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Perspectives for the therapeutic success

The scientific revolution that Endodontics is experiencing is spectacular. Many discoveries boosted the progress, promoting new and important resources which contribute to greater success in the endodontic treatment.

Strategies for navigating in cone beam computed tomography (CBCT) images brought new perspectives to the outcomes and monitoring of endodontic treatment. However, the estimates of success when using CBCT should be reassessed and treatment protocols should be reviewed and, if necessary, redirected. This is because, although the results are still preliminary, endodontic treatments were observed with higher failure rates when analyzed by CBCT than by means of periapical radiographs. However, regarding its radiation dose, cost-effective and proper recommendation, this imaging exam, routinely performed, still needs further and careful discussion.

There is a large number of specialization courses in Brazil, which demands greater care with teaching. For this reason, the adoption and critical monitoring of stricter therapeutic protocols, aiming at improving the quality of endodontic treatment should always be prioritized.

Every scientific progress brings a new challenge. Often, this advance can change a decision, but it must be grounded in effectiveness. This is one of the reasons for permanent updating with scientific knowledge, aiming at the use of a therapeutic protocol that brings greater expectation of successful treatment.

Carlos Estrela
Editor-in-chief

Endo *in* Endo

Original articles

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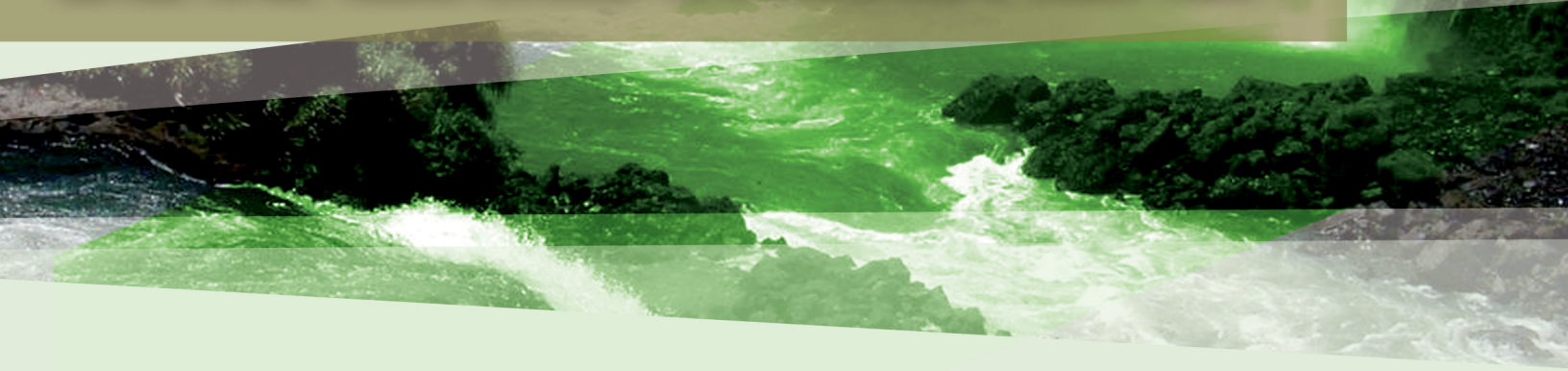

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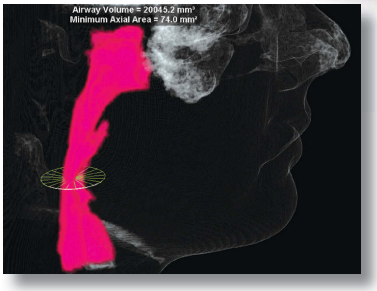

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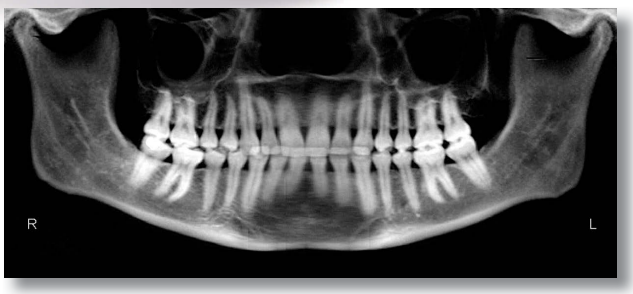

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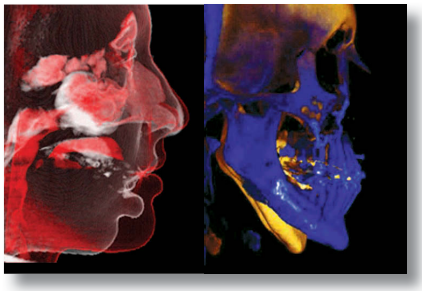
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Pulp repair: The reconstruction is done with granulation tissue — the pulp repairs itself, and does not regenerate itself!

Alberto **CONSOLARO**[1]
Renata Bianco **CONSOLARO**[2]

ABSTRACT

This paper aims at explaining, sequentially and in an integrated manner, pulp biology and its clinical applicability in understanding pulp repair after operative procedures have been carried out in the coronary region, as well as in the apical tissues. It also aims at substantiating what influences the choice of technique, the most opportune time for intervention and which material should be used. The dentin-pulp complex represents a single structure in the human body, with very specific characteristics. Pulp injuries are common in clinical practice and their repair involves the reorganization with new matrix production, forming mineralized tissue barriers and a new odontoblastic layer. The morphology of the newly formed dentin can be identified by several names, among which is tertiary dentin. As important as the coronary and apical pulp injuries that will be repaired, are the situations in which one wishes to repair teeth with incomplete root formation. The pulp biology and its implications in pulp repair are of great interest for all specialties, since they influence the outcome of every clinical case.

Keywords: Dental pulp. Pulp repair. Incomplete root formation. Dentin.

[1] Head Professor of Bauru Dental School and post-graduation courses, Ribeirão Preto Dental School / São Paulo University.

[2] PhD Professor, Adamantina Dental School, São Paulo – Brazil.

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3.	Dental pulp as dentin producer: the beginning of the production and fibronectin
4.	Origin and formation of dentin as mineralized tissue barrier
5.	Formation of reparative or tertiary dentin
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Introduction: repair and regeneration are conceptually different

The connective tissues (such as fibrous, osseous, adipose and other special ones) reconstruct themselves when damaged, forming in these areas a granulation tissue based on the fibrin network of a blood clot and/or an inflammatory exudate.[2] The granulation tissue is the precursor of connective tissues and, from the cells that invade it, can originate fibrous, osseous, cartilage, dentinal and cemental tissues, among others. The connective tissues are responsible for filling empty damaged spaces that may exist in the body.

Repairing characterized by the granulation tissue as a mediator of the reconstructive process is the last stage of a successful inflammation process and it is exclusive to vascularized tissues.[2] When repaired, the coating, glandular and visceral epithelia, as well as the peripheral nerves and muscles, do this directly from their remaining adjacent, with no interposition of the granulation tissue as precursor: this process is called regeneration. The dental pulp, as well as the periodontal tissues, has a specialized connective nature and its reconstruction is carried out by means of angiogenesis and cell migration, typical phenomena of a granulation tissue. Thus, the pulp repairs itself when damaged, instead of regenerating itself! This is neither better, nor worse, it just represents the mechanism of its reconstruction, which may or may not return it to its complete, or incomplete, anatomical and physiological normality, similar to what occurs in regeneration, depending on the conditions imposed on each clinical situation.

Pulp and dentin in the dentin-pulp complex context

The dental pulp is comprised of a specialized connective tissue with very specific morphological and functional characteristics (Figs 1 and 2). The pulp and the dentin constitute a structural and functional unit that interacts with the enamel and the cement. Apically, the pulp is naturally connected to the periodontal ligament tissues, with no structure or measure separating them.

The apical end of the dental pulp can be considered the point where the last odontoblast is found in the dentinal surface. Thereafter, in the mineralized tissue surface, there are cementoblasts incrementally depositing the cemental layers on the dentin. The most apical portion of the root canal is filled with periodontal tissue which, if left in place after the dental pulp has been removed, will be considered as periodontal remnant. The line represented by the limit between cementum and dentin, or CDC limit, tends to be three-dimensionally irregular in its occlusal-apical position.

Dental papilla originates the pulp and favors dentin formation (Figs 3 and 4). The dentin represents a product of synthesis of highly differentiated pulp cells — odontoblasts —, which remain inside it in cytoplasmic exten-

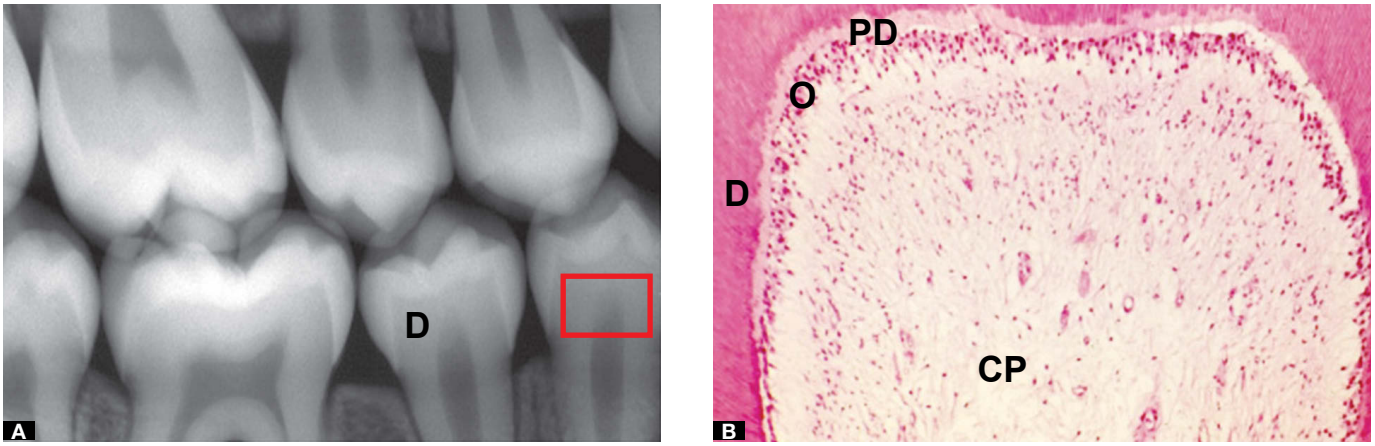

Figure 1. Dentin-pulp complex and its components: dentin (D), predentin (PD), odontoblastic layer (O) and central portion of the pulp (CP). (**B** = H.E., original mag. = 160X).

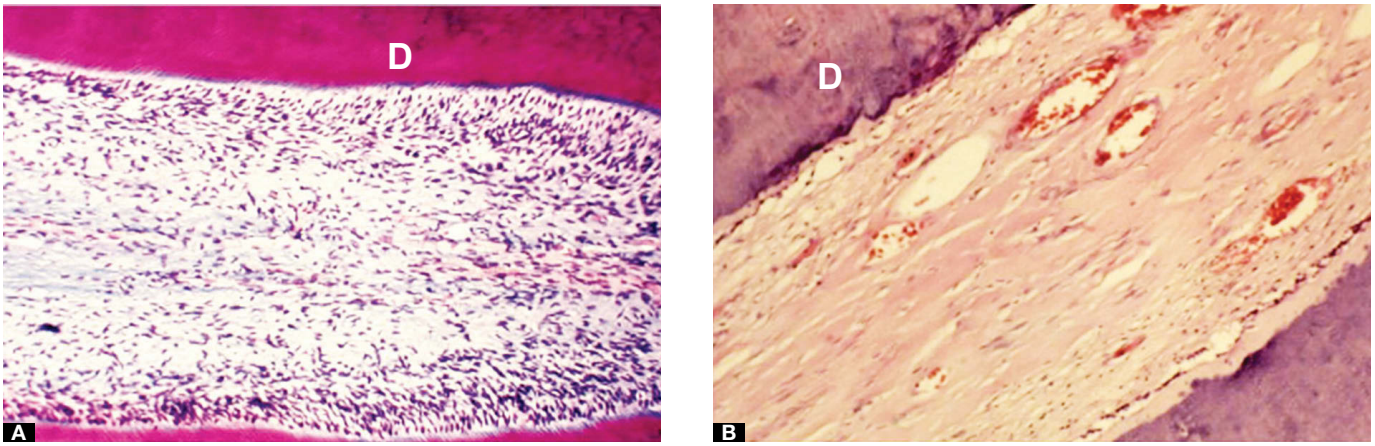

Figure 2. Young dental pulp, well cellularized, in **A**, and, aged pulp, in **B**, with exuberant fibrosis. D = Dentin (**A** and **B** = H.E., original mag. = 160X).

sion form, around which are formed the dentinal tubules. One of the purposes of these extensions is to maintain water and ionic balance of the dentin while providing defense mechanisms against external aggressors.

Studies concerning dental changes specific of the age and inflammatory diseases induced by physical, chemical and/or bacterial agents, prove to be impossible to separate the pulp from the dentin. Understanding these processes requires a concept of structural and functional unit known as "dentin-pulp complex".

However, in the diagnosis, treatment and prognosis of diseases affecting the tooth, one should consider the pulp separately from the dentin, in order to determine, i.e., the degree of depth of a cavity, the thickness of dentin remaining in the cavities, the retention or mechanical support capacity of the tooth structure and, also, the necessity and possibility of pulp protection in operating procedures.

Delineating and predicting the limits between dentin and pulp means to establish the parameters for certain therapeutic and prognostic conducts. Unreservedly replacing the terms "dentin" and/or "pulp" with the term "dentin-pulp complex" is unfeasible from a clinical and therapeutic point of view, because the dentin and the pulp are topographically distinct tissues. The term "dentin-pulp complex" should be applied when referring to embryonic and functional issues.

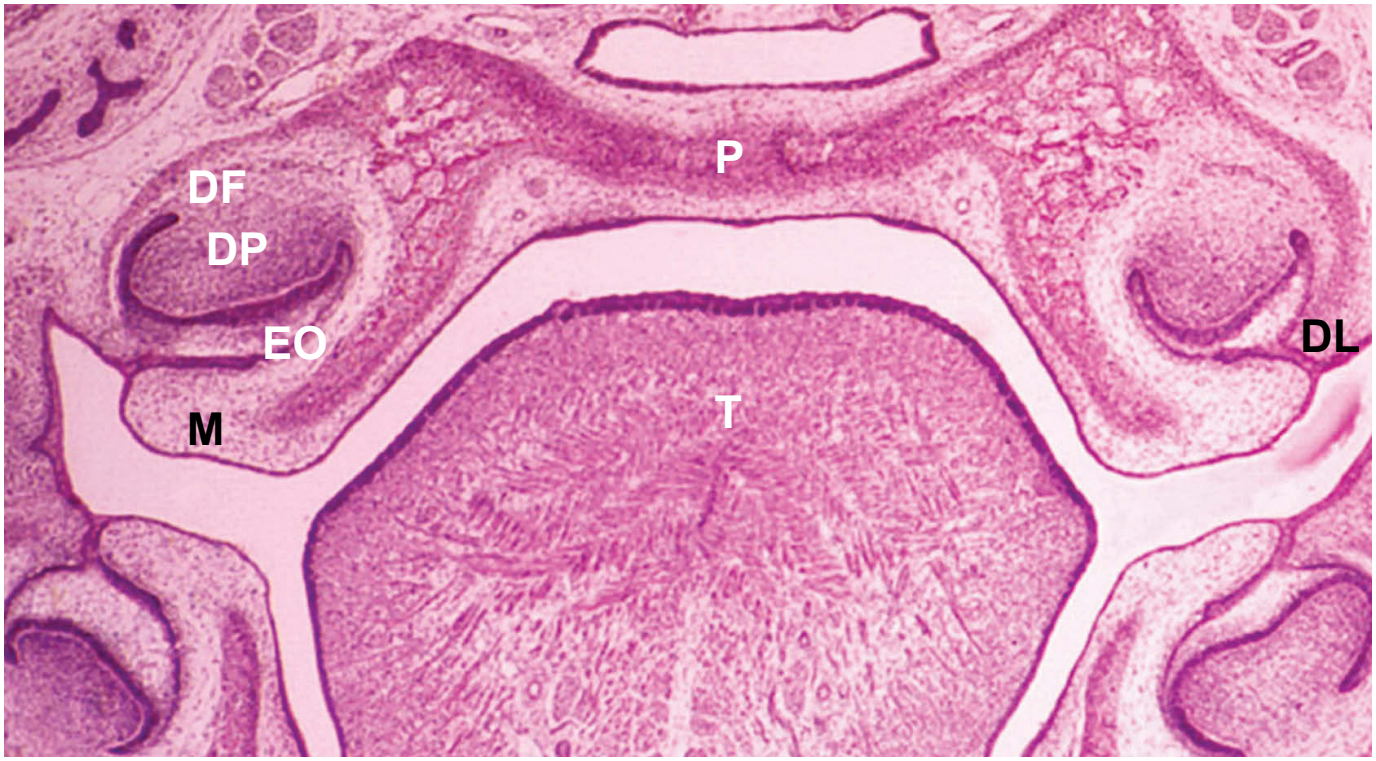

Figure 3. Frontal section of human embryo in oral and maxillofacial region, with four dental germs at bell stage. Dental lamina (DL) and primitive mesenchyme (M), enamel organ (EO), dental papilla (DP) and the dental follicle (DF) can be observed. T = tongue; P = palate. Covering the oral cavity, it is observed the ectoderm. (H.E., original mag. = 40X).

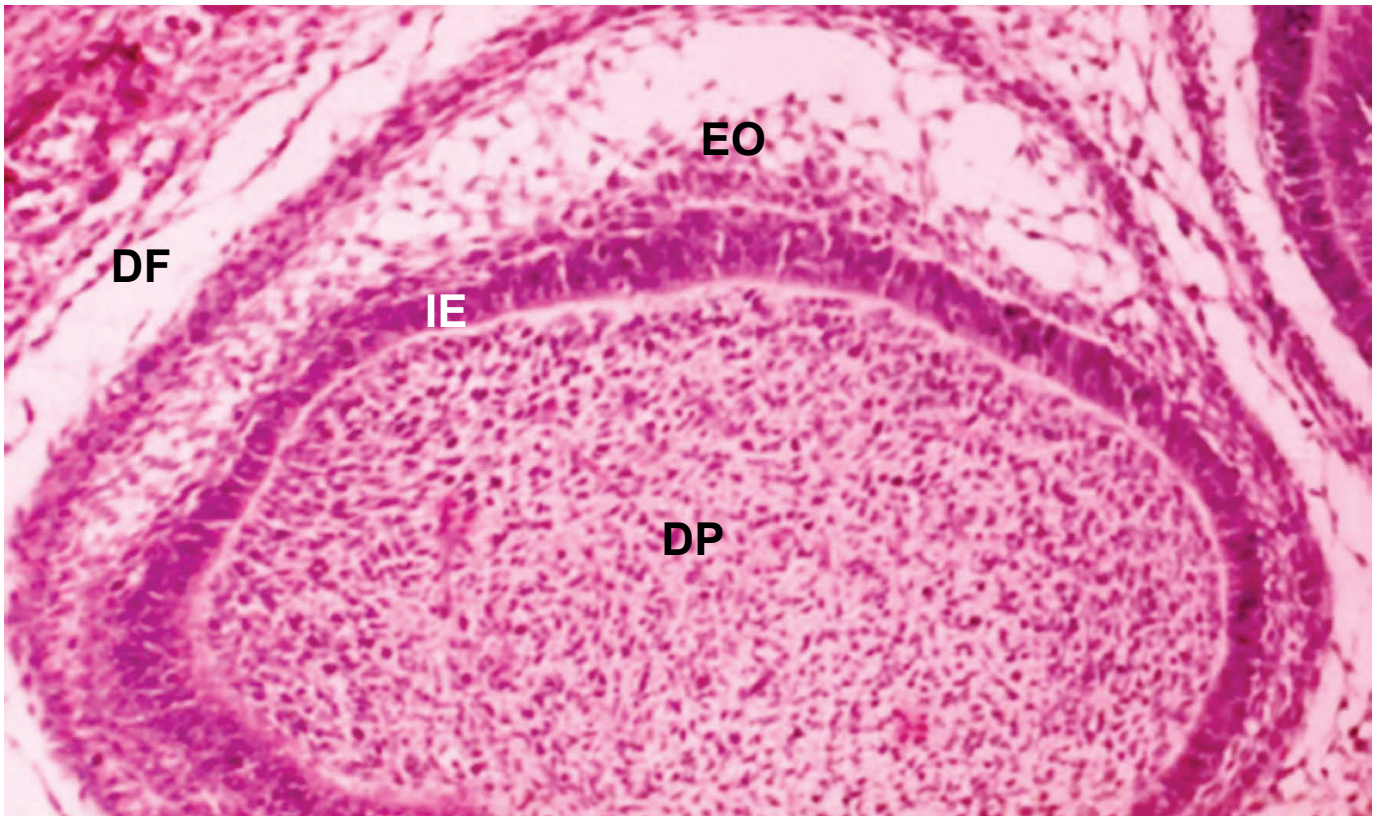

Figure 4. Dental germ at bell stage: EN = enamel organ; IE = Inner epithelium; DP = dental papilla; DF = dental follicle. (H.E., original mag. = 400X).

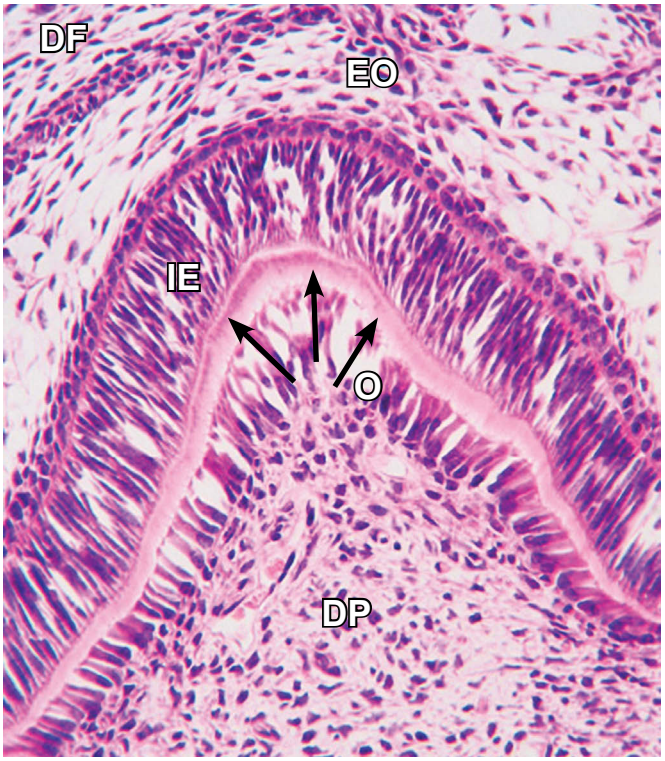

Figure 5. Dental germ with initial dentinogenesis (arrows). EO = enamel organ; IE = Inner epithelium; DP = dental papilla; DF = dental follicle; O = odontoblasts. (H.E., original mag. = 400X).

Dental pulp: a single structure in the human body

The dental pulp has ectomesenchymal origin and some peculiar aspects on the body. The use of the term "ectomesenchymal" implies saying that cells derived from the neural crest at an early stage of embryogenesis. At this stage, the neural crest rises from the ectoderm and "invades" the mesenchyme to go throughout the whole organism and originate many highly differentiated structures, such as melanocytes, peripheral nerves, neuroreceptors, dental papilla and dental follicle, among others.

The term "ectomesenchymal" means that the pulp and the dentin are formed by the mesenchyme with cells deriving from the neural crest, which invaded it in the primordial stages of life.

The dental papilla and its differentiation in pulp with dentin production requires for its formation a synchronized interrelation with the ectodermal tissues of the enamel organ and the enamel itself (Figs 4 to 7). In the root, the interrelation between papilla and dental pulp happens with the Hertwig's sheath, and with

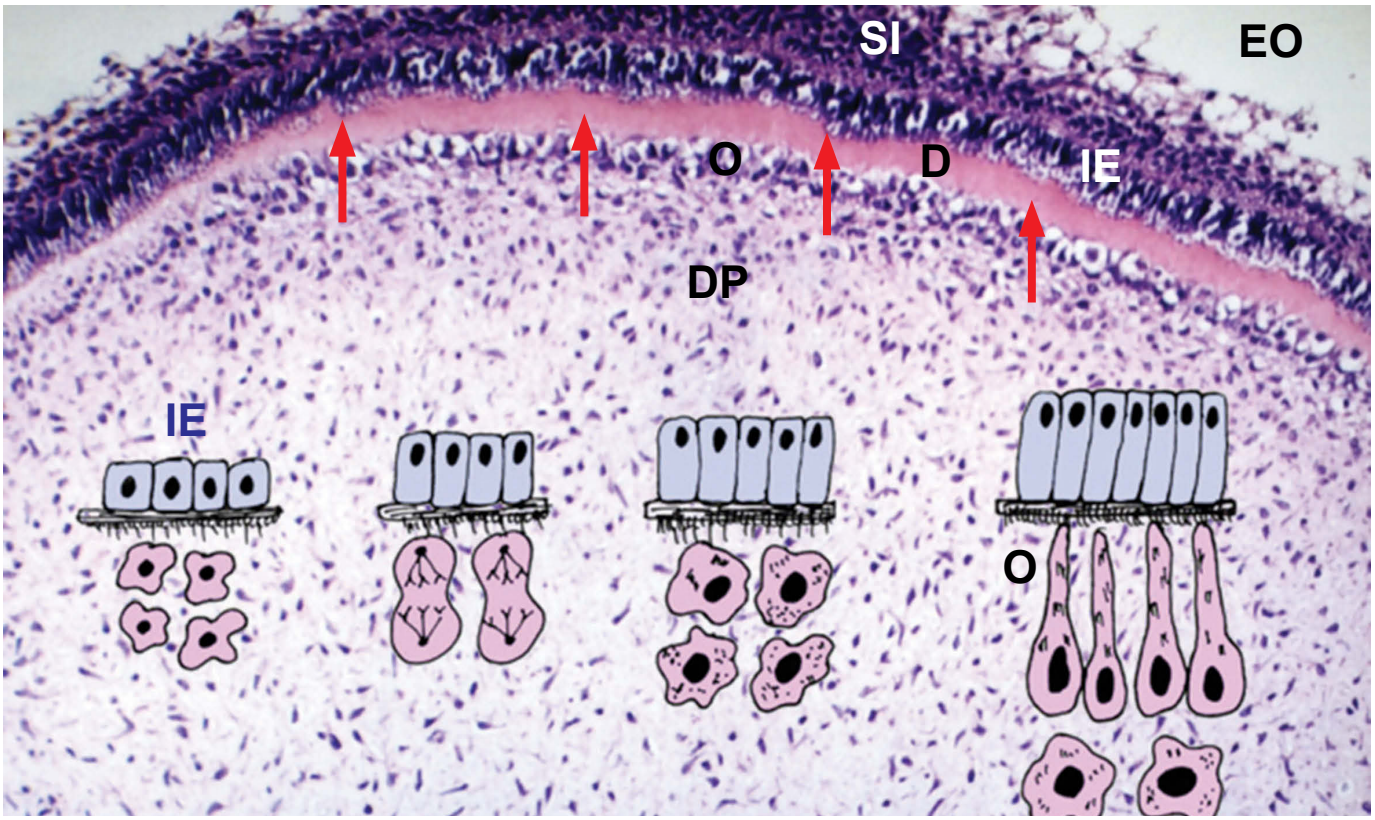

Figure 6. Dental germ with initial dentinogenesis (arrows). EO = enamel organ; IE = Inner epithelium; SI = stratum intermedium; DP = dental papilla; O = odontoblasts. In the scheme, the papillary cells get attached to the fibronectin layer next to the inner epithelium cells and assume the odontoblasts morphology and function (Adapted from D'Souza[8]). (H.E.; original mag. = 400X).

peripheral follicular tissues which will originate the cementum, periodontal ligament and bundle bone, also known as alveolar bone (Figs 23, 24 and 25).

During odontogenesis, dental tissues are interdependent with regard to their origin and function. This interrelation with several other cells for the formation of the pulp and coronal and radicular dentin makes it difficult for the stem cells to give rise to teeth which consist of several interdependent tissues in their formation and maturation.

The dental pulp has a formative function by dentin synthesis with cells located in the periphery. From the beginning, the odontoblasts keep themselves anatomically and physiologically connected to their secretion product, the dentin. Once the pulp is completely maturated, it slowly and steadily ages (Fig 2), because it is limited to a compartment outlined by hard dental tissue, with a minimum degree of elasticity, which is not enough to give rise to pulp expansion, if needed.

Besides being outlined with the dentin, the dentin-pulp complex is also externally protected by other mineralized tissues. In the coronal portion, it is isolated by enamel and, laterally, in the root, it is covered with cementum. Internally, the odontoblasts are so well arranged that the cell layer formed takes an epithelioid[35] aspect, effectively isolating the dentin (Fig 5), avoiding contact with other cells of the body, especially those immunologically competent.

The vascularity of the dental pulp, limited by a single access point — the apical foramen — weakens this tissue as for its defensive and repairing capacity. These characteristics may contribute to pulp aging a little faster than other connective tissues due to external factors. The exogenous aggressions promote

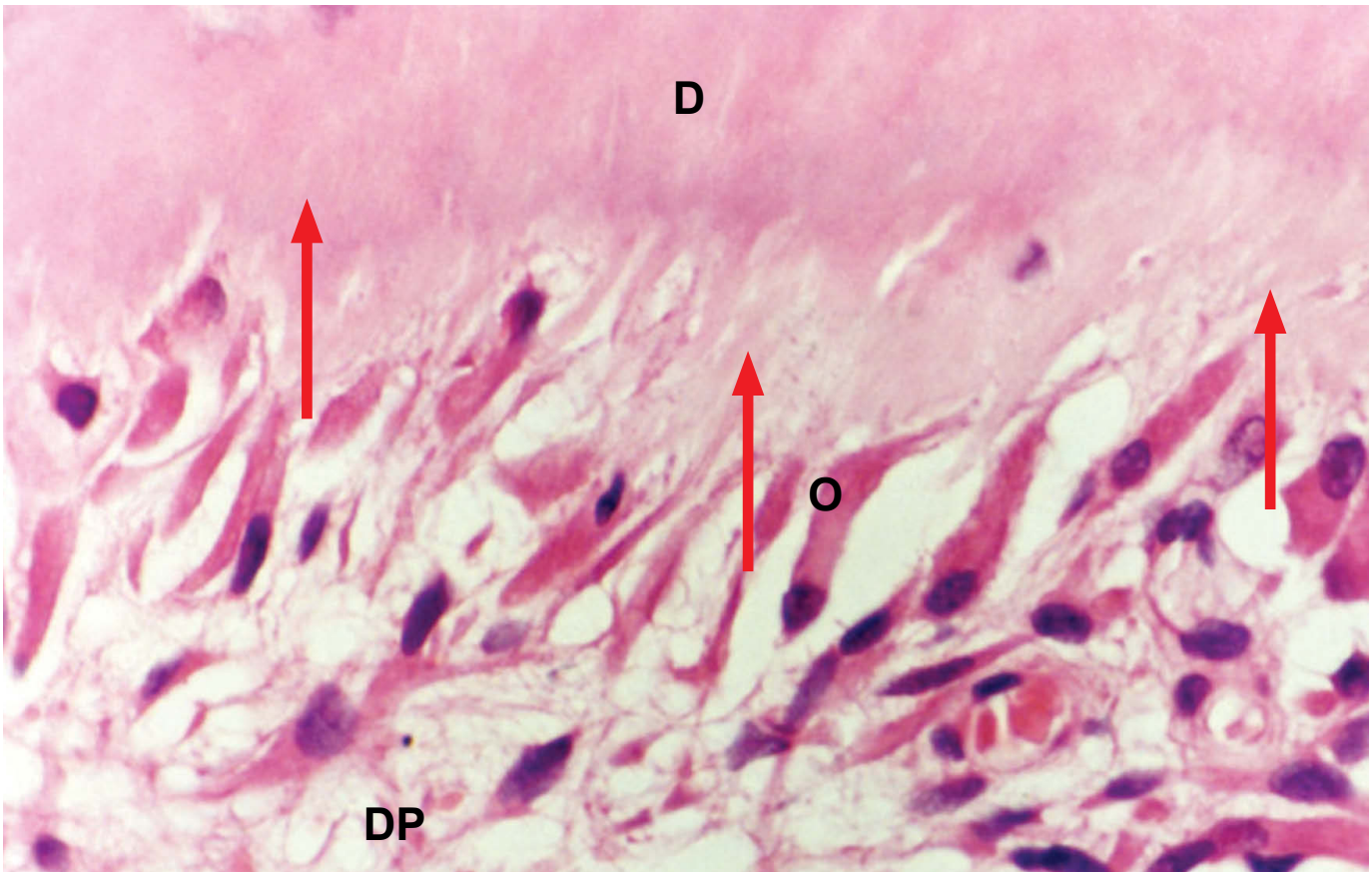

Figure 7. Odontoblasts (O) in dentinogenesis, forming a mineralized tissue barrier in pulpotomy with calcium hydroxide (arrows). D = dentin; DP = dental pulp. (H.E., original mag. = 400X).

a decrease in cellularity, vascularity and innervation; consequently, they diminish their reparative capacity and reduce their total volume.

Another peculiar aspect of the dental pulp, if compared to other tissues, is the fact that it is one of the rare tissues from which no type of neoplasia is originated, besides not allowing any infiltration of neoplastic cells from other tissues. Among the reasons that probably explain this peculiarity, is the blood supply coming from a single source, the low metabolism and the reduced blood volume per minute.

Where is the dental pulp originated?

The dental pulp comes from the dental papilla, a concentration of ectomesenchymal cells established under the enamel organ, since its initial stages (Figs 3 and 4).

In the bud of the enamel organ, derived from the dental lamina, the central and adjacent mesenchyme increases cell concentration, as if to fill the inner space of the ectoderm bell of the enamel organ. This cell concentration is already named dental papilla and will originate the dental pulp and its main product, the dentin (Figs 5 and 6).

The tissue environment of both the embryonic maxilla and mandible is isolated and externally covered with a covering tissue with two or three cell layers, representing the ectoderm. Internally, this maxillary and mandibular environment is comprised of a mesenchymal tissue, rich in cells and extracellular matrix; most of which have migrated from the neural crest of ectodermal origin. Thus, the mesenchyme that forms the tooth is also, very often, denominated as ectomesenchyme. The dental pulp is of ectomesenchymal origin and nature.

The newly formed dental papilla will receive influence from mediators, as well as from cells interaction, modifying itself and assuming new arrangements with the enamel organ. The interaction between cells and between a cell and the mediator in the dental papilla may happen among its cells and, especially among the cells and mediators from the enamel organ. The peripheral cells from the dental papilla in the enamel organ interface will reach organization and maturation, which means that the dental papilla is ready to begin dentin formation and, thus, originate the dental pulp (Figs 5 and 6).

The lesion to be repaired in the coronal and apical dental pulp

"Lesion" represents any physical change happening in a tissue or organ, which can be permanent or temporary, regardless of its nature, be it inflammatory, neoplastic or hyperplastic, for example.

In pulpotomy, the cut with sharped tools promotes a surface in the pulp tissue of the channels emergency where the connective tissue is exposed and gets irrigated, homeostasis is performed to minimize the clot and a material is applied to cooperate with, or even induce and improve pulp repair. In this sectioned

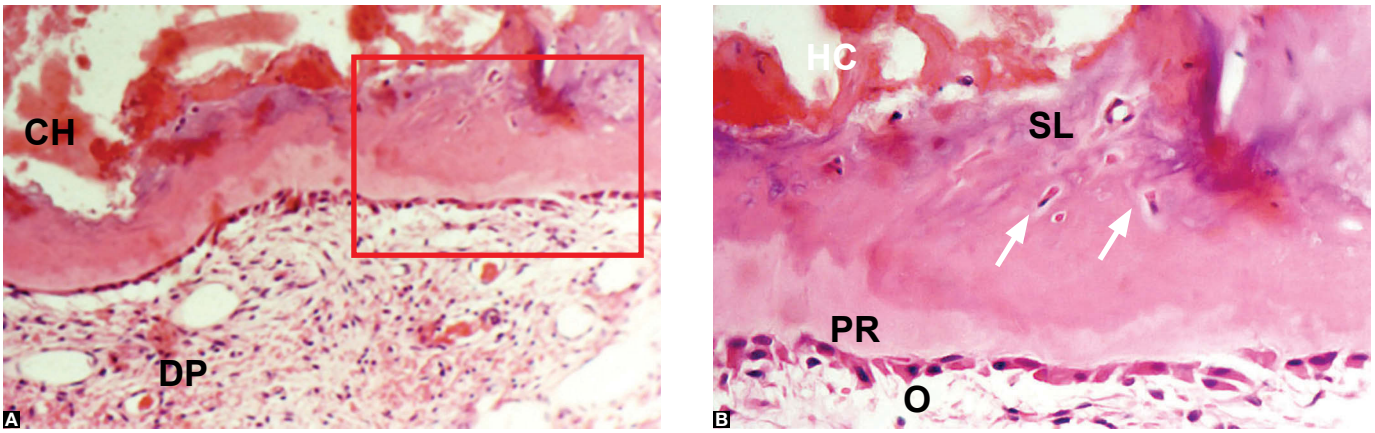

Figure 8. Mineralized tissue barrier formed after 45 days of direct application of calcium hydroxide to human dental pulp (DP). A disorganized superficial layer, basophilic (SL), is observed and reveals eventual cell inclusions (arrows). The deepest layer, facing the pulp, is eosinophilic . O = odontoblasts; PR = predentin; CH = calcium hydroxide. (Source: Lanza[17]). (H.E.; original mag.: in **A** = 160X and **B** = 400X).

tissue and material (normally calcium hydroxide) interface, reparatory phenomena occur.

In a few hours, subjacent to this cutting area, the pulp tissue will form a granulation tissue which is characterized by:

A) intense angiogenesis;

B) leukocyte infiltration; and

C) intense proliferation of undifferentiated young cells, young fibroblasts, preodontoblasts and pericytes.

After 24 hours, the great amount of newly-arrived neutrophils migrate or disappear by apoptosis, due to the fact that there is no staphylococci and streptococci bacteria to interact with and the macrophages predominate to clean the area and release mediators which, along with the platelet ones, will stimulate the proliferative and cell synthesis phenomena.

The same phenomena occur in accidental pulp exposures, in which direct capping is promoted, as well as in areas where curettage was purposely performed followed by this capping — from a therapeutic point of view, intentionally carried out. However, these same phenomena will be more discrete and faster, since the injured area is much smaller.

The quality of the material that will be placed in contact with the pulp is vital for repairing. If the material acts as a foreign body, at the interface with the material foreign body granulomas will be formed, where macrophages and derived multinucleated giant cells will indefinitely persist. With this type of organization, the inflammation does not develop into the reparative phase, despite the absence of signs and symptoms.

The material may be inert, i.e., cells do not recognize it as a foreign body, nor does it represent toxicity. The proliferation and cell synthesis happen normally in its surface, as if it did not exist, or as if it was part of the tissue where it is. On the other hand, the material may release substances and mediators that stimulate proliferation and cell synthesis, or even may induce cell and tissue reactions at their interface with the pulp, which stimulates repair in an organized and continuous way, until the tissue goes back to normal, also including a new odontoblastic layer and synthesis of new dentin.

In the apical region, when the pulp is cut off or necrosed, the periodontal remnant may remain vital or may be eliminated. In this situation, in the lesion or cutting plane of the periodontal tissues the same reparatory phenomena described for pulpotomy, for example, occur.

The difference in apical repair is in the cell type that migrates from the sectioned connective tissue to the interface surface with the filling material. Instead of pre and future odontoblasts, we will have pre and future cementoblasts. These cells disposed in palisade, side by side, may deposit a quite organized cemental matrix in the interface with the material, and this matrix will be gradually mineralized.

The connective tissue or periodontal ligament cells cannot change neither originate odontoblasts. When induced to proliferate and differentiate, they do as cementoblasts, fibroblasts and osteoblasts.

When empty spaces, free from infectious elements, remain — accidentally or intentionally — unfilled in the apical region after obturation, these spaces are filled with serous exudate with an exuberant fibrin net. Vessels and surrounding cells, i.e., those from the remnant or the periodontal ligament, use it as anchorage to migrate to these empty spaces and form the granulation tissue, occupying them and turning into fibrous connective tissue. On the walls of these empty spaces new cementum may be formed, but not dentin. Periodontal tissues only have precursors of cementoblasts.

Dental pulp as dentin producer: The beginning of the production and the fibronectin

The production of dentin by the organized dental papilla occurs before the induction of enamel production and it is determined by the action of biochemical mediators, through the enamel organ epithelium cell-to-cell contact, as well as through the interaction of pre-ameloblasts with dental papilla cells,[19] which may now be considered odontoblasts. The first dentin layer, also called mantle dentin, will represent one of the signs that allow the pre-ameloblasts to simultaneously begin the production of enamel (Figs 5 and 6).

In addition to the interactions mentioned, another phenomenon is necessary to begin dentin deposition: the formation of a biochemical, fibronectin-concentrated layer among the pre-ameloblasts and future odontoblasts of the dental papilla.[43] The contact of cells from the dental papilla with fibronectin is vital for the formation of dentin; without this glycoprotein, such phenomenon will be compromised (Figs 5 and 6).

Fibronectin is a normal integrating glycoprotein present in the extracellular matrix of the mesenchymal tissues and it is involved in several cell functions, including adhesion, migration and differentiation. It is usually found in the extracellular matrix of the connective tissues (the dental pulp, for example) and in constant and normal levels of blood plasma, being involved, among several phenomenon, in blood coagulation. The fibronectin tissue distribution is diffuse and not concentrated in interfaces, as occurring between the pre-ameloblasts and dental papilla layers. In this interface, its production and concentration are essential for the final differentiation of odontoblasts[8,43] (Figs 6, 7 and 8).

Even in adult pulp, dentin formation for repairing ulcerated areas requires the presence of fibronectin previously positioned in the place.[8,36-39] The differentiation and, especially, the arrangement of pulp cells in adult pulp depend on the previous presence of fibronectin.[37] For dentin deposition by odontoblasts, cell polarization is necessary. In this polarization (Figs 6, 7 and 8) of future odontoblasts, fibronectin and its interaction with the membrane receptors constitute one of the most important factors in dentinogenesis induction.[36,38,39,43]

When the fibronectin deposit is linear and regular, the overlap and polarization of future odontoblasts, or odontoblasts-like, will result in a cell layer in palisade and uniform[36-39,43] (Fig 9).

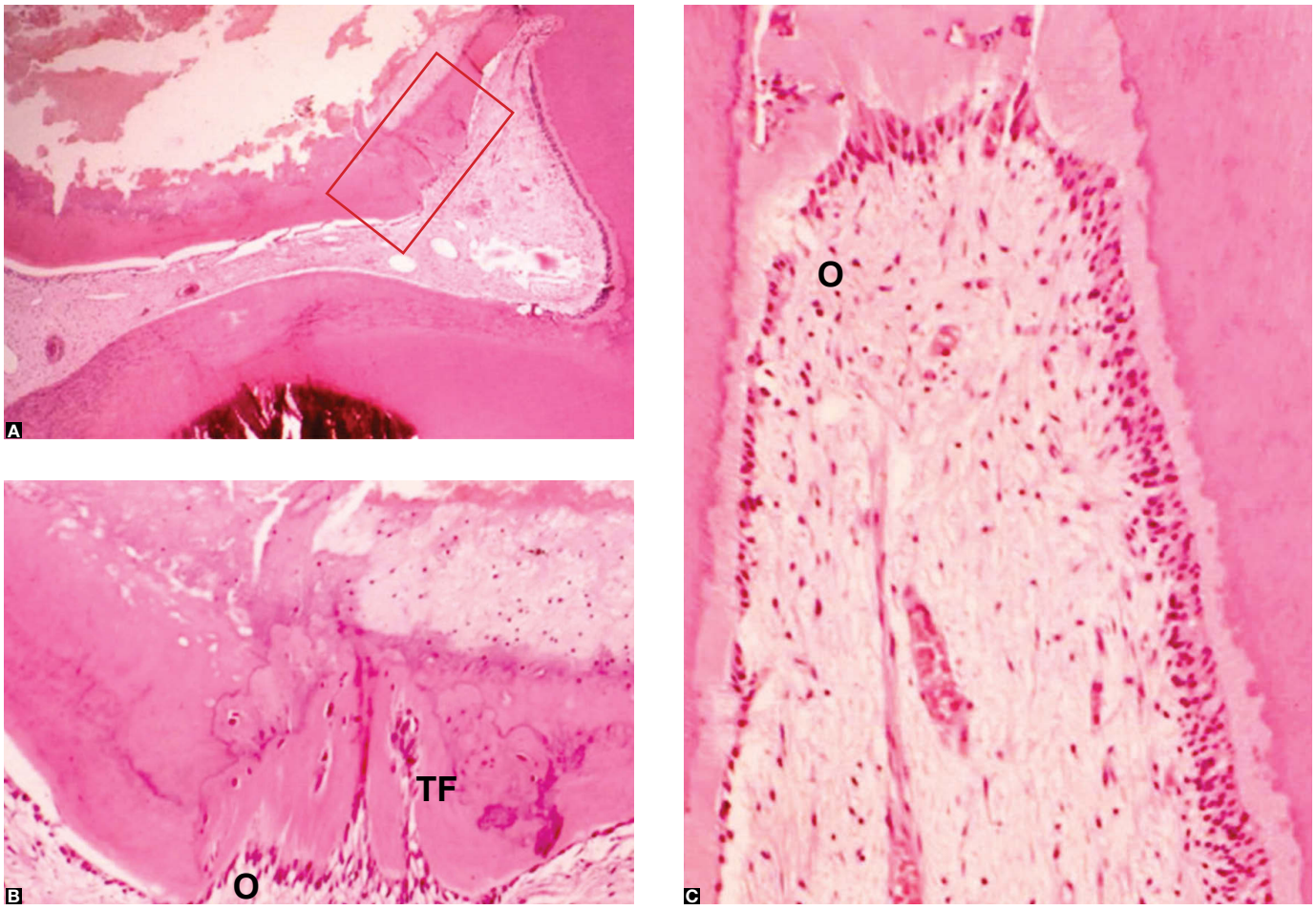

Figure 9. Organizational patterns of the odontoblastic layer in human dental pulps subjected to pulpotomies. The odontoblastic cells develop to a regular pattern in refined palisade, in **C**. O = odontoblasts; TF = tunnel formation (Source: Lanza[17]). (H.E. original mag. = 160X).

But, if we imagine a fibronectin deposition as if it were a line of irregular interface with recesses, or even, several interfaces around tiny areas determined by crystal structures, the overlap and polarization of future odontoblasts will occur in an irregular and random way (Figs 11 and 20), when analyzed in microscopic sections.

By analogy, we can compare a big parking lot, where the cars are oriented to stand in straight lines, to another big parking lot where the cars are oriented to stand in twisted lines or forming closed and irregular drawings, the lines would represent the fibronectin and the cars would be the odontoblasts.

When the odontoblasts begin their formation and deposition of dentinal matrix, they add to the large amount of collagen other non-collagen proteins and some peptides. Among these non-collagen elements are some cytokines and growing factors, such as TGF beta, a multifunctional cytokine.

The TGF beta is part of the BMPs, or bone morphogenetic proteins, inductors of osteogenesis, dentinogenesis and cementogenesis, when interacting with blast cells receptors. The release of this growing factor by the odontoblasts occurs during the intense phase of dentin production, aiming not only at cell activation, but also attraction, migration, proliferation and differentiation of new odontoblastic cells that will be incorporated during dentinogenesis. Part of the TGF beta remains incorporated in the dentin, being mineralized later.[40]

In dental caries, or any other procedure that result in clearing the dentin structure for cavity preparation when generating smear layer, TGF beta molecules will be released and, consequently, will interact with surface receptors of the odontoblastic extensions in its own body, or even in other pulp cells, activating them for odontogenesis or inducing them to migration, differentiation and posterior dentinogenesis, respectively. This mechanism leads to dentin sclerosis due to not only the acceleration of peritubular dentin synthesis, but also deposition of reactive dentin onto the pulp wall[5,19] (Figs 14-18 and 22).

Some researchers[12,13,23,26,27] directly apply particles of dentin or bone, demineralized matrix of dentin or bone and gel with TGF beta on the pulp, aiming at obtaining induction to dentinogenesis and, thus, getting a barrier of mineralized tissue in the operated pulp. The clinical feasibility of such experimental procedures has not been obtained yet, but applying the biological principle will result in improvements in pulp therapy.

Origin and formation of dentin as a mineralized tissue barrier after direct pulp therapy

OR

Formation of reparative dentin or tertiary dentin

The dentin that is formed in the pulp by new odontoblasts that are not from the original layer of primary and secondary dentin — called first and second generation odontoblasts — should be referred to as reparative or tertiary dentin. These new odontoblasts represent a third generation (Figs 7, 8, 11, 19 and 20)

The new odontoblasts depositing the tertiary or reparative dentin, of which new cell layer was reconstituted, originate from differentiation of cells from the center of the pulp as tissue stem cells, undifferentiated cells and/or from the dedifferentiation of fibroblasts and pericytes. Once the cells have been organized in the injured pulp surface, the odontoblastic cells, or odontoblasts-like, deposit a dentinal matrix with variable degrees of similarity with the original dentin. (Figs 7, 8, 11, 19 and 20).

In cases of pulpotomy and direct pulp capping, the material used directly on the pulp should not only facilitate the interaction between cells and mediators, but also, as far as possible, promote local environmental conditions favoring dentin production. One of these conditions may be the deposit of fibronectin (Fig 6) in the ulcerated pulp region.[36]

In pulp repair after pulpotomies with calcium hydroxide, several studies have demonstrated crystal structures formation, also known as calcites, right below the thin layer of necrosis caused by clotting induced by this material.[16] At first, its importance for pulp repair was known, but its contribution for the formation of a barrier with mineralized tissue was unknown.

Some experimental observations[31,36,39] revealed that this crystal structures, or calcites, formed in areas subjacent to calcium hydroxide application, favor the deposit of fibronectin around it. Overall, the numerous crystal structures determine the fibronectin concentration that will be deposited, creating an irregular interface with the underlying viable pulp tissue.

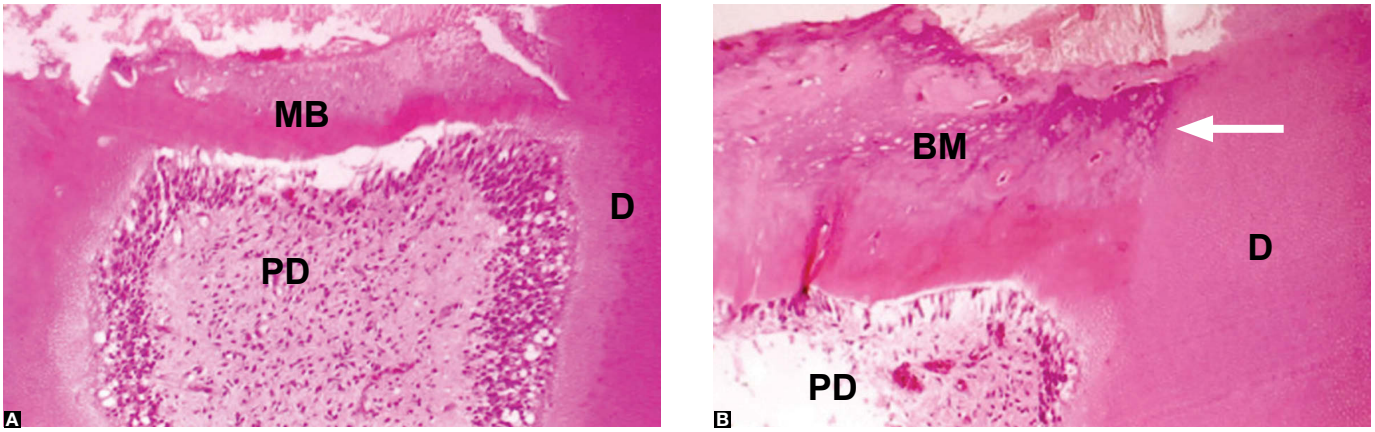

Figure 10. Relationship between the mineralized tissue barrier (MB) and the normal lateral dentinal walls in human dental pulps after direct application of calcium hydroxide, 90 and 120 days after pulpotomy. Juxtaposition is emphasized with linear or irregular interface on the superficial portion of the barrier (arrow). On the lower half of the barrier, it is emphasized the continuity of dentinogenesis of lateral walls as observed in the mineralized tissue barrier. (H.E., original mag. in **A** = 160X and **B** = 400X).

This explains why the odontoblastic cells layer juxtaposed in the region of pulp repair is quite irregular and, sometimes, seems to be randomly distributed. Cell inclusion and organizational irregularity of the superficial portion of mineralized tissue barriers can be now understood.

In the dental papilla, the fibronectin apposition is regular, i.e., the cells are in palisade and the received stimulus will promote a regular dentin layer. Around the calcites, the cell juxtaposition will happen in irregular and multiple plans, and many of these cells may remain in the dentinal matrix, deposited as follows: in this case, this reparative or tertiary dentin may be morphologically classified as "osteodentin" for having cell inclusions similar to what happens with bone and osteocytes.

The aforementioned explanations reinforce the need of care with regard to the regularity of the tissue cutting plans during pulp curettage, as well as the need of care when applying the material onto the ulcerated pulp. The more regular is the material-dentinal pulp interface, the better will be the structural and organizational quality of the mineralized tissue induced barriers.

In the mineralized tissue barrier formation, after the first dentin layers, dentinoid or osteodentin have been produced, , the subsequent layers tend to be regular, including being able to establish the formation of a tubular regular pattern, which cannot be compared with normal dentin, from a morphological point of view (Fig 9).

From a physiological point of view, the mineralized tissue barrier meets its functions similarly to those of the primary and secondary dentin, physiologically deposited. These functions include:

1. Isolation of the external environment: in both dentin structures, there will be a degree of permeability due to the great number of tubular structures and/or cell inclusion.

2. Dental pulp protection: preserving its sensory vitality and sensory normality as well as the secondary dentin forming function.

3. Maintenance of pulp reactive capacity against external agents

It cannot be expected that a mineralized tissue barrier of tertiary dentin would perform functions which not even the primary and secondary dentin have, including the impermeable isolation of the pulp against chemical and microbial agents. Chemical agents as well as bacteria also penetrate through the normal dentin, as it can be observed in the dental caries.

As for the primary and secondary dentin, it is expected that the enamel meet its protecting function, isolating the dentin from the aggressive external agents of the pulp. As for the mineralized tissue barrier or tertiary

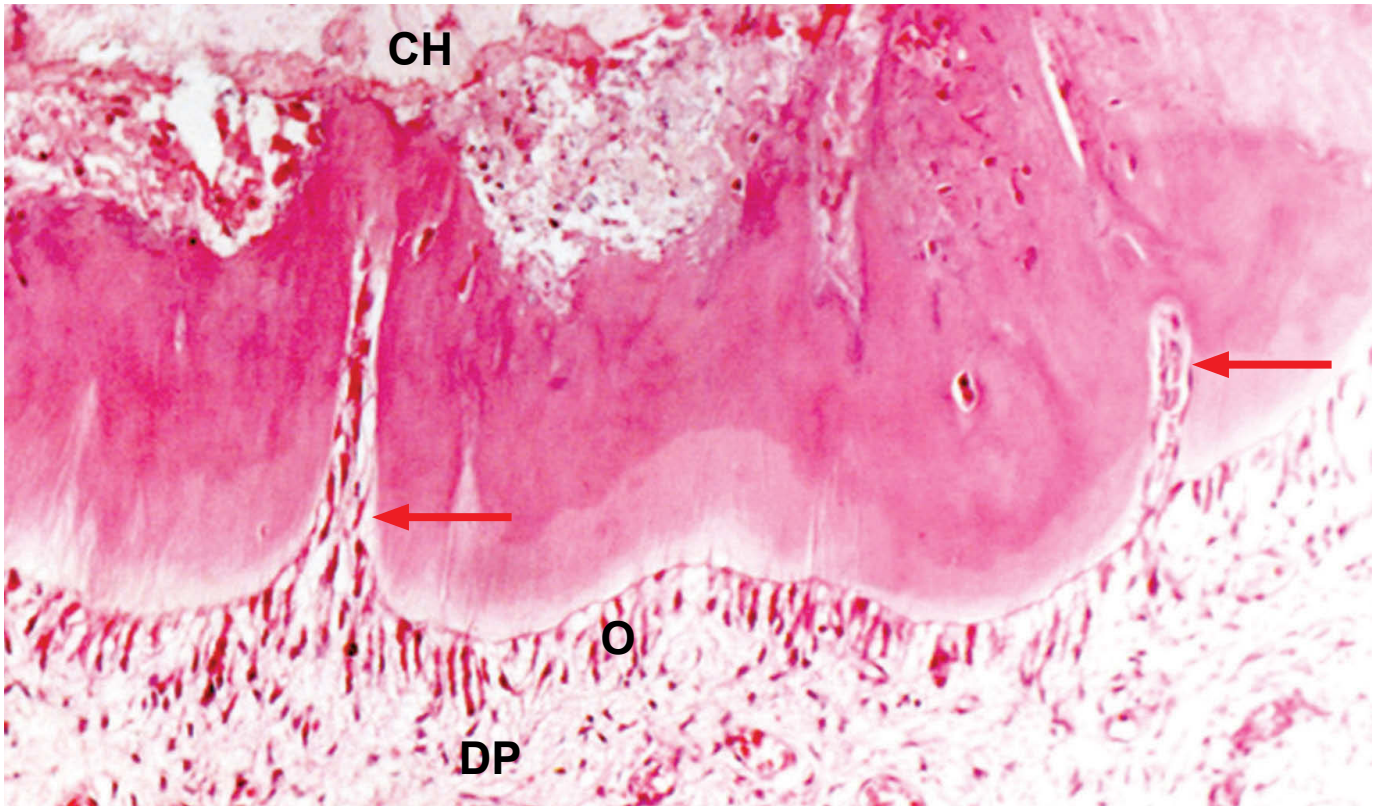

Figure 11. Mineralized tissue barrier showing canal or "tunnel" formations (arrows) filled with living pulp tissue, well cellularized and vascularized in human teeth, 90 and 120 days after pulpotomy with direct application of calcium hydroxide. DP = Dental pulp; O = odontoblasts; CH = calcium hydroxide. (Source: Lanza[17]). (H.E.; original mag.: 160X).

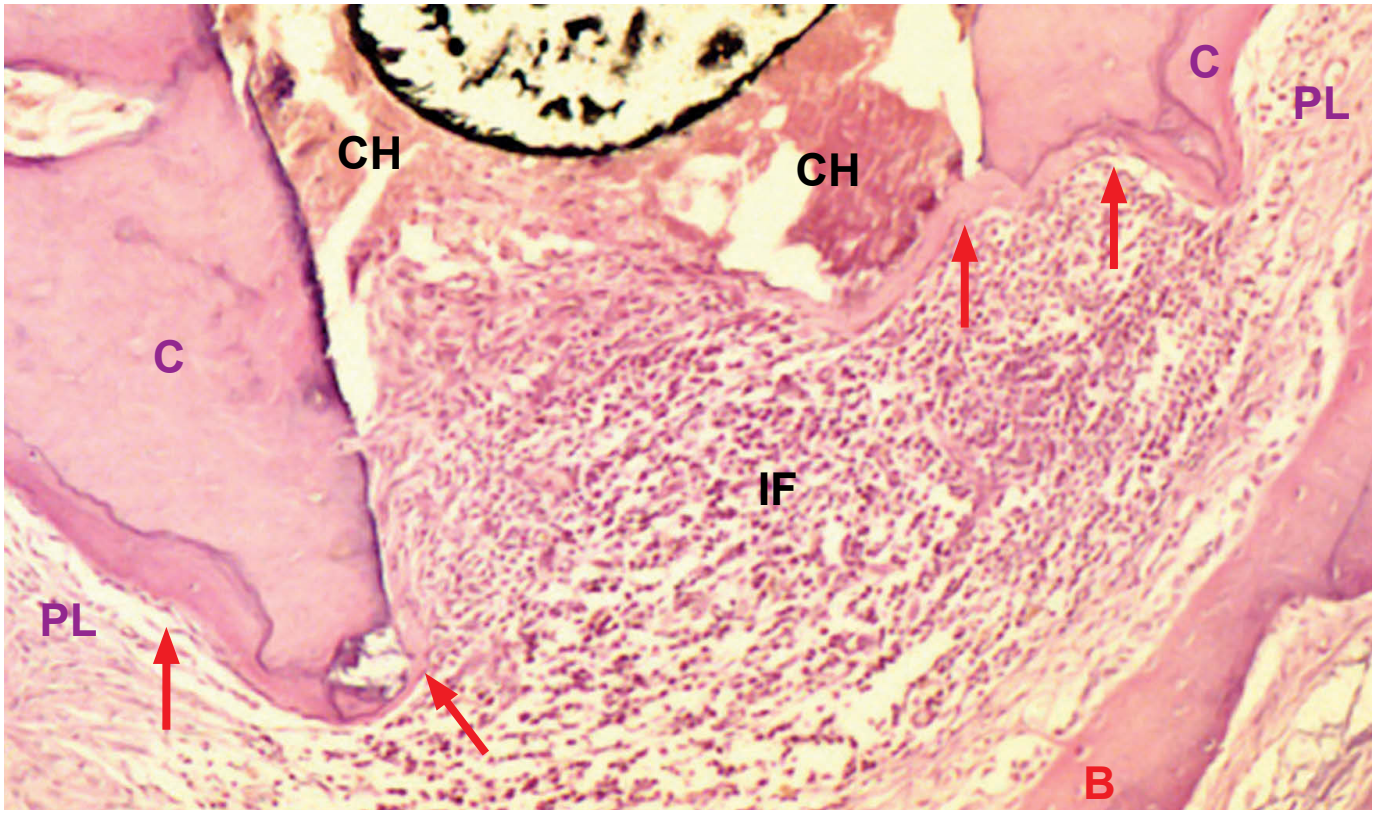

Figure 12. Mineralized tissue barrier (arrows) being formed over calcium hydroxide-based (CH) filling material. C = cementum; IF = inflammation; B = Bone; PL= periodontal ligament. (H.E., original mag.: 160X).

dentin, it is expected that the restorative material meet its function of isolating the dentin and the pulp from the external environment, since one of its basic functions is to "restore" the enamel functions lost due to cavity.

If bacterial penetration occurs in the pulp via the mineralized tissue, the failure was, first, of the restorative material that did not isolate the dentinal microorganisms. If this failure occurs in a restoration based on primary dentin, the bacterial or chemical penetration will also occur, though in smaller proportion and for a longer period of time, because it is thicker and more organized.

Two aspects regarding the mineralized tissue barrier and its functions are yet to be addressed: the integration of the newly formed tissue with the normal dentin walls (Fig 10) and the presence of pulp tissue papillary formations generating tunnel-shaped structures (Figs 11 and 20) when the barrier is analyzed in dried specimens and the living tissue is removed during the preparations.

Integration of newly formed tissue with normal dentin walls

On the formation of the first layers of the mineralized tissue barrier, the fibronectin deposit in the peripheries of the crystal structures, abundantly formed after the direct application of calcium hydroxide on the pulp, has been described. Additionally, the importance of regularity in fibronectin deposit, which on the dental germen is uniformly done on the pre-ameloblasts interface with dental papilla or future odontoblasts, precisely, has been highlighted.

In adult pulp, polarization and overlap of cells, which will perform the functions of odontoblasts in the production of mineralized tissue barrier, will happen around the crystal structures. Due to the irregularity of deposited fibronectin, the tissue similar to the dentin will be unorganized and full of cell inclusions; morphologically, there will be osteodentin.

In this new formation of odontoblastic cells there will not be reconstruction of an odontoblastic layer

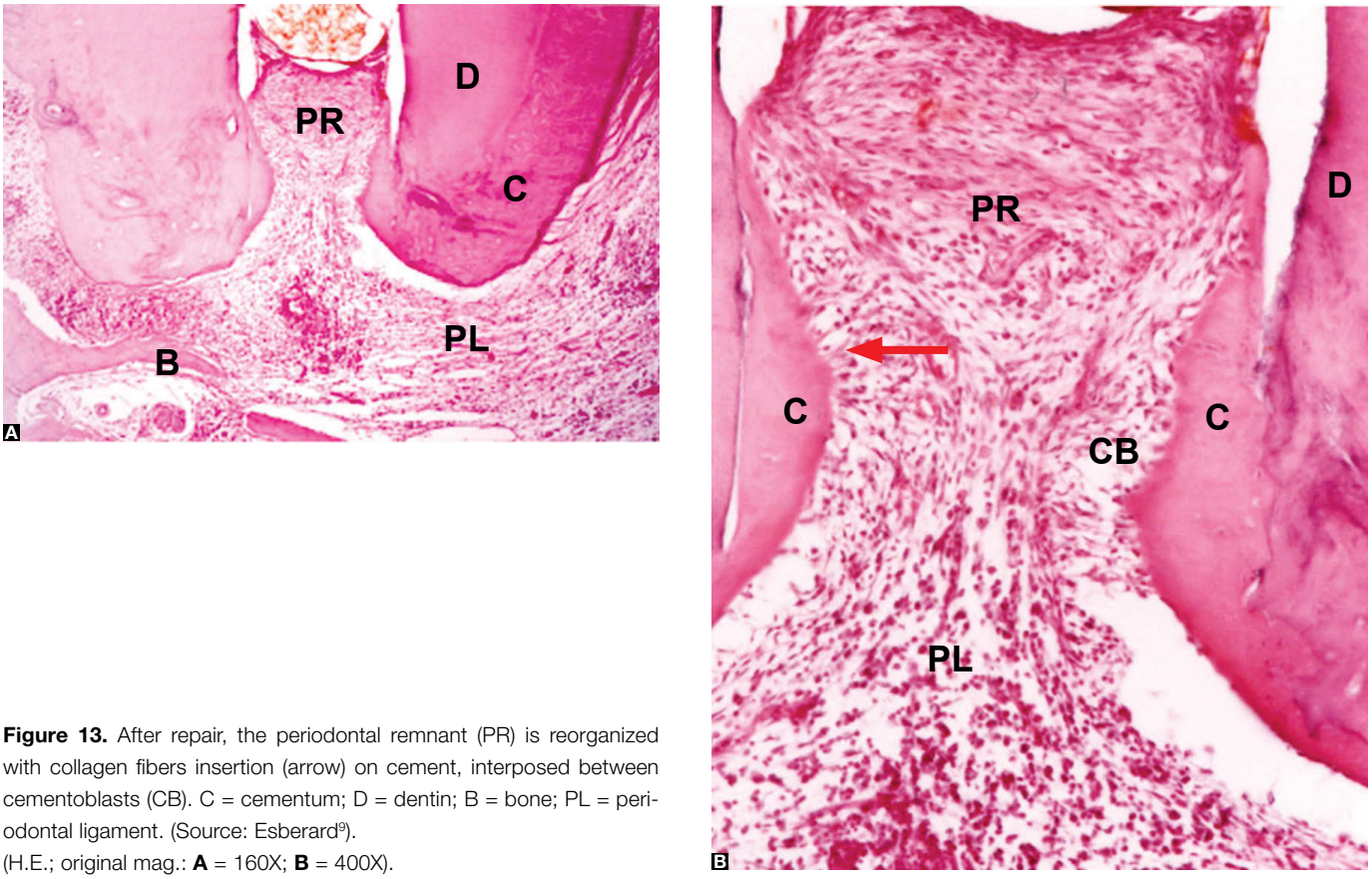

Figure 13. After repair, the periodontal remnant (PR) is reorganized with collagen fibers insertion (arrow) on cement, interposed between cementoblasts (CB). C = cementum; D = dentin; B = bone; PL = periodontal ligament. (Source: Esberard[9]).
(H.E.; original mag.: **A** = 160X; **B** = 400X).

in palisade, organized and linear as the one found in the lateral walls of the remaining dentin.[25] The new odontoblastic cells may originate from the pre-odontoblasts, from tissue stem cells, fibroblasts and perivascular pericytes.[11,36] The mature cells will be induced to a process of dedifferentiation before assuming a process of odontoblastic transformation. Thus, the first superficial layers of the barrier will not have structural integration with the preexisting lateral dentin.

As the production of mineralized tissue barrier and the elimination of the subjacent inflammatory process progress, this new layer of odontoblast-like cells becomes more organized, and the newly formed dentin begins to assume a tubular pattern[14] (Figs 9 and 11). At this stage, the new layer of odontoblast-like cells seems to be continuous and integrated in the odontoblastic layer of the lateral wall, while dentin production is happening integrally in continuity (Fig 10).

In previously analyzed hard tissue barriers, a lamina of hard tissue is newly formed by means of removing the pulp tissue; on the lamina, it can be observed that there is a juxtaposition interface on its most superficial portions, with no structural integration (Fig 10). However, in most of the thickness of the barrier formed, this interface is no longer observed, causing a structural and functional integration to occur with the preexisting normal lateral dentin.

Tunnel-shaped structures in the dentinal barrier

The application of material on the pulp does not necessarily generate a regular interface. The cutting plane and the material adaptation generate irregularities. In this context, pulp repair, as previously described, happens in the area determined by the healthy pulp tissue, right below it. In this process, small projections of living pulp tissue (papillary and fingerlike projections) (Fig 11, 20) may remain,[17,29] resulting in the formation of a tertiary or repairing dentin layer, similar to a osteodentin in the superior and lateral portion of these pulp projections. When completely formed and analyzed in a dried environment, tunnel-shaped formations are present in the structure of the newly formed hard tissue barrier.

The in vivo tunnel formations have in their interior viable, cellularized and vascularized pulp tissue (Figs 11 and 20) which will have its diameter and length decreased with time. These formations are not empty and passive spaces, as the analysis in dried specimens may suggest;[29,30] they react to external aggressors.

By analogy, the bone tissue also has cell inclusion and tunnel-shaped canals, and, even so, this tissue fully performs its biological and defensive functions. The tunnel formations would be better denominated as canal- or canaliculi-shaped formations, since “tunnel”

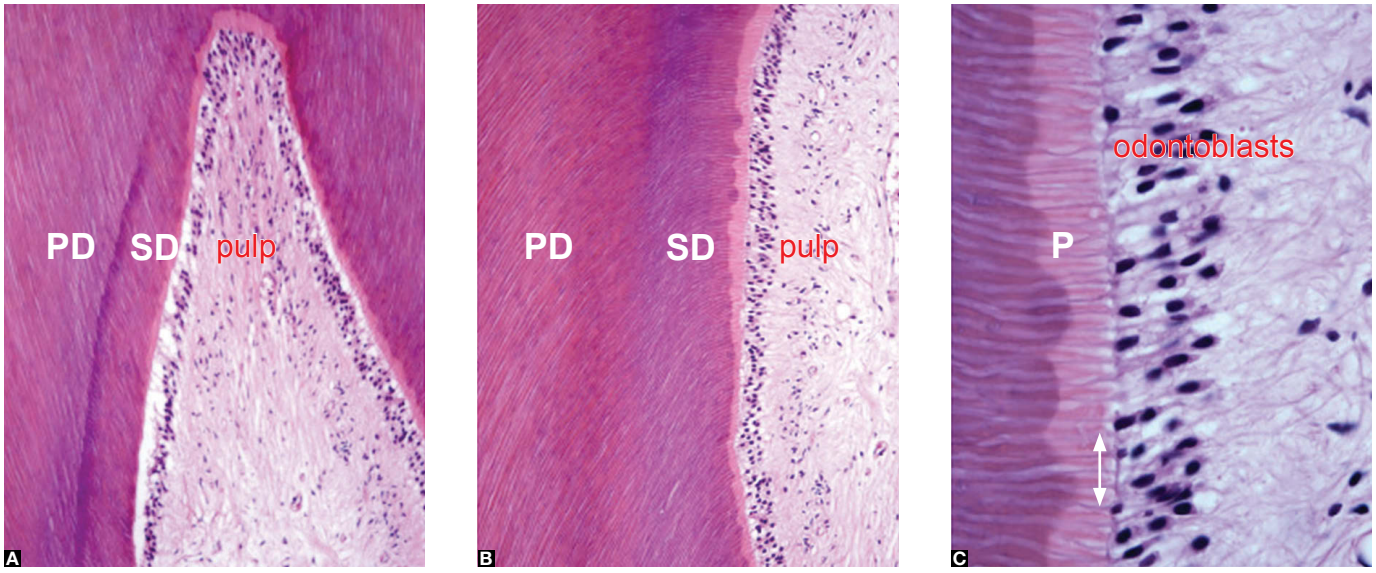

Figure 14. Dentin-pulp complex with primary dentin (PD) deposited before eruption and secondary dentin (SD), after eruption. The limits are only marked by a line with color alteration, but they are structural and functionally continuous. In **C** are shown the predentin (P) and the extensions in continuity with odontoblastics cells (arrow) (H.E.; original mag.: **A** = 40X; **B** = 160X and **C** = 400X).

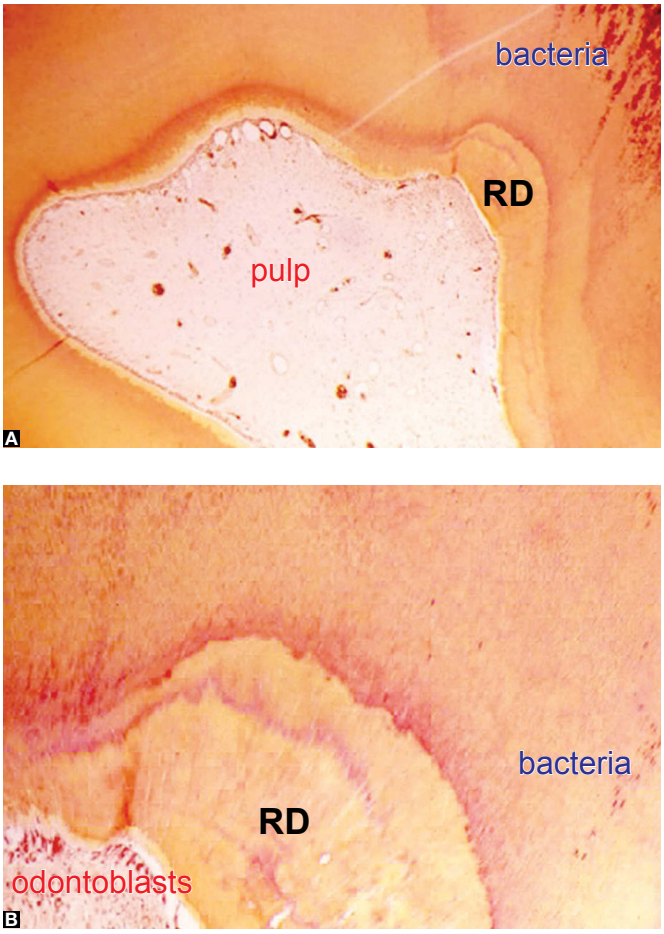

Figure 15. Dentin-pulp complex with reactive dentin (RD) subjacent to the cavity and its bacteria. Reactive dentin has lower number of tubules and less organized and mineralized structure, being originated by second generation odontoblasts (BB; original mag.: **A** = 40X; **B** = 160X).

can imply emptiness, while "canal" suggests that there is something occupying that space, in which case is the living pulp tissue.

Still by analogy, the canal-shaped formations of the mineralized tissue barriers may have the same behavior and progression as canal and pulp formations commonly found in the dental apexes, isolated or in an apical delta. In endodontic treatment, especially in biopulpotomies, these structures do not raise any concerns as for the case prognosis, since they have their diameter gradually reduced (Figs 12 and 13) and sometimes completely vanished.[9,15]

In short, the mineralized tissue barriers have superficial layers formed at the expense of dystrophic calcification of the necrosis thin area, by clotting induced by the high calcium hydroxide pH.[14] In this superficial layer, the initial irregular production of dentin, rich in cell inclusions, can also occur. Subjacently, the mid and deeper portions of the barriers are formed with dentin and/or dentinoid tissue, well organized and variable tubular pattern.

Dentin names: criteria and coherence in their use in reactions and pulp repair

From a functional point of view, the dentin deposited by the pulp represents a product of this special fibrous connective tissue located and protected in the central portion of the teeth. Dentin, in the dentin-pulp complex context, may be presented in different forms with regard to organization and structure.

In the intense scientific literature communication, during clinical practice and in didactic and pedagogical activities, some terms promote confusion instead of comprehension. Good examples are those meant to qualify the dentin.

Taxonomy is the science that studies and establishes guidelines for classifying and naming all things. Taxonomy comes from the Greek "*tassein*", which means "classify", along with "*nomos*", meaning "law, science, administration".

At first, taxonomy was a science of which aim was to classify the living beings. Later on, its use was extended to a broader sense, being applied to things, bringing the principles underlying any classification. Any living beings, inanimate objects, places and events can be classified according to some taxonomic scheme. Some philosophers believe that the human mind naturally organizes knowledge in such systems and criteria.

The taxonomic classification has gained support from computational biology (bioinformatics), applying the phylogenetic tree method, which facilitates the terminological normalization in a completely intercommunicating world. In a literature review, the more uniform is the name of a structure or process, the more accurate and fast the sources will be identified on papers websites and databases.

Even more specifically, nosology is the medical area that deals with the general aspects of diseases and classify them from an explanatory point of view according to their etiopathogenesis. The word "nosology" comes from the Greek, by the conjunction of "nosos" (disease) with "logos" (treaty or reason).

The names are selected based on criteria used to differentiate patterns, models, structures and situations. In dentin, it is not different,[32,33] being possible to classify it according to some criteria.

1st Criterion: Chronology of dentin formation

1A - *Primary dentin* corresponds to the one deposited before dental eruption. The first moment justifying the name "primary" corresponds to odontogenesis: its deposition is part of tooth formation performed by first generation odontoblasts (Fig 14).

1B - *Secondary dentin* presents a well-organized tubular structure which is morphologically different from the primary dentin with regard to a thin hyperchromatic delimiting line or zone (Figs 14 and 15). The secondary dentin is continuously and passively deposited throughout life after tooth eruption, when there is a slow and constant change of odontoblasts in the dentinal wall, a second generation or generation after odontogenesis of odontoblasts. The second moment justifying its name also naturally explains the reason for pulp volume decrease which happens slowly and gradually with age. For us, this secondary dentin, naturally deposited over life, is also referred to as *physiological secondary dentin*.

The physiological secondary dentin, from a morphological and functional point of view, is almost indistinguishable from the primary dentin, including the tubules continuity until the predentin (Fig 14). This information, although subtle, must be emphasized because it shows that the damage caused by bacteria and its products on cavity, as well as the damage caused by rotating tools and dressings, may reflect or have pulp consequences, regardless of the dentin being primary or secondary.

1C - *The tertiary dentin* does not present a uniform tubular pattern and its deposition results from aggressions and external stimuli that destroy the extensions and lead to the odontoblasts death (Figs 19 and 20). The third chronological moment would be determined by the severe injury of the odontoblastic layer, which requires differentiation of undifferentiated pulp cells, i.e., a new and third generation of odontoblasts would be necessary.

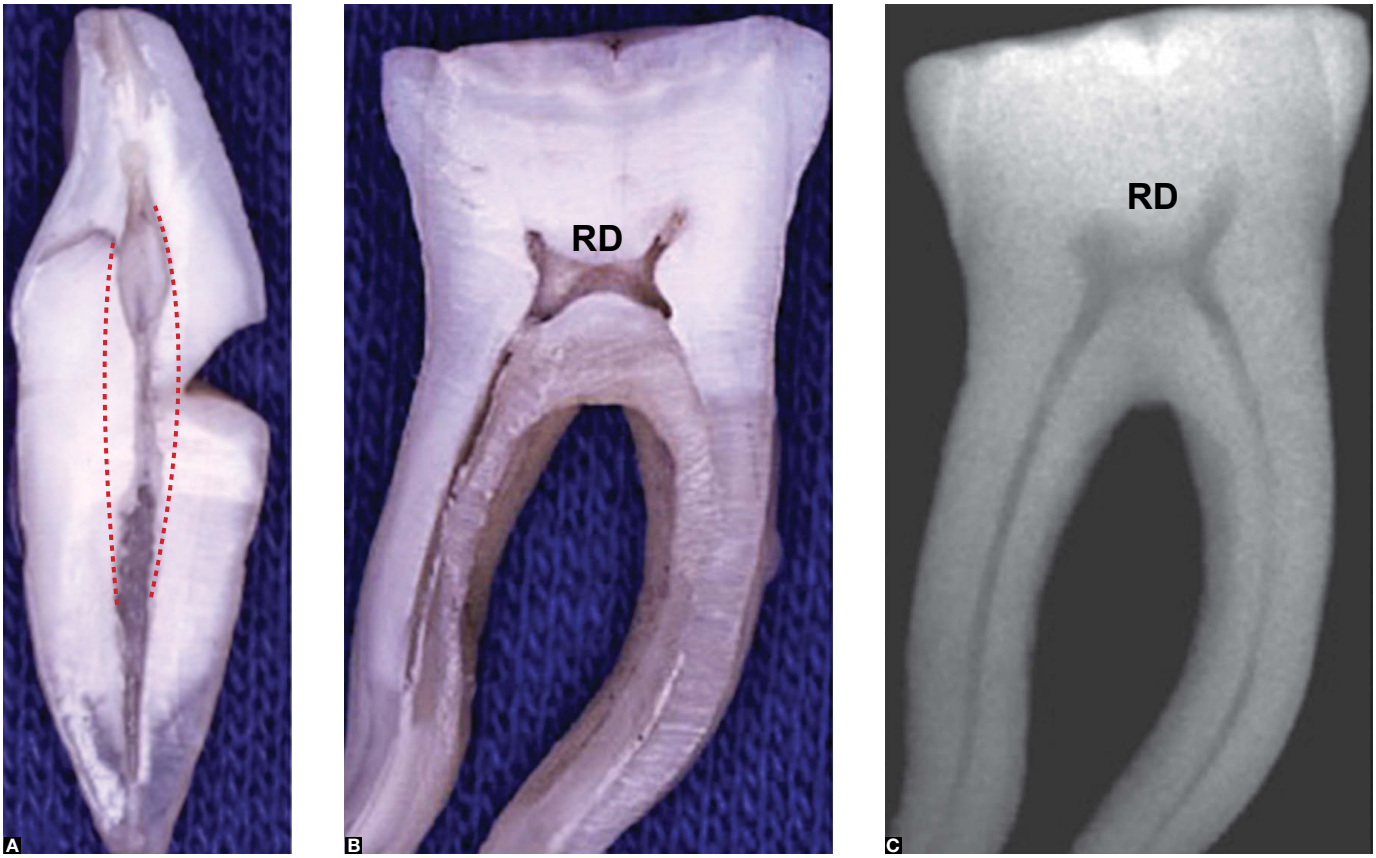

Figure 16. Pulp space reduction by excessive deposition of reactive dentin (RD) induced by abrasion in **A**, and by attrition in **B** and **C**.

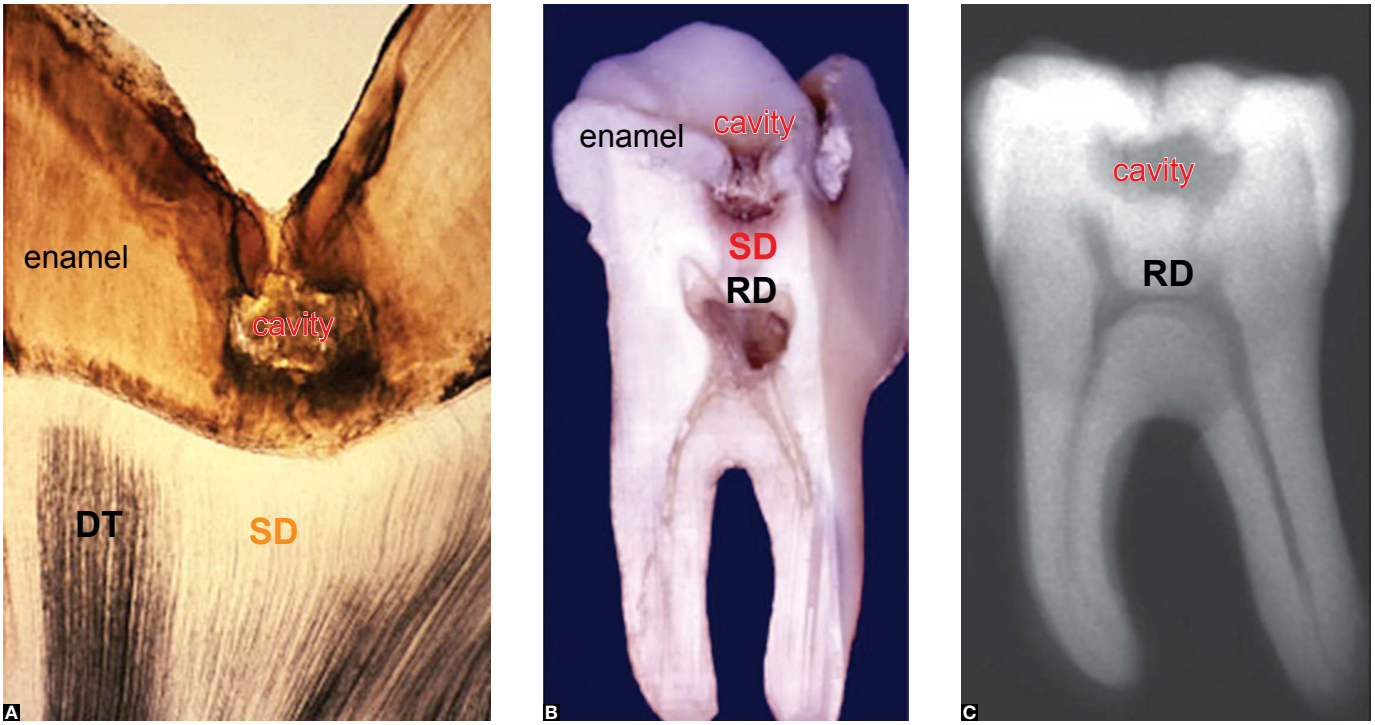

Figure 17. Subjacent to the cavity, sclerotic dentin (SD) is established with the closure of its tubules by peritubular dentin, and in the pulp walls reactive dentin (RD) is deposited, reducing the pulp space. The empty tubules are dark and named dead tracts of dentin (DT) (wearing, original mag.: **A** = 160X).

With no extensions being initially established, the dentin forming cells originated in the undifferentiated pulp cells cannot deposit matrix around an extension to form a tubular structure (Figs 9 and 11). These undifferentiated cells could be named as pulp tissue stem cells, although it is known that the pericytes may also originate odontoblasts in the injured pulp.[11]

The tertiary dentin structure irregularly deposited is amorphous and non-tubular, although it can be noticed one or two extensions and/or some islet-like pulp tissue cells trapped in between. After some time, uneven distributed tubules can be noticed in the amorphous dentin initially deposited. Should the aggressor disappear and the pulp be free from inflammation in this area, a better organization of the new odontoblastic layer and the formation of a well-organized tubular dentin can be noticed.

A conceptual synthesis of tertiary dentin may be: *the dentin deposited in areas where the original population of primary/secondary odontoblasts was lost, as a consequence, a third odontoblastic generation gets organized to deposit it, even if it is done irregularly.*

2nd Criterion: The nature of the stimulus or aggression, structure and organization

In tissue biology, the terms "stimulus" and "aggression" can be considered synonyms in several situations. The stimuli for dentinogenesis may be physiological, as in odontogenesis and when maintaining the normality of the tissues, but some pathological stimuli may occur as a result of external agents aggressions.

2A - *Physiological dentin:* It corresponds to the primary dentin deposited during odontogenesis and before dental eruption, and to the secondary dentin deposited after dental eruption, throughout life (Fig 14).

2B - *Reactive, irritation or pathological secondary dentin:* When phenomenas such as chronic cavity with its bacteria and products, abrasion, rotating tools with cutting action and heating release, as well as protective and restorative material occur in the dentin-pulp complex, the underlying odontoblastic layer has its extensions cut or retracted in the tubules. This kind of aggression or stimulus leads the odontoblasts

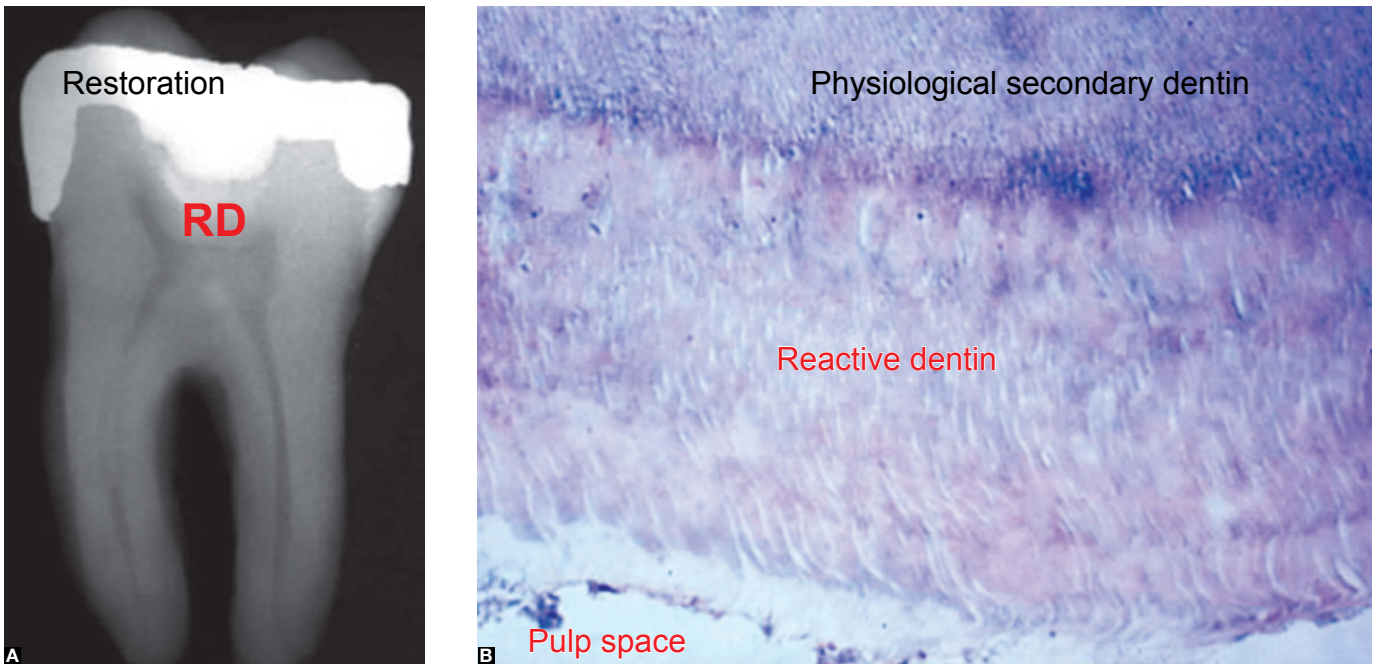

Figure 18. Subjacent to the amalgam restoration, pulp space reduction by reactive dentin (RD) can be observed. In **B**, the reactive dentin presents disorganized lower number of dentinal tubules, thus, a lower mineralized degree (H.E.: original mag.: **B** = 400X).

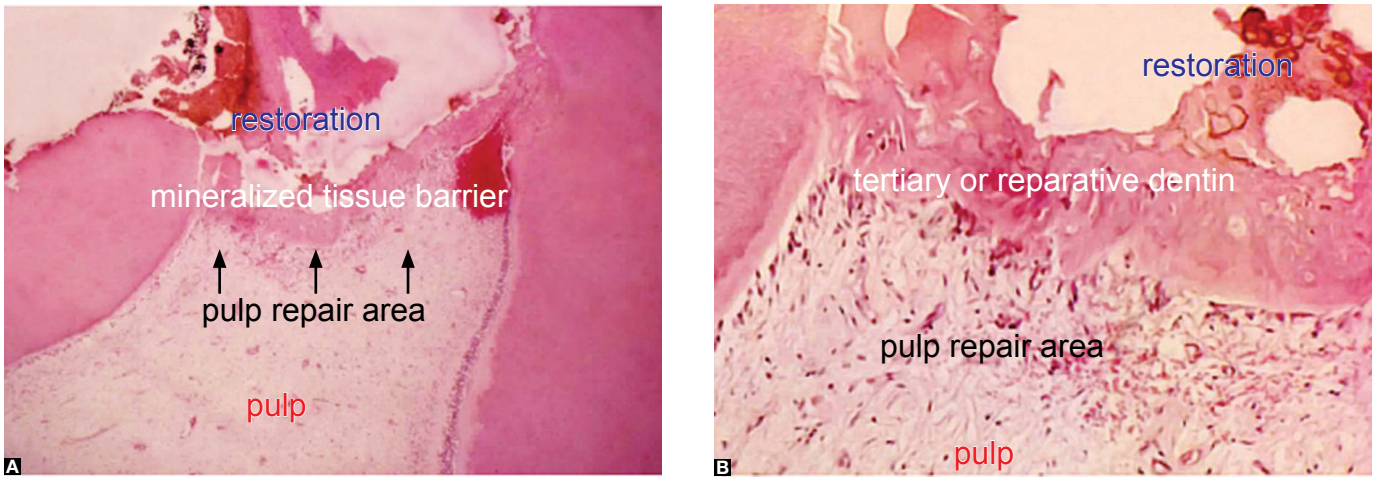

Figure 19. In the exposed human pulp area, at the interface with the calcium hydroxide-based material, pulp repair organized a new odontoblastic layer of third generation. A tertiary dentin was deposited, constituting a mineralized tissue barrier with cell inclusions and continuity with lateral walls (Source: Lanza[17]). (H.E.: original mag.: **A** = 40X and **B** = 160X).

to deposit faster, and in adverse conditions, deposit new dentin layers in the corresponding area of the pulp wall (Figs 15 to 18).

In such conditions, primary and/or secondary physiological dentins may have their tubules abruptly interrupted, and new tubules of reactive dentin create new parallel ways from this new layer in order to hinder the aggressors to reach the pulp.

The dentin deposited in the pulp wall when facing stimulus or external aggression to the dentin-pulp complex is named **reactive** or **irritation dentin**, which can also be identified as **pathological secondary dentin** (Figs 15 to 18).

The terms are exchangeable because the reactive dentin is deposited by the second odontoblastic generation which is characterized as the odontoblastic

population, formative of dentin after tooth eruption. The term "pathological secondary" would be used to explain that its acceleration and considerable lack of organization are due to harmful external stimulus or aggression to the dentin-pulp complex. In other words, it can be said that it was a reaction to an aggression — the reason for the name reactive dentin.

In turn, the term "irritation dentin", of German origin,[22] indicates the harmfulness of the external stimulus, making clear that this dentin, with changed tubular ways, lower mineralization degree and some lack of organization, does not reveal a physiological process, but a pathological, reactive process, caused by irritation or aggression to the dentin-pulp complex.

In short: reactive dentin, irritation dentin and also pathological secondary dentin can be considered synonyms of the same process.

2C - *Repairing, reparative or reparatory dentin:* these terms are used to identify the dentin, which in the dentin-pulp complex, was formed in areas where the odontoblastic layer was initially lost, destroyed and eliminated. If these exposed pulp areas, with no odontoblasts, are covered with low-aggression materials, not allowing chemical penetration in the pulp tissue, and not acting as foreign bodies in the interface of connective tissue, the tissue stem cells may originate a new third generation odontoblastic layer,[17,21] which will initially deposit an amorphous and non-tubular dentin (Figs 7 to 11, 19 and 20).

Later on, as the pulp assumes the repairing stage, this new layer of odontoblasts continues its matrix deposition in a more organized way, with organized tubules and parallelism.

In this dentinal matrix, deposited under these conditions, it is possible to notice some cell inclusion similar to osteocytes, and, thus, it can be referred to as ***osteodentin***. In addition to cell inclusions, it is possible to notice islet-like cells or projections of connective pulp tissue in some repairing dentins, with vases in their composition and, eventually, they may be named ***vasodentin*****,** found in teeth of some animals that are inferior in the biological evolution scale. These cell inclusions and projections of pulp tissue into the repairing dentin allow[1] us to compare it to a Swiss Cheese.

In order to justify this term, this dentin should be associated with an underlying pulp, free of any inflammatory process. Just like any other type of dentin, the repairing dentin also presents permeability and does not represent an impenetrable protection for the pulp tissues. Any repairing process represents the later stage of an inflammation process successful in eliminating the aggressor. Matrix deposition in a repairing process is part of the recovering process of connective tissues, including pulp tissues.

Some materials directly applied to the pulp in accidental expositions with the objective of pulp capping, or in pulpotomies, especially calcium hydroxide in its many formulations, are frequently associated with new layer formation of repairing or tertiary dentin, creating barriers of mineralized tissue newly formed. This is due to its low permeability in the pulp connective tissue, acting in the interface surface, as well as to its antimicrobial capacity, with high pH.

At first, calcium hydroxide creates a thin protein denaturation, or clotting necrosis, which acts as a primary matrix for minerals deposit. Subjacent to this layer in the pulp-material interface, undifferentiated cells organize and differentiate themselves in a new odontoblastic layer, starting the deposition of the repairing/tertiary dentin, since it is synthetised by a third generation of odontoblasts.

2D - *Dysplastic dentin:* dysplastic dentin corresponds to that deposited in different areas with a primitive organization (Fig 21), some unorganized tubules and several cell inclusions and/or islet-like pulp tissue, richly vascularized.

The dysplastic dentin often occurs in cases of "canal obliteration" or "pulp obliteration", known as "Calcific metamorphosis of the pulp", also referred to as "Calcific metaplasia of the pulp". This pulp situation is a consequence of dental traumas that did not fracture the root nor destroyed the neurovascular bundle inside the apical periodontium, but injured it and partially limited its blood supply. The most frequent clinical consequences in these cases are tooth darkening in higid teeth and, over the years, nearly 25% of the cases evolve to necrosis and chronic periapical lesion.

In these cases, pulp obliteration happens due to irregular and random deposition of dentin in the extracellular matrix of the intercellular spaces all over the pulp, since blood restriction induces this type of response in order to allow the cell to survive by lowering its metabolism when included in a matrix. Nearly all cells undergo transformation or metaplasia to

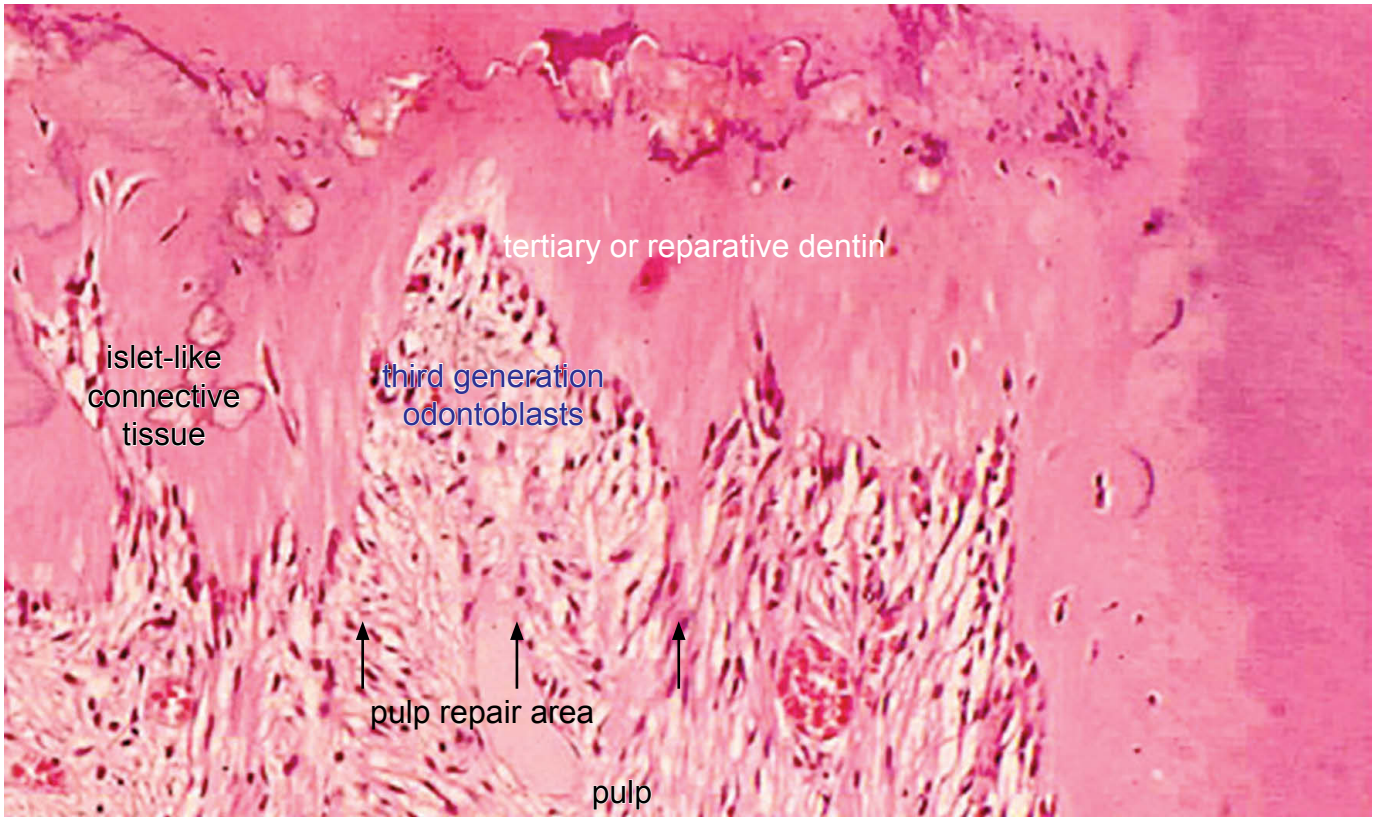

Figure 20. In the human exposed pulp, protected with calcium hydroxide-based material, pulp repair organized a new layer of third generation odontoblasts which deposited tertiary dentin, constituting a mineralized tissue barrier with cell inclusions and islet-like connective tissue in continuity with lateral walls (Source: Lanza[17]) (H.E. original mag.: 400X).

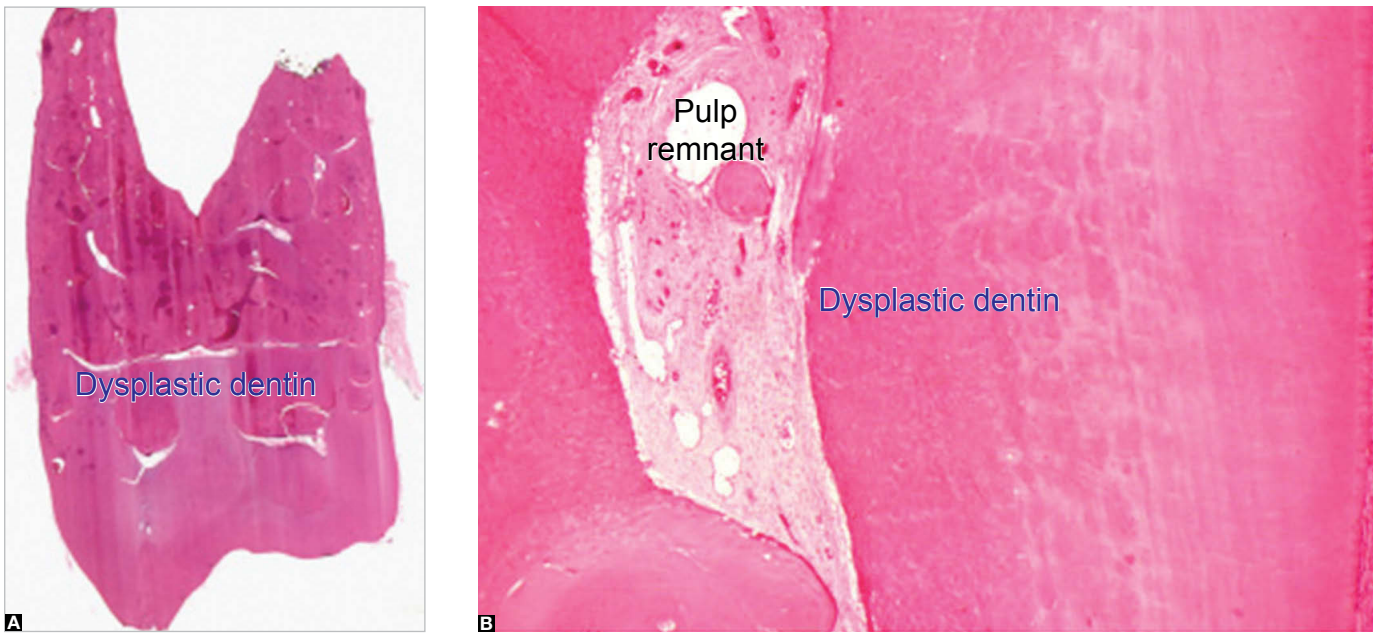

Figure 21. In human pulps, central cells may undergo metaplasia and assume odontoblasts morphology and functions, depositing randomly around it a disorganized and poorly mineralized dentin, also called dysplastic dentin. Dysplastic dentin occur in calcific metamorphosis, Induced by mild traumas, such as concussion and some other dental development alterations as dysplastic dentin in **A**, and dentinogenesis imperfecta in **B**. In almost every case there is partial or total obliteration of the pulp space (H.E. original mag.: **A** = 2,5X; **B** = 40X).

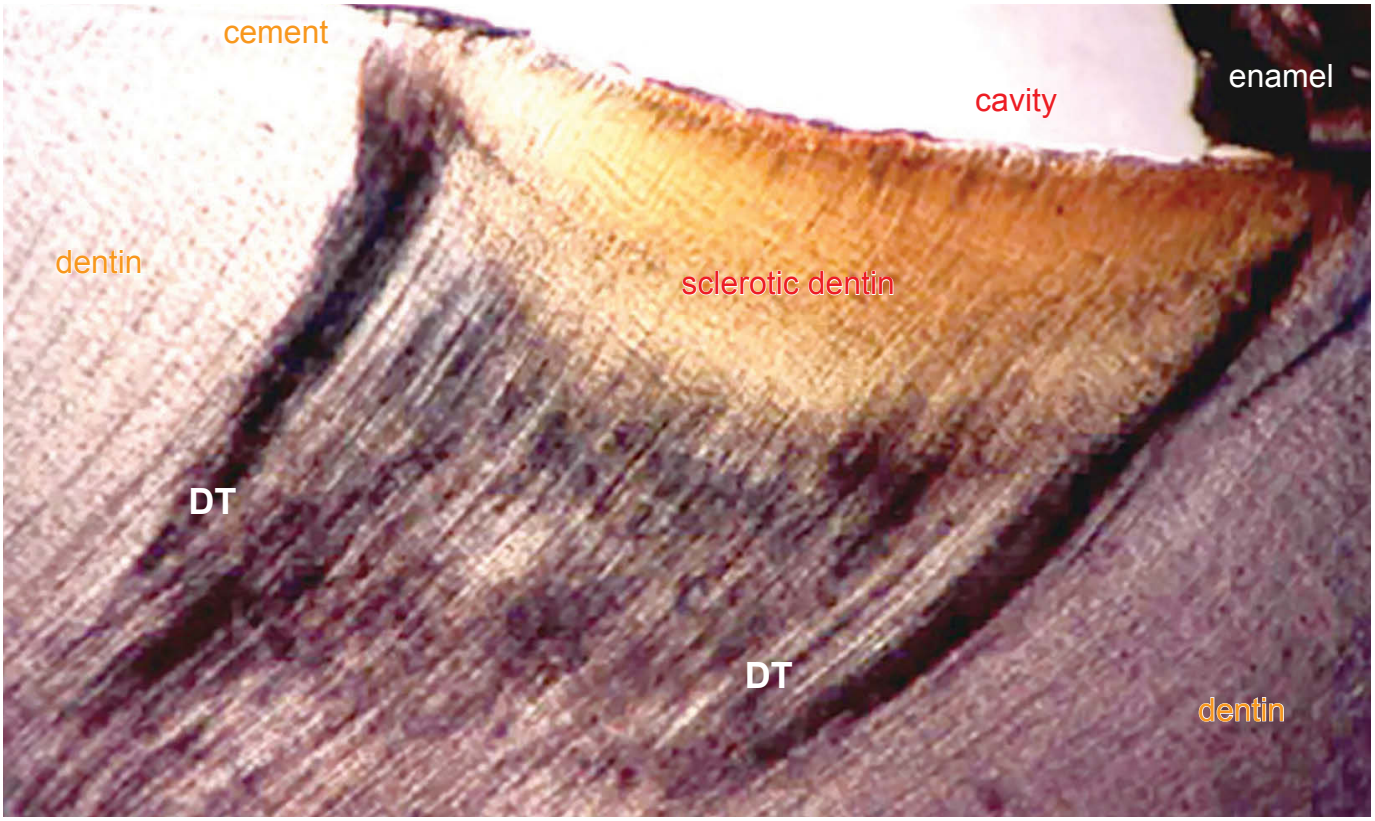

Figure 22. In cervical chronic cavity, sclerotic dentin is characterized by the closure of its tubules by peritubular dentin. The empty tubules are dark and named dead tracts of dentin (DT) (wearing, original mag.: 25X).

odontoblasts. Cell inclusion and/or islet-like aggregates in the malformed or dysplastic dentin lead it to be named as osteodentin and vasodentine, normally recognized as part of the tooth in some animals.

The dysplastic dentin can also be observed in certain development disturbs, such as dentinal dysplasia (Fig 21) and dentinogenesis imperfecta, as well as in certain odontogenic tumors, as in odontomas.

3rd Criterion: Light in dentinal tubules

The dentinal tubules may be with their lumen in normal middle diameter and partially filled with odontoblastic extensions amid the dentinal fluids.

3A - ***Sclerotic dentin or dentinal sclerosis.*** In aggressions caused by bacteria and its products in chronic cavities, as well as in those induced by rotating tools, heat, attrition and abrasion, and material applied to the dentin, the odontoblastic extensions may accelerate the deposition of peritubular dentin at the same time the reactive dentin is deposited in the pulp wall.[5,6] This can cause the lumen to partially or totally close, decreasing and hindering local dentinal permeability, obstructing the passage of bacteria, as well as of chemical products deriving from materials (Figs 17 and 22).

The narrowing and closure of the dentinal tubules does not characterize local dentin hardening,[24] but a reduction in permeability and penetration. As for the hardness degree, it remains similar to the normal dentin. The darkening of the sclerotic dentin tends to occur especially under cavities, due to incorporation of pigments derived from proteolysis. The sclerotic dentin is also referred to as ***transparent dentin*** because of its light refraction properties.

For a long time, dentinal sclerosis was considered as a consequence of precipitation of products deriving from dentin demineralization, such as calcium and phosphate ions, on the odontoblastic extensions in decay process, as in the dystrophic calcification of soft tissues.[5,34] But this understanding of dentinal sclerosis has only historical value.

3B - *Dentinal dead tracts:* in aggressions caused by bacteria and its products in chronic cavities, as well as in those induced by rotating tools, heating and material applied to the dentin, the odontoblastic extensions may retract, since they have contractile filaments in their cytoskeleton. Below these aggressions, many tubules may get empty (Figs 17 and 22) because the extension retraction may occur before new peritubular dentin layers have been deposited to close or sclerose the tubular light.

In the microscopy of dentin subjacent and adjacent to a cavity, or even to a cavity preparation, these empty tubules appear as dark lines, isolated or in group, and are identified as dead tracts of the dentin.[5] There is still no evidence defining the nature of this process, whether they represent a defense mechanism or if they facilitate dentin aggression. We only have observations with purely morphological implications based on microscopic analysis of an aggressed dentin.

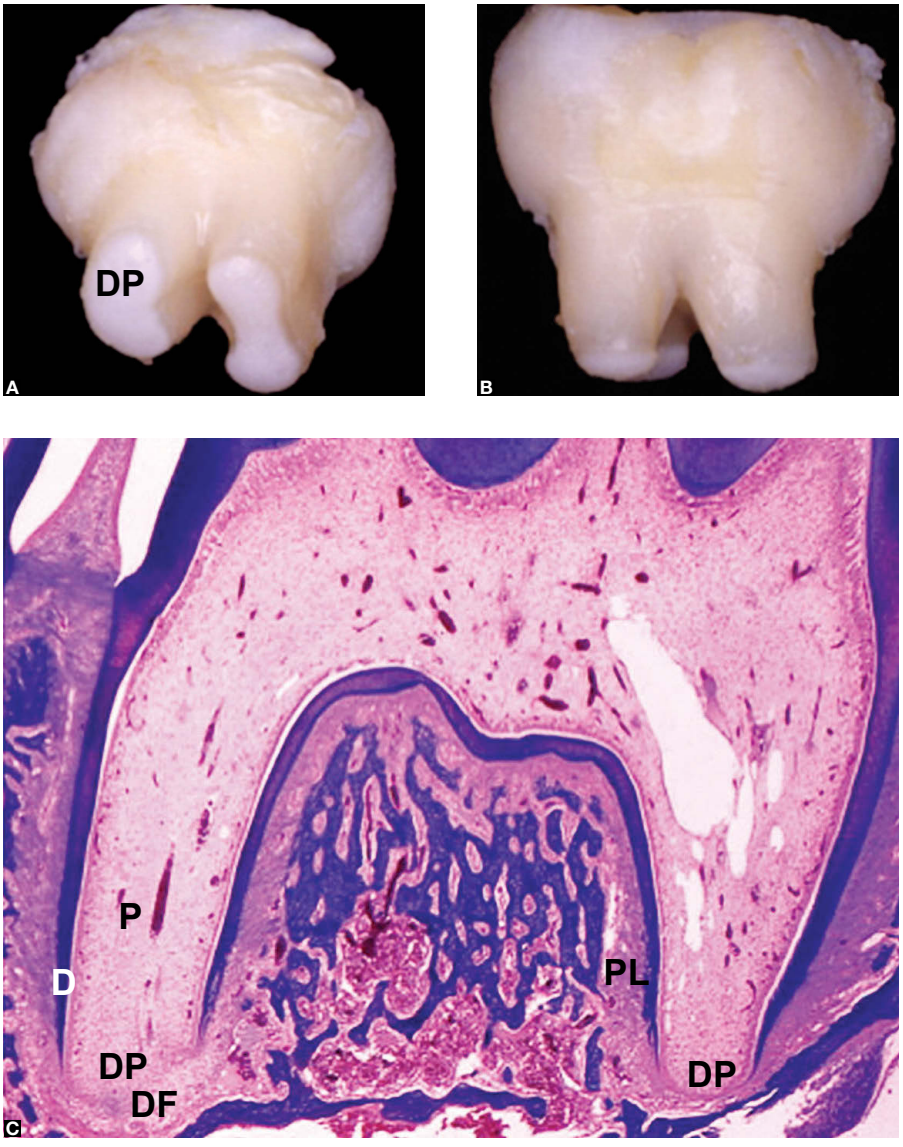

Figure 23. Tooth with incomplete root formation showing dental papilla (DP), dental follicle (DF), and also newly formed dentin (D) and dental pulp (P) (Masson trichrome: original mag.: in **C** = 40X).

The reparative ability of the pulp: determining factors

The repairing potential of a dental pulp is directly related to:

1. Cellularity degree: the younger or more preserved from external agents is the dental pulp, the bigger is the cellular source to replace the elements lost (Fig 2). The undifferentiated or differentiated precursors of specialized cells will be able to migrate and reposition the pulp structures, if needed. The cellular source is one of the basic requirements of a repairing process. Apoptosis is one of the mechanisms controlling the cell population of the dental pulp.[42]

2. Fibrosis stage: in the repairing process, the cells mobilized to differentiation and migration towards the place to be repaired require an extracellular matrix, poor in physical obstacles hindering this cell mobilization, as it probably happens to the dense collagen fibers, sometimes hyalinized in old pulps or frequently subjected to external agents (Fig 2). Conversely, an extracellular matrix poor in fibers may lead to an excessive permeation of chemical agents and bacterial products, spreading them all over the pulp tissue.

3. Abundant vascularization: maintaining rich cellularity and cell mobilization in the repairing process demands energy, because the level of activity of synthesis is high, especially during reparative dentinogenesis. With age, the natural aging of the pulp reduces the number of blood and lymphatic vessels,[6] a process that is accelerated by the excessive exposition of the tooth to external aggressors agents, such as caries, dental wearing, periodontal disease and restorative operative procedures.

4. Pulp volume: reduction in pulp size is often mentioned among the determining factors of low reparative capacity of the pulp. Pulp volume does not reduce the reparative capacity, but the defensive capacity of the pulp at acute inflammatory stage, of which exudate will end up compromising the circulatory dynamic of the pulp even before it effectively acts with the inflammatory infiltrate over the aggressor agent. Lower incisors or molars with a young pulp have similar reparative capacity, and this depends on the pulp volume.

5. Pulp nodules: these structures are formed where the pulp had been previously altered by circulatory focal deficiency or hyalinization of collagen fibers, as part of the pulp aging process. The pulp nodules may act as indicators of the degree of pulp aging, especially from a radiographic point of view. Alone, the nodules do not hinder repair, but pulp aging in which they are inserted and characterized by low pulp cellularity, rich fibrosis and decreased vascularization.

The increase in dystrophic calcification or pulp nodules frequency is erroneously mentioned as a consequence of calcium hydroxide directly used on the dental pulp. The only dystrophic calcification related to calcium hydroxide happens in the thin layer of necrosis caused by coagulation located in the pulp sur-

1st – As for the dentin formation chronology
a) Primary: Deposited during odontogenesis, before dental eruption.
b) Secondary: After eruption, for completing and keeping the tooth normal.
c) Tertiary: In pulp injuries, by a third generation of odontoblasts.
2nd – As for the stimulus nature, structure and organization
a) Physiological: Deposited during odontogenesis and maintenance of normal tooth.
b) Reactive, irritation or pathological secondary dentin: Deposited subjacent to dentinal aggressions which do not eliminate original odontoblasts.
c) Repairing, reparative or reparatory dentin: In repair of pulp injuries with odontoblasts death, and deposited by a third generation of odontoblasts, from the undifferentiated cells, or pulp tissue stem cells.
d) Dysplastic: In calcific metamorphosis of pulp, resulting from mild traumas, disturbs of dental development and in odontogenic tumors.
3rd - As for the light in dentinal tubules
a) Sclerotic dentin or dentinal sclerosis: Obliteration by accelerated deposition of peritubular dentin by extensions.
b) Dentinal dead tracts: Partial or total empty tubules, by contraction and retraction of odontoblastic extensions under dentinal aggressions.

Table 1. Nomenclature and classification of dentin in three criteria.

face, immediately under the calcium hydroxide directly applied on the pulp tissue. This phenomenon, by the way, is part of the initial formation of the mineralized tissue barrier,[29,30] being, thus, consciously expected.

Fibrosis and dystrophic calcification are natural consequences of the pulp aging process. They may occur in every tooth and may be accelerated by external agents, such as cavity, dental wearing, restoration and prosthetic preparation, among others.

Pulpotomy may momentarily and limitedly accelerate pulp aging. With time, the remaining pulp will also gradually age. The application of calcium hydroxide allows the remnant to continue with this aging process. When fixation substances of pulp tissue are used in pulpotomies, "mummifying" such tissue, this aging process ceases and fibrosis and dystrophic calcifications or pulp nodules will not occur, and, radiographically, the pulp space will be apparently kept.

It is not precise or true the statement about the calcium hydroxide accelerating pulp aging and increasing the nodules index. Aging occurs because of factors that led to the need for pulpotomy, and due to the remnant being still alive and biologically feasible.

6. Absence of aggressive elements. The presence of bacteria often requires neutrophilic and, occasionally, macrophagic mobilization of which interaction promotes an intense release of protolithic enzymes associated with local tissue destruction. Under these conditions the repairing process does not occur.

Regardless of their chemical and physical nature, the presence of foreign bodies that may indefinitely keep a chronic inflammatory process also avoid the repair in the operated pulp area, even if it is free of bacteria, because they induce the formation of granulomas, similarly to what happens with the microparticles of the adhesive systems directly applied to the pulp.[4,17]

7. Systemic alterations: general diseases normally do not result in specific alterations in the pulp. This low pulp reactivity against systemic diseases may be emphasized by the great number of cases

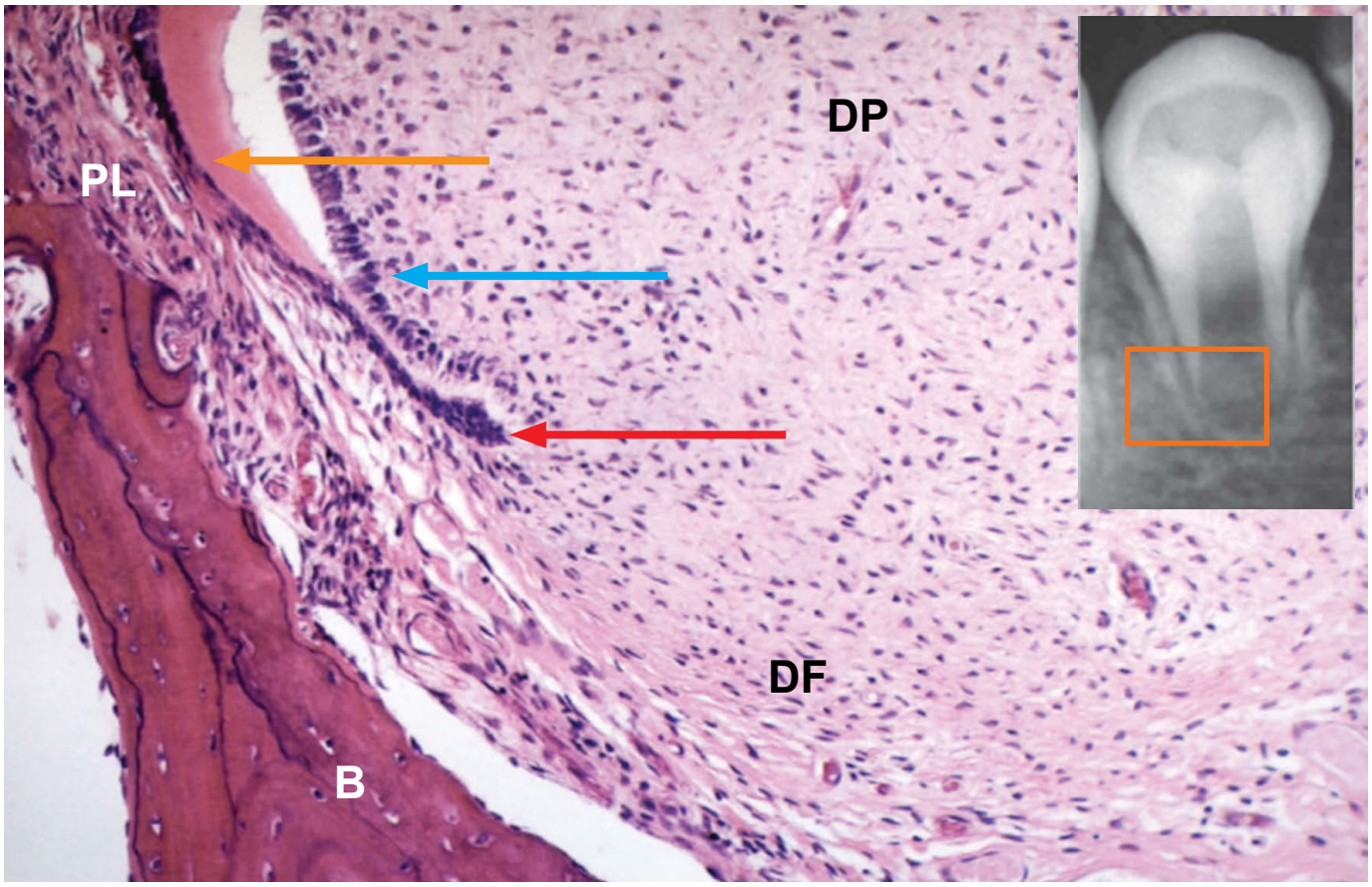

Figure 24. Hertwig's sheath cells (red arrow), odontoblasts (blue arrows) and dentin (orange arrow) in a tooth with incomplete root formation. B = bone; PL = periodontal ligament; DP = dental papilla; DF = dental follicle (H.E.: original mag. = 160X).

with severe organic impairment, with no increase in the pulp disease index for this specific population. This is probably due to isolation characteristics to which the pulp is subjected, and also due to the single source of blood in the apical foramen.

The clinical conditions which significantly increase the calcium level in the blood, promoting its precipitation on normal tissues, including the pulp, without the need of previous aggressions to the tissue, are among the manifestations of the pulp in cases of systemic diseases. This process is identified as metastatic calcification and may occur in hyperparathyroidism, in osteomyelitis, in cases of bone metastasis of malignancies, etc. When the metastatic and diffuse calcifications occur in the pulp, its reparative capacity decreases.

Dental papilla in formation and repair of root and pulp

Once the crown formation has been completed and the presence or absence of bi- or trifurcation of roots from the cervical loop of the enamel organ and horizontal invagination of the epithelial diaphragm have been

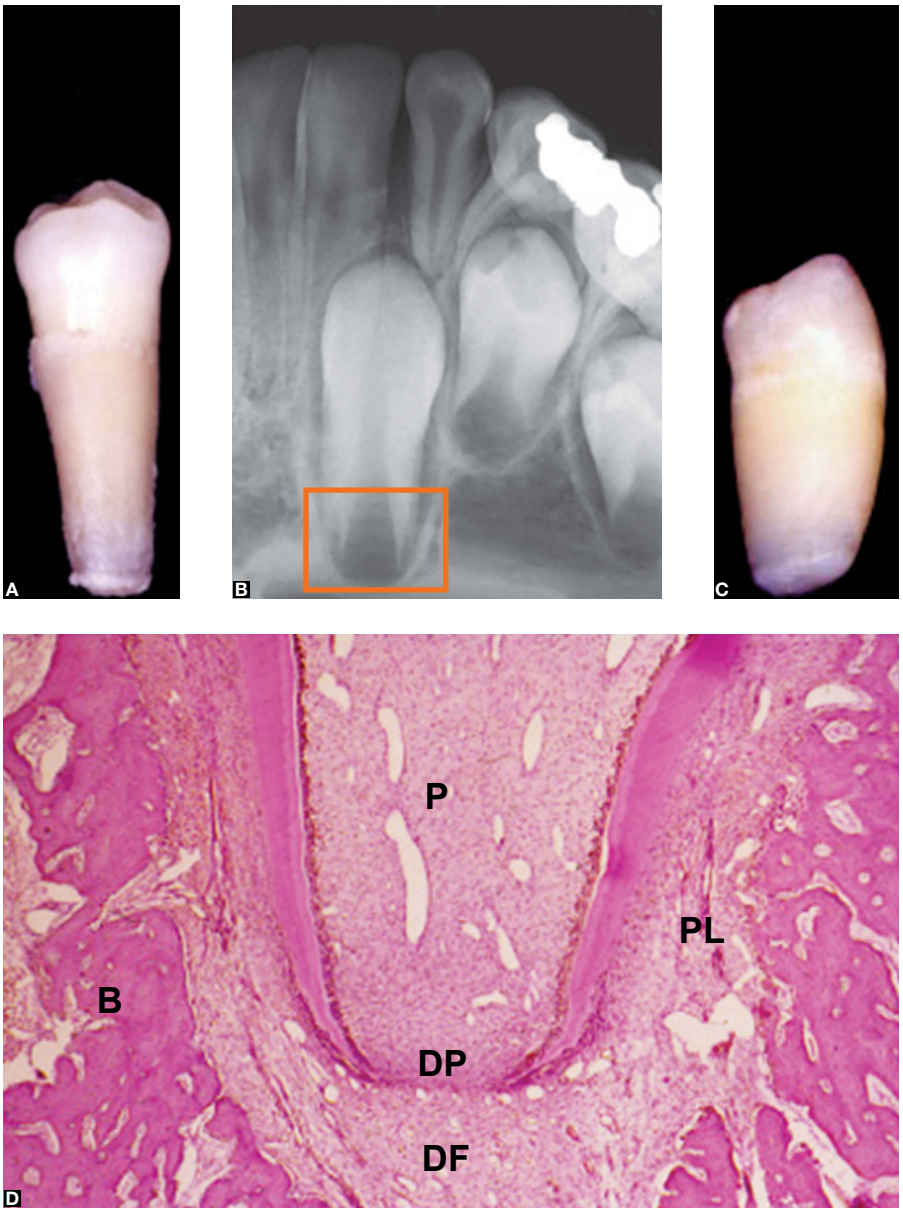

Figure 25. Teeth with incomplete root formation, specifying the root walls shape, the pulp space width and the apical area occupied by dental papilla.
DP = dental papilla; P = pulp; PL = periodontal ligament; B = bone (HE: original mag.: 40X).

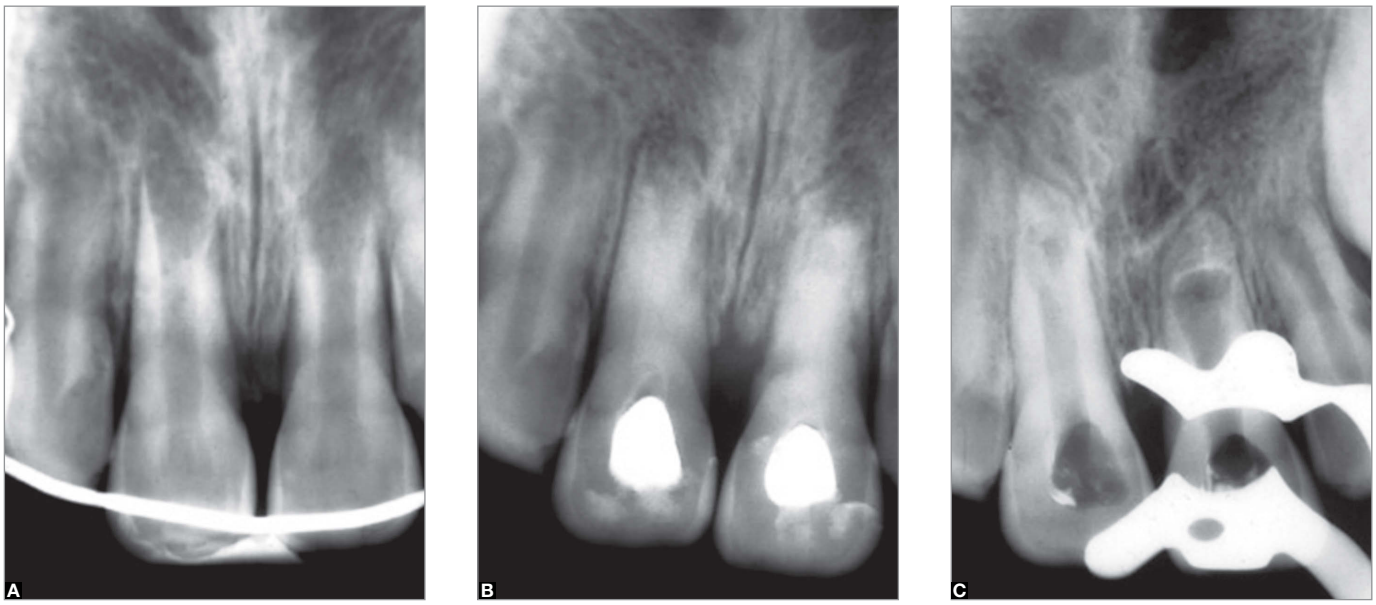

Figure 26. Clinical case with complete apical complementation of teeth with incomplete root formation, of which injury led to loss of root pulp vitality, without affecting the dental papilla and follicular tissues (from Dr. Júlio César Bento dos Santos, Limeira/SP).

defined, there will be a set of structures with specific and characterizing functions of a "tooth root formation organ" (Figs 23, 24 and 25). This set of structures comprises:

a) Dental papilla.

b) Hertwig's sheath.

c) Dental follicle.

Synchronously, the induction of root dentin formation is determined by cells of the intern layer of the Hertwig's sheath via biochemical mediators and cell-cell interaction. Thus, the peripheral cells of the dental papilla originate the root odontoblasts.

After the deposition of the first dentin layers, the cells of the sheath will position a thin protein matrix layer similar to the enamel, with no fibers, known as ***intermediate*** or ***afibrilar cementum***. Afterwards, the Hertwig's sheath is fragmented by apoptosis[18] and exposes the radicular surface, an event that starts the differentiation of follicular cells into cementoblasts and, as a result, cementogenesis to recap and insert the periodontal fibers (Fig 24).

At the same time, in small areas of the Hertwig's sheath, the absence of apoptosis will give rise to the epithelial cell rest of Malassez – discrete clusters of residual cells that did not disappear completely.

The Hertwig's sheath is very much alike an epithelial "ballet skirt", at the peripheral portion of the dental papilla. The progression of root formation happens vertically (Fig 24), and culminates in complete formation of the apex.

During root formation, the dentinal walls are cuneiforms and their thinner portion adheres to the Hertwig's sheath, delimiting the dental papilla. At this stage, this cuneiform characteristic of dentinal walls gives great amplitude to root canals (Figs 23 to 27) which gradually reduce due to constant dentin lateral deposition.

As the dentinal deposition process occurs, the dental papilla ceases to be a nearly exclusively cellular tissue characterized by an abundant jellylike extracellular matrix. The papilla assumes a loose connective tissue morphology, with minimal collagen fibers: a young dental pulp (Figs 24 and 25).

In the outer portion of root formation, the cementogenesis, periodontal ligament formation and alveolar osteogenesis are functions exerted by the dental follicle, a tissue of ectomesenchymal origin that plays a fundamental role in the formation, structuring and support of dental root.

Root formation depends on this set of structures comprised by the dental papilla, Hertwig's sheath and dental follicle. Some repairing evolution of teeth with incomplete root formation may be detected, and each one of them depends on:

a) The state of vitality as well as pulp and dental papilla reactive capacity;

b) The absence or presence of contamination in the region, since it leads to abscessation, destroying the local tissues and avoiding neoformative activities.

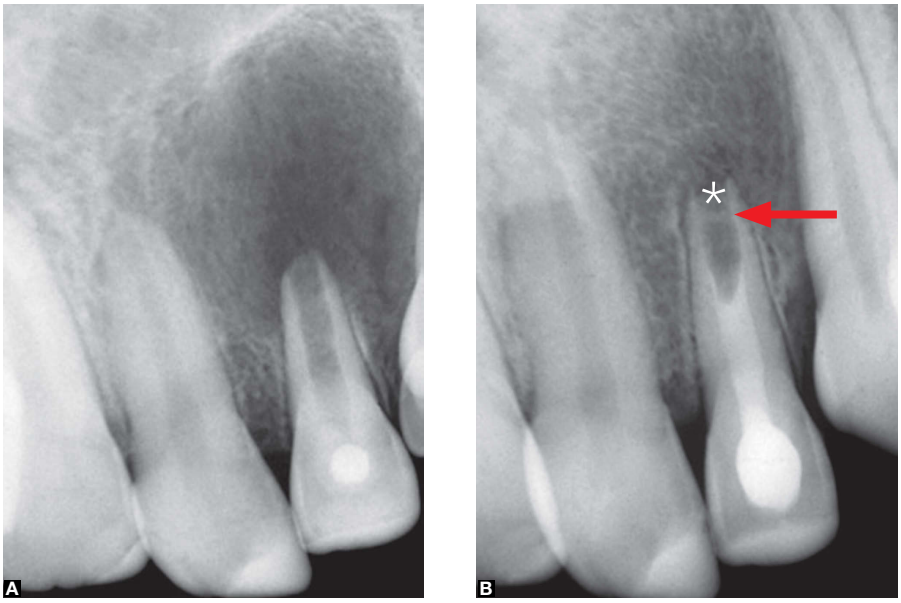

Figure 27. The apical complementation of upper lateral incisor with incomplete root formation, of which traumatic lesion led to loss of root pulp vitality, without affecting the dental papilla and follicular tissues, is being characterized by the likely pulp tissue formation (asterisk) from the surface of the material inserted on the canal, marked by the formation of a radiopaque tissue barrier (arrow) (from Prof. Dr. Eduardo A. Botoluzzi, Florianópolis/SC).

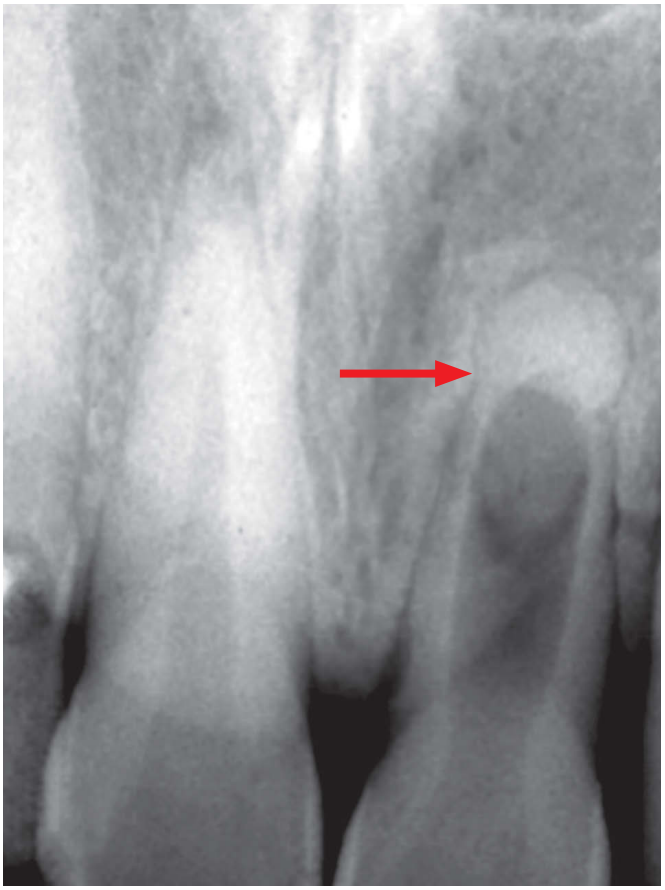

Figure 28. Periapical cap: Apical complementation of upper lateral incisor with incomplete root formation, of which injury led to loss of root pulp vitality disrupting and moving the dental papilla and follicular tissues from the root portion already formed, which continued originating dentin and cementum separately, until reaching each other and merging (asterisk). This was a radiographic sign observed in a routine exam, which was ignored by the patient.

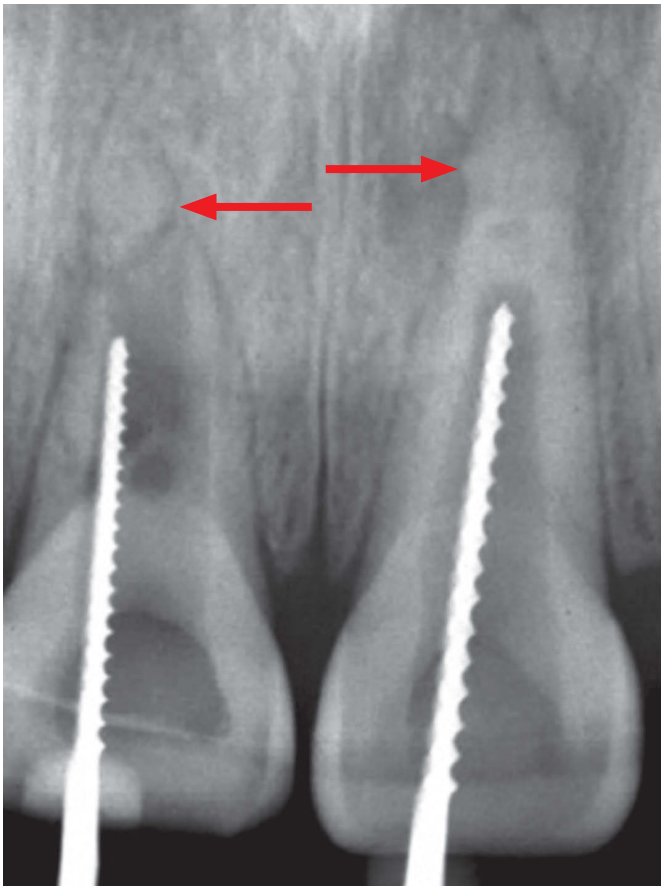

Figure 29. Both apical caps were formed after separation of the mineralized portion of the root, due to dental trauma. Over time, the periapical cap tends to merge with the formed portion, as observed in the upper left incisor and probably will happen on the right too, still apparently separated.

c) Integrity of the Hertwig's sheath and dental folicle,[10] since both are essential to the formation of apical and periodontal structures.

Pulp and root repair in teeth with incomplete root formation: seven ways and ten principles

Seven types of evolution (Fig 31), and ten biological principles should rule the endodontic therapy of teeth with incomplete root formation:

1. Whenever possible, preserve the root pulp portion alive, because it represents the "anteroom" of the dental papilla. Preserving the pulp implies in mandatory preservation of the dental papilla and, thus, the normal continuity of root formation and the original length of the tooth are kept (Fig 31).

2. When the radicular portion of the dental pulp is compromised and with no vitality, endodontic intervention should be the earliest possible in order to assure a greater possibility of preserving the vitality of the dental papilla, of the Hertwig's sheath and the dental follicle (Figs 26 and 27).

3. During the inspection and verification of the root length, do not manipulate or puncture the dental papilla with tools, because this will result in disorganization of this pulp formation organ, an embryonic tissue.

4. Dental papilla is well vascularized and full of cells, thus, its reparative capacity is high, but limited. The application of biocompatible material to the root canal is mandatory to keep it alive (Fig 27).

5. Without the dental papilla there is no complementation of root formation; "no poet, no poetry". Keeping the dental papilla alive, by means of endodontic intervention, will allow root apex complementation, in addition to keeping the original length of the tooth. This process may be named as apexogenesis (Figs 26 and 27).

6. Should there be no dental papilla and no Hertwig's sheath, the most peripherally located dental follicle, alive and under proper endodontic therapy, may promote cementum formation. Additionally, it may regularly deposit mineralized material in order to allow a round and anatomically accepted finishing for the dental apex, even though the tooth may present a definite shortening (Fig. 30). This process may be referred to as apexification.[10]

7. Should the dental follicle die because of the aggressor, an endodontic therapy will keep the root shape as it was in the moment the root formation stopped, there will be no apexification, let alone apexogenesis. The root will be paralyzed in its formation, resembling the upper part of an active volcano (Fig 31).

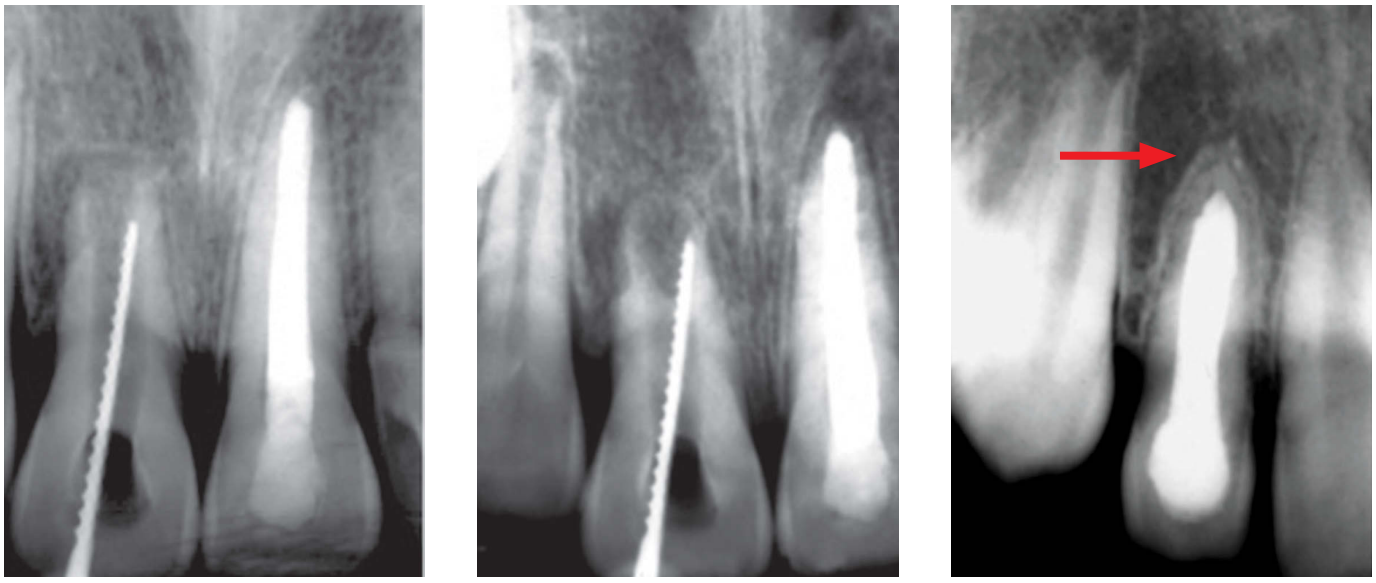

Figure 30. In some accidentally traumatized teeth, the apical complementation in teeth with incomplete root formation does not occur due to loss of root pulp vitality and dental papilla, only with the maintenance of follicular tissues, leading to the formation of cementum and ligament to form the apex with a shape similar to normal, but with shorter length of the root (from Prof. Dr. Francisco C. Ribeiro, Vitória/ES).

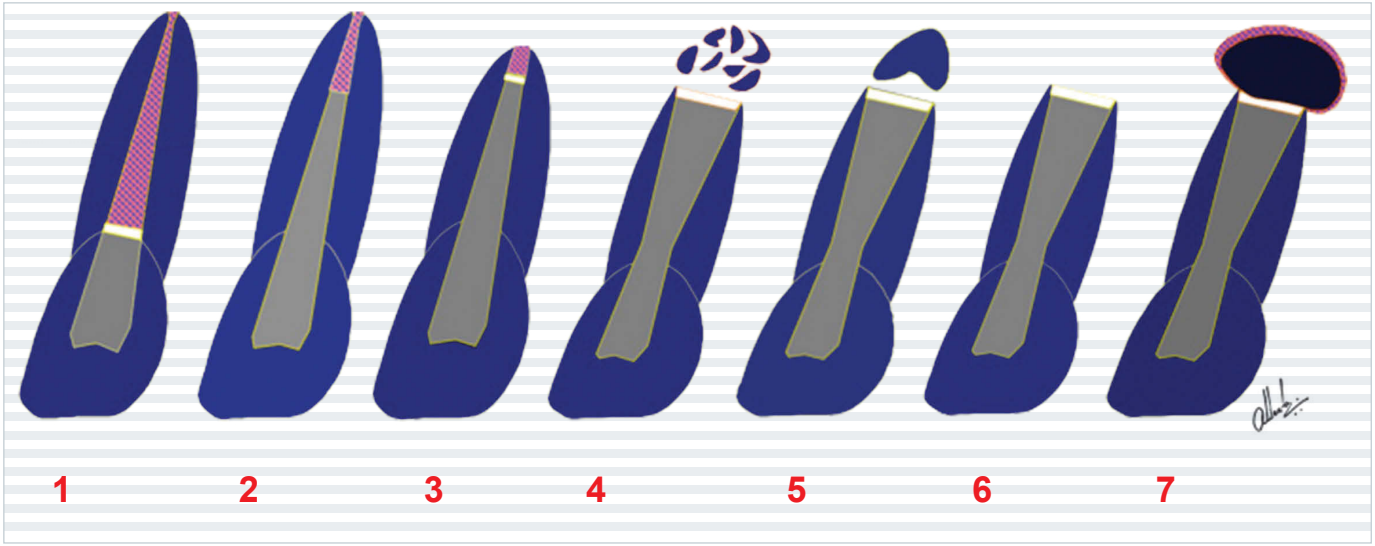

Figure 31. Different development of pulp repair in teeth with incomplete root formation injured by dental trauma (Source: Esberard and Consolaro[10]):
1 - apical complementation with impairment of coronary pulp, only;
2 - apical complementation with impairment of radicular pulp, only, but with dental follicle and papilla preserved.
3 - apical complementation due to impairment of vitality and dental papilla, and shorter length of the root.
4 - displacement of apical embryonic tissues and fragmentation forming isolated islet cells of dentinal and cemental tissue.
5 - apical cap due to displacement of apical embryonic tissue and isolated formation of apex, which will attach to the original mineralized tissue portion.
6 - total lack of apical complementation with regard to shape and length, due to papilla and dental follicle death;
7 - evolution to apical periodontal cyst, derived from the epithelial remnants of the fragmented Hertwig's sheath.

8. In trauma cases, the set of structures comprised by the dental papilla, Hertwig's sheath and dental follicle may move from the already formed portion which, by extrusion, will remain further away (Figs 28, 29 and 31).

Isolated and independently, this set will be able to "continue" with root formation in different degrees of organization. Over time, this will be radiographically detected as an apical "cap" formation, because the portion that had already been formed at the moment of the trauma remains with its apical area open and expulsive, since there was neither apexogenesis nor apexification.

In some cases, depending on the time and proximity, the apical cap may merge with the tooth by means of constant formation of cemental or cementoid tissue,[10] similarly to a champagne cork (Fig 28).

9. In trauma cases, fragmentation of the Hertwig's sheath may occur in addition to temporary disorganization of the dental papilla. In every viable fragment of epithelial sheath, points of dentin and cementum production will be formed. Radiographically, after some time, several points revealing this process will be identified in the apical portion of the tooth with incomplete root formation.[10] These points may grow, merge and give rise to an irregular "apical cap" (Figs 28, 29 and 31).

10. The fragmentation of the Hertwig's sheath and the unfeasibility of the dental papilla may lead the epithelial rests to originate apical periodontal cysts, even after correct endodontic therapy (Fig 31).

The apical pulp and the periodontal tissues of the cemental canal: two structures in continuity

Cell proliferation and lifetime are genetically programmed. This also applies to the Hertwig's sheath and the dental papilla. Once the Hertwig's sheath is inactive, with regard to proliferation, it ceases to induce the dental papilla to dentin formation. During this period, the dental papilla has its cellularity decreased. Without this induction, dentinogenesis is also ceased: it is the beginning of the last stage of root formation.

The dental follicle will still play its formation role to establish the periodontal ligament and promote a

regular apical surface, depositing cementum layers over the last portions of dentin deposited. This will happen lateral and apically to this dentin. It is, then, established a delimiting point between pulp and periodontium, which is known as the CDC limit.

The limit between dentin and cementum delineates — since the first moment of complete formation of the apex — the space denominated cemental canal, predominantly expulsive. The tissue occupying it has its origin at the dental follicle and keeps a reduced, but constant, cementogenic capacity. The connective tissue in the cemental canal is of periodontal nature and, when preserved during biopulptomies, should be referred to as periodontal remnant.

In microscopic sections of teeth with incomplete root formation, some blood vessels and nerve fibers coming out of the alveolar bone, passing through the dental follicle and reaching the papilla, can be observed. Dentinogenesis and cementogenesis are induced and performed by specialized and organized cells; the dentin and cementum matrixes deposited are fragile and non-mineralized, almost gel-like.

In the root formation in apical direction, when meeting these vascular-nerve bundles crossing the follicle and going into the dental papilla, the dentinal/cemental organic matrix surrounds them focal and linearly. Around these vessels and nerves, the most external layers required to the maintenance of the cells are found. This linear and focal surrounding, that the root formation process promotes on the vascular-nerve bundles, originates the lateral and accessory canals. Vessels and nerves release mediators which induce bone resorption and avoid their structures and lumens to be constricted.

The dental papilla is exposed to the dental follicle mainly in its apical portion; thus, it can be understood the reason why the accessory canals and canaliculi, or lateral ramification, are more commonly found in the apical third, sometimes establishing a real delta.

During the formation of mineralized tissue barriers in the dental pulps subjected directly to calcium hydroxide, this way of surrounding the vascular-nerve bundles of the underlying pulp may lead to canal-like formations, as found in several studies carried out with SEM.[29]

The gradual reduction in vascularization of the dental papilla may be one of the reasons that contribute to reduce its proliferative and productive capacity. It happens with the gradual reduction in the open apical space until the apical foramen is formed. This type of narrowing of the nourishing blood supply does not occur to the dental follicle, an embryonic element forming the periodontal ligament, a structure that, over time, can keep cementogenic, fibrogenic and osteogenic capacity, effectively participating in the apical repair after endodontic treatments.

In orthodontic movements performed in teeth with incomplete root formation, a reduction in diameter of vessels that keep the dental papilla active may occur if the movement is significantly excessive. This reduction will lead to premature maturation and formation of the apical third, resulting in tooth shortening if compared to a healthy equivalent one.

Dental pulp and other clinical specialties

Orthodontically induced tooth movement is often related to premature and accelerated pulp aging, usually revealed by radiographic images of clinical cases. Similar studies,[3,7,28,41] do not offer evidences or data allowing such assertion and, in orthodontic clinical practice, this type of complication is rarely detected (Fig 32).

In cases in which many pulp nodules and reduction of pulp volume are detected during or after the orthodontic treatment, it is necessary to perform a detailed anamnesis to recover the previous history of that tooth: dental trauma is probably involved. The documentation consisted of periapical radiographs taken before the orthodontic treatment, may reveal whether or not this aging already existed. The relation between orthodontic movement and pulp aging has not been experimentally confirmed[3,7,28,41] and, in clinical practice, it cannot be proved, just like it is not possible to prove that induced dental movement favors pulp necrosis or calcific metamorphosis of the pulp.

The ***chronic inflammatory periodontal disease*** does not age the pulp of endodontically healthy teeth, not even decreases their reparative ability. Pulp nodules, reduction in pulp volume and their spaces in radiographs are associated mainly with attrition, abrasion and extensive restorations.

Endo-periodontal injuries require independence and simultaneity of both pulp and periodontal processes. When both processes are directly associated,

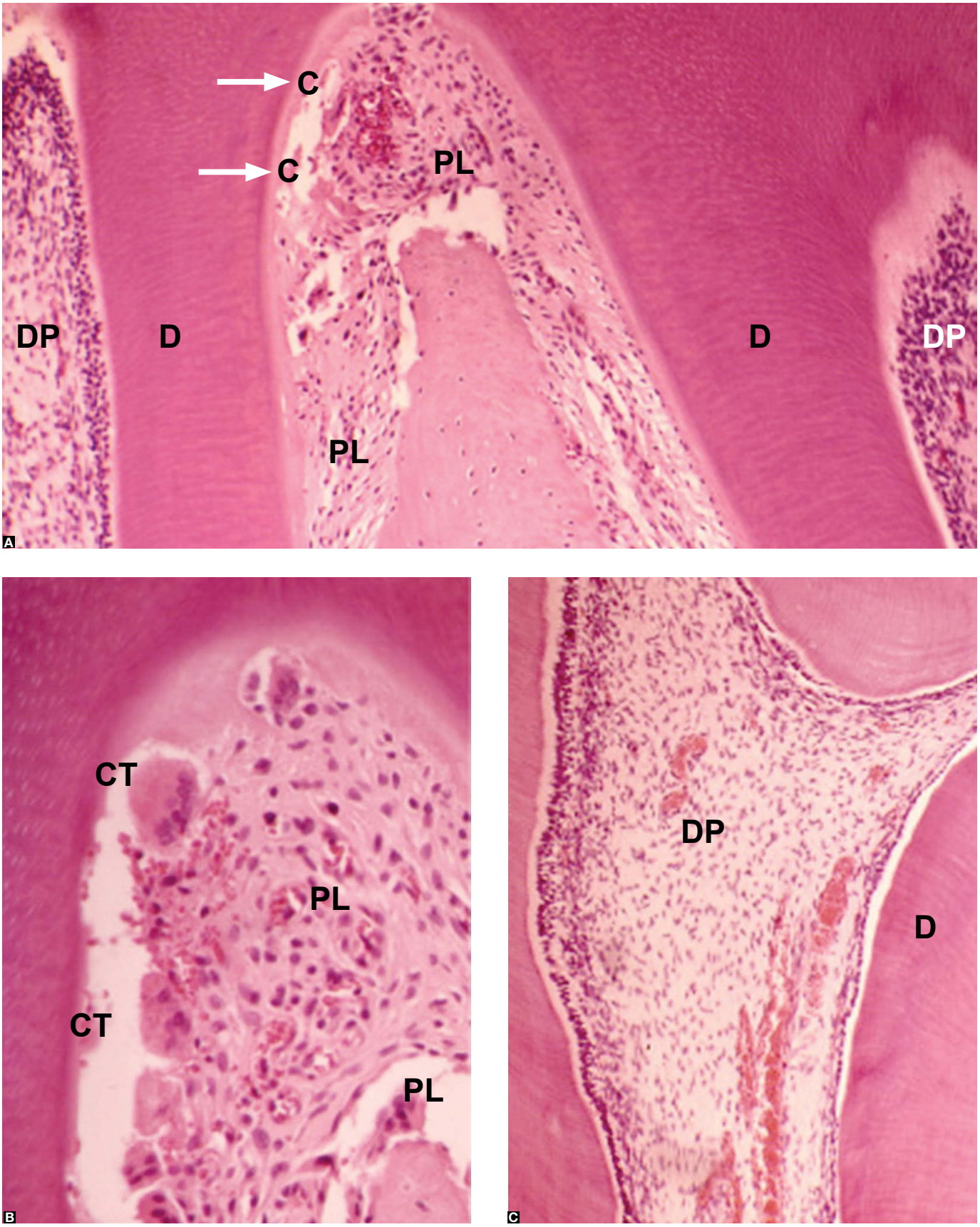

Figure 32. Orthodontically moved tooth, with cementoblasts death (arrows) in **A**, and root resorption with several clasts (CT), proving the effectiveness of the applied forces. The dental pulp (DP) kept its morphology indicating preserved vitality, despite the induced tooth movement. C = cementum; D = dentin; PL = periodontal ligament. (H.E., original mag.: **A** and **C** = 160X, **B** = 400X).

they cause a real endo-periodontal injury. When the pulp is alive, it positively exerts a pressure that leads the plasmatic and tissue fluids to originate the dentinal fluid which permeates the spaces between the odontoblastic extensions and the tubules.

The outward flux of dentinal fluid and its important biochemical and antimicrobial activities, make it almost impossible for the bacterial products in the root surface to reach the pulp during a chronic inflammatory periodontal disease. Despite the fact that the cementum is a very thin and poorly mineralized tissue, being easily eliminated in its buccal exposition or scraping procedures, the dentin-pulp complex mechanisms, including dentinal fluids and sclerosis, prevent the aggressors from reaching the pulp.

However, when pulp diseases involving bacteria, including necrosis, are present in the daily clinical routine, a secondary periodontal involvement is possible, as in cases of pulp necrosis with inflammatory involvement of the bifurcation region. With pulp necrosis, the dentinal fluid no longer exists and the tubules get empty, just like the lateral and accessory root canals.

Pulp evaluation should be part of radiographic reports, not only with regard to cavity and periapical injuries, but also with regard to the size, presence of nodules and diffuse calcifications. This piece of information allows the clinician, especially during surgical, restorative or prosthetic procedures planning, to establish prognosis regarding the reparative capacity of the tooth. A careful radiographic evaluation of the pulp may reveal conservative or radical treatment options which vary according to the clinical convenience of each case.

Knowing about the origin and the functions of the dental pulp is of paramount importance when preventive or curative procedures are performed in a tooth in root formation, determining the progress of the root formation. Conservative treatments, such as direct pulp capping and pulpotomy, involve the maintenance of a biologically viable pulp, or the continuity of an incomplete root formation.

Final considerations

Knowing the origin and formation of the process means to be aware of the moment and how to therapeutically interfere in the dental pulp. Once the clinical diagnosis and the treatment plan have been determined, the objective becomes the pulp/periapical structural restoration or repair. Knowing how a structure is formed increases the possibilities of reconstructing an injured structure. This enhances safety during procedures and accuracy in predicting the case progress, i.e., its prognosis. Based on the dental pulp origin and formation mechanisms, procedures and substances capable of inducing dentin formation in direct expositions can be found, and premature aging as well as pulp diseases onset can be avoided.

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Evaluation of EDTA, apple vinegar and SmearClear with and without ultrasonic activation on smear layer removal in different root canal levels

Clarissa Teles **RODRIGUES**[1]
Norberti **BERNARDINELI**[2]
Marco Antonio Hungaro **DUARTE**[2]
Clovis Monteiro **BRAMANTE**[2]
Flaviana Bombarda de **ANDRADE**[2]

ABSTRACT

Objective: This *in vitro* study evaluated the efficiency of EDTA, apple vinegar and SmearClear, with and without ultrasonic activation, on smear layer removal. **Methods:** Seventy extracted canines were randomly divided into eight groups and prepared by using ProTaper instruments. The final irrigation regimens used were: Group 1 (control) (SAL) and Group 2 (control) (SALUS): saline for 3 minutes without and with ultrasonics, respectively; Group 3 (EDTA) and Group 4 (EDTAUS): 17% EDTA for 3 minutes without and with ultrasonics, respectively; Group 5 (AV) and Group 6 (AVUS): apple vinegar for 3 minutes without and with ultrasonics, respectively; Group 7 (SC) and Group 8 (SCUS): SmearClear for 1 minute without and with ultrasonics, respectively. Specimens were then examined under scanning electron microscope and scored for smear layer removal on the coronal, middle and apical thirds. **Results and Conclusions:** Smear layer removal was most efficient when 17% EDTA and SmearClear were used, compared to apple vinegar. Ultrasonics did not improve the smear layer removal significantly in all groups. The poorest results were observed in the apical third of the root canal, with statistical differences between the coronal third in all irrigation regimens.

Keywords: Smear layer. Chelating agents. Ultrasonics.

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[1]MSc in Endodontics, FOB/USP.
[2]Professor of Endodontics, FOB/USP.

Contact address: Clarissa Teles Rodrigues
Rua Manoel Januário de Andrade, 308 – Recreio
CEP: 45.020-540 – Vitória da Conquista/BA – Brazil
Email: clarit@uol.com.br

Introduction

In endodontic therapy, after biomechanical preparations, an amorphous, granular and irregular layer known as the smear layer is formed and deposited on root canal walls.[1] The smear layer contains organic and inorganic substances derived from ground dentin, pulpal remnants, fragments of odontoblastic processes, necrotic materials and microorganisms in cases of infected root canals.[1,2,3]

It appears to be prudent to remove the smear layer because it occludes the tubules and hinders effective penetration of endodontic irrigants, intracanal dressing and sealers into lateral canals and dentinal tubules and may compromise the sealing between root canal filling and the root canal wall.[2,3,4]

No irrigating solution used in endodontic treatment is capable of acting on the organic and inorganic elements of the smear layer simultaneously. Sodium hypochlorite (NaOCl), in concentrations of 0.5% to 5.25%, is the main endodontic irrigant, but when used alone is ineffective in removing the entire smear layer.[1,2,3] Chelating agents are used in endodontics to aid in root canal irrigation and to remove the inorganic smear layer.[1,3,4] The ethylenediaminetetraacetic acid (EDTA) at a neutral pH has been recommended since 1957[5] and it is the one most frequently employed for the removal of the smear layer.[3,4,6]

Other substances have also been suggested to remove the smear layer, such as citric acid and apple vinegar.[7,8,9] Apple vinegar is composed of 5% acetic acid and 0,35% malic acid.[10] It has good cost-effectiveness and is a biocompatible substance.[7] Its antimicrobial potential has already been demonstrated,[11] but little published data is available regarding its cleaning ability.

The apical region is the portion of the canal most difficult to be cleaned because of the difficult of debridement and its complex anatomy. The chelating agents, such as EDTA, has been shown to be effective in achieving smear-free walls, mainly at the middle and coronal thirds.[12,13,14] However, the cleaning action is reduced toward the apex and is less efficient in the apical region of the root canal.[1,12-17] This could be attributed to the narrow dimensions of the apical third, which can prevent the effective distribution of irrigants, resulting in limited contact between the canal walls and the solutions.[15]

Some substances or methods have been proposed to improve the penetration of irrigants into the apical third of the root canal, such as the addition of surfactants to irrigating solutions and the use of ultrasonics,[18] SmearClear (SybronEndo, Orange, CA) is a product indicated for smear layer removal, containing 17% EDTA with 2 additional surfactants. The use of ultrasonics has been suggested to improve irrigation in the root canal because it employs an acoustic streaming effect along the length of the oscillating file,[19] and this effect could be beneficial in transporting irrigating solutions to the apical portion of the root canal.[18]

Various studies have been published on the use of ultrasonics for root canal irrigation,[15,18,20-23] but only one study suggests the use of ultrasonic associated with SmearClear.[18] There is no study evaluating the effectiveness of the combined use of apple vinegar with ultrasonics for removal of the smear layer from the root canal.

Therefore, the purpose of this scanning electron microscopic (SEM) study was to evaluate the effectiveness of 17% EDTA, apple vinegar and SmearClear, with or without ultrasonic activation in the removal of the smear layer at the coronal, middle and apical thirds of the root canal.

Materials and methods

Seventy extracted fully developed human canines with a single straight root were stored in saline solution after collection. An approval for this study was obtained from Ethics Committee of Bauru Dental School – Process nº 180/2009. The teeth were radiographed to observe the pulp chamber and root canal morphology and were selected based on uniform root canal width as determined by buccolingual and mesiodistal radiographs. The teeth were decoronated and the working length was determined by inserting a #10 K-File until the tip of the file was visible at the apical foramen and deducting 1 mm from this length. Warm wax was then used to close the apical foramen. The teeth were instrumented using a crown-down technique with ProTaper Universal rotary files (Maillefer, Ballaigues, Switzerland) with a constant speed of 300 rpm using a gentle in-and-out movement up to the F5 file corresponding to a 50/.04 size/taper. Between every instrument change, irrigation with 1 ml of 2,5% NaOCl (Rioquímica, São José do Rio Preto, São Paulo, Brazil) was performed by using a disposable syringe with a 27-G needle. After instrumentation, the teeth were randomly divided into six experimental groups (n = 10) and

two control groups (n = 5) to achieve different final irrigation sequences: Group 1 (control) (SAL): 3 ml of saline for 3 minutes without ultrasonics; Group 2 (control) (SALUS): 3 ml of saline for 3 minutes, activating the solution in the first minute with ultrasonics; Group 3 (EDTA): 3 ml of 17% EDTA (Biodinâmica, Ibiporã, Paraná, Brazil) for 3 minutes without ultrasonics; Group 4 (EDTAUS): 3 ml of 17% EDTA for 3 minutes, activating the solution in the first minute with ultrasonics; Group 5 (AV): 3 ml of apple vinegar (Castelo, Jundiaí, São Paulo, Brazil) for 3 minutes without ultrasonics; Group 6 (AVUS): 3 ml of apple vinegar for 3 minutes, activating the solution in the first minute with ultrasonics; Group 7 (SC): 3 ml of SmearClear for 1 minute, according to the manufacturer´s instructions, without ultrasonics; Group 8 (SCUS): 3 ml of SmearClear for 1 minute with ultrasonics. After these procedures, all groups received a final flush of 5 ml of 2,5% NaOCl followed by 5 ml of saline. When the ultrasonic was used in the final irrigation sequence, it was activated using a finger spreader B (Dentsply, Maillefer, Ballaigues, Switzerland) adapted to the standard unit Jet Sonic (Gnatus, Ribeirão Preto, São Paulo, Brazil) at a power setting of 2. The finger spreader was placed in the center of the canal, avoiding the contact of the instrument to the canal walls. The root canals were then dried with absorbent paper points and the teeth were split open to expose the root interiors. Two longitudinal grooves were made in a buccolingual direction along the root surface with a carborundum disc at low-speed and a wedge was used to split the roots into two halves. The samples were dried, mounted on metallic stubs, coated with gold, and evaluated under a scanning electron microscope (JEOL, JSM T 220 A, Tokyo, Japan) at the coronal, middle and apical levels. Each radicular third of all samples was first viewed at a magnification of 500 X in order to obtain an overview of the region analyzed. Subsequently, an image acquisition on the most typical zones of the sample was performed at a magnification of 750X to assess the presence or absence of smear layer. Three pictures were obtained from each sample, one for each radicular third, for a total of 210 pictures. The amount of smear layer observed was scored as follows: 1 – no smear layer (Fig 1A); 2 – few areas covered by smear layer, with many dentinal tubule openings visible (Fig 1B); 3 – most areas covered by smear layer, with few dentinal tubule openings visible (Fig 1C); 4 – all areas covered by smear layer, no dentinal tubule openings visible (Fig 1D).

Three examiners performed the blinded evaluations separately, after the calibration, which consisted of examining a few images together. The intra and inter-examiner´s reliability was verified by using the Kappa test.

The smear layer scores were calculated between the groups using the Kruskal-Wallis and Dunn test. The use of ultrasonics was calculated and evaluated by the Mann-Whitney test. The Friedman test was used to compare the cleaning of the thirds of root canals. The level of significance was set at $p < 0.05$.

Results

Intra-examiner and inter-examiner agreements evaluated with the Kappa test showed satisfactory values of 0.74 and above for the different categories.

Table 1 shows the median and the mean rank of all groups of irrigation.

At the coronal third, there were significant differences ($p < 0.05$) between groups 3 (EDTA) and 1 (SAL); groups 4 (EDTAUS) and 1 (SAL); groups 7 (SC) and 1 (SAL); groups 8 (SCUS) and 1 (SAL).

At the middle third, the significant differences ($p < 0.05$) were observed in groups 4 (EDTAUS) and 1 (SAL); groups 4 (EDTAUS) and 2 (SALUS).

At the apical third, the results showed significant differences ($p < 0.05$) in groups 4 (EDTAUS) and 1 (SAL); groups 4 (EDTAUS) and 2 (SALUS); groups 7 (SC) and 1 (SAL); groups 8 (SCUS) and 1 (SAL).

When the irrigating solutions were analyzed without considering the use of ultrasonics, there were significant differences ($p < 0.05$) in the following comparisons: EDTA and saline; and SmearClear and saline in all thirds evaluated; apple vinegar and saline at the middle and apical thirds; and EDTA and apple vinegar only at the coronal third.

When comparing the effects of ultrasonic activation, there were no significant differences ($p > 0.05$) between the groups with and without the use of ultrasonics, in all radicular thirds.

When comparing the cleaning in the different radicular thirds, there was significant difference ($p < 0.05$) at the coronal and apical thirds, independent of the irrigation regimen used.

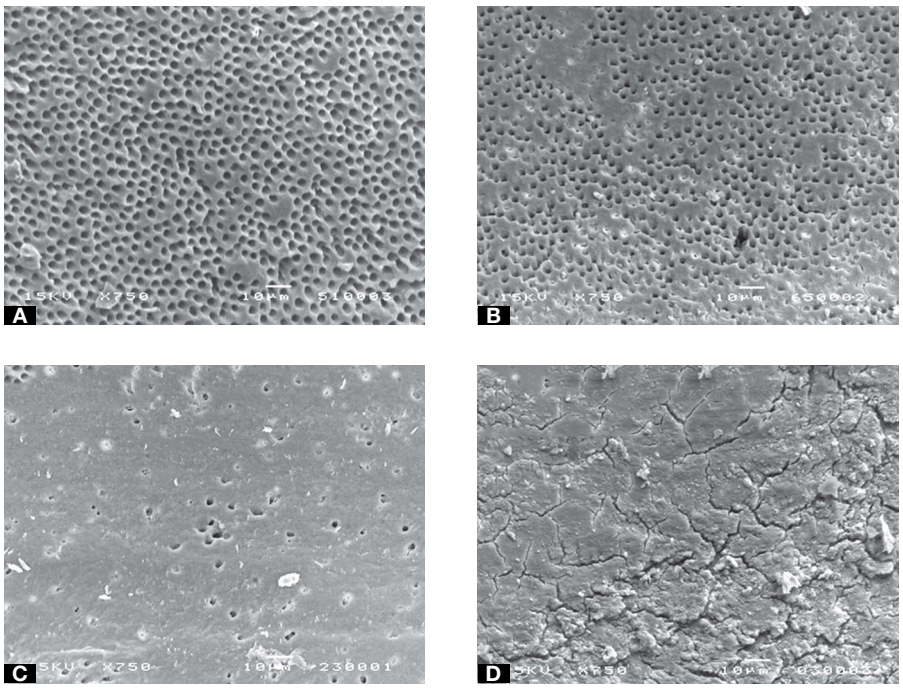

Figure 1. Representative SEM photomicrographs of specimens with score 1 (**A**), score 2 (**B**), score 3 (**C**) and score 4 (**D**) (750X).

Table 1. Median and the mean rank of all groups of irrigation.

Group	Cervical third		Middle third		Apical third		Nº specimens
	Median	Mean rank	Median	Mean rank	Median	Mean rank	
Saline solution	4.0	68.00	4.0	61.70	4.0	65.50	5
Saline solution + ultrasonics	3.0	46.40	3.0	58.80	4.0	58.50	5
EDTA	1.5	22.90	2.0	28.50	2.0	32.95	10
EDTA + ultrasonics	1.5	22.90	1.0	15.20	2.0	22.80	10
Apple vinegar	2.0	41.60	2.5	38.20	3.0	35.05	10
Apple vinegar + ultrasonics	2.5	39.40	2.0	37.70	3.0	41.35	10
SmearClear	2.0	32.30	2.0	33.60	2.0	26.65	10
SmearClear + ultrasonics	2.0	32.20	2.0	35.05	2.0	27.70	10

Discussion and Conclusion

In this SEM study, we attempted to evaluate methods to improve, especially in the apical third, the removal of the smear layer of prepared root canals. The results showed that EDTA could efficiently remove the smear layer from all canal thirds, whereas saline was not able to effectively remove the smear layer from any of the root canal's portions. Some authors[23,24,25] demonstrated that irrigation with EDTA is effective in removing the smear layer, which is in agreement with the finding of our study.

Reducing the surface tension of an endodontic solution improves its flow into narrow root canals.[26] The apical third is the most difficult portion of the root canal to be cleaned and this could be attributed to its narrow dimensions.[15] The results of this current study demonstrated that SmearClear had a better performance compared with EDTA in the apical third, but not statistically significant. In this study, SmearClear and EDTA had similar abilities to remove the smear layer from the root canal. These findings show that the addition of surfactants in SmearClear did not enhance the cleaning ability of the EDTA, which is in agreement with the findings of other studies.[17,18,25,27,28]

In our study we used apple vinegar as an experimental solution to possibly remove the smear layer in comparison with conventional chelators, such as EDTA. Apple vinegar has acids in its constitution,

especially acetic acid and malic acid.[10] Malic acid in apple vinegar confers its therapeutic properties.[11] Apple vinegar also has an antimicrobial potential against the endodontic microbiota.[11] In this study, apple vinegar was not able to completely remove the smear layer from the root canal, with significant differences between EDTA in the coronal third, and with some dentinal tubules remaining covered by a smear layer in all thirds. Besides, the pH of apple vinegar used in this study was 2.96, and it could cause damage on the root canal walls.

In analyzing the photomicrographs of all groups, we observed a better cleaning ability in the specimens with the use of ultrasonics, although there were no statistical differences between the groups with and without the ultrasonics. In our study, ultrasonic activation of irrigants did not improve smear layer removal and dentinal tubule opening, which is in accordance with the findings presented by other authors.[14,15,23,29] Ultrasonic activation in this study was performed during a 1 minute period. Cameron[20] reported better effects with ultrasonics when used for 3 minutes. Other researchers also achieved an effective smear layer removal when ultrasonic activation was performed for a longer period than 1 minute.[21,30] The diameter of the finger spreader used corresponded to a 25# file, and some authors[21] recommend the use of an instrument with a smaller diameter to avoid the contact of the instrument with the root canal walls.

Our results demonstrated that the removal of the smear layer was less effective in the apical third of the root canal, with a statistical difference in the coronal third, regardless of the irrigation regimen used. Torabinejad et al[6] attributed this fact to the inadequate penetration of the solution into the apical portion of the canal during the irrigation.

Based on the results of this experiment, we observed that the removal of the smear layer appears to be mostly influenced by the chemical action of the irrigating solution than the ultrasonic activation of irrigants.

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Comparison of the torsional fatigue resistance of PathFile nickel-titanium files with other small 0.02 mm taper files

Nicholas D. **SCHULTE**[1]
Timothy C. **KIRKPATRICK**[2]
John M. **YACCINO**[3]
Jon M. **DOSSETT**[4]

ABSTRACT

Objective: The purpose of this study was to compare the torsional fracture resistance of the following 0.02 mm taper files: PathFile size #13, #16, and #19, ProFile size #15 and #20, K3 size #15 and #20, Quantec LX size #15 and #20, and Liberator size #15 and #20. **Methods:** Eleven groups of files with 20 samples in each group were tested. The files were secured in the chucks of a torsiometer and rotated until fracture occurred. The maximum torque and degrees of rotation before fracture were recorded. Files of similar tip size were compared with one another for significant differences. One way ANOVA and Tukey's post hoc test were used to identify statistically significant ($p < 0.05$) differences between the groups. **Results:** The Liberator size #15 and #20 separated at significantly lower torque than all other similar sized files, while the PathFile size #16 separated at significantly higher torque than the size #15 files to which it was compared. **Conclusion:** The torsional fatigue resistance of PathFiles were better when compared to other small tip size #0.02 taper files.

Keywords: Endodontics. Fatigue. Rotary drilling.

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[1]DDS. Chief of Endodontics, RAF Lakenheath, UK.
[2]DDS. Program Director, Wilford Hall Endodontics Residency.
[3]DDS. Director Resident Education and Training, Wilford Hall Endodontics Residency.
[4]DMD. Director, Dental Biomaterials Evaluation USAF Dental Evaluation and Consultation Service.

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Contact address: Nicholas D. Schulte
Program Director, Endodontics Residency
Wilford Hall Ambulatory Surgical Center
59th Dental Training Squadron/SGDTN
2133 Pepperrell Street
Joint Base San Antonio - Lackland TX – USA – 78236

Introduction

Removal of bacteria and organic debris through chemomechanical cleaning and shaping of the root canal system is an essential step in root canal treatment.[1] Nickel-titanium (NiTi) rotary endodontic files are commonly used during root canal treatment and afford several advantages over stainless steel hand files. These advantages include the following: Improved ability to stay centered around curves, increased flexibility and torsional resistance, decreased transportation, ledging, zips and elbows and decreased instrumentation time.[2,3,4] Files occasionally separate in canals due to torsional or cyclic fatigue or a combination of the two.[5] If unable to be removed, the separated file can make further instrumentation of the canal difficult or impossible, especially if located in the apical third of the canal.[6] Small diameter files with small taper are more likely to fracture due to torsional fatigue than cyclic fatigue if the flutes of the file lock into dentin as the shaft continues to spin.[7,8]

It is important to create a glide path before instrumentation with rotary NiTi files to create a channel for the tips of the files to follow into the apical third of the root canal. This step has typically been accomplished using stainless steel hand files.[9] Most rotary systems recommend creating a glide path to at least a size #15 hand file before the use of NiTi rotary files.

The PathFile™ system (Dentsply Tulsa Dental Specialties, Tulsa, OK) has recently been introduced as a rotary method of establishing a glide path after the canal has been negotiated with a #10 hand file.[10] Berutti et al[11] reported that inexperienced clinicians produced more conservative glide paths with PathFiles than experienced endodontists with stainless steel hand files in plastic blocks.

The PathFile technique involves inserting rotary files to working length very early in the instrumentation sequence. While this has the potential of decreasing the time and effort required to instrument canals, these files could have sufficient engagement of the root canal wall which may exceed their threshold for fracture, especially in constricted canal spaces. A small separated instrument early in the shaping and cleaning process may inhibit the clinician from further access to the apical third of the root canal and may have a negative impact on the prognosis, especially in infected canals.[12] In addition to the PathFiles, there are several other 0.02 mm taper NiTi rotary file systems available with small diameter files. Even though these files have not been specifically made for the creation of a glide path, it is conceivable that they may be able to function in a similar manner to the PathFiles. To date, the torsional resistance to fracture of these various 0.02 mm taper small diameter files has not been compared. Therefore, the purpose of this study is to compare the torsional resistance of the following 0.02 taper files: PathFile size #13, 16, and 19 (Dentsply Tulsa Dental Specialties, Tulsa, OK), ProFile™ size #15 and 20 (Dentstply Tulsa Dental Specialties, Tulsa, OK), K3™ size #15 and #20 (SybronEndo, Orange, CA), Quantec LX™ size #15 and #20 (SybronEndo, Orange, CA), and Liberator™ size #15 and #20 (Miltex, York, PA).

Material and Methods

Torsional testing was accomplished in accordance with ANSI 58[13] and ISO 3630-1[14] standards using a torsiometer (Torsiometer/Memocouple, Maillefer, Ballaigues, Switzerland). Prior to file placement in the torsiometer, the handle was removed with wire cutters at the point where the handle was attached to the instrument shaft. The shaft end was then secured in the chuck of the torsiometer which was connected to a reversible, microprocessor-controlled rotating motor. The file's terminal three millimeters were secured into a digital torque meter. The NiTi files were then rotated in a clockwise direction as viewed from the shank end at a speed of 2 rotations per minute. The maximum torsional force and degrees of rotation at the moment of file fracture were recorded.

Eleven groups of 20 files each were tested. All files were 0.02 taper. Group 1: PathFile #13, Group 2: PathFile #16, Group 3: PathFile #19, Group 4: ProFile #15, Group 5: ProFile #20, Group 6: K3 #15, Group 7: K3 #20, Group 8: Quantec LX #15, Group 9: Quantec LX #20, Group 10: Liberator #15, and Group 11: Liberator #20.

Two variables were compared among the groups of files; maximum torque before fracture and degrees of rotation before fracture. One way ANOVA and Tukey's post hoc test were used to identify statistically significant ($p < 0.05$) differences between the groups. Files of similar tip size were compared with one another for significant differences. The size #13 and 16 PathFiles were compared with the size #15 group. The size #19 PathFiles were compared with the size #20 group.

Results

The results of the torsional testing are presented in Figure 1. The Liberator size #15 and #20 separated at significantly lower torque than all other similar sized files, while the PathFile size #16 separated at significantly higher torque than the size #15 files to which it was compared. The comparison of degrees of rotation before fracture is presented in Figure 2.

Discussion

Small stainless steel hand files are often used for creation of a glide path to size #15 or #20 before the use of rotary files. Allen et al[15] evaluated multiple types of hand files for this purpose. They compared the geometry, stiffness, efficiency and deformation during canal negotiation in plastic blocks and concluded that pitch, taper, cross-section, heat tempering, metal type, tip geometry, and operator skills all influence pathfinder efficiency when using hand files. When using PathFiles, the manufacturer recommends that the canal should be instrumented to working length to at least a #10 file before using PathFiles. It is therefore useful to consider these factors in selection of small hand files.

Pasquelani et al[16] conducted a study comparing PathFiles with stainless steel K files for the creation of a glide path using spiral computed tomography. They found that PathFiles stayed significantly more centered in the canal and created significantly less transportation in extracted human molars with moderate to severe curvature when compared with stainless steel hand files.

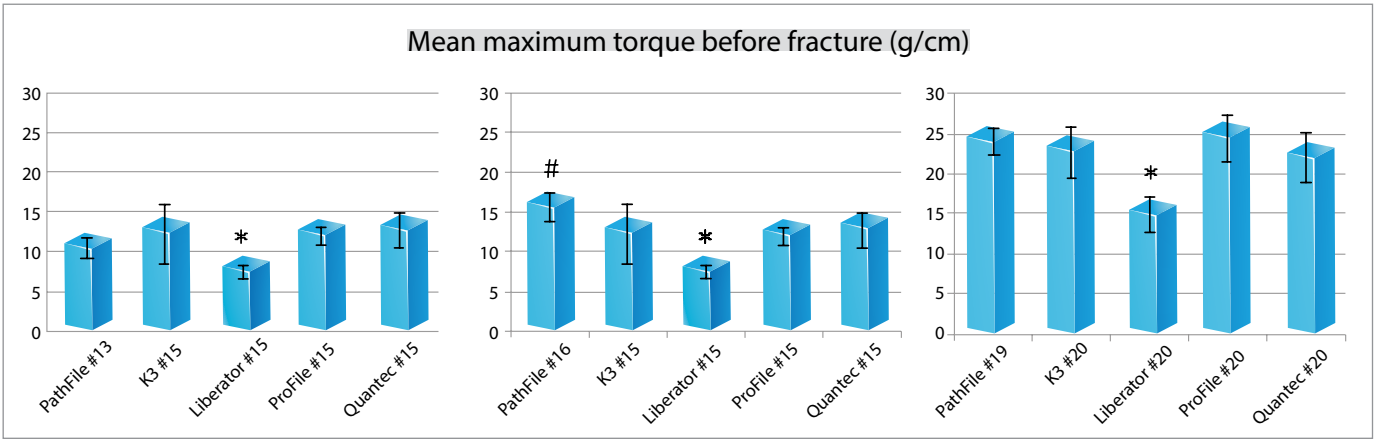

Figure 1. Mean maximum torque before fracture (g/cm). The asterisks indicate the file separated at significantly lower torque than all other files in the grouping. The (#) indicates the file separated at significantly higher torque than all other files in the grouping ($p < 0.05$).

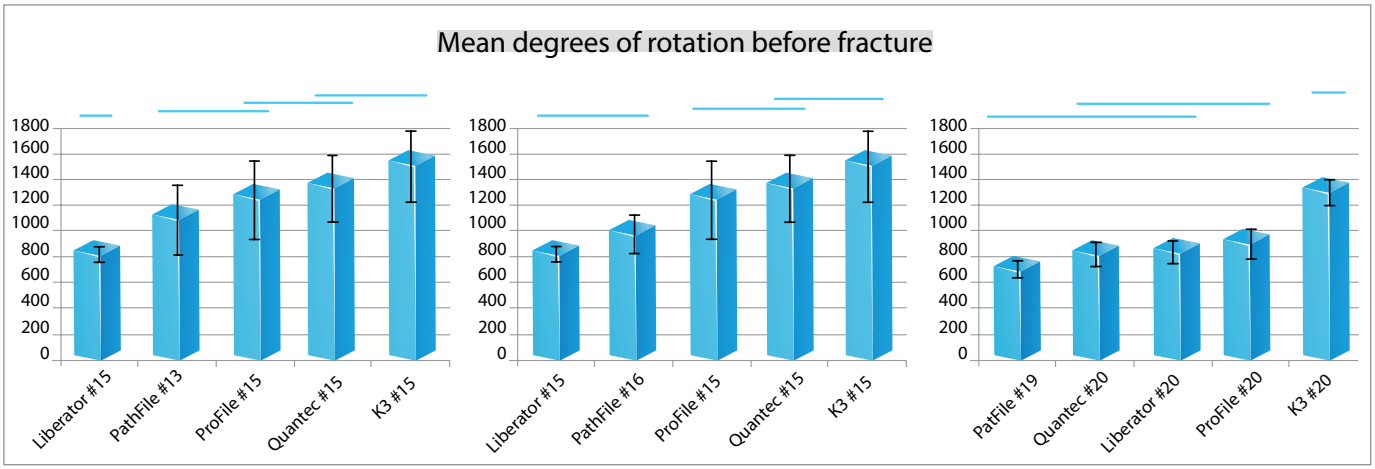

Figure 2. Within each grouping, the horizontal lines represent statistically similar groups. Two files are statistically different if they are not under the same horizontal line ($p < 0.05$).

Berutti et al[17] recently conducted a study showing that the creation of a glide path with PathFiles significantly reduced undesirable canal modifications when used in conjunction with the WaveOne system in plastic blocks.

The results of the present study indicate that rotary NiTi files with similar tip size and taper separated at a comparable torque, except for the Liberator groups. This result may be due to less bulk of metal at the breaking point of the Liberator compared to the other files or the specific type of NiTi metal used in the manufacturing process. Although there were significant differences in the degrees of rotation before fracture between some of the groups, the difference in rotation before fracture may not be clinically relevant as even the best group would have separated in less than 1 second spinning at 300 rpm with the file tip bound in dentin.

Using small rotary files to working length early in the shaping and cleaning process should be performed with caution as the torque required to break these files is very low. Many constricted canals are difficult to negotiate with a size #8 or #10 hand file, and small rotary files would surely encounter significant stress in tight canals. File separation due to torsional fracture early in the process could prevent further shaping and cleaning of the root canal space and could have a negative impact on outcome, especially in infected canals. Even though they are not currently marketed for development of a glide path, based on findings in this study, the 0.02 mm taper #15 and #20 K3, ProFile, and Quantec LX files may also be reasonable choices for development of a rotary glide path along with the PathFiles instruments. Although there was very little difference between the fracture resistance of the PathFiles and the size #15 and #20 NiTi rotary files compared in this study, the tip sizes of the PathFiles may offer a significant advantage when instrumenting tight canals. There is a 50% increase in size from a #10 to a #15 file whereas with PathFiles, the increase from #10 to #13 is only 30%. This smaller incremental increase may decrease binding of the file while developing a rotary glide path and help minimize file separation. This smaller percentage increase in size is also seen with the progression from the size #13 to #16 and from the size #16 to #19 PathFiles. Further research is needed concerning the use of small NiTi rotary files for the establishment of a glide path to determine their safety in clinical scenarios.

Conclusion

Under the parameters of this study, PathFiles were better when compared with other NiTi rotary files of similar diameter and taper.

Acknowledgment

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Human enamel colonization of *Candida albicans*

Tatiana Teixeira de **MIRANDA**[1]
Leonardo **RODRIGUES**[2]
Alexsander Ribeiro **PEDROSA**[3]
Carlos Augusto **ROSA**[4]
Ary **CORREA JUNIOR**[5]

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 °C, 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.

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[1]Post-Doc in Endodontics, Forsyth Institute. PhD in Microbiology, UFMG.
[2]Post-Doc in Phytopathology, UFMG.
[3]MSc in Science and Technology of Mineral and Material Radiation, Dentistry, UFMG.
[4]Post-Doc in Mycology, University of Western Ontario. Adjunct Professor, Department of Microbiology, UFMG.
[5]Post-Doc in Mycology, Cornell University. Associate Professor, Department of Microbiology, UFMG.

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Contact address: Tatiana Teixeira de Miranda - The Forsyth Institute - 245 First Street, Cambridge, MA, EUA 02142

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 mm^2 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 surface[12] 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

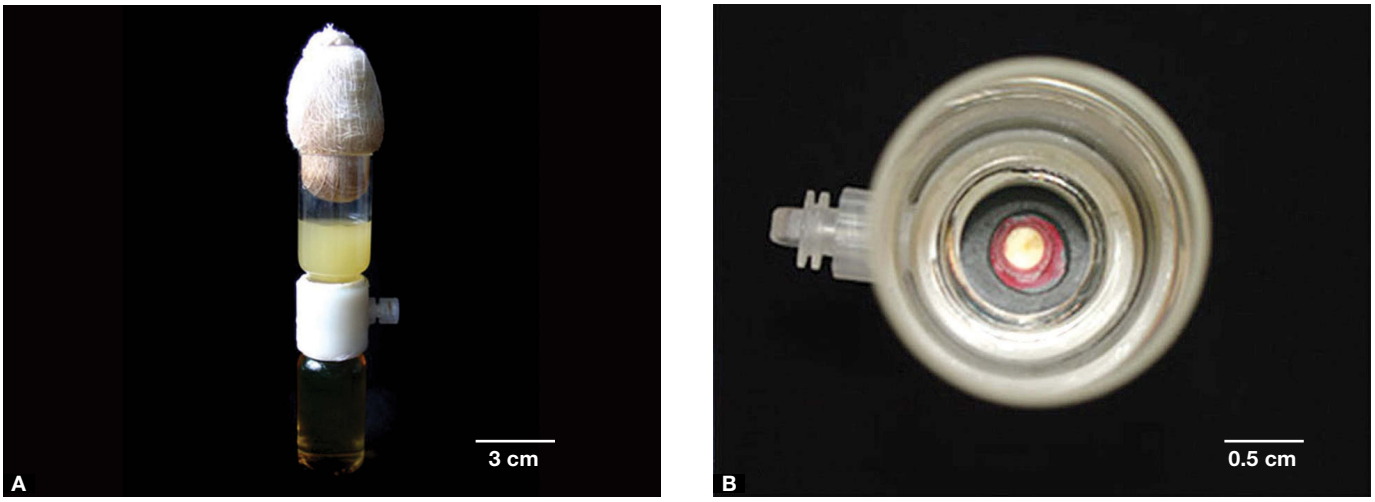

Figure 1. A) Side view of the apparatus for assessing dentinal colonization by *Candida albicans* 1015. **B**) Top view of an acrylic disc containing a dentinal fragment in its central portion.

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.[15] 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.

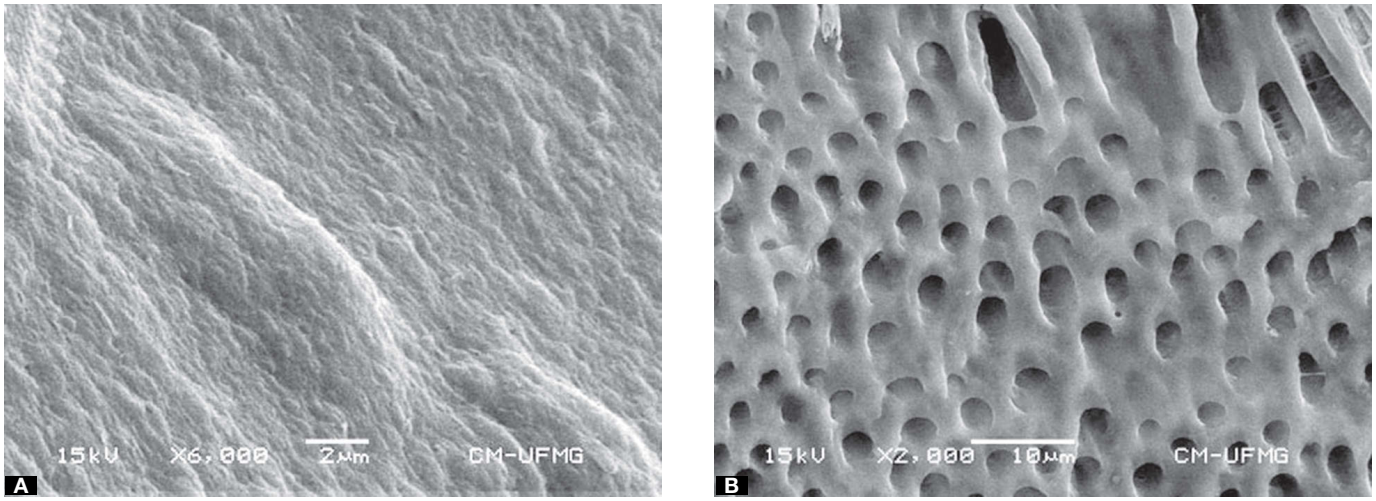

Figure 2. Dentinal fragments used as negative controls. **A**) Without smear layer. **B**) With smear layer.

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

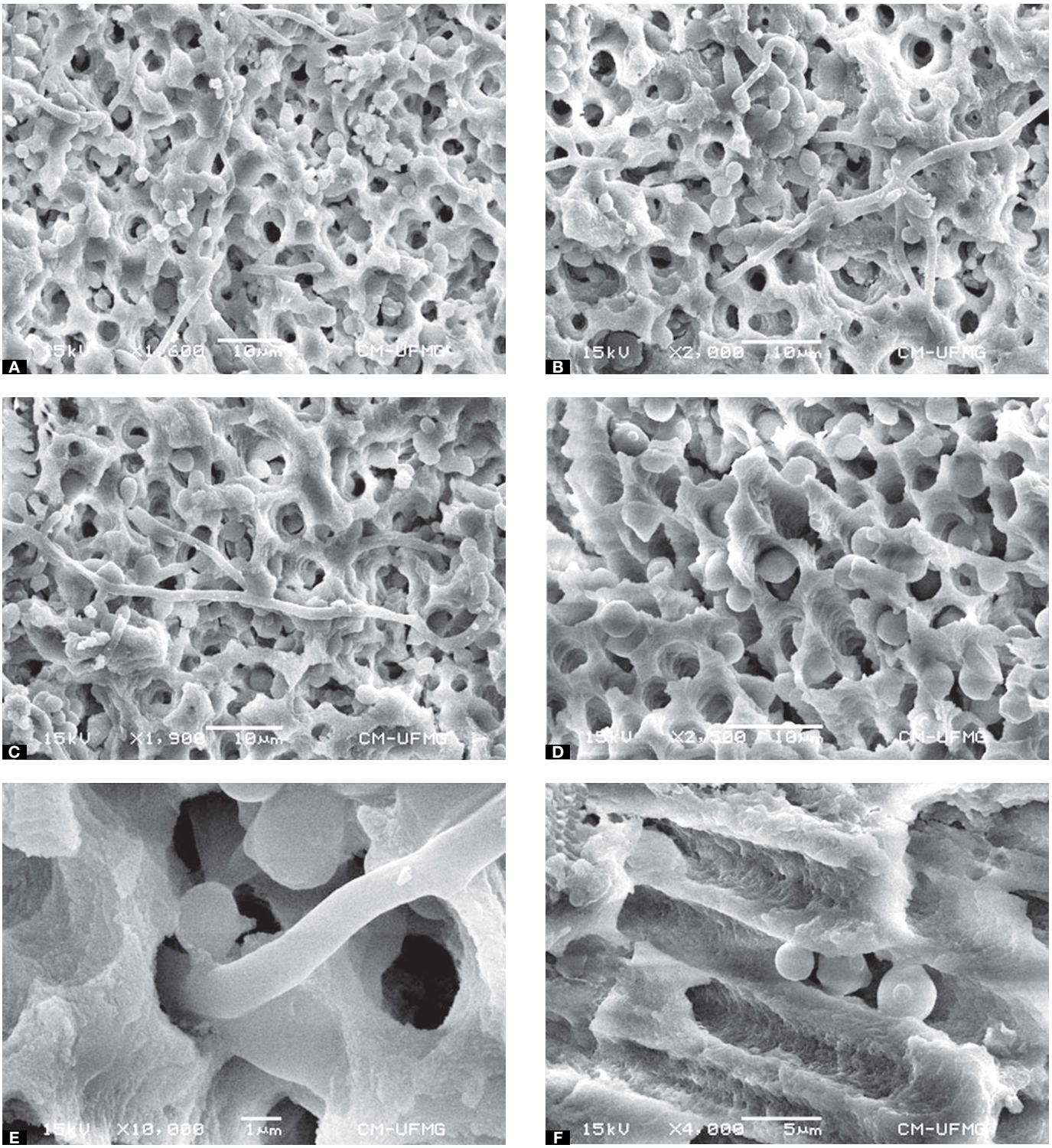

Figure 3. Electron micrograph showing colonization of dentinal tubules by C. *albicans* 1015 in dentin samples with the smear layer. **A**, **B**, **C**) Yeast cells and hyphae in the superficial dentin layer. (**D**) Yeast cells in dentinal tubules in the inner dentin layer. (**E**) Penetration of hyphae in dentinal tubules. (**F**) Yeasts attached to the dentinal tubule wall.

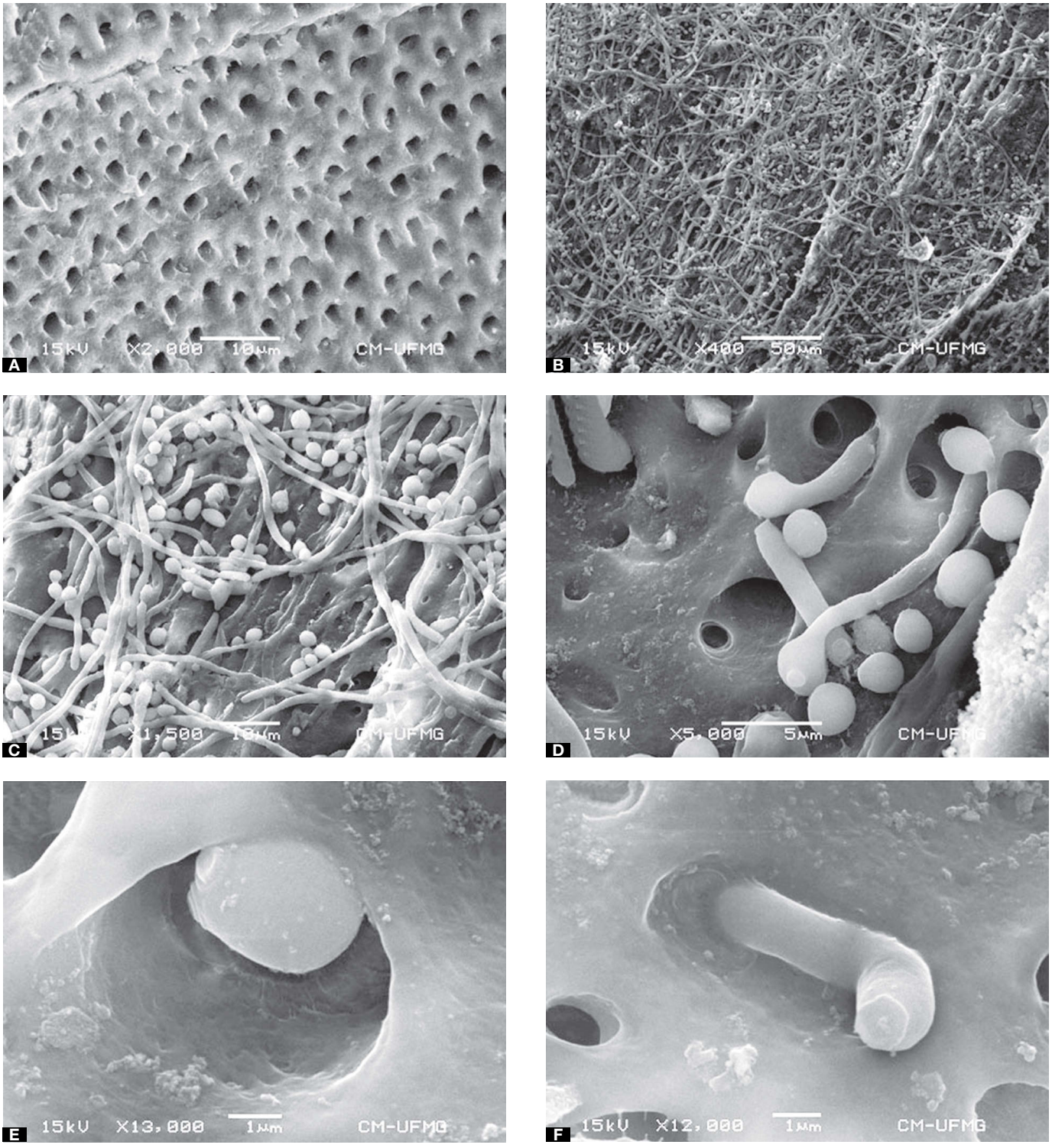

Figure 4. Electron micrograph showing C. *albicans* 1015 colonizing the dentinal tubules in dentin samples without the smear layer. **A**) Dentin sagittal section showing the absence of the smear layer in dentinal tubules (Negative control). **B**, **C**) Yeast cells and hyphae on the superficial dentin 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.[4] 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.[7,8] 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 findings[18,20] suggest that the yeast-hypha transition is required for *C. albicans* virulence. However, some studies[6,19,27] highlight that the yeast morphology that is observed in both superficial and inner most 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 Ca^{+2} ions and collagen. In the present study, removal of the smear layer did not affect microbial adhesion, suggesting that Ca^{+2} 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 periradicular infections.

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

Jefferson J. C. **MARION**[1]
Frederico Campos **MANHÃES**[2]
Tainá Celoria **BORTOLUZZI**[3]
Thaís Mageste **DUQUE**[4]

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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[1]PhD Student in Dental Clinic - State University of Campinas- UNICAMP, Piracicaba, SP, Brazil, Endodontic Department ABO, Maringá, PR, Brazil; Endodontic Department Ingá College – UNINGÁ, Maringá, PR, Brazil.

[2]PhD Student in Dental Clinic- State University of Campinas- UNICAMP, Piracicaba, SP, Brazil,

[3]Graduated in Dentistry – UNINGÁ, Maringá, PR, Brazil.

[4]PhD Student in Dental Clinic - State University of Campinas- UNICAMP, Piracicaba, SP, Brazil.

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Contact address: Jefferson José de Carvalho Marion
Rua Néo Alves Martins, 3176 – 6º andar – sala 64 – Centro
CEP: 87.013-060 – Maringá/PR - Brazil
Email: jefferson@jmarion.com.br / atendimento@jmarion.com.br

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.[1] According to Dotto et al,[2] 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.

Specifics studies show that bacteria represent one of the main factors of the pulp changes,making it necessary to use antimicrobial agents during endodontic therapy.[3] However, other studies show that dental trauma, extensive restorations and periodontal lesions also contribute to these pulp changes.[4,5,6]

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 presents 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[7] and potentially after the complete filling.[8]

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.[9] 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, polyethyleneglycol, propylene glycol, parachlorophenol, olive oil, lipiodol.[10]

Different methods have been described to carry the paste to the root canal. Some include the use of syringes with needles of different calibres,[11] guns[12] or amalgamators with endodontics condensers.[13] Others use Lentulo, McSpadden or similar,[14] endodontic instruments and gutta-percha.[15]

Despite the calcium hydroxide being used since 1920, its mechanism of action was first described by Holland et al,[16] 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, [17] in 1991, 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).[18]

Therefore this study was conducted using clinical case reports, in order to verify the effectiveness of the intracanal medication with calcium hydroxidein 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 radiolucent, 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, beside 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 radiolucent 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).

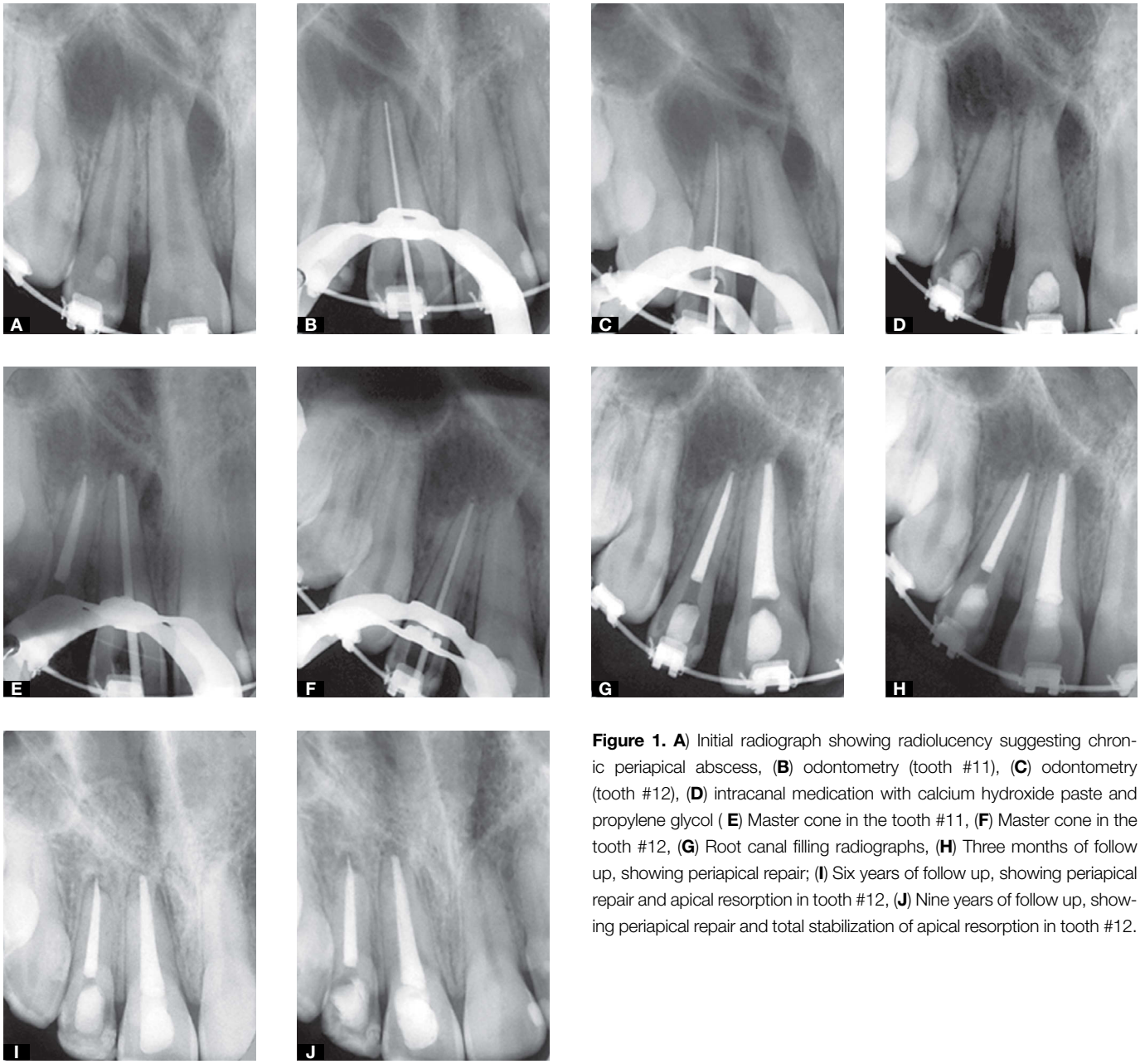

Figure 1. A) Initial radiograph showing radiolucency suggesting chronic periapical abscess, (**B**) odontometry (tooth #11), (**C**) odontometry (tooth #12), (**D**) intracanal medication with calcium hydroxide paste and propylene glycol (**E**) Master cone in the tooth #11, (**F**) Master cone in the 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.

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).

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]

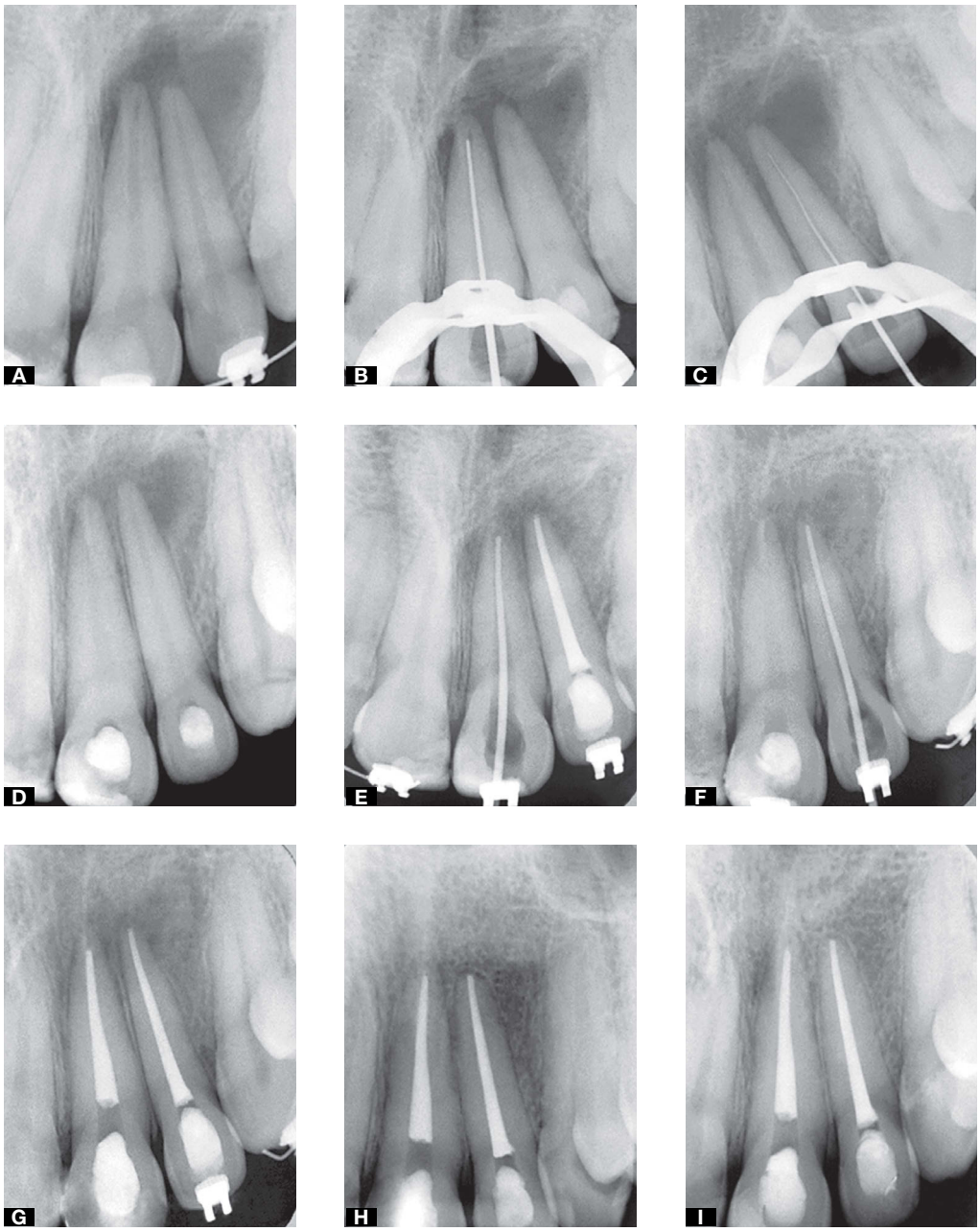

Figure 2. A) initial radiograph showing periapical radiolucent suggesting chronic abscess, (**B**) odontometry (tooth #21), (**C**) odontometry (tooth #22); (**D**) intracanal medication of calcium hydroxide paste with propylene glycol and partially reabsorbed, (**E**) gutta-percha points (tooth #22), (**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 canal[25] 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 Nakayama[28] 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.

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

Marili Doro Andrade **DEONIZIO**[1]
Alexandre **KOWALCZUCK**[2]

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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[1]PhD in Endodontics, USP. Adjunct Professor III, Dentistry, UFPR.
[2]Specialist in Endodontics, UFPR. Professor, Specialization courses in Endodontics, UFPR.

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Contact address: Marili Doro Andrade Deonizio
Av. Anita Garibaldi, 964 – 1403B – CEP: 80540-400 – Curitiba/PR, Brazil
E-mail: marilidoro@ufpr.br

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 sensitiveness 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® - Super Bonder Precisão - Henckel Ltda, São Paulo, SP, Brazil) was applied

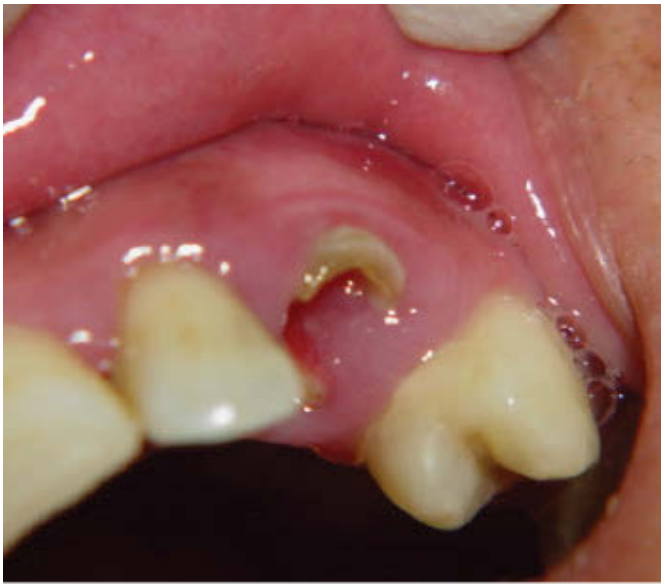

Figure 1. Clinical aspects of tooth #23.

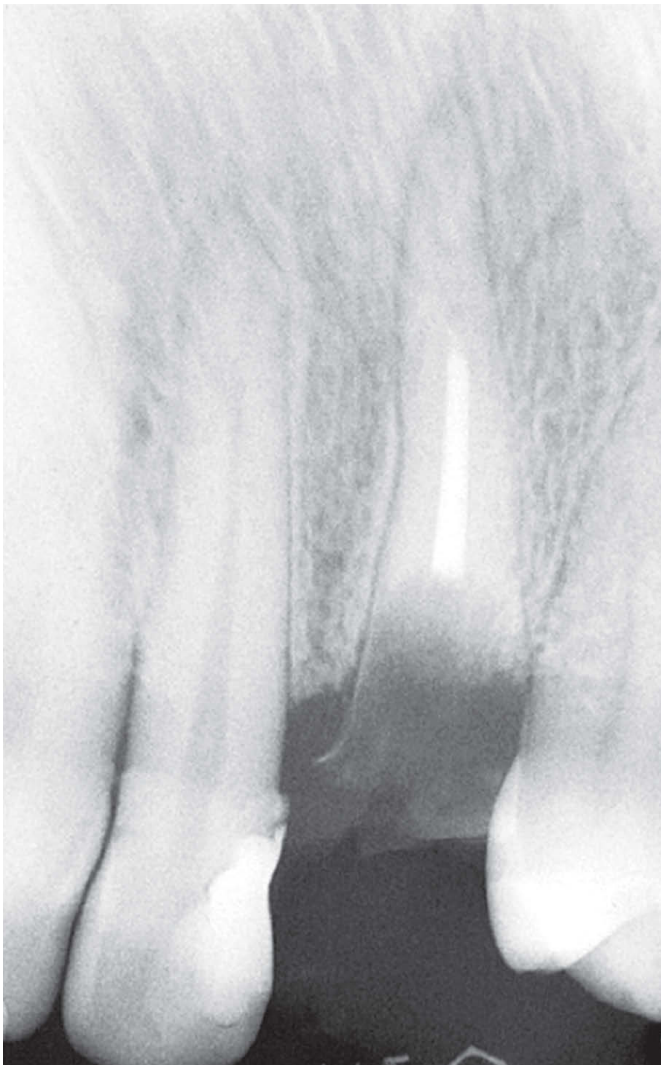

Figure 2. Radiographic aspects of tooth #23.

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,[6] 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.

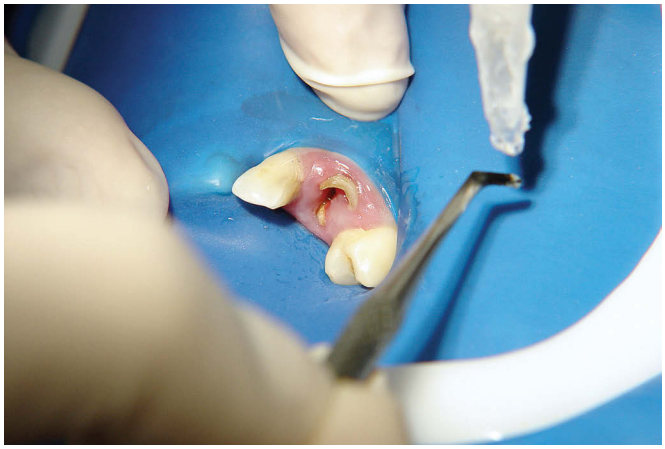

Figure 3. Applying ethyl cyanoacrylate between the rubber dam and the periodontium.

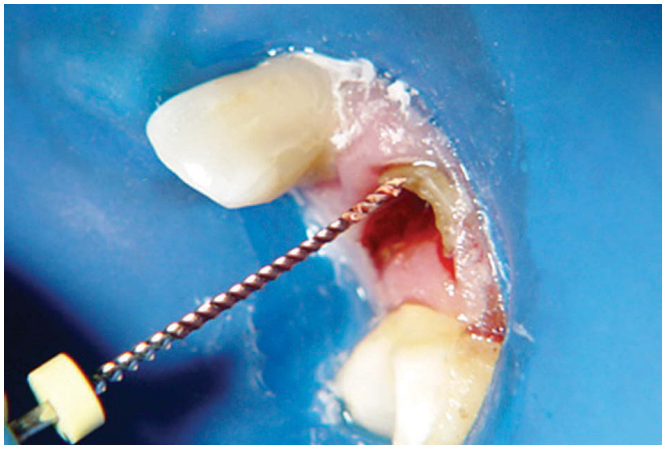

Figure 4. Removing of the filling material.

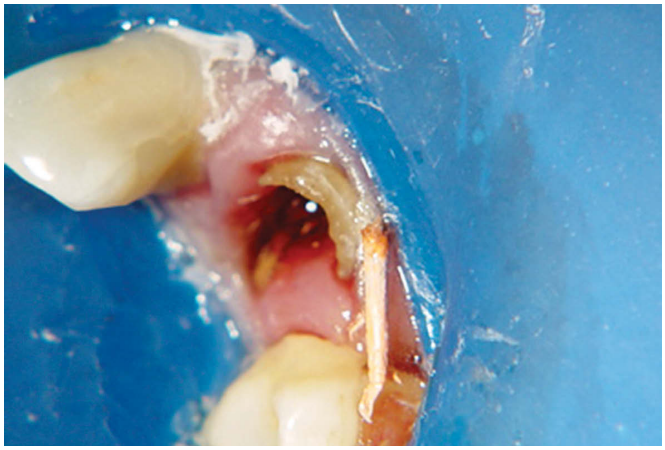

Figure 5. The filling material removed.

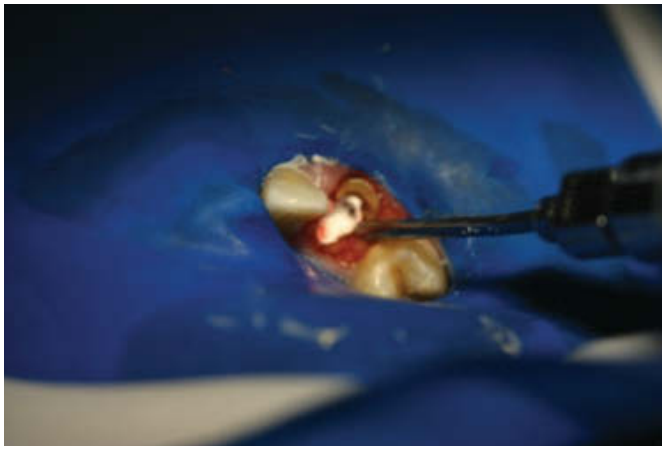

Figure 6. Zinc-oxide cement sealing the cavity.

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.[3] 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.[3]

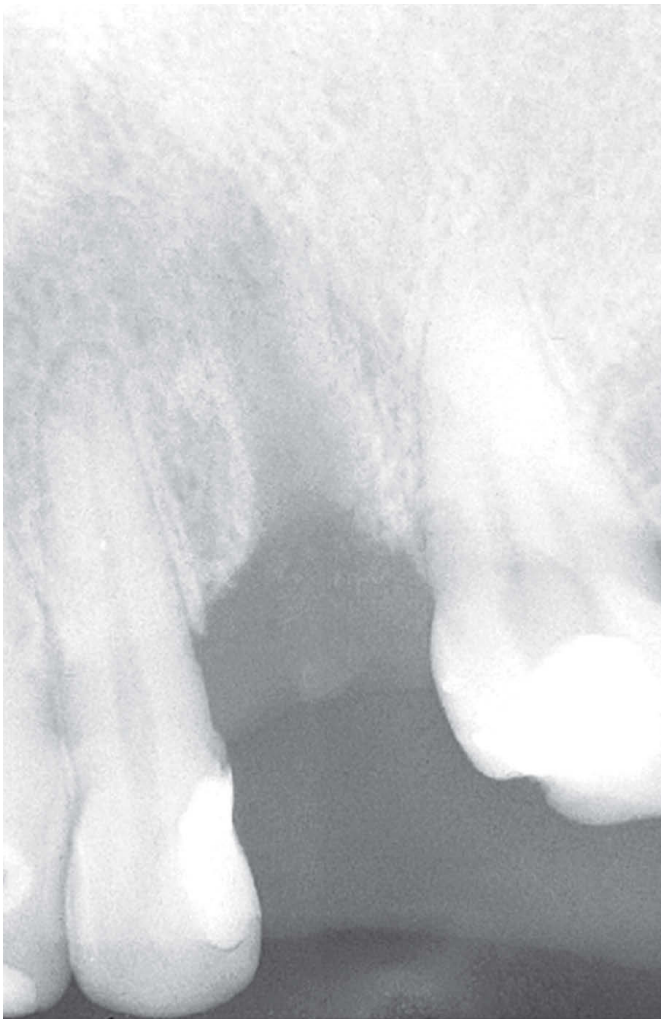

Figure 7. Radiograph after root extraction.

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[3] in order to avoid complications.

There are no restrictions regarding the use of vasoconstrictors, which provide more time and comfort for endodontic emergency procedures.[4,7] In infiltrative and intraligamentar anesthesia techniques, prior administration of coagulation factors is not necessary. The administration is recommended for the inferior alveolar nerve blocking,[8] due to the possibility of bleeding in the retromolar region, with the presence of trismus and the risk of asphyxia.[4] The administration of an antifibrinolytic was beneficial because it prevented excessive bleeding following trauma to the hyperplasic gum tissue.[7]

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.[9]

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.[10]

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.[4,11]

In the treatment of patients with hemorrhagic disorders, the interaction between professional and hematologist is a pre-requisite for safe procedures.[11]

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.

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Maxillary first premolar with three roots: Case report

Fausto Rodrigo **VICTORINO**[1]
Christine **MEN-MARTINS**[2]

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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[1]Professor of Endodontics, CESUMAR.
[2]MSc in Dentistry, UNESP. PhD student in Dental Sciences, UNESP.

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Contact address: Fausto Rodrigo Victorino
Rua Formosa, 489 – Centro – Marialva/PR – CEP: 86.990-000 – Brazil

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, a 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 al[15] 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 al[16] 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

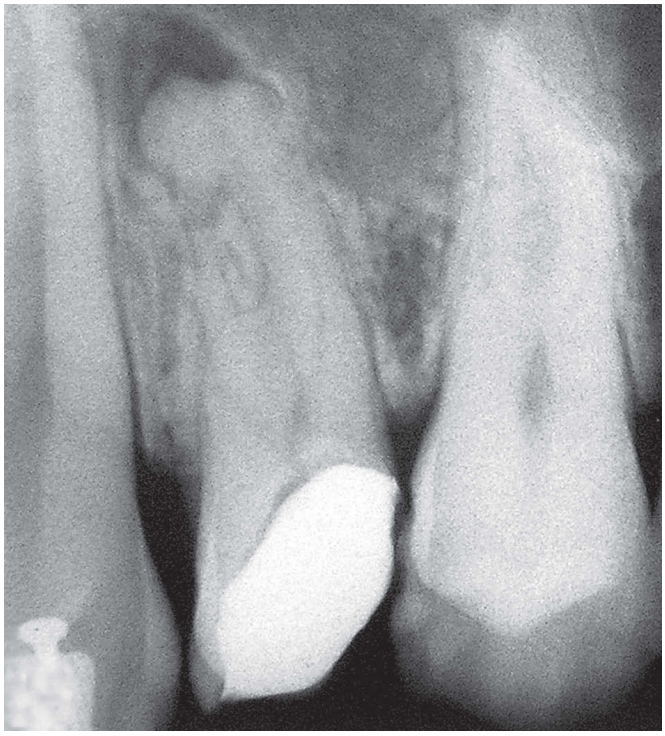

Figure 1. Initial radiographic examination of tooth #14, suggesting trirradicular premolar.

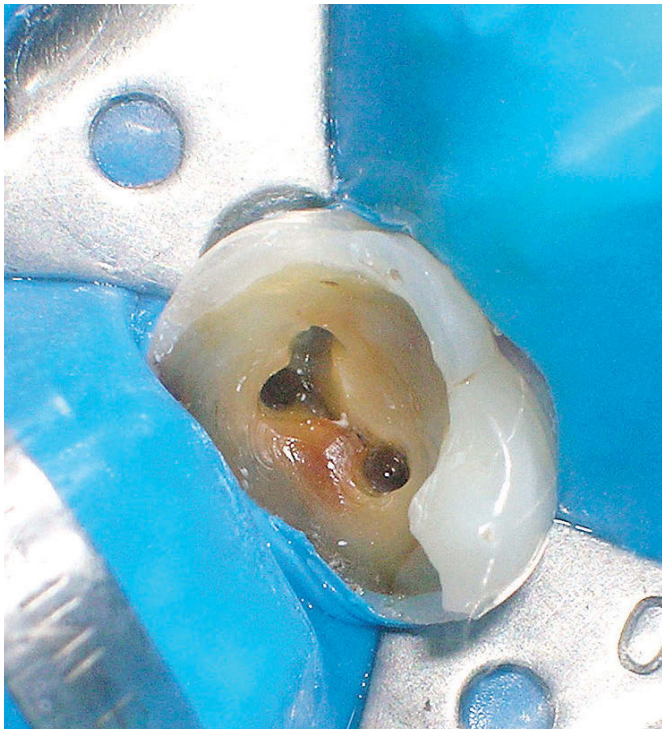

Figure 2. Triangular shape and presence of three roots.

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,[1,8,9,12,14,18,20] 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.[8]

Over the years, literature has reported the most varied anatomical changes of several teeth, including premolars.[1] These variations may be related to the number of root canal, its size, shape, divisions, fusions, directions and stages of development.[9]

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.[7] 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.[7,9,12] These roots may be well developed, not entirely separate or as a single and large root, with two canals in its interior.[7,9] The outline of the pulp chamber has an elliptical morphology, due to the mesiodistal flattening.[9]

However, the amount of roots and canals can vary in this dental group,[9,12,14,18,20] since the buccal root may divide into two,[7] evidencing mesiovestibular and distobuccal roots. In this case, the premolar may be

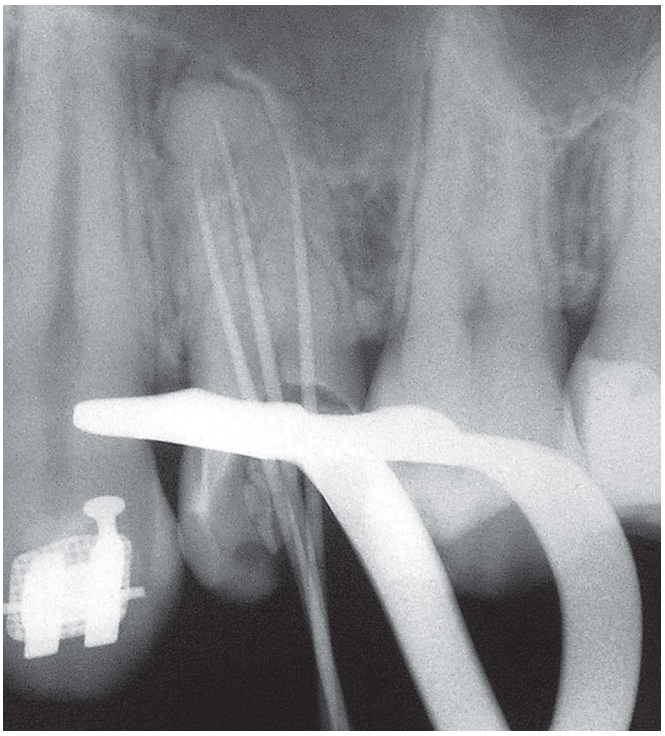

Figure 3. Root canal length, showing the presence of three root canals.

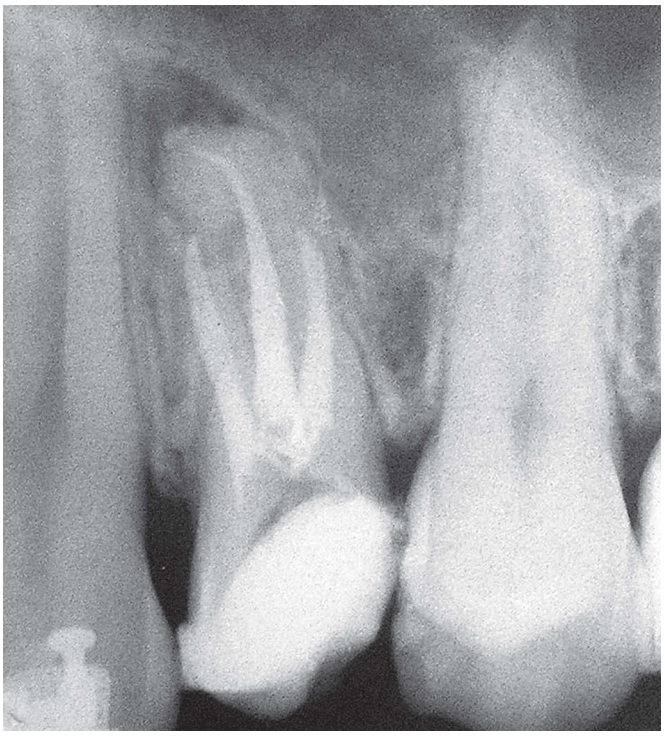

Figure 4. Final aspect of tooth #14 endodontic treatment.

referred to as a "minimolar".[12] According to Bellizzi and Hartwell,[2] 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[7,8,9] 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,[7,8,9] 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.[7,8,9,11]

The intracanal medication has the function of eliminate remaining microorganisms and prevent recontamination, prevent or reduce periapical inflammation, solubilize organic matter, neutralize toxins, control persistent exudation, control external inflammatory resorption and stimulate repair.[7,8,9] 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,[5-9,13,17] which justified the permanence of calcium hydroxide inside the canals for a month in this case presented.

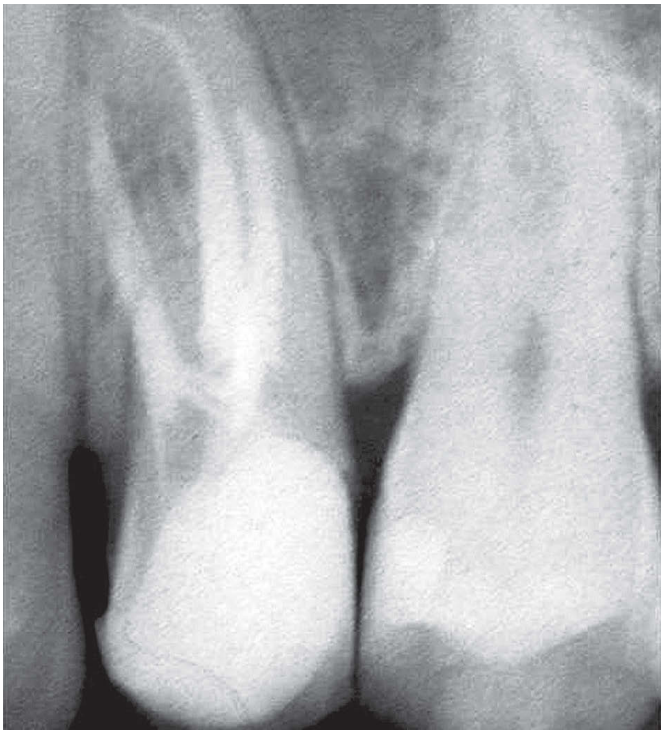

Figure 5. Radiographic control of endodontic treatment after 2 years, with appearance of normality.

The biological purpose of root canal filling is to provide favorable conditions for the body to repair tissue with no risk of relapse[3,7,8,9] and the used calcium hydroxide-based sealer (Sealer 26®) seems to achieve this goal[3] (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.

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Late treatment of dental trauma using apexification technique

João Eduardo **GOMES-FILHO**[1]
Camila Campos **MENDES**[2]
Simone **WATANABE**[3]
Carolina Simonetti **LODI**[4]
Marcelle **DANELON**[5]
Elói **DEZAN JÚNIOR**[6]
Mauro Juvenal **NERY**[6]
Luciano Tavares Angelo **CINTRA**[7]

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 coronary 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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[1]Full Professor, UNESP. Post-Doc in Endodontics, University of Michigan.
[2]Graduated in Dentistry, UNESP.
[3]PhD in Pediatric Dentistry / Endodontics, UNESP.
[4]Post-Doc in Pediatric Dentistry, UNESP.
[5]PhD Student in Pediatric Dentistry, UNESP.
[6]Full Professor and PhD in Endodontics, UNESP.
[7]Post-Doc in Endodontics, UEM.

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Contact address: João Eduardo Gomes-Filho
Endodontia – Faculdade de Odontologia de Araçatuba, UNESP
R. José Bonifácio, 1193 – Araçatuba/ SP – CEP: 16015-050 – Brazil
Email: joao@foa.unesp.br

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.[1] 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.[2] 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.[3] Several studies show that the best option is filling the root canal temporarily with medicinal substances, thus inducing the apical closure.[4,5,6] Zinc oxide and eugenol paste have been advocated for this purpose,[7] as well as the polyantibiotic[8] and iodoformized paste.[9]

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.[10]

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.[11-19]

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, Califórnia, 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

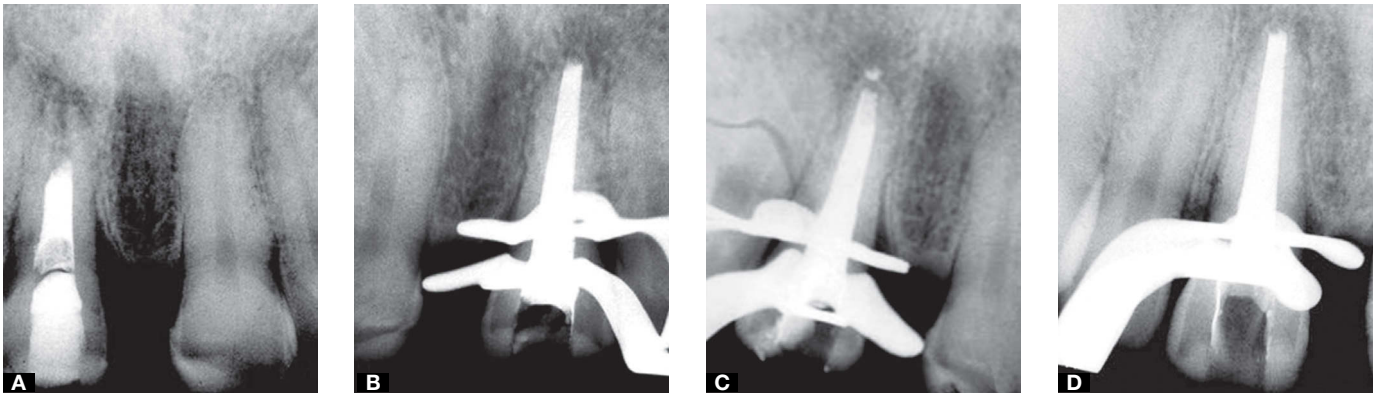

Figure 1. Periapical radiographs of the upper right central incisor. **A**) Diagnostic radiographic exam. Note the radiopaque intracanal dressing partially filling the root canal and the provisional restoration with radiopaque material and incomplete root formation. **B**) Radiographic exam performed 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.

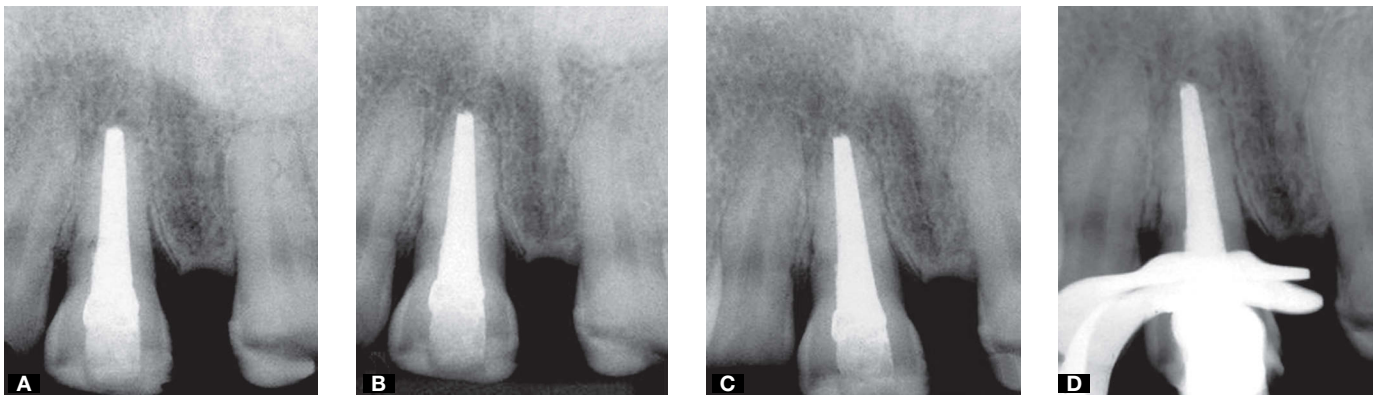

Figure 2. Periapical radiographs of the upper right central incisor. **A**) Radiographic exam at the fifth appointment, in the end of the treatment, consisting of careful biomechanical preparation and change of intracanal dressing with calcium hydroxide paste and iodoform. **B**) Radiographic exam at 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]

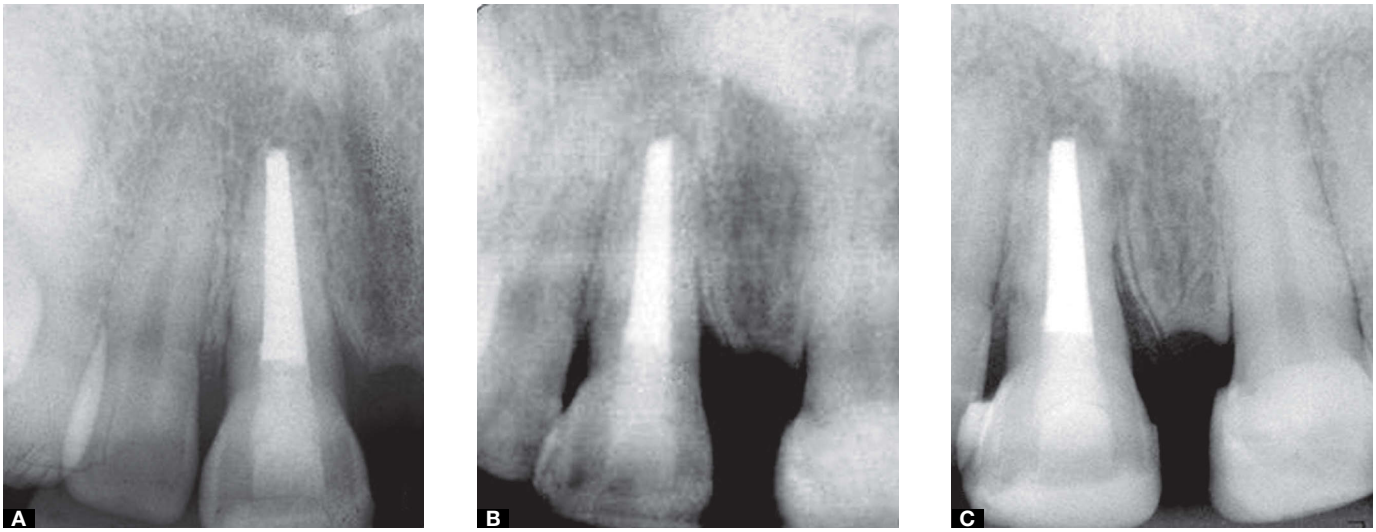

Figure 3. Periapical radiographs of the upper right central incisor. **A**) Radiographic exam at the eighth appointment, at the end of the obturation by the lateral condensation and Sealapex. **B**) Radiographic exam for 6-month postoperative control; note the total remission of the lesion and apical 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.[14,23,24,25]

As to the period of time to the exchange of calcium hydroxide, to induce apical closure and normal periapical tissues,[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.[20] Other authors believe that a single application of calcium hydroxide paste is sufficient to form the barrier.[11] Another indication is to change only if there is reabsorption of calcium hydroxide in the apical third of the root canal.[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.[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[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.[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.[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.

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Evaluation of response to pulp sensitivity test with cold in teeth with non-carious cervical lesion

Camile Dias **BEVILAQUA**[1]
Matheus **SOUZA**[2]
Fabiana **ZANETTE**[3]
Doglas **CECCHIN**[4]
Ana Paula **FARINA**[5]

ABSTRACT

Objective: To evaluate the response to cold pulpal sensitivity test in teeth with loss of dentinal structure by non-carious cervical lesion. **Methods:** Eighteen patients from School of Dentistry of University of Passo Fundo were selected for the present study. In these patients, were analyzed forty single-rooted teeth which filled the inclusion criteria, being divided into two groups: G1 = 20 teeth showing non-carious cervical lesions; and G2 (control) = 20 teeth without loss of dentinal structure. The patients were instructed regarding to pain level, through a visual analog scale which classified the painful response in mild, moderate and severe. From the obtained information, the data were statistically analyzed using nonparametric Kolmogorov-Smirnov test at 5% significance level. **Results:** The results of the present study have not showed statistically significant difference between Group 1 and Group 2, regarding to response to pulp sensitivity test with cold ($p < 0.05$). **Conclusion:** It was concluded that teeth with non-carious cervical lesion can demonstrate different levels of response to cold pulp sensitivity test, suggesting that teeth with loss of dentinal structure can or cannot show a response related to pulp sensitivity.

Keywords: Tooth abrasion. Tooth erosion. Dental pulp. Dental pulp test.

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[1]Graduated in Dentistry, Passo Fundo University (UPF).
[2]Professor of Dentistry, UPF.
[3]MSc in Endodontics, ULBRA.
[4]Professor of Endodontics, UPF. Post-Doc in Endodontics, UNICAMP.
[5]Professor of Dental Prosthesis, UPF. Post-Doc in Dental Prosthesis, UNICAMP.

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Contact address: Matheus Souza
Rua Fagundes dos Reis, 342 – Passo Fundo/RS – Brazil
CEP: 99.010-070 – matheus292@yahoo.com.br

Introduction

Regarding to global population it is possible to observe an increase of life expectancy as a result of improved quality of life. Thus, it is supposed that the number of people who expose their teeth to etiological factors related to progressive and non-carious loss of dental structures for a higher period of time has also increased.[1]

The non-carious cervical lesions are characterized by irreversible loss of mineralized tissue with no bacterial involvement, located in areas close to cementoenamel junction, resulting in dentin exposure. The exposure of dentinal tubules is, obviously, followed for an episode of hypersensitivity, which becomes the main complaint of patients.[2,3] The hydrodynamic theory proposed by Brännstrom and Aströn,[4] is able to explain, in a reasonable way, the painful phenomenon of dentin.

Regarding to incidence of non-carious cervical lesions, it can be more visible on buccal face of teeth from adult patients, knowing that prevalence and severity of these lesions increase over time. The more committed teeth are premolars.[1,5] The most common injuries into the non-carious cervical lesion are dentinal abrasion, abfraction and erosion.

In other hand, not all exposed dentin tissue is sensitive to thermal stimulation, since, with advancing age, there is deposition of reactionary dentin below the affected area and is likely to appear sclerotic dentin, which will create a barrier in these areas.[6] Furthermore, the reduction of pulp chamber volume through physiological deposition of dentin, as well as appearance of acellular and fibrosis zones, decrease the sensitivity of dental element to painful stimulation. For this reason, the incidence of dentinal hypersensitivity decrease in elderly patients.[9]

Nowadays, the thermal tests of pulpal sensitivity represent an important resource which will help in the diagnosis of pulp tissue condition into different clinical situations. The cold tests have been the more used for this finality. Despite to promote a temperature reduction on pulp-dentin interface, the thermal tests by cold have not been deleterious to healthy pulpal tissue.[7]

The thermal stimulation is a standard way of evaluation of pulpal vitality. Several methods of cold application are available, including ice and ethyl chloride. More recently, dichlorodifluoromethane and carbon dioxide snow (CO_2) have demonstrated to be more reliable than any other method.[8]

The aim of this study is to compare the response to pulpal sensitivity test with cold in teeth with loss of dentinal structure by non-carious cervical lesion and teeth with normal dental structure, identifying a standard response to sensitivity test with cold in patients who have this kind of lesions.

Material and Methods

This study was approved by Ethics Committee of University of Passo Fundo (064/2012). The patients were selected from School of Dentistry of University of Passo Fundo. The selected patients signed an informed consent term to perform the research.

Forty teeth were selected for the present study, being divided into two groups (n = 20), as follow: G1 = teeth with loss of dental structure by non-carious cervical lesions; G2 = teeth with no loss of dental structure (control).

Before the test performing, a visual inspection was promoted in order to evaluate morphological aspect, extension and depth of non-carious cervical lesion, identifying as abrasion, abfraction or erosion. The lesions should demonstrate a wedge aspect, with at least 1 to 3 mm of extension and 1 to 2 mm of depth.

Single-rooted teeth (lower and upper canines and premolars specifically) of patients of both gender (male and female), in adult age and with significant loss of dental structure by non-carious cervical lesions were included into the experimental group of the present study. Teeth with cervical restoration, decay or periodontal disease were excluded from the sample.

The patients were instructed regarding to pain level through a visual analog scale which classified the painful response in mild, moderate and severe (Fig 1). The first tooth to be tested was a dental element which has not showed any change of loss of dental structure. On this way, the tolerance to pain of each patient was recognized.

The pulpal sensitivity test with cold was performed according Soares and Goldberg[9] technique: relative isolation of the region to be tested with cotton rolls; application of refrigerant gas (Endo-Ice – Coltène/Whaledent, Inc., Langenau, Germany) with the help of a cotton pellet which was gently placed on the buccal surface of the dental element crown, above the area of the patient lesion until to have a sensitivity response. Otherwise, the cotton pellet was

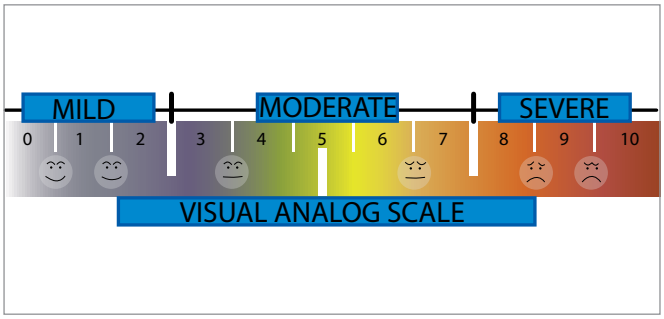

Figure 1. Visual analog scale classifying the painful response in mild, moderate and severe.

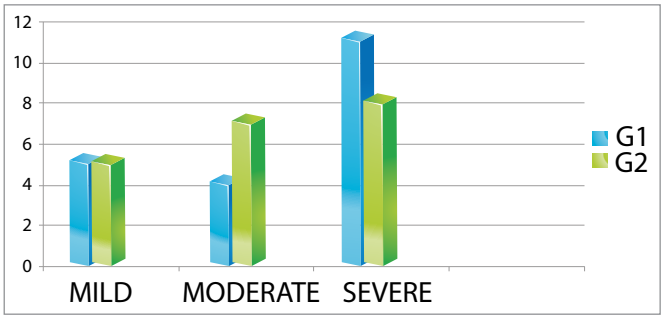

Figure 2. Graph showing results of G1 (teeth with non-carious cervical lesion) and G2 (teeth with no non-carious cervical lesion) according to response of sensitivity test.

maintained for until 10 seconds when it was removed. In this moment, the patient was questioned about the sensitivity level filling the visual analog scale.

The data were obtained and statistically analyzed using nonparametric Kolmogorov-Smirnov test at 5% significance level.

Results

The results of the present study did not show statistically significant difference between Group 1 and Group 2, regarding to response to pulpal sensitivity test with cold ($p < 0.05$). Regarding to group 1 (teeth with non-carious cervical lesion), five[5] teeth showed severe response (25%), four teeth showed moderate response (20%) and eleven teeth showed mild response (55%), according to visual analog scale used to perform this evaluation. Regarding to Group 2 (teeth with no non-carious cervical lesion), five teeth showed severe response (25%), seven[7] teeth showed moderate response (35%) and eight[8] teeth showed mild response (40%), according to visual analog scale used to perform this evaluation. These data can be visualized in Figure 2.

The non-carious cervical lesions were more frequents in people older than fifty years old (77.6%). There was no difference between genders regarding to presence of these lesions. According to the present study could be detected three kinds of non-carious cervical lesions, being abfraction the most common (50%), followed by abrasion (35%) and erosion (15%) respectively. Furthermore, lower premolars (40%) was the most injured dental group, followed by upper canines (30%), upper premolars (25%) and lower canine (5%).

Discussion

The increase of life expectancy can be observed as result of improved quality of life. Thus, it is supposed that the number of people who expose their teeth to etiological factors related to progressive and non-carious loss of dental structures for a higher period of time has also increased.

According to the present study, it was possible to realize that non-carious cervical lesions are found more frequently in people older than fifty years old. However, this kind of lesion also was found in young patients. According to Negoro et al,[10] the loss of dental structure is higher in patients younger than twenty-six years old, making a comparison with patients ranging between thirty and fifty-five years old. In this context, there are scientific evidences showing that age of patient is not related with the appearance of non-carious cervical lesions.[11]

The lower premolars was the most injured teeth by this kind of lesion, which is in accordance with previous study.[1,5] This result can be explained due its location into the oral arch which induces loss of dental structure by action of acids, traumatic action by tooth brushing, and occlusal interferences.[1,5]

The abfraction was the most common kind of non-carious cervical lesions in the present study (50%), followed by abrasion (35%) and erosion (15%) respectively. These results are not in accordance with Molena et al,[12] who verified that abrasion was the most common kind of non-carious cervical lesions (46%), followed by abfraction (42%) and erosion (12%) respectively.

The abfraction lesions are V-shaped, usually deep, with well-defined edges and sharp angles. These lesions are usually located in the cervical zone

of buccal faces, being more frequents in premolars, followed by molars and canines respectively.[3,13,18] The etiology of these lesions is attributed to occlusal trauma. The occlusal overload and eccentric occlusal forces that generate load in the axial direction lead to tooth flexion.[2,13,19,20]

The abrasion is the pathological attrition of buccal faces on the cervical level, provided by external mechanical process involved in harmful habits like horizontal and aggressive tooth brushing or use of tooth brush with hard bristles and abrasive substances.[3,12-16]

The erosion is known as result of irreversible loss of mineralized tissue by chemical action of acids with no bacterial involvement. This kind of lesion can be distinguished from abfraction and abrasion lesions through the extension of lesion which reach all dental surfaces beyond the cervical area. Clinically, the erosion lesions are characterized by loss of normal bright of teeth, being the lesion surface very smooth and polished, U-shaped, wide and with no sharp angles.[17]

According to Addy[21] patients who have teeth with non-carious cervical lesion develop painful symptoms of dentinal hypersensitivity due to loss of dental structure and consequently dentinal exposure. However, the results of the present study showed several level of pain after application of sensitivity test with cold. On this way, teeth with this kind of lesion can or cannot demonstrate pulpal sensitivity.

According to limitation of the present study, it was concluded that teeth with non-carious cervical lesion can demonstrate different levels of response to pulp sensitivity test with cold, suggesting that teeth with loss of dentinal structure can or cannot show a response related to pulp sensitivity.

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Horizontal root fracture in the middle third: Case report

Jefferson J. C. **MARION**[1]
Juliana Yuri **NAGATA**[2]
Ketrin Kimberly **RAMOS**[3]
Frederico Campos **MANHