Human enamel colonization of Candida albicans

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ABSTRACT

Introduction: Candida albicans may be a commensal member of the oral microbiota, and may colonize the endodontic environment. Using an in vitro dentin infection model, the objective of this study was to evaluate the pattern of dentin colonization by C. albicans and the influence of thigmotropism on the colonization. Methods: An apparatus was designed being composed of two glass flasks connected by a silicone manifold. Internally, they were separated by a dental fragment protruding an acrylic disk. The upper and bottom flasks were filled with Sabouraud broth and C. albicans was inoculated in the upper flask. After 72 h at 37 ºC, the device was aseptically dismounted and the dentinal fragment was prepared for scanning microscopy. Results: Candida albicans 1015 strain actively penetrated dentinal tubules and hyphae were the mainly growth form for the primary yeast invasion of human dentin. Yeast cells were observed in inner dentin layers. Conclusions: The direction of the hyphal tip was not influenced by the tubular nature of the dentin. In his view, only the pleomorphism has a significant role in the fungal colonization of human dentin.

Keywords: Infection. Periapical diseases. Dentin.
Introduction

Under physiological conditions, the pulp tissue and the surrounding dentin are protected by enamel and cementum. Any factor that causes loss of these protective structures, such as caries, fracture, attrition, abrasion, scaling, and root planning, exposes dentin and eventually the pulp tissue to detrimental effects due to mechanical, chemical, and particularly microbial irritants.25,26

Exposed dentinal tubules are the main routes microorganisms have to the endodontic environment. The number of dentinal tubules per mm² of dentin ranges from 15,000 at the cementoenamel junction to 45,000 near the pulp.5,11 Intratubular dentin deposition results in narrowed tubules as it is more advanced in the superficial dentin when compared to the dentin adjacent to the pulp and resulting in tubules with a uniform conic appearance. The largest depositions were observed around the pulp (approximately 2.5 µm diameter) and progressively decreased as they approached the cementoenamel junction (approximately 0.9 µm diameter).3

A reduced tubule diameter in the superficial dentin layers might hinder opportunistic yeasts of the genus Candida from penetrating the pulp environment. However, some studies have demonstrated the ability of these microorganisms to infect this dental tissue.15,16,21,22,23,26,29 Furthermore, this ability has been suggested to be closely linked to the pleomorphic growth patterns that are most commonly exhibited by isolates of C. albicans.

Regarding the tubular nature of dentin and also of thigmotropism, the latter is defined as a directional response of a cell or tissue to topographic modifications of a surface12 and is supposedly regarded as an important factor for the colonization of the dental pulp by Candida species. Nevertheless, this inference is based on models of oral and vaginal mucosal infections, where the thigmotropic response has already had a defined role. In the present study, we aimed to assess the pattern of dentin colonization by C. albicans and the influence of thigmotropism on the colonization using an in vitro dentin infection model.

Material and Methods

The apparatus used consisted of two glass flasks of equal volume (10 ml) and size (7 cm x 1 cm radius) connected by a hollow silicone ring (2.5 cm x 2.5 cm diameter). Flasks were connected to each end of the ring, and a small hole (0.5 mm) was created in the side of the ring to remove air bubbles and to allow us to insert the desired volume of microbiological culture medium. A plastic connector was attached to the hole in order to seal the system. This apparatus was made airtight using an acrylic disk, and all connections were sealed with rubber rings (0.7 mm thick) (Fig 1).

A dental fragment was added to the acrylic disc. Apices and crowns of recently extracted human permanent incisors were sectioned perpendicularly along the axis of the tooth using carboril disc with a profuse irrigation with distilled water. Two cylinders were
made from each of the remaining root portions, and each cylinder was approximately 5 mm in diameter and 2 mm thick. Cementum was removed using a diamond bur. Then, dentinal cylinders were individually placed into the central hole that was previously made in the acrylic disc and fixed using thermopolymerizable acrylic resin (Fig 2). The smear layer was removed from some dentinal cylinders by immersing them in 17% EDTA and 5.25% NaOCl for 3 min.

Microorganisms and Culture Medium
After sterilizing the entire apparatus with gamma radiation, the top flask was filled with modified Sabouraud broth (2% glucose, 1% peptone, 0.5% yeast extract) containing $10^7$ CFU/mL of the strain *C. albicans* 1015, which had been isolated from a necrotic root canal. The density of the inoculum was standardized at an absorbance reading equivalent to 1.2 using spectrophotometry (O.D. 560 nm).

Sterile Sabouraud broth was placed into the bottom flask, which removed all of the air (Fig 1). The apparatus was incubated at 37°C until the culture medium in the lower flask was visibly cloudy, indicating microbial growth. Flasks without microbial inoculum were used as a negative control. Assays were persistently repeated, at least thirty times.

Scanning Electron Microscopy
As soon as microbial growth was observed in the bottom flask, the apparatus was dismantled under aseptic conditions. An optical microscope was used to analyze 10 μL aliquots of Sabouraud broth from the bottom portion. The acrylic discs containing dentinal fragments were washed three times with 0.1 M PBS and fixed for 1 h in a solution of 2.5% glutaraldehyde and 0.1 M PBS, pH 7.4. After the fixation period, acrylic discs were washed again in 0.1 M PBS and then sprayed with gold while under vacuum. Specimens were examined with a scanning electron microscope, model JEOL JSM-6360 LV (Tokyo, Japan), operating at an accelerating voltage of 15 kV.

Results
In experiments conducted on dentinal fragments both with and without the dentinal smear layer, the culture medium in the lower bottle was visually turbid after 72 h of incubation. Microscopic analysis of the aliquots from the culture medium in the lower compartment revealed growth of yeast cells morphologically identical to those previously inoculated in the top bottle.

In Figure 2, negative controls are seen with and without a smear layer.

