open as a result of its viscosity that keeps debris in suspension and reduces the formation of smear layer, which does not occur with chlorhexidine liquid. Furthermore, the active ingredient of chlorhexidine gel establishes long-term contact with microorganisms and, as a consequence, inhibits their growth.²⁷ When chlorhexidine gel is used for the mechanical preparation of a root canal, the irrigant solution of choice must be saline solution or distilled water.

In this context, this study aims at conducting a literature review that presents the properties of chlorhexidine used as an auxiliary chemical substance for endodontic instrumentation.

Literature review

Microorganisms have been broadly recognized as the main etiologic factor of periapical bone lesions.²⁸ Their persistence in the apical area of obturated root canals is responsible for the majority of endodontic treatment failures.^{29,30} Thus, microbial control is of paramount importance for an effective endodontic treatment,²⁸ of which success relies on the elimination of microorganisms from infected root canals.¹

Most bacteria found in infected root canals can be removed by the simple mechanical action of endodontic instrumentation. However, despite thorough mechanical instrumentation and the several techniques available, organic residues and bacteria located deeply inside the dentin tubules cannot be reached due to the anatomic complexity of root canals.^{2,31} For this reason, chemical treatment of the root canal system proves necessary.

According to several authors,^{11,32,33,34} the ideal auxiliary substance must have the property of: leaving debris in suspension, lubricating endodontic instruments, dissolving organic tissue, developing antibacterial activity during instrumentation, substantivity, exerting chelating action, promoting cleaning of inaccessible areas, being biocompatible at concentrations that fulfill these properties within a viable clinical time, removing the smear layer formed during instrumentation, having low surface tension, neutralizing action and bleaching effect, having no color alterations, being of easy application, removal, handling and storage, accessible, inexpensive and of extended useful life.

Several substances have been used to irrigate the root canal system, namely: sodium hypochlorite (NaOCl), chlorhexidine gluconate — also known as chlorhexidine digluconate or simply chlorhexidine —, 17% EDTA, citric acid, MTDA and 37% phosphoric acid solution.⁵ Sodium hypochlorite, at different concentrations, is the most commonly used substance due to its triple mode of action: necrotic tissue dissolving ability attributed to its high alkalinity; antibacterial properties related to hypochlorous acid formation in chlorine solution; and fat saponification.³⁵

Sodium hypochlorite is a halogenated compound of which first use was registered in 1972 under the name of “Javele’s water”. It was obtained by mixing NaOCl with potassium. In 1820, Labarraque obtained sodium hypochlorite at a concentration of 2.5% of active chlorine. In the early XX century, during World War I, sodium hypochlorite was used to treat infected wounds. In 1915, Dakin³⁶ proposed a new concentration for the solution (0.5%) because, according to the author, wounds treated with 2.5% sodium hypochlorite took too long to heal due to the high content of sodium hydroxide.^{36,37} In Endodontics, its use was

first proposed by Coolidge, in 1919; first employed by Walker, in 1936, due to its excellent tissue dissolving ability as well as its antimicrobial efficacy,³⁹ and disseminated by Grossman.^{38,40} It has been employed in Endodontics for more than 60 years as an irrigation solution during chemo-mechanical preparation of the root canal system.⁹ Despite NaOCl excellent antimicrobial activity and tissue dissolution ability, it causes irritation to periapical tissues,⁴¹ it is caustic and causes clothes stain and instruments corrosion,⁴² especially at high concentrations.¹¹ According to Ramos and Bramante,⁴³ biocompatibility is one of the main desirable properties of an irrigation solution. For this reason, the search for another irrigation solution with lower potential in inducing adverse effects proves feasible.^{2,12}

Among different alternatives, chlorhexidine has proved to be an effective antimicrobial agent acting inside root canals, showing a great potential to be used as irrigant or intracanal dressing. It is also recommended for cases of incomplete root formation or hypersensitivity to sodium hypochlorite due to its low toxicity. Chlorhexidine is found in the form of liquid (water solution) or gel, at concentrations that vary from 0.2 to 2%.^{35,44}

It is characterized as a cationic detergent of the biguanide class. It is available as acetate, hydrochloride and digluconate which is the most used format.⁴⁵ Chlorhexidine was first introduced in the late 40s when scientists were searching for new agents against malaria.^{20,21} In 1954, it was first used as an antiseptic to treat skin wounds⁴⁶ under the trademark Hibitane registered by the Imperial. Ltd. (Macclesfield, United Kingdom).²²

In Dentistry, chlorhexidine has been proved effective and safe against bacterial plaque since 1959. It was first tested by Løe and Schiott⁴⁷ who demonstrated that 0.2% chlorhexidine mouthwash twice a day is effective to decrease biofilm growth and gingivitis development for a period of 21 days.⁴⁵ Initially, it was commercialized in Europe, in the 70s, as a 0.2% mouthwash solution and in 1% gel.^{21,25}

Due to its broad antibacterial spectrum, it has been widely used in Periodontology. In Endodontics, it has been recommended as digluconate salt, liquid or gel at different concentrations, as well as root canal irrigant^{13,15,18,23,48,50,51} or as intracanal dressing.^{13,53-57}

In this context, this literature review highlights 11 major points related to chlorhexidine, so as to facilitate understanding. The extensive literature on chlorhexidine determined that discussions should be restricted to factors commonly focused by *in vivo* studies and literature reviews. To this end, the following databases were used for research: MEDLINE, PubMed, BBO, Lilacs, SciELO, websites available on the internet and the library archives of the School of Dentistry / Piracicaba (FOP-UNICAMP).

Structure and mechanism of action

The structural formula of chlorhexidine consists of two symmetric 4-chlorophenyl rings and two biguanide groups connected by a central hexamethylene chain.²² Classified as a cationic detergent, this biguanide is a strong base which is practically insoluble in water. For this reason, it is prepared in the form of salt,²³ which increases its solubility. In Dentistry, its most commonly used form is chlorhexidine digluconate salt in water solution.^{13,22} The bactericidal effect of the drug is due to its cationic molecule binding to extra-microbial complexes and negatively charged microbial cell walls, entering in the cell by active or passive transportation.⁵⁸ At high concentrations (2%), chlorhexidine has a bactericidal effect due to precipitation and/or coagulation of the cytoplasm of bacterial cells, probably caused by protein cross-linking, resulting in cell death.^{59,60} At lower concentrations (0.2%), chlorhexidine has a bacteriostatic effect, which causes inhibition of the membrane function. This effect remains for several hours after application due to its excellent substantivity (residual effect).⁴⁹ Solutions are usually colorless as well as odorless.

When aqueous, chlorhexidine seems to be more stable for pH varying from 5 to 8. pH values above 8 lead to precipitation. In an acidic pH, chlorhexidine solution loses stability and, as a consequence, deterioration of its properties occurs. Its antibacterial effect is excellent for pH values varying from 5.5 to 7.^{48,61} Chlorhexidine is found in the form of solution, dentifrices, varnishes and gel.⁶²

Tasman et al⁶³ assessed the surface tension of different irrigation solutions: distilled water; 2.5% sodium hypochlorite; 5% sodium hypochlorite; 17% EDTA; 3% hydrogen peroxide; 3% citanest-octapressin and

0.2% chlorhexidine. The ring method was employed to this end. The authors yielded the following results in ascending order: chlorhexidine; 2.5% hypochlorite; 5% hypochlorite; 17% EDTA; 3% citanest-octapressin; hydrogen peroxide; saline solution and distilled water. The authors concluded that the low surface tension of chlorhexidine favors its penetration into the dentin tubules.

According to Ferraz et al,² chlorhexidine gluconate had lower surface tension in comparison to sodium hypochlorite and EDTA. The use of chlorhexidine associated with a gel vehicle provides dentin walls free of waste produced by instrumentation as a result of the mechanical properties of gel.

Substantivity

According to Hortense et al,⁶⁴ substantivity is the capacity chlorhexidine has to remain active in the surface where it is applied (tooth, gingiva and oral mucosa surfaces negatively charged). It is slowly released, avoiding salivary flow to neutralize its action. Substantivity is an important property for treatment of dental plaque infections, since antimicrobial agents need some time to neutralize/kill a microorganism.²²

In Endodontics, the residual antibacterial effect of chlorhexidine is due to its capability to bind to hydroxyapatite.⁶⁵ Therefore, a gradual release of chlorhexidine could maintain a constant level of molecules, which is enough to create a bacteriostatic scenario inside the root canal for a long period of time.

Parsons et al⁴⁸ conducted one of the first studies recommending the use of chlorhexidine for endodontic purposes. The authors observed the adsorption and release of chlorhexidine solution by bovine pulp and dentin samples, as well as its antibacterial properties after a deliberate contamination caused by *Streptococcus faecalis*. Results revealed that, after the samples were treated with chlorhexidine, no contamination was observed within 48 and 72 hours of bacterial exposure. This confirmed the residual effect of chlorhexidine.

Other studies have been conducted to assess the substantivity of chlorhexidine. Their results showed that this activity can last 48 hours,¹⁸ 72 hours,¹⁶ 7 days (chlorhexidine liquid and gel),⁶⁶ 21 days¹⁷ or 4 weeks.⁶⁷ Rosenthal, Spangberg and Safavi⁶⁸

assessed the substantivity of 2% chlorhexidine in root canal system and its long-term efficacy in comparison to its antimicrobial effect. Their results revealed that chlorhexidine remains in the dentin of root canals with its antimicrobial effect for more than 12 weeks.

According to Messer and Chen,⁶⁹ this property differs chlorhexidine from other disinfectants that quickly dissipate and have no residual antibacterial effect. Khademi, Mohammadi and Havaee⁶⁷ highlight that only chlorhexidine and tetracycline have the aforementioned property.

Tissue dissolving effect

Several studies have searched for a product that meets the properties necessary for a root canal irrigant: antimicrobial activity, non-toxic to periapical tissues, soluble in water and organic matter dissolving ability.³¹ In 1941, Grossman and Meiman⁷⁰ demonstrated the importance of tissue dissolving ability of an endodontic irrigant, determining that success of endodontic treatment relies on pulp tissue elimination from the root canal. Zehnder¹⁹ corroborates Grossman and Meiman⁷⁰ and asserts that the ideal cleaning of root canals is crucial for endodontic treatment, given that removal of tissues and bacterial residue would prevent the tooth from becoming a source of infection. Therefore, the necrotic tissue dissolving ability of irrigation agents was assessed. An *in vitro* study revealed that 1% sodium hypochlorite had a substantial dissolution capacity, unlike 10% chlorhexidine.⁷¹ According to Moorer and Wesselink,⁷² tissue dissolution depends on the frequency of agitation, the amount of organic matter in relation to the irrigant, and on the tissue surface area available for contact with the irrigant. Okino et al⁷³ assessed the tissue dissolving ability of sodium hypochlorite at different concentrations, 2% chlorhexidine digluconate water solution, chlorhexidine gel and distilled water. Fragments of bovine pulp were submerged in 20 mL of each solution. Both distilled water and chlorhexidine solutions did not dissolve the pulp during the six hours of the experiment.

Considering the experiments performed, it can be concluded that a disadvantage of chlorhexidine is its inability to dissolve tissues.³¹

Interaction between chlorhexidine and sodium hypochlorite

An *in vivo* study conducted by Zamany⁷⁴ employed two therapeutic protocols in which, after chemo-mechanical preparation with NaOCl, a final irrigation with 4 mL of saline solution or 2% chlorhexidine was performed during 30 seconds. Evaluation was carried out by means of culture mediums and biological indicators collected from tooth canals. The chlorhexidine protocol produced a positive culture in one out of 12 cases, whereas the saline solution protocol produced a positive culture in seven out of 12 cases. The use of 2% chlorhexidine digluconate as an extra irrigant used after biomechanical preparation improved the efficiency of endodontic therapy with regard to antimicrobial activity.

For treatment before root canal filling, Zehnder¹⁹ recommends irrigation with sodium hypochlorite to dissolve organic tissue, irrigation with EDTA to eliminate the smear layer and irrigation with chlorhexidine to increase antimicrobial spectrum and substantivity. Despite the visible increase in antimicrobial efficacy produced by the combination of irrigants,⁴¹ chemical interactions, such as precipitation and color change that result from a combination between NaOCl and chlorhexidine, must be taken into account.^{19,26,75} This corroborates the study conducted by Basrani et al⁷⁶ who sought to determine the minimum concentration of sodium hypochlorite causing pigmentation and precipitation when associated with 2% chlorhexidine. The resultant precipitate was qualified and quantified. All sodium hypochlorite solutions in combination with 2% chlorhexidine digluconate led to color alterations, even with NaOCl at low concentrations (0.023%). The formation of precipitate was also observed until the sixth dilution (0.19%). Both pigmentation and precipitation were directly proportional to the concentration of sodium hypochlorite. By-products were formed in the mixtures with 3% and 6% sodium hypochlorite. One example is the formation of parachloraniline, a fragment that results from hydrolysis of chlorhexidine digluconate. In other words, a by-product that theoretically forms another by-product. Fragmentation occurs in the bond between carbon and nitrogen (guanidine group) of which dissociation requires little energy. The clinical importance of these findings

relies on the pathological potential of parachloraniline, as well as on other by-products that result from the mixture. Parachloraniline has a carcinogenic potential and causes methemoglobinemia and cyanoses, being cytotoxic.⁷⁷ Other by-products might have pathological action related to their own molecular character, as it is the case of action exerted by higher reactivity (free radicals). The formation of precipitate may be explained by the acid-base reaction that results from mixing sodium hypochlorite and chlorhexidine.³¹

The precipitate that results from mixing sodium hypochlorite and chlorhexidine is also known as fluorination.⁷⁸ Basrani et al⁷⁶ observed that it produces an orangish-brown solution which, once in the pulp chamber, chemically stains the dentin tubules and, as a consequence, changes tooth color⁷⁸⁻⁸¹ and interferes in root canal filling.^{28,82} A spectrophotometric analysis revealed the presence of calcium, iron, magnesium, copper, zinc and manganese in the precipitate.⁷⁸ According to Heling and Chandler,⁸³ associating chlorhexidine with EDTA also forms a milky-white precipitate. When combined with saline solution and ethanol, they produce salt. Thus, when sodium hypochlorite is used as an irrigation solution during mechanical preparation, chlorhexidine may be used as a final irrigant or intracanal dressing only after sodium hypochlorite is completely removed from the root canal,⁸² so as to avoid interaction between solutions.⁵ As complementary irrigation solutions, distilled water and saline solution are recommended.

Cytotoxicity

Chlorhexidine is stable and has low cytotoxicity.⁶ It is minimally absorbed by the mucosa and skin, it is well tolerated in animals, when administered via parenteral and intravenously, it seems not to cross the placental barrier, it does not cause systemic toxic side effects or alterations in the oral microbiota.⁸⁴⁻⁸⁸ With regard to the metabolic pathways of chlorhexidine, whenever ingested, it reduces plasma levels and is excreted in feces (90%) and urine (10%). The frequency of metabolic segmentation by oral intake is also low, with no evidence of parachloraniline formation. When carried in the bloodstream of dogs, it is metabolized by the liver and kidney, producing polar metabolites, while chlorhexidine remains intact in the bile.⁸⁷

Tanomaru Filho et al⁶ assessed the inflammatory response of different endodontic solutions used in rats. 0.5% sodium hypochlorite, 2% chlorhexidine digluconate and saline solutions were injected in the peritoneal cavity of the animals which were killed after 4h, 24h, 48h and seven days. Results revealed that sodium hypochlorite induced inflammatory response, whereas chlorhexidine digluconate did not provoke any significant response. In 2005, Ribeiro et al⁸⁹ assessed the genotoxicity (potential damage to DNA) of formocresol, paramonochlorophenol, calcium hydroxide and chlorhexidine against the ovary cells of Chinese hamsters. The results revealed that none of the agents damaged the DNA. Faria et al⁹⁰ assessed the cytotoxicity of chlorhexidine digluconate by means of observing tissue lesions (edema/inflammation) in rats' paws. Assessment was complemented by histopathological examination and analysis of cell death and stress in culture of fibroblasts. Edema (inflammation) was observed as a result of exposing the lesions to chlorhexidine digluconate at different concentrations (0.125; 0.25; 0.5 and 1%). Edema subsided after 14 days at the two lowest concentrations. At 0.125%, no tissue necrosis was observed despite mild inflammation, whereas at 0.25%, small foci of necrosis were found. Edema persisted after 14 days at the two highest concentrations. Inflammation and larger foci of tissue necrosis were also observed. The authors concluded that chlorhexidine digluconate may produce an adverse effect on the resolution of apical periodontitis. Additionally, their results point to higher biocompatibility in concentrations equal to or less than 0.25%. Furthermore, lower concentrations are characterized by promoting cell apoptosis, whereas higher concentrations cause stress and cellular necrosis.

Thus, the concentrations of chlorhexidine clinically used have acceptable biocompatibility,³¹ with relative absence of cytotoxicity.¹⁵

The first studies about the toxicology of chlorhexidine were conducted by Foukes⁹¹ who established the lethal dose of chlorhexidine orally and intravenously taken, and tolerance to chronic administration. The author concluded that chlorhexidine has unusually low toxicity for both, animals and humans. Additional research conducted by Davies and Hull⁸⁴ confirmed the findings of other authors, determining

the lethal dose of 50 (LD 50) for chlorhexidine applied by intravenous injection (22 mg/Kg/day), and LD 50 (1800 mg/kg/day) for oral administration. These results were obtained from experiments carried out with species of rodents (rabbits and mice) and ruminants (cattle). Hugo and Longworth⁹³ found no harmful effect for chlorhexidine digluconate orally taken. To test the carcinogenic potential, four groups with 224 rats each were used. The animals received doses of 5, 25 or 50 mg/kg of body weight and were tested for two years. By the end of the dosage, peak levels dropped by half within one to two weeks. Chlorhexidine levels in the brain, lung, liver, kidney, mesenteric nodes and other lymph nodes, as well as in the blood were determined at regular intervals during the experiment and after the end of administration during three, six and nine weeks. No histological changes were found. The concentration of chlorhexidine in the liver was high in the final controls, but decreased to half after one and two weeks. There was no incidence of neoplasm in the control and treated groups. The extremely low acute oral toxicity found in animals has been confirmed in humans in the last 30 years of experience, with unrestricted use. Pereira⁹⁴ conducted a research on acute and chronic toxicity of chlorhexidine digluconate orally taken by mice and found an increase in weight gain in comparison to the control group, significant reduction in the number of deaths attributable to the inhibition of intercurrent infections in the treated groups and absence of teratogenic effects. Case⁸⁵ and Rushton⁸⁶ concluded that percutaneous absorption is practically null.

Action over biofilm

According to Costerton, Stewart and Greenberg,⁹⁵ biofilm is a structured community of microorganisms surrounded by a matrix of polysaccharides produced and adhered to live or inert surfaces. The cells comprising the biofilm structure are phenotypically different from planktonic cells (microorganisms presented in a free and disorganized form), since they are less susceptible to antimicrobial substances.⁹⁶

Biofilm control occurs as a result of the anti-septic property of chlorhexidine associated with adsorption (ability to be retained on an oral surface and be slowly released), assuring an extended

antimicrobial environment.^{60,97} Adsorption is explained by electrostatic interaction. Due to its cationic characteristic, chlorhexidine has a strong affinity for anions, such as phosphate ions from the cell wall of oral microbiota which normally colonizes the tooth surfaces,⁹⁸ thus reducing adherence and colonization of tooth surfaces. This process enhances cell wall permeability and, as a consequence, leads to cytoplasm rupture and causes cell death.⁹⁸ Due to its bactericide and bacteriostatic effect, chlorhexidine inhibits the development of microbial plaque development.⁶⁴ This anti-plaque effect is probably the most significant property of chlorhexidine.⁹⁹

One of the major mechanisms of resistance of biofilm is associated with failure of agents in penetrating its extension. Polymeric substances, such as those found in biofilm matrix, reduce the diffusion of chemical substances and antibiotics. Solutes tend to diffuse more slowly. The speed of penetration varies according to the type of microorganism and the composition of the exopolysaccharide matrix. A second mechanism of resistance is associated with the ability of a microorganism present in biofilm to survive after long periods of food shortage which decreases its growth rate. Microorganisms with reduced growth rate, or no growth, are less sensitive to chemical substances.^{95,99,100,101} Mohammadi and Abbott³¹ reported that a microorganism growing in biofilms is two to 1,000 times more resistant than its correspondent planktonic form.

Studies conducted with biofilm composed by a single species^{102,103} and apical dentin biofilm¹⁰⁴ revealed that an increase in sodium hypochlorite concentration (varying from 2.25 to 6%) and 2% chlorhexidine solution were effective against the microorganisms tested. Mechanical agitation enhances antimicrobial activities of chemical substances, particularly favoring liquid agents such as 5.25% sodium hypochlorite and 2% chlorhexidine.¹⁰² Chlorhexidine has a significantly lower effect on microbial biofilm in comparison to hypochlorite.³¹

Tyler et al¹⁰⁵ assessed the distribution and transport of chlorhexidine digluconate and glucose in *Candida albicans* biofilm. Their results confirmed the diffusion capacity of chlorhexidine digluconate through biofilm, which is not uniform, thus suggesting that chlorhexidine preferentially binds to sites

of microbial cells and/or passes through microcanals present in biofilm. The presence of microcanals suggests that biofilm is somehow organized or at least has a complex structure, since microcanals allow the entrance of nutrients and excreta output. Additionally, the authors concluded that the action of chlorhexidine is directly proportional to concentration that tends to decrease as chlorhexidine goes deeper into the biofilm. Glucose does not diffuse uniformly either, which results in areas with nutrients shortage.

Clegg et al¹⁰⁴ assessed the efficacy of disaggregating and removing polymicrobial biofilm produced by sample collected from teeth of patients diagnosed with periapical lesion 3-mm in diameter associated with pulp necrosis and who were not treated by antibiotic drugs. The samples were seeded in culture medium and evaluated microscopically. 2% chlorhexidine proved not to affect biofilm or eliminate bacteria. Nevertheless, it generated absence of microbial growth (culture medium). 6% sodium hypochlorite was the only substance that favored absence of bacteria, removed biofilm and promoted absence of microbial growth (culture medium).

Antibacterial activity

Its antibacterial activity is explained by the ability of chlorhexidine to be rapidly attracted by the negative charge of bacterial surface, and adsorbed to the cell membrane by electrostatic interactions, probably by hydrophobic bindings or hydrogen bridges. Adsorption is concentration-dependent. In higher concentrations, it causes not only precipitation and coagulation of cytoplasmic proteins, but also bacterial death; whereas in low concentrations, cell membrane integrity is altered, resulting in extravasation of low molecular weight bacteria components.^{60,93,106} Thus, the molecule cationic end binds to the pellicle with negative charge (anionic), whereas the other cationic end is free to interact with bacteria that aim at colonizing the tooth.⁴⁵ In Endodontics, chlorhexidine is recommended for root canal irrigation during chemo-mechanical preparation,¹⁰⁶ since it inhibits bacterial growth in endodontic infections.^{51,56,107} The action of chlorhexidine depends on the susceptibility of microorganisms; Gram-positives have higher susceptibility to chlorhexidine in comparison

to Gram-negatives.¹⁰⁷ Some species of *Streptococci* seem to retain an additional amount of chlorhexidine in their extracellular polysaccharide capsules, which might be related to the high sensitivity of *Streptococci* to chlorhexidine.¹⁰⁸

In 1982, Delany et al¹³ conducted an *in vitro* study on the antimicrobial action of 0.2% chlorhexidine gluconate solution used as irrigant and intracanal dressing on root canal microbiota of recent extracted necrotic pulp of human teeth. Bacterial growth was observed by inoculation of dentin debris on agar, which caused a significant reduction in the number of bacteria in both endodontic procedures.

Heling et al⁵³ conducted an *in vitro* study to assess the antibacterial effect of 2% chlorhexidine gluconate at 20% used, in a in a slow release system, as intracanal dressing in bovine incisors contaminated with *S. faecalis*. The slow release system consisted of strips containing glutaraldehyde as vehicle and 1.2 mg of 20% chlorhexidine as active agent. The microbiological analysis of dentin removed from canal walls revealed that both forms of dressing were effective for depth of 0.5 mm in experimental periods of 24, 48 hours and seven days.

Siqueira Jr. and Uzeda⁵⁶ assessed the antibacterial activity of 0.12% chlorhexidine digluconate gel, 10% metronidazole gel, calcium hydroxide with distilled water, calcium hydroxide with PMCC camphorated paramonochlorophenol and calcium hydroxide with glycerin applied on strict and facultative anaerobic bacteria commonly found in endodontic infections. Their results revealed that calcium hydroxide paste with PMCC and chlorhexidine were effective for all species of bacteria tested (strict anaerobic — *Porphyromonas endodontalis*, *P. gingivalis*, *Actinomyces israelis*, *Fusobacterium nucleatum*, *Propionibacterium acnes* and *Campylobacter rectus*; and facultative anaerobic — *Staphylococcus aureus*, *Streptococcus mutans*, *S. sanguis*, *S. salivarius*, *Enterococcus faecalis* and *Actinomyces viscosus*). Metronidazole inhibited the growth of all strict anaerobic species, whereas calcium hydroxide with distilled water or glycerin were ineffective.

Lindskog, Pierce and Blomlöf⁵⁷ assessed the effect of 10% chlorhexidine gluconate gel used as intracanal dressing during one month on inflammatory root resorption induced in monkeys. The authors

found a reduction in the resorption process due to the antimicrobial action of chlorhexidine inside dentin tubules and on periodontal ligament cells.

Ferraz⁵¹ conducted an *in vitro* research on chlorhexidine gel used as endodontic irrigant in comparison to other irrigants commonly used in Endodontics. The author concluded that 2% chlorhexidine gel or solution showed the highest averages of inhibition halos against all microorganisms tested by the agar diffusion test. Chlorhexidine gel produced, *in vitro*, higher inhibition halos of microbial growth when compared to chlorhexidine solution at equivalents concentrations. However, with no statistically significant differences. Similarly to 5.25% sodium hypochlorite, 2% chlorhexidine solution produced negative cultures after 45 seconds of contact with *Enterococcus faecalis*, acting more rapidly than other irrigants. Teeth irrigated with 2% chlorhexidine gel had a higher number of negative microbiological cultures (80%); after *in vitro* instrumentation, 2% chlorhexidine gel significantly reduced smear layer in comparison to 2% chlorhexidine solution and 5.25% sodium hypochlorite.

Menezes et al⁵² conducted an *in vitro* study to assess the efficacy of sodium hypochlorite and 2% chlorhexidine used as irrigation solution. Teeth had been contaminated by *Enterococcus faecalis*. The authors concluded that chlorhexidine was more effective.

Haapasalo et al⁴⁴ conducted a literature review in which they highlight that the use of chlorhexidine at 0.2 to 2% might offer an additional advantage against resistant microorganisms disseminated by the root canal system. This is due to the ability of chlorhexidine to increase bacterial cell or cell wall permeability; act inside fungi cytoplasm membrane; cause coagulation of intracellular constituents at high concentrations. Other advantages include residual antimicrobial action and substantivity; relatively low toxicity, wide spectrum of action and efficacy against *Enterococcus faecalis* and *Staphylococcus aureus*. According to the authors, chlorhexidine efficacy decreases in contact with organic matter, mycobacteria, bacterial spores and virus, all of which are resistant. Additionally, chlorhexidine has cytotoxicity at high concentrations; chlorhexidine gel is less effective against *Enterococcus faecalis* in comparison to solution; chlorhexidine combinations

are so or less effective than its compounds alone; when in contact with tooth dentin (organic compounds), chlorhexidine efficacy decreases, but is not completely neutralized; albumin from bovine plasma neutralizes chlorhexidine action and does not act as a tissue solvent.

Dametto et al⁶⁶ conducted an *in vitro* study to assess the antimicrobial activity of 2% chlorhexidine gel against *Enterococcus faecalis* in comparison to other endodontic irrigants (2% chlorhexidine solution and 5.25% sodium hypochlorite). 2% chlorhexidine gel and 2% chlorhexidine solution significantly reduced *E. faecalis* at post-treatment and final phases. 5.25% sodium hypochlorite also reduced *E. faecalis* immediately after root canal instrumentation. However, it did not completely eliminate *E. faecalis* from the root canal. The authors concluded that 2% chlorhexidine gluconate (gel and solution) had higher antimicrobial capacity against *E. faecalis* in comparison to 5.25% sodium hypochlorite seven days after biomechanical preparation.

In 2006, the results of a research conducted by Fachin, Nunes and Mendes⁹² agreed with Jeansonne et al¹⁵ who affirmed that 2% chlorhexidine is an effective antimicrobial that produces results statistically similar to 5.25% sodium hypochlorite, and of which substantivity increases antimicrobial performance.

Wang et al¹⁰⁹ assessed the clinical efficacy of 2% chlorhexidine gel with regard to the reduction of intracanal bacteria during root canal instrumentation. The additional antibacterial effect of calcium hydroxide associated with 2% gel used as an intracanal dressing was also assessed. The authors concluded that 2% chlorhexidine gel effectively decontaminates the root canal, and, when used as intracanal dressing, does not produce additional significant effects on bacterial reduction.

Pretel et al¹¹⁰ concluded that 2% chlorhexidine is a feasible irrigation solution due to its specific characteristics of substantivity and high antibacterial effect. According to the authors, chlorhexidine proves more effective considering its penetration and substantivity inside dentin tubules.

Its bactericidal activity is faster than its fungicide activity and strongly depends on pH. Its maximum activity can only be achieved with pH 8 (Neobrax¹¹¹).

Antifungal activity

Chlorhexidine digluconate has a wide spectrum of action^{59,112} with potent antifungal action against *Candida albicans*.^{113,114} Fungi (or yeast) represent a small portion of oral microbiota. *Candida* is the species of fungi most commonly found in healthy (30 to 45%) as well as in medically compromised individuals (95%).¹¹⁵ These fungi might be involved in cases of persistence and secondary infection associated with relapse of periapical lesions, given that they are microorganisms strongly associated with therapeutic failures.^{17,59,65,74,75,114,116-119} For this reason, endodontic irrigants should include these microorganisms in within their spectrum of activity.³¹ According to Waltimo et al,¹¹³ the presence of fungi in infected root canals varies between 1 to 17%.

In 1999, Sen, Safavi and Spangberg¹²⁰ assessed the antifungal effects of 0.12% chlorhexidine and 1 to 5% sodium hypochlorite on root canals. They performed root sections and removed smear layer in half of the specimens. Root canals were inoculated with *Candida albicans* for 10 days. Subsequently, root sections were treated with 3 mL of the irrigation solution during one, five, 30 and 60 minutes. The authors observed that, in the presence of smear layer, the antifungal activity of all irrigants started after 60 minutes, only. Antifungal activity was higher in teeth of which the smear layer was removed. After 30 minutes, 5% sodium hypochlorite showed antifungal activity of 70% and after one hour, it was totally effective. 0.12% chlorhexidine and 1% sodium hypochlorite proved to be totally effective after an hour.

Waltimo et al¹¹³ assessed the antifungal action of calcium hydroxide, 0.5% chlorhexidine acetate, 0.05% iodinated potassium iodide and sodium hypochlorite, alone and in combination. To this end, they used absorbent paper points contaminated with *Candida albicans*, directly exposed to the disinfectants, for periods of 30 seconds, five minutes, one and 24 hours. In comparison to calcium hydroxide associated with distilled water, 0.5% and 0.05% chlorhexidine proved more effective. After 24h, the association of 0.5% chlorhexidine with calcium hydroxide P.A. was also more effective than calcium hydroxide associated with distilled water and less effective than 0.5% chlorhexidine alone.

Alexandra et al¹²¹ conducted an *in vitro* study in which the efficacy of four chemical substances

used as intracanal dressing were compared: calcium hydroxide, chlorhexidine gel, PerioChip (Asstra Zeneca) and chlorhexidine gel associated with calcium hydroxide. Saline solution was used as the control group. The substances were tested in three different periods (three, eight and 14 days) using human teeth previously contaminated with *E. faecalis*. Calcium hydroxide eliminated *Enterococcus faecalis* within three to eight days, but it was effective in the 14-day group, probably due to a pH drop. The different formulations of chlorhexidine were effective in eliminating *E. faecalis* from dentin tubules, with chlorhexidine gel showing the best results.

Siqueira Jr. et al¹²² assessed the efficacy of four intracanal dressings in decontaminating the root canal of bovine teeth experimentally infected with *Candida albicans*. Infected dentin cylinders were exposed to four different dressings: calcium hydroxide and glycerin; calcium hydroxide and 0.12% chlorhexidine digluconate; calcium hydroxide with camphorated paramonochlorophenol and glycerin; 0.12% chlorhexidine digluconate with zinc oxide. Specimens were in contact with the dressings during 1 hour, 2 and 7 days. *Candida albicans* viability after exposure was evaluated by means of incubating the sample in culture medium to compare the efficacy of the dressing in dentin disinfection. Results revealed that specimens treated with calcium hydroxide associated with camphorated paramonochlorophenol and glycerin, or with chlorhexidine combined with zinc oxide were completely decontaminated after 1-hour exposure. Calcium hydroxide with glycerin eliminated *C. albicans* after 7 days, only. Calcium hydroxide associated with chlorhexidine proved ineffective to disinfect dentin, even after one week of exposure. Calcium hydroxide with camphorated paramonochlorophenol and glycerin, as well chlorhexidine digluconate associated with zinc oxide proved to be the most effective in eliminating *C. albicans*.

Ruff, McClanahan and Babel¹²³ compared the antifungal efficacy of 6% sodium hypochlorite, 2% chlorhexidine, 17% EDTA and MTDA BioPuro with final rinse as canal preparation, in which teeth were contaminated with *Candida albicans*. Teeth were divided into four groups: Group 1 – 1 mL of 6% sodium hypochlorite for 1 min; Group 2- 0.2 mL of 2% chlorhexidine for 1 min; Group 3 -5 mL of MTDA

BioPuro for 5 min, following the manufacturer's instructions; Group 4 – 1 mL of 17% EDTA for 1 min. Results showed that 6% sodium hypochlorite and 2% chlorhexidine were equally effective and significantly superior to the other groups. MTDA was significantly superior to 17% EDTA.

Ballal et al¹²⁴ analyzed the antiseptic action of different intracanal dressings. They used *Candida albicans* and *Enterococcus faecalis* as microbiological indicators and conducted an observation on inhibition halos of microbial growth in solid medium culture. All intracanal dressings tested exhibited inhibition halos. Within 24 hours of action against *C. albicans*, calcium hydroxide paste in water proved to be the most effective, whereas against *E. faecalis*, 2% chlorhexidine gel had the best action. After 72 hours, 2% chlorhexidine gel was the most effective dressing against *C. albicans* and *E. faecalis*, whereas the combination of the two substances yielded the worst results against both biological indicators. The authors concluded that 2% chlorhexidine gel is more efficient than calcium hydroxide paste, whether associated with water or 2% chlorhexidine gel.

Intracanal dressing

Chemo-mechanical preparation significantly reduces microbiota in infected root canals. However, Bystrom, Claesson and Sundqvist,¹²⁶ Sjögren et al¹²⁷ as well as Ando and Hoshino¹²⁵ highlighted the need for intracanal dressing use to prevent those bacteria surviving to chemo-mechanical preparation in a sufficient number and adequate environment from multiplying between treatment sessions. Thus, the need for root canal disinfection through chemo-mechanical preparation is clear. It can be achieved not only by the proper use of an intracanal dressing that has antimicrobial properties and functions as a physical barrier,^{3,127-130} but also by proper filling of the root canal system and appropriate coronal sealing.¹³² Additionally, intracanal dressing aims at reducing periapical lesions, solubilizing organic matter, neutralizing toxic products, controlling persistent exudate, controlling inflammatory external root resorption and stimulating repair by means of mineralized tissue.¹³³

Chlorhexidine has been highly recommended as intracanal dressing due to its immediate antimicrobial action; wide antibacterial spectrum of action

against Gram-positive and Gram-negative bacteria, whether anaerobic, facultative and aerobic; yeast and fungi;^{20,23,59,112} (especially *Candida albicans*);^{113,120} relative absence of toxicity;^{49,86} dentin adsorption capacity and slow release of its active substance, which extends its residual antimicrobial activity.^{15,16,53,54,134}

Delany et al¹³ demonstrated the effect of 0.2% chlorhexidine gluconate used as intracanal dressing on the reduction of remaining antimicrobial population after root canal instrumentation. Due to its wide antimicrobial spectrum, chlorhexidine has been largely used in Endodontics. It has been recommended as digluconate salt, liquid or gel at different concentrations, as well as intracanal dressing.^{13,53-57}

Ohara et al¹⁴ assessed the antimicrobial effects of six irrigants against anaerobic bacteria and highlighted that chlorhexidine was the most effective. With regard to the elimination of *E.faecalis* from inside of dentin tubules, chlorhexidine used as intracanal dressing yielded better results than calcium hydroxide.⁵³

Lenet et al¹³⁵ conducted an *in vitro* study to compare the residual antimicrobial activity of 0.2 and 2% chlorhexidine gel in a system of controlled release, and calcium hydroxide associated with saline solution used as intracanal dressing in bovine incisors, during seven days. After the experimental period, the specimens were inoculated in *E.faecalis* during 21 days. Results revealed that 2% chlorhexidine gel had no viable bacteria in all dentin depths.

According to Vianna,¹³⁴ 2% chlorhexidine gel had higher antimicrobial activity. The combination between calcium hydroxide and 2% chlorhexidine gel decreased the antimicrobial activity of chlorhexidine, however, it increased the activity of calcium hydroxide.

Gomes et al¹³⁶ assessed the efficacy of 2% chlorhexidine digluconate gel and calcium hydroxide used as intracanal dressing at different time intervals (one, two, seven, 15 and 30 days). To this end, roots from bovine teeth previously infected with *E.faecalis* were used. 2% chlorhexidine gel; calcium hydroxide associated with polyethylene glycol 400; and 2% chlorhexidine gel associated with calcium hydroxide were used as intracanal dressing. The authors observed that 2% chlorhexidine gel inhibited bacterial growth in the infected dentin samples in all periods tested. The combination of calcium hydroxide and polyethylene glycol 400 was inefficient in

eliminating bacteria during all periods. Absence of dentin contamination was found in periods of one and two days for samples comprising the association of 2% chlorhexidine gel and calcium hydroxide. As for periods of seven and 15 days, there was a decrease in antimicrobial activity and, after 30 days, all samples from this group were contaminated. In conclusion, 2% chlorhexidine gel has a wide antimicrobial activity against *E.faecalis*. However, the authors highlighted that this property might decrease with time if the medication is used for long periods.

Pinheiro et al¹³⁷ conducted an *in vitro* study to assess the antimicrobial activity of 50% calcium hydroxide and 2% chlorhexidine gel used alone or in combination. The following microorganisms were tested: *Enterococcus faecalis*, *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermis* and *Pseudomonas aeruginosa*. After 24 and 48 hours, they assessed the inhibition halos. The halos formed against *E. coli*, *S. aureus* and *S. epidermis* were discrete and of similar dimension. Calcium hydroxide and 2% chlorhexidine gel used alone showed antimicrobial activity against all microorganisms tested. When combined, the substances showed higher inhibition halos against *E.faecalis* and *C.albicans* in comparison to calcium hydroxide used alone. However, the combination of substances showed smaller halos, for both microorganisms, in comparison with 2% chlorhexidine gel used alone.

In 2006, Montagner et al¹³⁸ assessed the antimicrobial action of intracanal dressings on external surface root against different microorganisms. 288 roots extracted from upper canines were divided into two groups, with and without cementum. The following microorganisms were isolated from clinical samples and analyzed: *Enterococcus faecalis*, *Candida albicans*, *Actinomyces viscosus* and *Porphyromonas gingivalis*. 2% chlorhexidine gel; 2% chlorhexidine gel and calcium hydroxide (1:1); 2% chlorhexidine gel, calcium hydroxide and zinc oxide (1:1:1); calcium hydroxide and saline solution; saline solution (positive control) were used as intracanal dressings. The best antimicrobial effect was produced by 2% chlorhexidine gel, followed by 2% chlorhexidine gel and calcium hydroxide; 2% chlorhexidine gel, calcium hydroxide and zinc oxide; and calcium hydroxide and saline solution. *A. viscosus* (2.85 mm)

was most sensitive to the medications, followed by *E. faecalis* (1.84 mm), *C. albicans* (0.95 mm) and *P. gingivalis* (0.82 mm). Presence or absence of cementum did not interfere in the substance capacity of reaching the outer root surface and exerting its antimicrobial action. The authors concluded that intracanal dressings associated with chlorhexidine were able to diffuse through the dentin and reach the outer root surface. The combination between calcium hydroxide and saline solution did not show antimicrobial activity in the outer root surface within 72 hours. Conversely, 2% chlorhexidine gel associated with calcium hydroxide and zinc oxide revealed rapid diffusion capacity in root dentin, causing inhibition of bacterial growth.

Gomes et al¹³⁹ investigated the antimicrobial activity of intracanal dressings by means of the agar diffusion test as well as by direct contact. The following biological indicators, which represent endodontic infection, were included: *Enterococcus faecalis*, *Candida albicans*, *Staphylococcus aureus*, *Porphyromonas endodontalis*, *Porphyromonas gingivalis* and *Prevotella intermedia*. Agar diffusion and direct contact tests revealed that 2% chlorhexidine digluconate gel (1% Natrosol "hydroxyethyl cellulose" with pH 7.0) had the highest efficacy; calcium hydroxide in 2% chlorhexidine digluconate gel, intermediate efficacy; and calcium hydroxide with sterile water as vehicle, the worst. The latter did not produce inhibition halos. There was susceptibility of *Enterococcus faecalis* and *Candida albicans* to intracanal dressings, following the order previously related, as well as inactivity of calcium hydroxide in water in the agar diffusion test. The authors explained that the inability of calcium hydroxide in water to diffuse throughout agar is due to the low solubility of hydroxide, as well as the buffer effect and protein coagulation action occurring in the agar. These effects are liable to occur *in vivo*, which avoids penetration of the intracanal dressing into the dentin tubules and irregularities of the root canal. The antimicrobial action of 2% chlorhexidine digluconate gel is reduced when the substance is associated with calcium hydroxide.

Fachin, Nunes and Mendes⁹² assessed the efficacy of four intracanal dressings (camphorated paramonochlorophenol, calcium hydroxide, 2% chlorhexidine gel and 1% sodium hypochlorite)

in cases of pulp necrosis with periapical lesion, by means of clinical and radiographic control. All solutions were effective to decrease the size of apical lesions. Initial results reveal that, after three months, the highest percentages of reduction in lesion diameter occurred with 2% chlorhexidine gel.

The results of this research are encouraging with regard to the use of 2% chlorhexidine gel as intracanal dressing in cases of pulp necrosis. Thus, these results corroborate Heling et al,⁵⁴ Barbosa et al,⁵⁵ Lenet et al¹³⁵ and Rosa et al¹⁴⁰ and confirm the efficacy of 2% chlorhexidine used as intracanal dressing.

Ballal et al¹²⁴ analyzed the antiseptic action of different intracanal dressings. They used *Candida albicans* and *Enterococcus faecalis* as microbiological indicators and conducted an observation on inhibition halos of microbial growth in solid medium culture. All tested intracanal dressings exhibited inhibition halos. Within 24 hours of action against *C.albicans*, calcium hydroxide paste in water proved to be the most effective, whereas against *E.faecalis*, 2.0% chlorhexidine gel had the best action. After 72 hours, 2.0% chlorhexidine gel was the most effective medication against *C.albicans* and *E.faecalis*, whereas the combination of the two substances yielded the worst results against both biological indicators. The authors concluded that 2% chlorhexidine gel is more efficient than calcium hydroxide paste, whether associated with water or 2% chlorhexidine gel.

Marion et al¹⁴¹ reported a case conducted by means of a new therapeutic protocol, in which calcium hydroxide was associated with 2% chlorhexidine gel and zinc oxide and used as filling paste for avulsed tooth. The combination between calcium hydroxide, 2% chlorhexidine gel and zinc oxide was also assessed by Souza-Filho et al,¹⁴² Almeida et al¹⁴³ and Montagner et al¹⁴⁴ in an *in vitro* study that revealed the antimicrobial action and capacity to keep an alkaline pH of the substance. Other case reports found in the literature^{138,145} reveal that this association has a fast diffusing capacity in root dentin, causing inhibition of bacterial growth on the outer surface of the root canal. The case report conducted by Marion et al¹⁴¹ revealed absence of signs and symptoms in tooth treated with filling paste, which remained after a three-year follow-up, thus proving the efficiency of this medication in the treatment of traumatized permanent teeth.

Rheological action

This action is found in chlorhexidine gel, since it refers to the capacity of maintaining debris in suspension inside the root canal.⁵

When the pulp chamber and root canal are flooded with chlorhexidine gel and mechanical preparation of root canal system is initiated (instrumentation), both inorganic and organic debris (smear layer) — detached from root canal walls — accumulate in the amorphous mass of gel which captures and keep them suspended. Subsequently, active irrigation with saline or distilled water removes the debris, preventing them from accumulating in the root canal walls and, as a result, exposing the entrance of dentin tubules. In other words, it considerably reduces the formation of smear layer, thus improving the efficacy of EDTA as a chelating substance and increasing treatment prognostic.^{2,5,27,146,147}

Ferraz et al² investigated the antimicrobial action of chlorhexidine gel and solution over *Enterococcus faecalis* and its capacity of cleaning the root canal wall, in comparison to 5.25% sodium hypochlorite. To this end, 70 recently-extracted single-rooted teeth were selected. They were prepared up to the apical foramen with file #40, submitted to a 17% EDTA wash with ultrasound, sterilized and infected. Subsequently, root canals underwent instrumentation with 2% chlorhexidine gel, chlorhexidine solution or 5.25% sodium hypochlorite. Water and Natrosol gel were used as control. As for suppression of bacterial growth, no statistical differences were found between groups. Nevertheless, with regard to cleaning, the highest number of open dentin tubules was found in chlorhexidine gel, followed by chlorhexidine solution and 5.25% sodium hypochlorite, which confirmed the capacity of chlorhexidine gel in preventing smear layer formation, probably as a result of the mechanical action of Natrosol gel.

Allergic reactions

No adverse effects have been published regarding the use of chlorhexidine as irrigant or intracanal dressing.⁵ On the other hand, animal studies have shown that 2.0% chlorhexidine used as intracanal dressing did not induce intense inflammatory response when injected into the peritoneal cavity of mice.^{148,149} Chlorhexidine has a limited number of

adverse effects, such as desquamative gingivitis, tooth and tongue discoloration or dysgeusia (distortion of the sense of taste). Contact sensitivity to chlorhexidine was first described by Calnan.¹⁵⁰ Contact with the conjunctiva may cause permanent damage, whereas accidental contact with the tympanum might cause ototoxicity.¹⁵¹ It may also cause contact urticaria, photo-sensibility, fixed drug eruption and occupational asthma. Patients with leg ulcers and eczema have particular risks of contact allergy (besides doctors and dentists). Contact sensitivity to chlorhexidine seems to be generally rare. Some studies have demonstrated a high rate of sensitization, around 2%.^{152,153} Ohtoshi, Yamauchi and Tadokoro¹⁵⁵ described even rarer reactions caused by chlorhexidine, in which case immediate anaphylactic reactions were observed and IgE antibodies were found in patients' serum.

The major side effects of chlorhexidine are as follows: tooth discoloration (in the cervical third and proximal surfaces),¹⁵⁶ restorations, prosthesis and tongue; dental calculus accumulation, taste alteration (especially to salt), oral desquamation, supragingival calculus formation and occasional parotid gland swelling dyspnea and anaphylaxis.¹⁵⁷⁻¹⁶¹ Among these effects, tooth discoloration stands out as patients' chief complaint,¹⁶² since it affects 30 to 50% of patients.^{88,153} It is considered as the main limiting factor of chlorhexidine when used for long periods of time. Concentration and volume of chlorhexidine interfere in the prevalence and severity of discoloration. Thus, despite having similar efficacy and effectiveness,¹⁶⁴ lower concentrations, in larger volumes, proved to cause less tooth discoloration.¹⁶⁵ However, these unpleasant effects are reversible once the use of chlorhexidine is suspended.¹⁶¹

Although sensitivity to chlorhexidine may be rare, the possibility of complications should be kept in mind during its application.¹¹⁸

Final considerations

Based on this literature review on the applications of chlorhexidine for endodontic purposes, it is reasonable to conclude that:

- » Chlorhexidine, liquid or gel, may be used during all phases of root canal preparation, in which case the concentration of 2% is most frequently used.

- » Its wide antimicrobial spectrum (Gram-positive and Gram-negative bacteria), including fungi, is improved due to substantivity, which may last from 48 hours to 12 weeks.
- » Chlorhexidine does not solve organic tissue, however, chlorhexidine gel may favor it as a result of rheological reaction and lubrication of endodontic instruments during mechanical action.
- » Sodium hypochlorite associated with chlorhexidine results in an orangish-brown solution (parachloraniline) that requires further investigation.
- » Chlorhexidine has been recommended as an alternative to sodium hypochlorite. It is considered a biocompatible solution, however, the possibility of further complications should be taken into account during its application.

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Pulp revascularization: a literature review

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ABSTRACT

Permanent teeth with incomplete root formation are one of the greatest challenges of endodontic practice. These teeth need a type of treatment that is different from conventional endodontic therapy. The most common causes of incomplete root formation are: dental trauma and deep tooth cavity, both of which may lead to pulp necrosis. Teeth with incomplete root formation and pulp necrosis were usually treated by apexification. In other words, treatment comprised multiple sessions to replace calcium hydroxide-based root canal dressing and the fabrication of a MTA apical plug to create an apical barrier. Nevertheless, with this method, root dentin walls are thin and fragile. Thus,

revascularization becomes a new treatment option that aims at promoting the completion of root formation, with invagination of a new connective tissue in the inner space of the pulp cavity. This study conducts a literature review comparing some case reports that focus on pulp revascularization in immature permanent teeth with pulp necrosis. Based on this review, it is reasonable to conclude that pulp revascularization is a feasible method for root maturation with thickening and, as a consequence, strengthening of young permanent teeth root walls.

Keywords: Pulp revascularization. Apexification. Blood coagulum.

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Introduction

Histologically speaking, whenever the root apex of a tooth does not have apical dentin covered by cementum; or, radiographically speaking, should the apical ending not reach Nolla's stage 10 (tooth with complete root apex), the tooth is diagnosed with incomplete root formation.¹ According to Nosrat et al,² treating immature teeth by means of pulp necrosis is a challenge for Endodontics.

A traumatic injury of an immature permanent tooth may lead to loss of pulp vitality and, as a consequence, interrupted root development mainly due to bacterial contamination caused by tooth cavity or traumatic injury.³ As a result, the tooth is left with an open apex and poor crown-root ratio, given that it tends to have thinner-wall root subjected to a higher risk of fracture. Should an endodontic intervention be necessary, the clinician is about to face a difficult situation, since biomechanical preparation is complicated in these cases.⁴

Conventional treatment of young permanent teeth with incomplete root formation and pulp necrosis includes long-term application of calcium hydroxide to induce apexification.⁵ According to Ding et al,³ this treatment method has several disadvantages, namely: variable treatment time, patient's cooperation and increased risk of tooth fracture after long-term preparation. The MTA plug technique has also been employed to create an artificial apical barrier. Although those techniques prove to be clinically effective, they do not contribute to root strengthening. As a consequence, the absence of continuous root development leads to thin and fragile root dentin walls.⁶

A new concept of endodontic therapy for immature teeth with incomplete root formation and pulp necrosis has been introduced by Iwaya et al.⁷ It consists of pulp revascularization, aiming at continuous root development; and invagination of a new connective tissue in the inner space of the pulp cavity. Revascularization has many advantages, among which is the reduction in treatment time, completed within one or two sessions after infection control; favorable cost/benefit, given that a few appointments and little additional material are necessary; and, last but not least, the greatest advantage of all: stimulus for complete root formation, which, as a result, provides root wall strengthening. According to Thibodeau and Trope,⁵ as well as Keswani and Pandey,⁸ the procedure involves root canal passive decontamination (with sodium

hypochlorite, EDTA or chlorhexidine solution) and the use of intracanal dressing which, according to Shin et al,⁹ Iwaya et al¹⁰ and Cehreli et al,¹¹ may be formocresol, triple antibiotic paste (metronidazole, ciprofloxacin and minocycline) or calcium hydroxide paste. Thus, according to Nosrat et al,² in the absence of clinical signs and symptoms, treatment goes on by removing the paste and inducing bleeding of apical tissues with a sterile instrument. Once blood coagulum is formed, the root canal opening is sealed at the cervical third with the aid of a MTA barrier. The process of inducing blood coagulum to go into the root canal is justified by the studies conducted by Ostby.¹² The author observed that blood and blood coagulum were essential to form fibrous connective tissue inside an empty root canal. According to Nosrat,² the tissue formed in the root canal is not pulp and does not function as pulp tissue. This means that revascularization is similar, but not equivalent to regeneration during the process of wound repair.

Thus, this paper aims at conducting a literature review to assess a series of case reports, published between 1961 and 2013, about young permanent teeth with incomplete root formation and pulp necrosis treated by pulp revascularization.

Literature review

This literature review focuses on clinical cases which, according to some studies, would directly influence the basic criteria required for a successful revascularization treatment. We believe that this methodology increases one's understanding about the study. The extensive literature on pulp revascularization determined that discussions should be restricted to some factors commonly focused by *in vivo* studies and literature reviews. To this end, the following databases were used for research: MEDLINE, PubMed, BBO, LILACS, SciELO and the library archives of the School of Dentistry / Piracicaba (FOP-UNICAMP).

Root formation begins after enamel and dentin achieve the enamel-dentin junction. Tooth root development relies on the inner and outer epithelia of the enamel organ which are connected and apically immersed into the mesenchyme that surrounds them. The connection between both epithelia is known as Hertwig's epithelial root sheath. These cells induce differentiation of inner connective tissue cells into odontoblasts, responsible for depositing the first layer

of root dentin. At this point, the continuity of the root sheath is disrupted, and its lower portion remains immersed into the mesenchyme, which induces differentiation of odontoblasts. From this point on, the epithelial rests of Malassez are formed. As odontoblasts differentiate to form the dentin, and as the sheath degenerates, dentin contacts the surrounding mesenchyme. The mesenchymal cells in contact with the dentin differentiate into cementoblasts to form the cementum that is deposited over the root dentin.¹³

According to Torneck,¹⁴ at the beginning of root formation, initial dentin formation causes the disruption of Hertwig's epithelial root sheath, providing it with a lacy aspect in the area permeated by dental follicle. Complete root formation of permanent teeth happens three to five years after tooth eruption.

"Tooth with incomplete root formation", "immature apex", "open apex" or "young foramen" are terms that have been widely used to identify the endodontic processes in which root has not been completely formed.¹⁵

An open root apex occasionally results from extensive resorption of a mature apex after orthodontic treatment, from periapical inflammation or as part of trauma repair.¹⁶

In young patients, permanent incisors are commonly affected by traumatic lesions of which type and intensity might cause pulp alterations. The incidence of lesions, particularly those caused by falls, collisions and stumbles, is higher for individuals aged between 8 and 10 years old.¹⁷

According to Soares et al,¹⁸ the apical constriction of a mature tooth, found near the CDC (cementum, dentin, canal) limit facilitates endodontic preparation and filling. However, the root canal of immature teeth with incomplete apexes is usually hourglass-shaped, with the diameter of the foraminal opening greater than the canal found in the cervical and middle thirds.

Completely formed teeth have a cone-shaped root canal with the major base towards the pulp chamber and the minor, towards the apical third. Conversely, teeth with incomplete root formation do not have the root completely formed. They have a frustum-shaped canal, with the major base towards the apical third. Thus, an open foramen does not provide anatomic buttress which, as a consequence, hinders filling,^{15,16} given that biomechanical preparation cannot establish the "apical seat" necessary for gutta-percha cone placement.

For this reason, there might be a risk of root canal overfilling (even if cones of greater diameter are used) due to the absence of a physical barrier that prevents condensation necessary to establish complete hermetic apical sealing.¹

To overcome the aforementioned difficulties, several procedures have been used. All efforts should be made to preserve a vital pulp. Conventional treatment allows the physiological process of root formation (apexogenesis) to happen without further complications.¹⁸ Pulp necrosis, on the other hand, requires especial endodontic intervention.

Traditionally, the absence of pulp vitality and presence of an open apex require an intervention that aims at promoting apexification. The procedure includes cleansing and filling the root canal with a temporary paste (calcium hydroxide monthly replaced) that stimulates the formation of calcified tissue in the apex. Nevertheless, calcium hydroxide-induced apexification has its limitations, for instance, the time required for the formation of an apical barrier (from 6 to 24 months for a porous barrier). Additionally, the technique does not promote complete root formation, but apical closing instead. In addition to the aforementioned technique, the method of placing an apical barrier composed of mineral trioxide aggregate (MTA) has also been used as buttress during condensation for definitive filling.^{5,15,19}

Apexification procedures have some disadvantages, including the fact that root canal walls remain thin and that the finishing process of root development might not occur. As a result, teeth are more likely to fracture after endodontic treatment.²

Revascularization procedures are recommended to treat young permanent teeth with pulp necrotic tissue and/or apical periodontitis.²⁰ This method increases thickness of root canal walls as a result of hard-tissue deposition, and stimulates complete root development of young permanent teeth.²¹

According to Camp,²² Shin et al,⁹ Banchs and Trope,²³ Nosrat et al² and Chueh et al,²⁴ before endodontic treatment, correct pulp and periapical diagnoses are essential for teeth with incomplete root formation, since there is risk of pulp vitality loss. Diagnostic tests are essential for obtaining as much information as possible before exploring all treatment options.

Diagnosis starts with patient's complete medical history and the potential implications related to the

treatment of choice. Patient's dental history and associated pain are useful to determine the conditions of the pulp.^{9,21-24}

Aspects such as nature, type, length and distinction between provoked and spontaneous pain must be registered. Pain provoked by thermal, chemical or mechanical irritants usually suggests a minor degree of pulp inflammation which, most of the times, is reversible. Spontaneous pain, however, is commonly associated with extensive and degenerative irreversible pulp inflammation or necrosis.^{2,2,24}

Medical as well as dental history is determined by a complete clinical examination. Areas of redness, swelling, fluctuation, tissue sensibility, dental cavity, defective restorations or fractured teeth are recorded. Crown discoloration, or parulis, might be indicative of pulp necrosis.^{2-5,22,24}

Electrical and thermal pulp tests are limited due to variability in response. Occasionally, they might not be elucidative because, in teeth with incomplete root formation, the parietal layer of nerves is not completely developed and, as a consequence, the pulp responds less to those stimuli.^{18,21-23} Furthermore, invalid data might be obtained as a result of less reliable responses (fearful child, conduct-related issues, lack of understanding or poor communication). Therefore, more sensitive methods and tools must be developed.^{22,25} As a consequence, most diagnoses are made by observation of clinical symptoms and pathologic radiographic evidence.

Radiography is of paramount importance to the diagnosis of pulp pathologies in teeth with developing apex. Apical radiograph of high quality is used to assess root development, periapical radiolucency and root resorption.^{2,6,9,21,22,24,26,27}

Given that revascularization occurs in immature teeth with open apex and pulp necrosis, this situation is frequently found in young patients. For this reason, patient's age is key to help immature teeth attain complete root maturation.

Most studies focusing on pulp revascularization analyze the clinical-radiographic results of treatment performed on immature teeth of patients aged between 6 and 16 years old.^{2-5,6,9,12,21,23,24} Four researches also report tests conducted with 6-month young dogs.^{12,28,29,30}

According to Chueh et al,²⁴ young children have great healing capacity and better potential of stem cell regeneration. Furthermore, immature teeth have faster apical bone healing and continuation of root development,

which reveals the great potential for regeneration in young children's teeth.

Most case reports and studies reveal that lower premolars with pulp necrosis and upper incisors, which are more likely to fracture, are the type of teeth most commonly treated by revascularization.^{3,4,5,9,23,24,28,31,32} However, two researches report revascularization of immature molars.^{2,32} Radiographically speaking, these teeth have immature apex, large canal, parallel walls and open apex.^{3,4,21,23,24}

These teeth are often diagnosed with chronic periapical abscess.^{4,9,21,23} Recent studies reveal that revascularization is predictable in ideal conditions and with chemical decontamination of root surface.^{2,4,21,23,24,32}

According to Nosrat et al,² Banchs and Trope,²³ Cotti et al,⁴ Thibodeau and Trope,⁵ Wang et al,²⁸ Ding et al,³ Chen et al²¹ and Shin,⁹ the ideal conditions comprise root canal biomechanical preparation carried out after local anesthesia and isolation with rubber barrier. In case of remaining restorations or dental cavity, the barrier is removed and a cavity access is prepared with a fissured diamond bur and abundant water. The pulp chamber entrance is copiously irrigated with an auxiliary chemical substance. Root canal mechanical instrumentation is not carried out by the majority of revascularization treatment procedures not only because immature teeth have thin dentin walls and, for this reason, are more likely to fracture; but also because smear layer formation could obstruct dentin walls and tubules.^{3,4,5,9,21,24} Therefore, chemomechanical preparation aims at cleaning, extending and shaping the root canal, since infection control promotes a favorable environment that allows pulp and periapical cells to participate in repair and regeneration.²¹

To this end, irrigation solutions are used in root canal irrigation-aspiration due to their minor viscosity coefficient and reduced superficial tension, requirements that favor increased jet reach, turbulence formation and liquid reflux towards a coronary direction, all of which allow a more effective root canal cleaning.

The literature reveals that some researchers use sodium hypochlorite irrigation similarly to conventional root canal preparation, which is widely used at different concentrations: 5.25% NaOCl,^{2,3,4,21,23} 2.5% NaOCl,²⁴ 1.25% NaOCl,^{5,28} 1% NaOCl + EDTA,³² 6% NaOCl + saline solution + 2% chlorhexidine gluconate.⁹ This is due to the potent antibacterial and proteolytic

activity of sodium hypochlorite which dissolves organic material, eliminates microorganisms and removes necrotic tissue. Higher concentrations increase the effect of sodium hypochlorite, however, such efficacy is neutralized by the increase in toxicity, given that sodium hypochlorite leads to inflammatory response and severe damage when in contact with vital tissues.³² Nevertheless, the cases reported to date reveal that the application of sodium hypochlorite do not produce any post-operative sequelae. Once the procedure is complete, the root canal is gently dried with sterile paper points.

Revascularization success depends on the absence of bacteria inside the root canal, since the new tissue ceases its development as bacteria are found in the root canal space.^{30,33} Thus, the technique most commonly employed for revascularization includes the use of a triple antibiotic paste applied to complement canal decontamination. Several topical antibiotic combinations are able to decontaminate carious dentin and necrotic root canal. One of the combinations used is the triple paste composed of metronidazole, ciprofloxacin and minocycline. It has proved effective in fighting pathogens usually found inside the canal root system,^{2-6,9,21,23,28} and in eliminating bacteria found deeper in the root dentin (which can survive even after endodontic treatment).

Sato et al³¹ concluded that the combination between ciprofloxacin, metronidazole and minocycline enters the dentin tubules and eradicates bacteria from infected root dentin. This strongly suggests that root canal treatment allows infected root dentin to be decontaminated by the topical application of those drugs.

Considering that most bacteria found in deeper layers of infected root dentin walls are strict anaerobic, metronidazole was the first choice of antibacterial drug. It has been reported that metronidazole enters deeply into carious lesions, decontaminates lesions *in vivo* and spreads through the dentin. However, alone, it cannot neutralize all bacteria. For this reason, other drugs are necessary to sterilize infected root dentin. Therefore, ciprofloxacin and minocycline, combined with metronidazole, were necessary to decontaminate infected root dentin because, together, they generate fibroblasts that are viable for toxicity test and biocompatible with the tissues.²⁹

On the other hand, calcium hydroxide and formocresol have also been effectively used as intracanal dressing, decontaminating the root canals of young permanent

teeth with pulp necrosis and apical periodontitis/abscess during revascularization procedures.^{3,4,6,21,23,24,26} Nevertheless, some authors describe that a retrospective evaluation of radiographic results reveal that revascularization with triple antibiotic paste significantly increases root wall thickness, greater than what is produced by calcium hydroxide or formocresol. Additionally, radiographic results reveal that disinfection by formocresol caused minor improvements in root length and thickness. Furthermore, a series of cases concerning revascularization treatment with calcium hydroxide as intracanal dressing reveal that a 10 to 29-month follow-up is necessary to judge radiographic evidence of root development.^{2,6}

The polyantibiotic mixture is applied with a K-file #25 instrument, 3 mm shorter than the length radiographically estimated. Afterwards, the tooth is temporarily restored. After a period of time, the patient returns and the tooth is once more anesthetized with anesthetic without vasoconstrictor, which facilitates isolated and accessed bleeding. Subsequently, the antibiotic paste is removed by means of a new irrigation procedure and, afterwards, canal is dried with paper sterile points.

Apical tissues are irritated using a K-file #40 instrument, so as to obtain bleeding and blood coagulum formation inside the root canal, as well as to guide tissue invagination.^{2-5,21,23,25,28,29,32} Blood coagulum consists of a fibrin network that functions as a path for cell migration, including macrophages and fibroblasts in the periapical region. Blood coagulum, however, not only forms an inactive scaffold, but it also provides growth and differentiation factors that are important for the process of repair.^{2,3,5,28} Additionally, it contributes to tissue inner growth.^{2-5,23,28}

In 1961, Ostby¹² reported that laceration in granulation tissue adjacent to the foramen might be beneficial to the process of repair. Moreover, blood has inherent antibacterial properties such as cells that develop into phagocytes. Blood invagination into the root canal might have two effects: destruction of remaining microorganisms and phagocytosis of necrotic remnants. Last but not least, the most important benefit: blood coagulum organization and the formation of fibrous tissue in the apical third.

Thus, it is safe to suppose that blood coagulum — inside the decontaminated and empty root canal space which contains growth factors derived from platelets, as well as growth factors derived from dentin

walls — plays the role of a scaffold rich in proteins, crucial to a successful population, as well as to cell differentiation and root development.^{2,3,5}

According to Ding et al,³ during revascularization treatment, blood coagulum formation should remain until it reaches 3 mm below the level of enamel-dentin junction. Subsequently, an MTA plug is fabricated over the blood coagulum, preventing bacteria from entering into the root canal before revascularization. This procedure is performed to develop a coronary sealing against bacteria and to prevent potential root canal recontamination.^{2-6,9,21,26,28} MTA has been used to promote effective pulp sealing. Oppositely to calcium hydroxide, it is biocompatible with adjacent pulp tissue and is able to induce pulp cells proliferation. Moreover, MTA is able to maintain a higher pH for long periods of time, in addition to having excellent marginal adaptation. It has also been used as coronary plug, due to the beneficial properties it has during therapy.^{2,9,26}

Nosrat et al² also report that the use of CEM cement is an alternative to the cervical plug. This tooth-colored, water-based cement has the same clinical applications of MTA, but with a different chemical composition. The sealing capacity, cytotoxicity and biocompatibility of CEM cement are comparable to MTA. However, unlike the latter, the characteristics of CEM cement surface resemble the human dentin which is able to form hydroxyapatite, even in normal saline solution, in addition to promoting the differentiation into stem cells and inducing the hard tissue formation.

The MTA plug is usually protected by a glass ionomer layer, 2 mm thick, placed over the cement which,

in turn, is covered by a resin barrier placed in the coronary portion to seal the tooth against potential infiltration.^{2-5,21,23,28} Young permanent teeth submitted to this process should be clinically and radiographically preserved from 6 to 26 months.^{2-6,9,21,23,26,28} Follow-up is necessary to verify whether the tooth had a good response to treatment, continued its root development in a physiological manner, and whether it is asymptomatic and with apex is closed, all of which represent a successful case of revascularization.

Final Considerations

Based on this literature review on revascularization, it is reasonable to conclude that:

» Apexification remains as the first choice of treatment for young immature permanent teeth with incomplete root formation and pulp necrosis.

» Revascularization is advantageous over apexification due to the number of sessions and, as a consequence, the treatment time required. Revascularization requires one to three sessions performed within a short period of time, approximately one or two weeks. Additionally, it is advantageous for increasing dentin wall thickness and favoring continuous root development.

» There are new possibilities (revascularization) for pulp treatment of young permanent teeth with open apex and pulp necrosis.

» Additional studies are warranted to further investigate the topic of revascularization, given that the benefits yielded by this technique are greater in comparison to the results achieved by apexification (thin root walls more susceptible to fracture).

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Planning and diagnosis predictability by means of cone beam CT before endodontic treatment: clinical resolution

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ABSTRACT

Introduction: Diagnosis and planning of new interventions are crucial when implementing a treatment. One method routinely used to assist diagnosis is the periapical radiograph, however, with this technique, anatomical structures are compressed into two-dimensional images.

Objective: The aim of this study was to show the importance of CBCT in endodontic diagnosis before endodontic retreatment of buccal perforation of which clinical resolu-

tion was planned, guided and executed after image visualization with cone beam computed tomography (CBCT). After diagnosis, immediate clinical therapy comprising retreatment via canal, sealing the perforation with MTA, root rehabilitation with fiberglass post and crown shielding with composite resin was carried out in a single session.

Keywords: Cone beam computed tomography. Imaging diagnostic. Combined therapy.

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Introduction

A second intervention is recommended in cases of unsuccessful endodontic treatment, especially those in which accidents and complications occur and, as a result, hinder endodontic therapy to the fullest extent of the root canal. Nevertheless, it is important to highlight that in case of failure, retreatment is the first procedure employed.^{1,2} In planning an endodontic reintervention, the affected area and surrounding anatomical structures must be carefully assessed. Assessment of imagiologic examination should not lead to imaging diagnosis limited to observe three-dimensional anatomical structures compressed into two-dimensional images, resulting in overlapping structures of diagnostic interest.^{3,4,5} Although panoramic and periapical radiographs reproduce details that are acceptable in meiodistal direction, buccal-lingual observation is considered inadequate.^{1,6} One should opt for imaging techniques that provide more information in the region, for instance, computerized tomography.

Computed tomography is a subsidiary diagnostic method. It allows a real three-dimensional overview of pathological lesions which are not radiographically visible, as well as observation of adjacent teeth and surrounding involved anatomical structures, which allows planning and procedures to be carried out with great precision.^{7,8}

A clinical approach guided by images obtained by CBCT provides appropriate diagnosis that can act against a particular circumstance, which in this case, is root perforation.

Endodontic perforations are iatrogenic and can be related to lack of anatomical and radiographic knowledge of the possible anatomical variations of a tooth, including dentin thickness and root curvature, as well as the specificities of each case. A perforation prognosis mainly depends on time factor, location, removal of the offending agent, and the material used for sealing, which should be biocompatible and dimensionally stable.^{6,9}

Mineral trioxide aggregate (MTA) has been considered an ideal repair material due to its excellent remedial and osteoinductive properties.^{10,11} For this reason, it is the first choice among the different materials used for direct sealing.¹²

Thus, well performed root rehabilitation and coronal sealing are of paramount importance for the

success of endodontic therapy.¹³ Fiber glass posts and composite resin restorations are easy to perform and yield good cosmetic and functional results. For this reason, they are recommended for endodontic reconstruction.^{14,15,16} The restorative technique, when appropriately performed with composite resin and fiber glass post, is a useful tool for biomechanical restoration of teeth impaired in color and strength.

The objective of this study is to report a case of endodontic reintervention via root canal, in which the diagnostic hypothesis and proper treatment plan were confirmed by cone beam computed tomography images, which enabled integrated multidisciplinary rehabilitation treatment to be carried out in a single session.

Case report

A 35-year-old male patient attended the Endodontic Clinic of the Army General Hospital of São Paulo (HGESP), complaining about severe pain in the buccal region of tooth #22. The patient reported a history of endodontic retreatment of tooth #22, with the last intervention being performed four months before. At clinical examination, the patient reported pain on palpation and vertical percussion of tooth #21 and 22. The examination also revealed the presence of edema in the buccal region and tooth mobility level I in #22, absence of periodontal pockets at probing and negative response to pulp vitality tests of teeth #21 and 22. The diagnostic radiograph showed a circumscribed radiolucent apical image that suggested a periapical lesion. In spite of that, a radiopaque image, compatible with root filling material, was observed in the middle and apical thirds of the canal (Fig 1). The patient was not subject to unnecessary diagnostic procedures.

A CT scan was requested for detailed assessment of the case. The result of the high resolution digital CT, yielded by means of the 3D-image visualization software (i - Dixel 2.0 - One Volume Viewer, Accutomo 80 - J. Morita Mfg. Corp., Kyoto, Japan) was clear. The 3D MIP plan of volumetric reconstruction did not reveal vestibular cortical bone loss (Fig 2). Assessment of the frontal plane revealed inappropriate root filling.

In the sagittal and axial planes, the exact location of the root perforation with slight overflow of

radiopaque material on the buccal surface could be observed (Figs 3, 4).

After analysis of the tomographic images, diagnosis and endodontic reintervention plan were carried out. Treatment plan comprised immediate clinical intervention that included retreatment via canal, perforation sealing with MTA, root rehabilitation with fiber glass post and crown shielding, all of which were performed in a single session.

After anesthesia and isolation of the operating field, the endodontic access surgery began via root canal, with odontometry obtained by means of coronary and sagittal tomographic images. The filling material was removed with engine-driven rotary NiTi files (Protaper retreatment D-1, D-2 and D-3) (Maillefer / Dentsply, Ballaigues, Switzerland). Afterwards, gutta-percha was removed for apical finishing of the chemical-surgical preparation, for which Protaper

Universal F3, F4 and F5 files were used (Dentsply Maillefer, Ballaigues, Switzerland). Throughout the canal preparation procedures, ultrasonic irrigation with saline solution was carried out. After canal preparation, ultrasonic irrigation was performed by alternating saline solution and EDTA (Odahcam Herpo Prod. Dent. Ltda. - Brazil) during 30 seconds, within the root canal. Subsequently, new irrigation/aspiration procedures were performed with saline solution. At last, the root canal was dried with absorbent paper points (Dentsply Ind. Com. Ltda., Petrópolis/RJ — Brazil) compatible with the diameter of the last instrument used in the real working length.

Filling was performed with gutta-percha and AH PLUS sealer (Dentsply/Maillefer) by means of the continuous wave condensation technique. Obturation was performed from the apical third to the level of root perforation so as to allow sealing with MTA



Figure 1. Initial radiograph.



Figure 2. Tomographic 3D reconstruction.

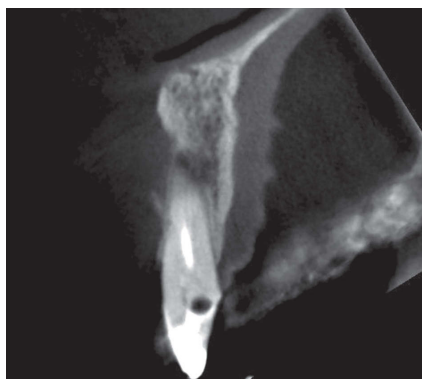


Figure 3. Sagittal tomographic view.



Figure 4. Axial tomographic view.

(Angelus, Londrina/PR Brazil) (Fig 5) and intracanal anchorage with fiber glass post. In this case, an esthetic fiber glass post (Reforpost/Angelus) was selected. The middle and cervical thirds were conditioned with 37% phosphoric acid gel, and one-bottle adhesive system (Single Bond®) was applied. The post was cemented with resin cement (Rely X® - 3M). Afterwards, the fiber glass post was coated, and coronary filling was carried out with light-cured resin Z100 (3M Brazil). At treatment completion, radiographic images were retaken, revealing correct crown shielding (Fig 6).

The patient was informed of the need for periodic follow-up appointments. After 6 months of endodontic treatment (Fig 7), the patient reported having neither pain nor symptoms, and the periodontal examination revealed that the periodontal tissue surrounding the tooth had no inflammatory signs.

After 12 months of follow-up, a CT scan was requested in order to assess, in the sagittal and axial planes, the sealing of the perforation in the buccal-palatal direction (Fig 8).

In the last follow-up appointment, 22 months after treatment, the periodontal ligament and lamina dura surrounding the tooth were intact, whereas periapical radiolucency was absent (Fig 9).

Discussion

Imageology is considered an important tool for endodontic diagnosis. Information obtained by clinical and image examinations directly influence clinical decisions,^{1,4,5} given that accurate data allow appropriate decisions and a more favorable prognosis. Within this context, CBCT allows clinicians to have more relevant information which could not be obtained by conventional radiographs. Conventional radiography



Figure 5. Obturation of the apical third and perforation sealing with MTA.

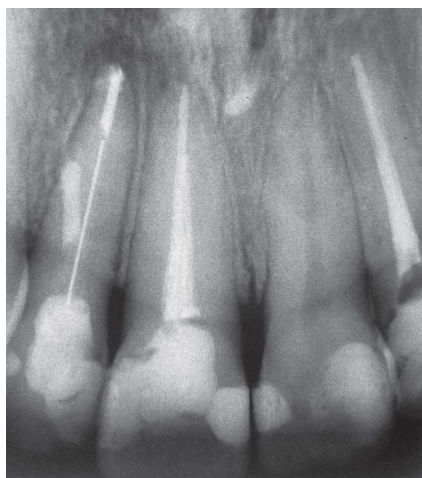


Figure 6. Final radiograph. Root and crown shielding with fiber glass post and composite resin.

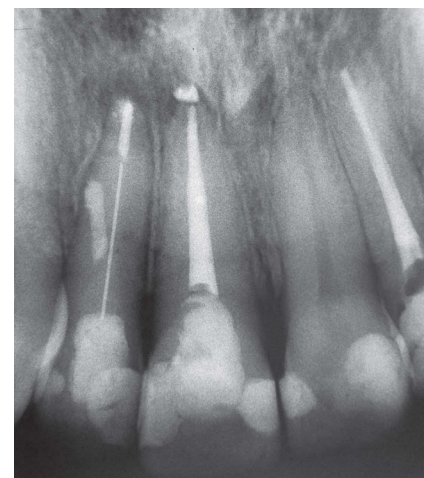


Figure 7. Follow-up radiograph after 6 months.

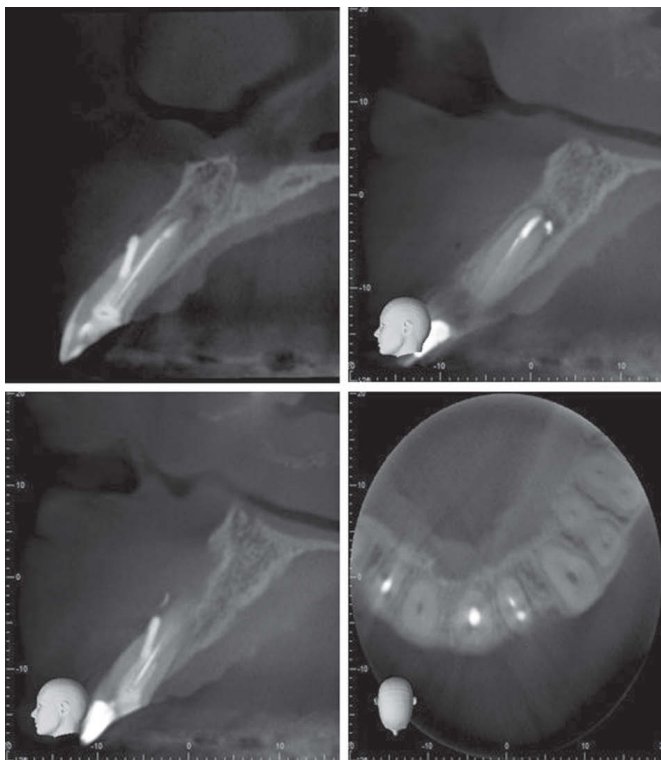


Figure 8. Annual follow-up tomograph taken to assess, in the sagittal and axial planes, the perforation sealing in the buccal-palatine direction.

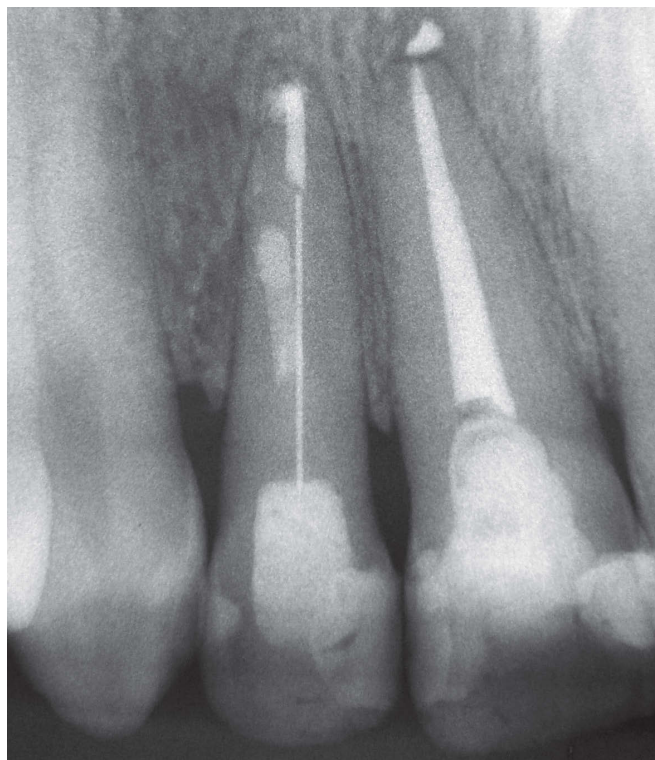


Figure 9. 24-month follow-up tomograph. Note intact periodontal ligament, absence of periapical radiolucency and intact lamina dura surrounding the tooth.

is the most used method due to being practical and providing images that can assist the professional in solving a large number of cases. However, the limitations of this method on the difficulty in viewing these images in certain regions and in two dimensions can hinder treatment planning of specific cases. Computed tomography has been used to overcome these limitations, since it enables the visualization of anatomical regions and identifies the presence of diseases that are not often diagnosed by conventional radiography.^{5,7,8} Patel et al² stated that the major advantage of CBCT is its three-dimensional geometric accuracy, which eliminates overlap in the area of interest. Images obtained with CBCT are more detailed, which enables one to detect potential bone imperfections more easily in comparison to conventional radiographs. Scarfe et al¹⁷ were able to prove not only the accuracy of 3D images, but also that these images closely resemble real measurements.

With regard to the images obtained in the case reported herein, the periapical and panoramic radio-

graphic images allowed an overall visualization of the quality of obturation, limits of the lesion and adjacent structures; not showing, however, the actual details, the type of pathology and its relation with anatomic structures. The initial clinical planning, based on the radiographs, included retreatment via canal, exposing the patient to an uncertain and subjective reintervention and an invasive diagnostic scanning, which could have led him to a situation of stress and discomfort due to a symptomatic clinical presentation and the limited information provided by the periapical radiographs.

The analysis of images obtained with cone beam computed tomography in a cross section plane allowed the professional to plan the endodontic reintervention via root canal. The diagnosis and proper treatment plan were confirmed by the images and enabled integrated multidisciplinary rehabilitation treatment to be carried out in a single session. The iatrogenic perforation could not be identified by conventional radiography, which proved it to be a difficult

and inaccurate diagnostic method, especially when the defect is located in the buccal or lingual surface of the root.^{1,6,18} The prognosis of a perforation directly depends on the location of this perforation, the contamination exposure time, the feasibility of sealing and accessibility of the main canal. The site of perforation, middle third of the root canal and the small diameter of the perforation may have contributed to the good performance of the repair material.^{6,9} Tsesis et al⁶ found worse prognosis of root perforations in humans when the former were located in the cervical third of the root where contamination can occur.

In this clinical case, the sealing material of choice was the MTA. It is considered a suitable material for having a good sealing effect, being biocompatible and cementogenic, as well as for being attached in the presence of moisture.^{10,11,12} Many authors agree that MTA is a material with excellent physical-chemical properties and good acceptance by the periodontal tissues, i.e., biocompatibility^{10,11}, which was confirmed by studies that evaluated the material's cytotoxicity and tissue inflammatory response.^{11,12}

Restoration of endodontically treated teeth must follow the biological principles of endodontic treatment and prevent contamination of the root canal system

caused by weakness of the remaining tooth structure, which is a natural consequence that compromises the structural unit of the tooth and its mechanical strength. Hepburn¹⁹ shows the importance and the need for functional rehabilitation of teeth endodontically treated with intraradicular posts. The author also asserts that the main function of the post in the root canal is to create a connection between the root portion and the coronary, in addition to mechanical stabilization of the latter. Advances in adhesive Dentistry allowed the use of composite resin associated with intra-radicular fiber glass posts to be considered a good therapeutic method for the reconstruction of anterior teeth endodontically treated.^{14,15,16}

Conclusion

Based on the results of this study, it is reasonable to conclude that the detailed three-dimensional dental structures obtained by CBCT guided the treatment planning and therapeutic decision. CBCT was essential to avoid doubtful diagnosis and unnecessary clinical intervention. The combination of imageology and clinical practice provides a high degree of treatment predictability, a key factor for the success of integrated multidisciplinary rehabilitation root treatment.

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Apical root resorption of vital and endodontically treated teeth after orthodontic treatment: A radiographic evaluation

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ABSTRACT

Introduction: Root resorption, generally expressed in a round apical shape, is one of the most common findings in the orthodontic clinic. **Objective:** To radiographically evaluate whether vital and endodontically treated teeth present similar severity of apical root resorption in response to orthodontic treatment. **Methods:** This study was conducted with twenty-eight patients who had one upper central incisor endodontically treated (experimental group) and its vital counterpart untreated (control group)

before orthodontic movement. Measurements were made by means of periapical radiographs taken before and after orthodontic treatment. **Results:** There were no statistically significant differences ($P > 0.05$) in apical root resorption levels between endodontically treated and vital teeth. **Conclusion:** Endodontic treatment does not interfere in apical root resorption after orthodontic treatment.

Keywords: Root resorption. Orthodontic treatment. Endodontics. Apical root resorption.

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Introduction

Movement of endodontically treated teeth has become more and more common due to an increase in the number of adult patients seeking orthodontic treatment. Many studies have been conducted to assess root resorption of vital teeth. They reveal that practically all teeth undergoing orthodontic treatment present some degree of external root resorption,¹⁻⁹ however, not too many researches have focused, in a controlled manner, on orthodontic treatment of endodontically treated teeth.^{10,11} Many variables have been described as being predisposing factors of root resorption, namely: Genetics, anatomic factors, treatment time and applied mechanics.^{5,12} The importance of local factors, such as root and alveolar bone crest morphology, has also been highlighted,^{6,13,14,15} but its correlation with endocrine disorders and individual susceptibility has not yet been proved.^{13,14,15} Conversely, intense forces and long-term treatment are directly related to an increase in root resorption associated with Orthodontics, of which risks are widely known by orthodontists.^{4,5,12,15}

Although endodontically treated teeth respond to the application of forces similarly to how vital teeth do during orthodontic therapy, the literature investigating the theme is controversial and scarce. Wickwire et al¹³ reported that devitalized teeth were subjected to a higher degree of root resorption in comparison to vital, during orthodontic movement. On the other hand, a radiographic study conducted by Spurrier et al¹⁰ described that some endodontically treated teeth were less susceptible to root resorption, although no differences were found between groups. In animal models, there seems to be no significant difference between external root resorption of vital and devitalized teeth during orthodontic therapy.^{17,18,19} In a previous study, we did not find differences between root resorption during orthodontic treatment of maxillary central endodontically treated and untreated teeth.¹¹

The aim of this study was to radiographically assess whether there is any significant difference in apical root resorption found in vital and endodontically treated teeth subjected to orthodontic therapy.

Material and methods

The study comprised twenty eight patients with a vital maxillary central incisor and its endodontically treated counterpart. The latter had intact periodontal ligament

in the apical region. Patients' selection involved a review of nearly 3,500 orthodontic records of three private orthodontic clinics. Thus, 56 incisors were assessed, 28 of which had been endodontically treated (experimental group) and 28 were vital teeth (control group). All patients were subjected to orthodontic movement with fixed appliance for at least six months. The endodontic interventions had been performed on the incisors at least one year before orthodontic treatment onset.

Patients whose crown presented alterations in size due to restorative procedures as well as patients with a history of trauma in the studied area were excluded from the study.

Pre and post-treatment periapical radiographs used for measuring the teeth were digitized (scanner HP Photo Smart, USA) and assessed by means of CorelDraw 11 parallel dimension tool. The images were under magnification of 350% for better visualization. All teeth had their longest length measured, from the incisal edge to the root apex, before and after orthodontic movement (Fig 1A).

In order to minimize any potential radiographic distortions before and after treatment, the longest distance from the incisal edge to the enamel-cementum junction of all radiographs were also measured (Fig 1B). Additionally, the differences for determining the foreshortening/elongation factors were calculated as suggested by Spurrier et al.¹⁰

The correction calculation was carried out as follows: initial total length, initial crown length, X (total expected length), final crown length, X – final total length = apical root resorption.

All measurements were repeated four times with a week interval in between. Arithmetic mean was used among the four assessments for comparison between groups. Student's t-test was employed for paired data, with a significance level set at 5%.

Results

Table 1 shows the results of apical root resorption assessment expressed in millimeters. Twelve patients (42.8%) presented greater apical root resorption in the endodontically treated tooth in comparison to its vital counterpart; whereas the remaining sixteen patients presented greater root resorption in the vital tooth. Nevertheless, the data did not reveal statistically significant differences (Table 2).

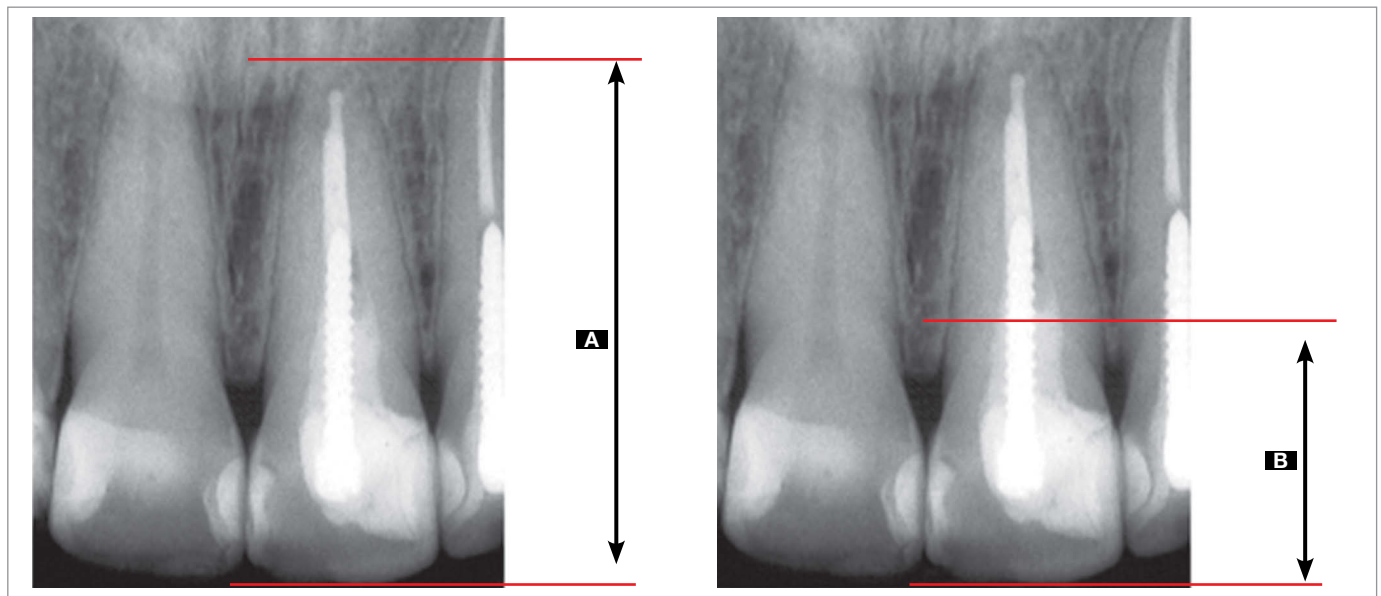


Figure 1. Radiograph representing the dental measurements carried out. **A.** Incisal-apical distance used to calculate root resorption. **B.** Incisal-enamel-cementum junction distance used to calculate the radiographic foreshortening/elongation factor.

Table 1. Apical root resorption (mm)*.

Patient	Endodontically treated	Vital (control)
1	0.45	0.34
2	0.64	0.63
3	3.07	2.86
4	0.31	0.80
5	0.21	0.11
6	4.42	1.65
7	0.69	0.00
8	0.92	1.26
9	0.16	3.50
10	0.00	0.40
11	0.35	0.20
12	0.52	0.30
13	0.79	1.60
14	0.20	0.30
15	0.20	1.40
16	0.20	1.20
17	0.92	0.09
18	1.18	2.37
19	0.25	0.44
20	0.13	1.82
21	0.77	1.03
22	0.56	0.07
23	0.77	0.97
24	2.65	4.00
25	0.35	0.48
26	0.89	1.82
27	0.17	0.31
28	0.31	0.23

Table 2. Comparative results of radiographic measurements between vital and endodontically treated teeth (n = 28).

	Mean	Standard deviation	Standard error	P value	Significance*
Vital T ₁ ** - T ₂ *** (mm)	1.08	1.06	0.20	0.00005	S
Endo T ₁ - T ₂ (mm)	0.79	0.99	0.18	0.00001	S
Endo T ₁ - T ₂ x Vital T ₁ - T ₂ (mm)	-0.29	1.03	0.19	0.14	N.S.

*Statistic significance set at $P \leq 0.05$. ** T₁ - Pre-treatment. *** T₂ - Post-treatment.

All means, standard deviation, standard error and p values obtained from measurements of radiographic examinations of vital and endodontically treated teeth are expressed in Table 2. Comparison between pre and post-treatment radiographs (T₂-T₁) of both vital and endodontically treated teeth revealed an increase in root resorption as a result of orthodontic treatment ($P < 0.001$). However, comparison of root resorption between groups does not reveal any statistically significant differences ($P > 0.05$).

Discussion

Root resorption of vital teeth expressed as a complication of orthodontic therapy has been widely discussed and studied. According to some authors,^{4,5,12,14,15,16} etiological factors that may contribute to external root resorption include, but are not limited to: anatomic factors, amount of applied force and type of movement.

Only two out of the 56 teeth subjected to orthodontic treatment and assessed by this study (patient 7 vital tooth and patient 10 endodontically treated tooth) did not present apical root resorption (Table 1). Comparison between pre and post orthodontic treatment means revealed statistically significant differences in both groups (Table 2), not only proving the occurrence of apical root resorption as a result of induced tooth movement, but also corroborating the literature.^{1,7,19} It is worth noting that the comparison of homologous incisors conducted in the present study resulted in a unique sample, given that both teeth underwent the same type of movement during the same period of time and presented similar intra-individual anatomy, thus reducing the influence of these variables.

No significant differences regarding apical root resorption as a result of orthodontic movement were radiographically observed in endodontically treated and untreated teeth (Table 2). These findings corroborate those of Spurrier et al¹⁰ who assessed 43 patients by means of a similar radiographic methodology, although

these authors described that some cases of endodontically treated teeth presented little root resorption as a result of greater mineral density as well as greater hardness, without, however, testing the teeth so as to verify such characteristics.¹⁵ Mattison et al¹⁸ also concluded that absence of vital pulp in endodontically treated teeth is not a predisposing factor of root resorption, even when submitted to orthodontic forces.

Likewise, no differences regarding macro and micro aspects of vital and endodontically treated teeth undergoing orthodontic movement were found in animal models.^{17,18} Mah, Holland and Pehovich¹⁹ reported that a microscopic analysis of weasels' teeth revealed greater loss of cementum in endodontically treated teeth in comparison to vital ones, while a radiographic analysis revealed no statistically significant differences in the root length of both groups.

Steadman²⁰ reported that histological slices of areas with root resorption in devitalized teeth were similar to those found in foreign body reactions, suggesting that endodontically treated teeth would react as a foreign body and cause chronic irritation. Therefore, endodontically treated teeth would be more susceptible to root resorption. Such reaction is not commonly found in teeth correctly treated, but may happen in cases of over-filled teeth or those which were filled at the limit of the apical foramen.¹⁵ Wickwire et al¹³ also reported a higher incidence of root resorption in devitalized teeth in comparison to vital teeth. However, the methods employed by these authors were subjected to criticism, given that the endodontically treated teeth of their study had already been traumatized, which naturally causes increased susceptibility to root resorption.^{15,18,21}

Based on the results of this study as well as on data found in the literature about apical root resorption during induced orthodontic movement,^{1,9,22,23} it is suggested that a further study on the etiology of endodontic treatment be carried out, thus rejecting the connection

between root resorption and previous trauma. Furthermore, additional studies should be conducted to investigate the history of trauma in vital teeth,^{15,21} or the orthodontist might otherwise perform orthodontic movement without worrying about further root damage.

Conclusion

Endodontically treated and untreated teeth (without history of trauma) undergoing orthodontic therapy present apical root resorption similar to teeth subjected to induced tooth movement.

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Mandibular first premolar with three canals and two roots: A case report

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ABSTRACT

Introduction: The possibility of additional root canals should be considered even in teeth with a low frequency of abnormal root canal anatomy, therefore demanding more attention of the clinician during root canal treatment. **Objective:** This article reports a relatively uncommon clinical case of a mandibular first premolar with two roots and three canals which was successfully treated with root canal therapy. **Methods:** After initial radiograph, the presence of two roots was detected. Additional care was taken to explore the root canals,

confirming the presence of three canals with the aid of a microscope. The root canals were instrumented using a hybrid rotary technique advocated by the School of Dentistry from Piracicaba, and obturated using Sealer 26 and cold lateral compaction. **Conclusion:** In order to achieve the best possible outcome in root canal treatment it is important to have a good knowledge of the root canal system morphology in addition to appropriately using the diagnostic tools.

Keywords: Root canal therapy. Root canal preparation. Premolar.

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» The patients displayed in this article previously approved the use of their facial and intraoral photographs.

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Introduction

The success of nonsurgical endodontic therapy depends on a thorough knowledge of the root and root canal morphology to adequately locate all canals and properly clean, shape and obturate the canal space in its three dimensions.^{1,2,3} The internal anatomy of a canal system may reveal isthmuses, lateral and accessory canals or diverse canal shapes, all of which can complicate the cleaning and shaping procedures. Incomplete cleaning and shaping of these areas may leave tissue, bacteria or necrotic debris in the canal. The presence of these irritants can result in persistent periapical inflammation and failure of the root canal treatment.⁴

Some authors have suggested that mandibular premolars may represent the greatest difficulty in performing successful endodontic treatment due to wide variation in root canal morphology.⁵ Additionally, the mandibular first premolar was found to have the highest failure rate (11.45%).³ Previous studies have reported many variations in the internal anatomy of this tooth. The majority of all mandibular first premolars have a single root (97.9%), and only 1.8% of them presented two roots.⁶ As for the internal morphology of this group of teeth, Vertucci⁷ revealed that 74% had one canal at the apex, 25.5% had two canals and 0.5% had three. Thus, this article reports a case of a mandibular premolar with an unusual morphology: with two roots and three canals.

Case report

A 37-year-old male patient sought the school of Dentistry from Piracicaba (FOP-UNICAMP) with a chief complaint of having spontaneous pain in the posterior right mandibular tooth for one week. The patient was in good health without any systemic involvement. Intraoral clinical examination revealed the presence of a localized edema in the buccal gingiva, the tooth was tender on percussion and sensitive on periapical palpation, no periodontal pockets were found. Furthermore, the cavity had a temporary restorative material and, according to the patient, a local dentist had performed an emergency procedure before referring the patient to the FOP/UNICAMP. A bifurcation could be seen at buccal sight, suggesting the presence of more than one root. Radiographic examination confirmed an unusual anatomy of two roots, as well as widening of the periodontal ligament and apical lesion (Fig 1).

The clinical and radiographic examinations led to a diagnosis of acute periapical abscess. After anaesthetic procedure carried out with 2% lidocaine containing 1:100000 epinephrine (DFL, Rio de Janeiro, Brazil), the tooth was isolated with a rubber dam. For endodontic access, a round #1014 diamond bur (KG Sorensen, Barueri, Brazil) was chosen. Pulp chamber inspection and localization of canal orifices were performed with the aid of a clinical microscope (DM Pro, Opto Eletrônica SA, São Paulo, Brazil) and a sharp explorer (SSWhite, Rio de Janeiro, Brazil). All orifices were in opposite directions (buccal, lingual and distal). Apical foramina of the canals were also separated. Cleaning and shaping of the canals were done by the hybrid rotary technique advocated by the endodontic department of FOP/UNICAMP. Apical patency was obtained for each canal with a #10 K-file and measurement of the working length was electronically taken with Romiapex A-15[®] apex locator (Romidan, London, UK). Root canal instrumentation was done using 2% chlorhexidine gel as auxiliary chemical substance. Canals were dried with absorbent paper and an intracanal dressing consisting of paste of calcium hydroxide and 2% chlorhexidine gel was placed. Seven days later, obturation of the root canals was not possible due to the presence of purulent exudate. Instrumentation was repeated and a medication consisting of amoxicillin 500 mg and metronidazole 400 mg was prescribed for 7 days. After this period, the tooth was completely asymptomatic, the edema had regressed and the root canal treatment was completed. To this end, the root canals were irrigated with EDTA 17% for three minutes, dried with sterile absorbent paper and obturated with gutta percha and Sealer 26[®] using cold lateral compaction (Dentsply Maillefer Ind, Petrópolis, Brazil). A final radiograph was taken to access the quality of obturation (Figs 2 and 3).

Discussion

One of the main reasons for endodontic failures is incomplete instrumentation and disinfection of the root canal space.^{5,8,9} Knowledge of the complexity of root canal anatomy is essential when performing endodontic therapy and should be recognized before or during treatment. Anatomical variations such as additional canals or root may be missed, resulting in possible treatment failure. This fact becomes important since authors have demonstrated that the mandibular premolar has



Figure 1. Preoperative radiograph of mandibular first premolar.



Figure 2. Radiograph of root canal filling quality.



Figure 3. Postoperative radiograph of the root canal obturation.

a disproportional number of flare-ups and endodontic failures.⁵ Anatomic variations can result in inadequate debridement which may leave irritants within the canal system and produce an unfavorable environment for healing periapical tissues.⁴

Due to the presence of such anatomic variations, some suggestions can help the clinician identify multiple root canal systems in the mandibular first premolar and, therefore, achieve a successful treatment outcome. In this sense, methods such as multiple preoperative parallel radiographs, as well as mesial or distal shift radiographs, can help to determine the type of canal system present. If the root anatomy has an indistinct definition after several radiographs, it probably indicates a second root or even a possible third.⁵ Additionally, it is important to have adequate access opening in order to allow a proper access to all potential root canal systems.¹⁰ Magnification and illumination are absolute prerequisites for evaluating color

changes and for working deep inside the tooth. Moreover, clinicians should be prepared to properly use the diagnostic tools available, as it is the case of microscopes. The introduction of magnifying glass and surgical microscopes has revolutionized the practice of Endodontics. The advantages of using these surgical magnifying glasses for conventional Endodontics include enhanced visualization of root canal intricacies, which enables the clinician to investigate the root canal system and to clean and shape it more efficiently.

Conclusion

It is extremely important to use all diagnostic tools available to locate and treat the entire root canal system. Additionally, careful interpretation of angled radiographs, proper access preparation and a detailed exploration of the interior of the tooth, ideally under magnification, are essential prerequisites for a successful treatment outcome.

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1. Registration of clinical trials

Clinical trials are among the best evidence for clinical decision making. To be considered a clinical trial a research project must involve patients and be prospective. Such patients must be subjected to clinical or drug intervention with the purpose of comparing cause and effect between the groups under study and, potentially, the intervention should somehow exert an impact on the health of those involved.

According to the World Health Organization (WHO), clinical trials and randomized controlled clinical trials should be reported and registered in advance.

Registration of these trials has been proposed in order to (a) identify all clinical trials underway and their results since not all are published in scientific journals; (b) preserve the health of individuals who join the study as patients and (c) boost communication and cooperation between research institutions and with other stakeholders from society at large interested in a particular subject. Additionally, registration helps to expose the gaps in existing knowledge in different areas as well as disclose the trends and experts in a given field of study.

In acknowledging the importance of these initiatives and so that Latin American and Caribbean journals may comply with international recommendations and standards, BIREME recommends that the editors of scientific health journals indexed in the Scientific Electronic Library Online (SciELO) and LILACS (Latin American and Caribbean Center on Health Sciences) make public these requirements and their context. Similarly to MEDLINE, specific fields have been included in LILACS and SciELO for clinical trial registration numbers of articles published in health journals.

At the same time, the International Committee of Medical Journal Editors (ICMJE) has suggested that editors of scientific journals require authors to produce a registration number at the time of paper submission. Registration of clinical trials can be performed in one of the Clinical Trial Registers validated by WHO and ICMJE, whose addresses are available at the ICMJE website. To be validated, the Clinical Trial Registers must follow a set of criteria established by WHO.

2. Portal for promoting and registering clinical trials

With the purpose of providing greater visibility to validated Clinical Trial Registers, WHO launched its Clinical Trial Search Portal (<http://www.who.int/ictrp/network/en/index.html>), an interface that allows simultaneous searches in a number of databases. Searches on this portal can be carried out by entering words, clinical trial titles or identification number. The results show all the existing clinical trials at different stages of implementation with links to their full description in the respective Primary Clinical Trials Register.

The quality of the information available on this portal is guaranteed by the producers of the Clinical Trial Registers that form part of the network recently established by WHO, i.e., WHO Network of Collaborating Clinical Trial Registers. This network will enable interaction between the producers of the Clinical Trial Registers to define best practices and quality control. Primary registration of clinical trials can be performed at the following websites: www.actr.org.au (Australian Clinical

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WHO proposes that as a minimum requirement the following information be registered for each trial. A unique identification number; date of trial registration, secondary identities, sources of funding and material support, the main sponsor, other sponsors, contact for public queries, contact for scientific queries, public title of the study, scientific title, countries of recruitment, health problems studied, interventions, inclusion and exclusion criteria, study type, date of the first volunteer recruitment, sample size goal, recruitment status and primary and secondary result measurements.

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Consequently, authors are hereby recommended to register their clinical trials prior to trial implementation.

Yours sincerely,

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