**Figure 2.** Dentinal fragments used as negative controls. **A)** Without smear layer. **B)** With smear layer.
Dentin colonization by *C. albicans* 1015 in the dental fragment with smear layer is shown in Figure 3. Yeast cells and hyphae were observed in the superficial layer of the fragment (Figs 3A, B and C). Hyphae showed branching and linearly extended into the substrate; however, the hyphae seemed randomly oriented and some

Figure 3. Electron micrograph showing colonization of dentinal tubules by *C. albicans* 1015 in dentin samples with the smear layer. **A, B, C** Yeast cells and hyphae in the superficial dentin layer. **D** Yeast cells in dentinal tubules in the inner dentin layer. **E** Penetration of hyphae in dentinal tubules. **F** Yeasts attached to the dentinal tubule wall.
Figure 4. Electron micrograph showing *C. albicans* 1015 colonizing the dentinal tubules in dentin samples without the smear layer. **A** Dentin sagittal section showing the absence of the smear layer in dentinal tubules (Negative control). **B, C** Yeast cells and hyphae on the superficial dentin layer. **D** Yeast cells and germ tubes in the inner dentin layer. **E** Yeast adhered to the dentinal tubule wall. **F** Hypha in a dentinal tubule.
headed toward the opening of the dentinal tubules. The predominant yeast growth was observed in the inner dentin layers (Figs 3D and F). Yeasts were present in various extensions of the dentinal tubules, and some cells exhibited budding, indicating growth potential.

Figure 4 shows dentinal colonization by *C. albicans* 1015 in the dental fragment that had been treated to remove the smear layer. After removal, the diameter of the dentinal tubules ranged from 1.27 µm to 5.50 µm. The superficial dentin layer was densely colonized by budding cells and hyphae (Figs 4B and C). Invasion of dentinal tubules by germ tube formation (Fig 4D) or by mycelium growth (Fig 4F) was evident in the inner most portions of the substrate. Yeast adherence to the dentinal wall by matrix secretion may be observed in the micrograph (Fig 4E).

**Discussion**

Colonization of dentinal tubules by microorganisms is considered a significant risk factor for early and persistent endodontic infection. In this study, *C. albicans* 1015 was found to be able to invade dentinal tubules. Visualization of a large number of hyphae in the superficial dentin layers of dentinal fragments with and without the smear layer indicates that this invasion results from a morphological differentiation of the yeast cells that grow into mycelial form, thus entering the tubules. The presence of *C. albicans* in carious dentin fragments has been reported in clinical studies and confirms that these microorganisms are able to penetrate dentin *in vivo*.2,9,10

*C. albicans* cells usually have a spherical or oval cellular shape. Their blastospores may start forming hyphae that linearly extend and sometimes branch.18 Pleomorphic growth patterns, described for this species, are related to their different morphologies, including germ tubes, blastospores, pseudohyphae, true hyphae, and chlamydospores.14,19,30 All of these growth patterns, except chlamydospores, may assume other forms based on the following environmental conditions: pH, temperature, and nutrient supply.6,19,28 Therefore, in addition to need to colonize the innermost dentin layers, the temperature of 37°C used in our study favored *C. albicans* 1015 pleomorphism on this substrate.

Multiple findings18,20 suggest that the yeast-hypha transition is required for *C. albicans* virulence. However, some studies6,19,27 highlight that the yeast morphology that is observed in both superficial and innermost layers of dentin fragments, with and without a smear layer, are critically important for microbial adhesion and rapid dissemination in various tissues and for biofilm formation, which are both processes indirectly related to virulence. The presence of the smear layer did not influence adhesion of *C. albicans* 1015 to the substrate in contrast to the other findings.22

In another study,24 it was concluded that the presence of the smear layer enhanced the adhesion of *C. albicans* to human dentin due to a higher availability of Ca²⁺ ions and collagen. In the present study, removal of the smear layer did not affect microbial adhesion, suggesting that Ca²⁺ ions are less important for microbial colonization. Furthermore, the presence of germ tubes in the dentinal tubules in samples without a smear layer supports this conclusion because these structures are very substrate-adherent.13,17

The methodology used in the present study allowed us to determine whether dentinal tubules may be used as a route to colonize a sterile ecological niche, as it may occur *in vivo*. Although the penetration of dentinal tubules by *C. albicans* has been shown to be directly related to the polymorphism of this species, it seems that the growth direction of *C. albicans* hyphae was not influenced by the substrate topography. Hyphae were randomly oriented in both of the analyzed samples. Thus, yeast penetration of the dentinal tubules and colonization of the innermost dentin layers seems to be a natural consequence of the process and therefore is not due to active recognition of topographic changes, as it occurs during the thigmotropic response. Differentiated infection structures and structural modifications to the hyphae were not evident either.

As observed in the present study, *C. albicans* is able to invade dentinal tubules, like bacteria. However, the behavior of this yeast in this ecological niche should be further studied *in vivo*. *In vivo* and in the presence of other microbial groups and oral fluids, yeasts may show different morphological responses that may have varied impact on the pathogenesis of endodontic and periapical infections.

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References


The influence of calcium hydroxide paste change on repairing of extensive periapical lesions: Cases report

ABSTRACT

Introduction: In this paper we describe the endodontic treatment of teeth with extensive periapical lesions through case reports. Objective: Analyze the effectiveness of change the intracanal medication with calcium hydroxide, reducing or eliminating the surgical procedures and still observe, by follow up, the periapical repair. Results: After clinical and radiographic examination and found the need for endodontic treatment, was performed the coronal opening, irrigation with sodium hypochlorite 1% and biomechanical preparation with manual endodontic files. The EDTA 17% was used for 3 minutes with manual shaking before application of the medication in all the sessions as well as all sessions before the final filling. Thus, the medication with calcium hydroxide and propylene glycol was inserted in the root canal and replaced whenever the medication had been partly resorbed. After the beginning of periapical repair, the filling of the root canals was performed by the technique of horizontal and vertical condensation and radiographic controls were performed according to the availability of the patients. Conclusion: In these case reports, the renovation of calcium hydroxide as root canal dressing showed efficient in the treatment of extensive chronic periapical lesions, repairing the bone and periodontal tissues and eliminated the need for surgical intervention.

Keywords: Calcium hydroxide. Periapical abscess. Propylene glycol.

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Introduction

In the last decades, the evolution of biological knowledge has been a remarkable phenomenon in healthcare. This development occurs in both the scientific and technological areas and intensifies in dentistry with the enhancement of existing resources and creating new ones, whose goal is the preservation of the dental element in its original position, allowing it to exercise its functions accordingly. According to Dotto et al., endodontics aims to cleaning, disinfecting and shaping of the root canal system to obtain the desired sanitization and provide conditions for the involved tissues to return to its normal state maintaining the health of the periapical tissue.

Specific studies show that bacteria represent one of the main factors of the pulp changes, making it necessary to use antimicrobial agents during endodontic therapy. However, other studies show that dental trauma, extensive restorations and periodontal lesions also contribute to these pulp changes.

The biomechanical preparation, aided by copious irrigation, represents the stage of greatest impact on the root canal microbiota, but its antiseptic efficiency is partial and temporary. The microorganisms present in the root canal system, represented by the secondary and accessories canals, isthmus, dentinal tubules, apical cemental gaps or cementoplasts must be eliminated or inactivated, whereas recolonize the root canals after biomechanical preparation and potentially after the complete filling.

Within the biological advances of endodontic treatment, there is a greater concern in selecting substances that provide the best type of repair. Thus, add with the need of decontamination, not only dentinal canal but also cemental canal, the use of an intracanal medication has been considered important by some researchers. These studies analyzed histologically the results and showed that it favors the periapical repair. Thus, the intracanal medication most widely used since 1920 is the calcium hydroxide, associated with various vehicles, which requires cleaned canals and biomechanically prepared for effectiveness.

The calcium hydroxide medication has been prepared with various vehicles, such as methyl cellulose aqueous solution, distilled water, saline solution, anesthetic, polyethylene glycol, propylene glycol, para-chlorophenol, olive oil, lipiodol. Different methods have been described to carry the paste to the root canal. Some include the use of syringes with needles of different calibres, guns or amalgamators with endodontics condensers. Others use Lentulo, McSpadden or similar, endodontic instruments and gutta-percha.

Despite the calcium hydroxide being used since 1920, its mechanism of action was first described by Holland et al. in 1978, which claimed that the calcium hydroxide in contact with periapical tissue, which has water and carbon dioxide, dissociates into calcium and hydroxyl ions. The calcium ions react with the carbon from tissues, originates calcium carbonate in the form of calcite crystals. And this mechanism is complemented by Seux et al., who claim that these granulations has a great accumulation of fibronectin that provides adhesion and cell differentiation with subsequent hard tissue deposition.

Therefore the calcium hydroxide biological action is closely related to the ionic dissociation in Ca++ and OH- that occurs in the presence of water and its high alkalinity, which allows change the dentin pH and preventing the survival of most endodontic microorganisms (bacteriostatic power promoted by enzymatic inhibition of these microorganisms).

Therefore this study was conducted using clinical case reports, in order to verify the effectiveness of the intracanal medication with calcium hydroxide in extensive chronic periapical lesions, reducing or eliminating surgical procedures and still observe through follow up the periapical repair.

Case reports
Case 01

A 20-year-old girl was referred for endodontic treatment of teeth 11 and 12, in May 2002, by her orthodontist. During the interview there was no history of systemic disease, but in dental history, the patient reported being in orthodontic treatment and that about 5 years ago, had an accident with skateboard and broke the crown of these elements. At the time, after being examined by the dentist, there was no need of endodontic treatment, being realized only dental esthetics. The intraoral clinical examination showed absence of swelling, sinus, percussion or palpation pain, tooth mobility. The teeth did not respond...
to pulp sensitivity tests and it was possible to note color change of dental crown. The periapical radiographs showed the presence of a periapical radiolucency, with approximately 20 mm, involving the apical third of both dental elements and suggested a clinical diagnosis of periapical chronic abscess (Fig 1A).

The patient was informed about the various treatments for this case and the option chosen was endodontic treatment without surgery with only frequent changes in intracanal medication in order to obtain periapical repair. Therefore, it was requested disrupting orthodontic treatment in these dental elements, i.e., it was not applied orthodontic force until it was observed early or complete repair of the periapical region.

After anesthesia, rubber dam and canals access, the shaping was done with manual endodontic files and sodium hypochlorite 1%. During the preparation the odontometry was performed (Figs 1B and C). Because it was a necropulpectomia case, then patency was done. After biomechanical preparation of each canal, it were dried with sterile paper cones and the EDTA 17% was used for 3 minutes, with manual agitation for better cleaning of the canals. After EDTA removal with new hypochlorite irrigation and new drying canals, a calcium hydroxide with propylene dressing was applied (Fig 1D), and that being replaced whenever it was radiographically verified that it had been partially removed.

After a period of 6 months it was radiographically observed early repair apical neoformation of periapical bone tissue. Then there was a last irrigation of the root canals with hypochlorite 1% and EDTA 17% as described above. In November 2002, the canals were filled with gutta-percha points (Figs 1E and F), and cement based on calcium hydroxide, by lateral condensation followed by vertical condensation (Fig 1G). The pulp chamber was cleaned and temporarily sealed with sterile cotton pellet and Coltosol, asking the patient to return to her dentist so that it accomplished the definitive coronal restoration after endodontic treatment.

After 3 months of the conclusion of the case, the patient was asked to perform the first follow up radiographic, to check the progress of periapical repair and restart orthodontic treatment. In the intraoral periapical radiograph was possible to observe the repair evolution (Fig 1H) and thus released the orthodontic treatment.

In the second control, after 6 years, the periapical repair was complete, but it was possible to observe a mesial apical resorption of the dental element #12, probably resulting from orthodontic treatment (Fig 1I).

In the third control performed after 9 years, besides total repairing, the root resorption present in tooth #12 had stabilized (Fig 1J).

Case 2

A 16-year-old patient attended the private practice with his legal guardian (biological father) in July of 2004, because his orthodontist asked him to perform endodontic treatment in teeth #21 and #22. During the interview, there was no history of systemic disease and in the dental history, the patient reported being in orthodontic treatment. The intraoral clinical examination showed absence of swelling, sinus, palpation or percussion pain, tooth mobility. The pulp sensitivity test showed negative results. In the radiographic periapical intraoral exam it was observed a periapical radiolucency with size approximately 16 mm involving the apical third of both dental elements, suggesting probable clinical diagnosis of chronic periapical abscess (Fig 2A).

The patient and the responsible were informed about the different ways to conduct this case, and it was chosen to perform endodontic treatment with regular change of calcium hydroxide dressing. For this case, it was also asked to the orthodontist to stop with the orthodontic forces on these dental elements until it was observed the start or complete repair of the periapical region.

After anesthesia, rubber dam and canals access, the shaping was done with manual endodontic files and sodium hypochlorite 1%. During preparation the odontometry was performed (Figs 2B and C). In this case it was also made apical patency after biomechanical preparation and before the insertion of each intracanal medication and filling procedures, each canal was dried with sterile paper points and flooded with EDTA 17%, for 3 minutes with manual agitation. After EDTA removal with new irrigation of hypochlorite and another canals drying, an intracanal medication composed with calcium hydroxide and propylene glycol was applied, being replaced whenever its resorption was determined radiographically (Fig 2D).
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After a period of 6 months a partial healing evolution of the apical third was seen. The root canal filling was performed in January 2005, through gutta-percha points and calcium hydroxide cement (Figure 2E and 2F), by lateral condensation followed by vertical condensation vertical (Fig 2G). The pulp chamber was cleaned and temporarily sealed with sterile cotton pellet and Coltosol, asking the patient to return to his dentist so that it accomplished the definitive coronal restoration after endodontic treatment.

In the first follow up performed after 3 years, the periapical repair was complete (Fig 2H).

In the second follow up performed after 6 years, the periapical repair was complete (Fig 2I).

Figure 1. A) Initial radiograph showing radiolucency suggesting chronic periapical abscess, B) odontometry (tooth #11), C) odontometry (tooth #12), D) intracanal medication with calcium hydroxide paste and propylene glycol (E) Master cone in the tooth #11, (F) Master cone in the tooth #12, (G) Root canal filling radiographs, (H) Three months of follow up, showing periapical repair, (I) Six years of follow up, showing periapical repair and apical resorption in tooth #12, (J) Nine years of follow up, showing periapical repair and total stabilization of apical resorption in tooth #12.
Discussion

In necro-pulpectomy cases usually the pulp stub is necrotic or severely compromised and therefore the main concern during the treatment is bacteria elimination of dentinal tubules and in the periapical zone.\(^{19}\)

To complement the root canal disinfection during biomechanical preparation it was used sodium hypochlorite 1% as auxiliary chemical substance irrigation, because according to Star et al,\(^{20}\) the hypochlorite has antimicrobial properties, ability to dissolve organic tissues as well as low surface tension. But there are studies that use chlorhexidine gel as auxiliary chemical substance due to its substantivity properties, antimicrobial properties, broad spectrum of action and good lubrication of the root canal.\(^{21}\)

Figure 2. A) initial radiograph showing periapical radiolucent suggesting chronic abscess, (B) odontometry (tooth #21), (C) odontometry (tooth #22); (D) intracanal medication of calcium hydroxide paste with propylene glycol and partially reabsorbed, (E) gutta-percha points (tooth #22), (F) gutta-percha points (tooth #21), (G) root canal filling radiographs, (H) radiographs after three years of follow up, showing total periapical repair; (I) radiographs after six years of follow up, showing complete periapical repair.
The EDTA was used before the application of the medication in all sessions as well as before the final filling of the root canal in order to increase the permeability of dentin and facilitate the calcium hydroxide ions diffusion in the dentin, it is justified because in the literature is large the number of papers that use of EDTA in different concentrations and associations in order to promote a greater cleaning of the root canal walls, with the removal of the residual layer of dentinal magma.22,23

Regarding the root canal sealers, supported on the calcium hydroxide properties, some cements have it as main active component. In our work it was used the Sealapex™ (SybronEndo - SDS) in the root canal filling because of its biological properties and its ability to stimulate deposit of mineralized tissue in the apical third, according to Holland and Souza.24

The choice of propylene glycol is due to the fact that this vehicle give a good fluidity that facilitates its handling and deposition within the canal25 and also because, according to O’Neil,26 it has a large capacity of solubilization of the organic materials and still because Seidenfeld and Hanzlik,27 the propylene glycol has approximately the same density as water, and when used as a solvent and vehicle is less toxic and causes no noticeable cumulative effect. In contrast to these results Safavi and Nakayama28 found that calcium hydroxide is not dissociated in contact with propylene glycol because the calcium hydroxide needs water to dissociate.

Our choice for the treatment of reported cases was relied on evidence presented by Holland et al.,29 and intracanal dressing changes were performed until the final filling of the root canal.

Although various substances have been shown to be intracanal dressing, calcium hydroxide has become widely used, mainly in endodontic treatment of infected teeth, because of its antimicrobial potential, for stimulating periapical repair and also by paralyzing the osteoclasts destructive action found in resorption areas.29

Furthermore, its antimicrobial activity quickly eliminates bacteria that come into direct contact with this substance,30 however, its effect on the microorganisms presented in the dentinal tubules takes longer.31 According to Oguntebi,32 the infection in these sites would favor the development of certain types of bacteria that could constitute an important reserve for reinfection of the root canal, during and after endodontic treatment.

Although the highest dentin alkalinization occurs only after 3-4 weeks,33 in our reports, the dressing was renewed when reabsorption in the root canal was seen radiographically, in agreement with Katebzadeh et al.34

The filling of the root canals was performed only when the lesion showed a considerable reduction in its diameter, which disappeared in follow up. This result is very close to that obtained by Souza et al.35 which noted the repair of large lesions between 6 and 8 months.

The follow up of this work were made with large amount of time due to the difficulty of the patients return to the office.

**Conclusion**

The results of the reported cases show that the technique of the calcium hydroxide as root canal dressing is an effective alternative for the treatment of teeth with extensive chronic periapical lesions, because it was possible to observe the biological repair of the periapical region, by means of the radiographic follow up reducing or eliminating the necessity for surgical procedures.
References


Emergency endodontic care of patient with inconclusive diagnosis of von Willebrand disease

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ABSTRACT

Introduction: Patients presenting bleeding disorders need special care when submitted to dentistry procedures. Objectives: The aim of this case report is to provide information on how to handle a patient with a probably diagnosis of von Willebrand disease and acute periapical abscess in tooth #23. Methods: The patient was a white female, 35 years old, who presented to the emergency program of the School of Dentistry - Federal University of Paraná, Brazil, with extensive decay below gum level, projecting into the palate, and crown fracture exposing the root canal to the oral environment. Attention was focused on isolating the operative field, which could not be done in the conventional manner due to the extension of the caries, the proliferation of gum tissue and the patient’s systemic conditions. Conclusion: The strategy used in this case was effective in management of coagulopathy and allowed for emergency care to be carried out without complications.

Keywords: Von Willebrand factor. Endodontics. Periapical abscess.


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Introduction

Von Willebrand disease (VWD) is a hereditary bleeding disorder caused by deficiency or dysfunction of a protein called von Willebrand factor.\(^1\) Diagnosis is performed in different steps.\(^2\) Specific tests for a complete laboratory characterization are needed to confirm or exclude the diagnosis.\(^3\) Meanwhile, patients are susceptible to emergency endodontic care.\(^4,5\) Endodontic procedures can be developed safely and with predictable results as long as a correct treatment plan is established.

The aim of this clinical case is to describe endodontic emergency care for a patient with acute periapical abscess and VWD disorder suspect.

Case history

A 35 year-old woman presented to the Emergency Care Center of the School of Dentistry of the Federal University of Paraná, with pain in the left upper region. A discomfort in the canine fossa for approximately two weeks, culminating in fever and continuous, spontaneous and long-lasting pain that was not relieved by common analgesics was related. The patient presented history of relentless bleeding following dental extraction which required hospitalization six months ago. She presented a letter from the Medical Care Center of the Federal University of Paraná relating suspect of VWD, requiring dental treatment and reporting that 1.0 g of y-aminocaproic p.o. acid had been administered every 6 hours, for 7 days.

Clinical examination showed crown fracture and extensive decay, exposing the root canal to the oral environment for the last 4 months. There was sensitivity to palpation and no extraoral edema. The radiographic examination showed a poor endodontic treatment limited to the middle third of the root canal and apical radiolucency (Figs 1 and 2).

The treatment was performed using anesthesia with 2% mepivacaine with 1:100,000 epinephrine (DFL Indústria e Comércio S.A., Rio de Janeiro, Brazil). The rubber dam could not be done in the conventional manner, due to the extension of the carious process, the proliferation of gum tissue and the patient’s conditions. It was sequentially perforated three times in order to involve teeth #22, #23 and #24. After positioned, ethyl cyanoacrylate (Loctite\textsuperscript{®} - Super Bonder Precisão - Henckel Ltda, São Paulo, SP, Brazil) was applied...
between the rubber dam and the periodontium on the vestibular and palate surfaces (Fig 3), irrigation with 1% sodium hypochlorite solution was used to disinfect the operative field and as an irrigating solution. Special instruments were prepared using files #15 and #20 according to Kobayashi, to remove the filling material in association with orange oil (Citrol® – Fórmula & Ação – São Paulo, SP, Brazil). Instrument #70 (Dentsply-Maillefer, Ballaigues, Switzerland) penetrated a few millimeters in the apical direction, followed by instrument #60 (Dentsply-Maillefer, Ballaigues, Switzerland) which, when rotated and pulled, removed the filling material at once (Figs 4 and 5). As soon as the filling material was removed, drainage was present for few minutes. The root canal was completely emptied using instruments #15, #20 and #25 (Dentsply-Maillefer, Ballaigues, Switzerland) and filled with paramonochlorophenol 2% (PRP®- Fórmula&Ação- São Paulo, SP, Brazil) intracanal dressing.

An auxiliary suction tip controlled the slight bleeding of the gum tissue, originated from the inevitable trauma caused by the teeth conditions. Zinc oxide-eugenol cement (IRM® - Dentsply Indústria e Comércio Ltda, Petrópolis, Rio de Janeiro, Brazil) was used to seal the cavity (Fig 6).

Paracetamol 750 mg, 8/8 hours for 2 days and Amoxicillin 500 mg, 8/8 h for 7 days were prescribed and the patient referred to the Surgery department (Fig 7), where appropriate evaluation was performed in order to remove the remaining root.
Discussion

VWD is a heterogeneous clinical entity, with variable degrees of bleeding manifestations. It presents different clinical phenotypes, being the most common mucocutaneous bleeding, especially epistaxis and menorrhagia. Bleeding in other regions, such as the genitourinary tract and the digestive tract have also been reported, although less frequently. The patient presented a chronic ulcer with active bleeding in the lower left limb, urinary bleeding, daily epistaxis and menorrhagia.

Complementary exams are necessary to confirm the disease. Screening tests for initial evaluation of hemorrhagic coagulopathies, specific tests for diagnostic confirmation, and discriminatory tests that allow the classification of the disease, which may take some time.

The patient was conducted to the emergency care of the Dental School from the University Hospital presenting pain and infection, but the diagnosis of VWD was still not conclusive. The patient was under prescription of an antifibrinolytic. Extra care must be taken during dental procedures in order to avoid complications.

There are no restrictions regarding the use of vasoconstrictors, which provide more time and comfort for endodontic emergency procedures. In infiltrative and intraligamentary anesthesia techniques, prior administration of coagulation factors is not necessary. The administration is recommended for the inferior alveolar nerve blocking, due to the possibility of bleeding in the retromolar region, with the presence of trismus and the risk of asphyxia. The administration of an antifibrinolytic was beneficial because it prevented excessive bleeding following trauma to the hyperplasic gum tissue.

The use of rubber dam is almost mandatory in modern endodontic practice to provide aseptic operating field and to protect the patient against foreign body aspiration or ingestion.

When the margins of the root are submerged by gingival ingrowths, it is necessary to excise sufficient tissue to expose the margins, not possible in this case. An alternative method for retaining the dam was applied. The rubber dam was perforated three times in order to involve teeth #22, #23 and #24, the holes were connected using a scissor and ethyl cyanoacrylate was applied between the rubber dam and the gum tissue on the vestibular and palatal surfaces. No clamp was necessary and the procedure should be carried out in the least traumatic way possible.

The endodontic procedure was performed removing the filling material with specials instruments and a solvent in order to permit some drainage and relieve of pain.

Systemic medication for managing pain and infection must be careful for these patients. Painkillers derived from acetylsalicylic acid as well as nonsteroidal anti-inflammatory drugs (NSAIDs) should be avoided because they affect platelet aggregation when used for prolonged periods. There are no restrictions regarding antibiotics.

In the treatment of patients with hemorrhagic disorders, the interaction between professional and hematologist is a pre-requisite for safe procedures.

Figure 7. Radiograph after root extraction.
During dental emergencies, knowledge of the risks is essential in the decision-making process, once local bleeding control measures may not be enough.4,12,13

**Conclusion**

Emergency endodontic care may be necessary while VWD diagnosis is still not confirmed. The emergency procedures must be defined for safe and predictable emergency dental procedures in patients with bleeding disorders. An alternative method for retaining the dam could be provided and the procedure should be carried out under the least traumatic course management.

**References**

Maxillary first premolar with three roots: Case report

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ABSTRACT

Introduction: The maxillary first premolar may rarely present with three roots, two buccal and one palatal, demanding more attention during endodontic intervention. Objective: This paper reports the case of a maxillary first premolar with three roots and three root canals, highlighting the difficulties and special care during endodontic treatment. Methods: After initial radiography and coronal opening, the presence of three roots and three root canals was detected. The exploration of the canals was performed with #10 K-file and the root canal length was measured by means of radiographic technique, which made it possible to confirm the anatomical variation and to assure that the buccal canals were independent. The instrumentation was mixed, with K-type hand files, until #35 file, automatized with ProTaper® system (Dentsply). The filling of the canals was performed with the lateral compaction technique with sealer Sealer 26®. Conclusion: Professionals should always carefully consider the diagnostic radiograph and perform all steps of root canal treatment properly, so that possible changes can be detected, not compromising the success of treatment.

Keywords: Anatomy. Bicuspid. Dental pulp cavity.

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Introduction

The thorough knowledge of dental anatomy is extremely important to the success of endodontic treatment, which is composed of several interdependent steps.1,8,9,12,14,18,20 Roots and root canals can vary in number, size, shape, divisions, fusions, directions and stages of development.9 The periapical radiographs help in the study of internal anatomy and its variants,8,9 and, for better viewing, we recommend the use of two diagnostic radiographs, an orthoradial and another mesially or distally displaced.10

Often, the first premolar presents itself with two conical roots, one buccal and one palatal, and two respective canals.9,12 These roots may present themselves independent, not entirely separate or as a single root.9

However, this dental element may have many variations, especially in the amount of roots.9,12,20 The buccal root, sometimes, is divided into two: Mesiovestibular root and distobuccal root.9,12 In this case the tooth is called “minimolar”.12 This format was described with a frequency of 2.5% by Pécora et al15 and 3.3% by Chaparro et al.4 The presence of the third canal in first premolars regardless of the number of roots was reported in 1979, by Vertucci and Gegauff,19 which examined 400 extracted teeth and found a prevalence of 5%. Recently, in 2008, Rózylo et al16 described the presence of the third canal in 9% of the cases.

Thus, the objective of this paper is to present, by means of a clinical case, a rare anatomical variation of the first premolar with three roots and three root canals, and its implications in endodontic treatment.

Case report

Male patient, 32-years-old, attended the service of endodontics, School of Dentistry of Bauru/SP, conveyed from the public health system to perform endodontic treatment of tooth #14.

Radiographically, it was observed unusual root anatomy, suggestive of two buccal roots and one palatal root (Fig 1). After performing anesthesia, access cavities and rubber dam, it was confirmed the presence of two vestibular sockets, making the final shape triangular, with a base facing the buccal aspect (Fig 2). The exploration of the canals was performed with #10 K-file and the root canal length was measured.
by means of radiographic technique, enabling to confirm the variation in morphology and to assure that both canals were independent (Fig 3). The instrumentation was mixed, and the apical preparation was performed with K-type hand files, until #35 file; cervical and middle thirds were prepared with ProTaper® automated system (Dentsply).

Due to the presence of apical lesions, we used calcium hydroxide (Ca(OH)₂) as an intracanal medication for a month. After this period, it was performed the filling of the canal with the lateral compaction technique with Sealer 26® (Fig 4).

In the X-ray control, achieved after two years, it was observed a normal aspect of periapical region structures (Fig 5).

**Discussion**

The thorough knowledge of dental anatomy is of utmost importance for the completion of endodontic treatment, because is through this science that the dentist can estimate a three-dimensional situation of the canal system, from a two-dimensional image provided by radiographs. Over the years, literature has reported the most varied anatomical changes of several teeth, including premolars. These variations may be related to the number of root canal, its size, shape, divisions, fusions, directions and stages of development.

Premolars are a group of teeth exclusive of the permanent dentition, and their predecessor are the first deciduous molars. They also have the basic function of crushing and grinding food, in addition to helping the speech and aesthetics. The first premolar has an average length of 21.5 mm, ranging from 17 to 25.5 mm. In most cases, they feature two conical roots — one buccal and one palatal — with one canal each one, and the vestibular root often presents a curvature towards the palatine aspect. These roots may be well developed, not entirely separate or as a single and large root, with two canals in its interior. The outline of the pulp chamber has an elliptical morphology, due to the mesiodistal flattening.

However, the amount of roots and canals can vary in this dental group, since the buccal root may divide into two, evidencing mesiovestibular and distobuccal roots. In this case, the premolar may be

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**Figure 3.** Root canal length, showing the presence of three root canals.  
**Figure 4.** Final aspect of tooth #14 endodontic treatment.
referred to as a “minimolar”. According to Bellizzi and Hartwell, when this change in morphology occurs, the roots can be classified into three groups. In group 1 the three roots are merged or there is only two buccal roots, and the palatal root is semifused or free. In group 2 buccal roots present separate, from middle or apical third. In group 3 the three roots are separate from the cervical third. In this case reported, there was no certainty about the classification, since the radiographic images allow a subjective understanding on roots separation (Figs 1, 3 and 4).

Endodontic treatment of maxillary premolars with this morphology should be adjusted to such a situation, by locating all existing root canals and making the outline change from elliptical to triangular, with the base toward the buccal aspect and the apex to the lingual aspect. In the case reported, it could be seen that by making the coronal opening, due care has been taken regarding the location of the canals, which was more difficult due to the great loss of coronal structure, in particular at the distal aspect of the tooth (Fig 2).

Regarding the instrumentation employed, the use of hand files is established for confection of apical stop, and ProTaper® system consists of rotary instruments that have proven effective, improved and simplified, allowing the professional to perform more effectively and quickly the root canal treatment.

The intracanal medication has the function of eliminate remaining microorganisms and prevent re-contamination, prevent or reduce periapical inflammation, solubilize organic matter, neutralize toxins, control persistent exudation, control external inflammatory resorption and stimulate repair. Calcium hydroxide is the most appropriate medication for endodontic purposes, being a substance with antimicrobial activity, inhibiting root resorption and inducing formation of hard tissue. However it is necessary a long time in contact with the tissue so that its action is successful, which justified the permanence of calcium hydroxide inside the canals for a month in this case presented.

The biological purpose of root canal filling is to provide favorable conditions for the body to repair tissue with no risk of relapse and the used calcium hydroxide-based sealer (Sealer 26®) seems to achieve this goal (Fig 4). In the case reported, the repair could be confirmed in the control radiograph, two years after completion of endodontic treatment (Fig 5).

Conclusions
Changes in shape and number of roots and root canals are likely to occur, and sometimes in a rare form, as in the premolar with three roots and three canals. Thus the professional is required to pay more attention to the diagnostic radiographs during the course of endodontic treatment, so such alterations do not affect prognosis.
Late treatment of dental trauma using apexification technique

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ABSTRACT

Introduction: A 37 years old male patient was admitted to the clinic of endodontics. After anamnesis it was found that the tooth #11 had coronal open access and the presence of calcium hydroxide with dental trauma history. Radiographically, the tooth had incomplete root formation, thin and fragile dentin walls and foraminal divergence associated with periapical radiolucent image.

Objective: To report a clinical case of apexification, performed with calcium hydroxide dressing. Methods: The treatment chosen was the apexification that began in the second session, after 15 days, through chemomechanical debridement of the entire root canal, with K files and irrigation with 2.5% sodium hypochlorite solution. Then, the calcium hydroxide paste (calcium hydroxide, iodoform and propylene glycol) was applied and changed every 15 days over four months. The radiographic exam demonstrated the complete closure of the foraminal opening and regression of periapical radiolucency. The root canal was filled using a cone made from the union of three master cones #60 and lateral condensation technique with Sealapex®. Results: Six months after the filling, tests revealed normal periapical tissues and absence of symptoms. Conclusion: It was concluded that the treatment of dental trauma associated with dental pulp necrosis and periapical lesions with successive changes of calcium hydroxide paste was adequate to obtain the regression of periapical lesion, formation of a mineralized barrier and promotion of patient’s health.

Keywords: Incomplete root formation. Calcium hydroxide. Apexification. Immature teeth.

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Introduction

Trauma in young permanent teeth can produce effects such as pulp necrosis. When the pulp necrosis is linked to incomplete root formation there is a difficulty in performing a treatment, because the process of root formation by deposition of dentin ceases. The dental trauma with pulpal involvement, as well as dental caries, are generally in the main etiological factors of necrosis. Permanent teeth with incomplete root development exhibit root canal and foramen diameter extremely large and exaggerated. The canal walls are not always parallel and present apical differences and the open foramen does not promote suitable bulkhead for filling material. Thus, it becomes difficult to keep the endodontic treatment within the limits of the root canal and especially to obturate it.

Various techniques have been described in literature to treat teeth with incomplete root formation associated with pulp necrosis. The techniques vary as a function of time and research on different authors. The apexification is the induction of the apical foramen closing through the deposition of hard mineralized tissue at the apex in teeth with pulpar necrosis. It has been shown that mineralized tissue comprises osteocementum, osteodentine or bone, or a combination of all three in the apical region, with thickness variation. Several studies show that the best option is filling the root canal temporarily with medicinal substances, thus inducing the apical closure. Zinc oxide and eugenol paste have been advocated for this purpose, as well as the polyantibiotic and iodoformized paste.

Currently, the cases of apexification are being treated by most professionals with the use of calcium hydroxide in resorbable pastes. The calcium hydroxide has been the material of choice for apexification since 1964, when Kaiser reported for the first time the ability to induce the biological closure of immature pulpless teeth.

Calcium hydroxide has been nominated for apexification because of its alkaline pH and for presenting a high antibacterial effect, inhibiting osteoclastic activity and preventing the entry of exudate and granulation tissue. Several long term studies have shown a success rate of 74-100% for apexification cases using calcium hydroxide.

The aim of this study was to report a case of apexification performed with dressing changes of calcium hydroxide.

Case report

The patient, 37 year-old, entered the clinic of endodontics, School of Dentistry of Araçatuba - UNESP, with a history of dental trauma in the permanent central incisor and lack of sensitivity. On clinical examination, it was observed the presence of coronary opening sealed with temporary material, lack of mobility and normal periodontal probing. Radiographs (Fig 1A) showed the presence of radiopaque material compatible with calcium hydroxide paste and iodoform filling the root canal with incomplete apex. The proposed treatment was apexification using calcium hydroxide paste.

In the second appointment, odontometry was performed with the file dimensioned to the temporary work length that was obtained based on previous radiographs. The patient was anesthetized with Citanest (Dentsply, Rio de Janeiro, Brazil), the tooth was isolated with rubber dam and the temporary restoration was removed. The root canal was irrigated with a solution of 2.5% sodium hypochlorite and the calcium hydroxide that was in the canal root was removed. Following was done biomechanical preparation around the root canal with K files and irrigation with a solution of 2.5% sodium hypochlorite, caring not to reach the periapical tissues and not excessively wear out the root walls. The canal was instrumented to K file #80 and the working length was set at 20 mm, coinciding with the radiographic apex, always being careful not to wear out the walls, irrigating thoroughly with 2.5% sodium hypochlorite. The smear layer was removed with 17% EDTA and was performed the final irrigation with sodium hypochlorite. The canal was dried with absorbent paper points sterilized and then filled with calcium hydroxide paste (calcium hydroxide, iodoform and propylene glycol) using a lentulo spiral calibrated to 3 mm of work length, to avoid leakage of the paste (Fig 1B).

The exchange of calcium hydroxide paste was carried out each 15 days until complete formation of calcified tissue barrier, detected by clinical and radiographic examination (Figs 1C and 1D; Figs 2A-C). The time required for formation of the apical barrier was four months (Fig 2D).

The tooth was filled with the sealer Sealapex (SybronEndo, Glendora, California, USA) and lateral condensation technique (Fig 3A). For this, the gutta-percha master cone was made from the union of three cones #60. The cone was taken to the work length and...
Late treatment of dental trauma using apexification technique

Figure 1. Periapical radiographs of the upper right central incisor. **A** Diagnostic radiographic exam. Note the radiopaque intracanal dressing partially filling the root canal and the provisional restoration with radiopaque material and incomplete root formation. **B** Radiographic exam performed in the second appointment, in the end of the treatment, consisting of biomechanical preparation and intracanal dressing with calcium hydroxide paste and iodoform. **C** Radiographic exam at the third appointment, in the end of the treatment, consisting of careful biomechanical preparation and change of intracanal dressing with calcium hydroxide paste and iodoform. **D** Radiographic exam at the fourth appointment, in the end of the treatment, consisting of careful biomechanical preparation and change of intracanal dressing with calcium hydroxide paste and iodoform.

Figure 2. Periapical radiographs of the upper right central incisor. **A** Radiographic exam at the fifth appointment, in the end of the treatment, consisting of careful biomechanical preparation and change of intracanal dressing with calcium hydroxide paste and iodoform. **B** Radiographic exam at the sixth appointment, in the end of the treatment, consisting of careful biomechanical preparation and change of intracanal dressing with calcium hydroxide paste and iodoform. **C** Radiographic exam at the seventh appointment, in the end of the treatment, consisting of careful biomechanical preparation and change of intracanal dressing with calcium hydroxide paste and iodoform, note the apical closure. **D** Radiograph proving obturation by lateral condensation technique and Sealapex, in the eighth appointment.

A periapical radiograph was performed to proof length (cone test). A signal was made on the cone with clinical tweezer marking the reference point. After cone test, the sealer was prepared from a portion of homogenized base paste with a portion of the catalyst paste on a glass plate sterilized. The cone was smeared with sealer and settled in the root canal. The length was observed by the coincidence of the mark on the cone with the reference point. Spacing was initially carried out with spacers A30 (Maillefer Instruments, Switzerland) and removed with semicircunferencial clockwise and counter-clockwise movement with slight pressure towards incisal. Immediately after the spacing, a B7 cone (Dentsply, Rio de Janeiro, Brazil) was settled in the space obtained. The process was repeated in order to set four cones B7. Then, new spacings were made with spacer A40 (Maillefer Instruments, Switzerland) and settlement of B8 cones (Dentsply, Rio de Janeiro, Brazil) until complete filling of the canal. In this moment a radiograph was performed to confirm the filling.
Then, the cones were cut at the height of the orifice of the canal using heated Paiva condenser #4. A cotton ball soaked in alcohol was used to clean the remnants of filling material in the pulp chamber and coronal sealing was accomplished with glass ionomer cement (Vidrion R, SS White). The final periapical radiograph revealed dense and homogeneous obturation, associated with the filling of apical branching in the root segment newly formed (Fig 3A).

Six months after the final treatment, a new periapical radiograph was performed and revealed complete disappearance of the lesion and absence of signs and symptoms (Fig 3B). At 18 months of postoperative control, there was again complete disappearance of the lesion and apical closure, confirming the successful treatment (Fig 3C).

**Discussion**

Immature permanent incisors with open apices can lose their vitality, as a result of trauma, which leads to root development stoppage. The root canal treatment in such cases becomes difficult due to the open apex. However, with the apexification technique, which consists in inducing the closing of the apical foramen through the deposition of hard mineralized tissue, root canal treatment may be well conducted.20

Immature permanent teeth and pulp necrosis require the use of a dressing for the occurrence of hard tissue deposition in the apical region for a period of time.14,21 Traditionally, the most commonly used material for apexification is calcium hydroxide, which stimulates formation of mineralized and fibrous tissue by cells of the granulation tissue in the apical portion of the root.18,22

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**Figure 3.** Periapical radiographs of the upper right central incisor. **A** Radiographic exam at the eighth appointment, at the end of the obturation by the lateral condensation and Sealapex. **B** Radiographic exam for 6-month postoperative control; note the total remission of the lesion and apical closure. **C** Radiographic exam for 18-month postoperative control; note the total remission of the lesion and apical closure.
In the present clinical case it was chosen the use of calcium hydroxide, following the example of various studies which demonstrated a high degree of clinical success.\textsuperscript{14,23,24,25}  

As to the period of time to the exchange of calcium hydroxide, to induce apical closure and normal periapical tissues,\textsuperscript{18} there is no consensus among the authors. Some suggest that the change is initially made in a month and subsequently each interval of three months.\textsuperscript{20} Other authors believe that a single application of calcium hydroxide paste is sufficient to form the barrier.\textsuperscript{11} Another indication is to change only if there is reabsorption of calcium hydroxide in the apical third of the root canal.\textsuperscript{19,26} In this clinical case was chosen to make the exchange of calcium hydroxide each 15 days, until there was a complete barrier formation of calcified tissue.

A study on the index of apical barrier formation showed that it was directly proportional to the frequency of renewal of the paste.\textsuperscript{27} However, another study, in dogs, reported that replacement of the calcium hydroxide paste was not required to occur the apexification in teeth with incomplete root formation, however, it significantly reduced the intensity of the inflammatory process\textsuperscript{14} and a simple application of calcium hydroxide paste with monthly applications or renewals every three months showed that the amount of calcified tissue formed was similar in the three groups.\textsuperscript{28}

Calcium hydroxide is capable of inducing the formation of a hard tissue barrier, and in addition, is also capable of stimulating tissue repair. When placed in direct contact with the pulp tissue, there is an immediate and short-term reaction of the tissue, supposedly caused by high alkalinity. This effect is alkaline due to the release of hydroxyl ions, which, in contact with vital tissues, produce morphological alterations that are characterized histologically by the presence of self-limiting superficial necrosis in its initial phase. It has also been reported that the alkaline environment prevents the growth of bacteria, which is very important, as tissue repair and deposition of mineralized tissue only occur in the absence of an infectious process.\textsuperscript{26}

### Conclusion

It was concluded that treatment of dental trauma associated with necrosis of the pulp tissue and periapical lesions with successive changes of calcium hydroxide paste was adequate to obtain the regression of periapical lesion, formation of a mineralized barrier and health promotion for the patient.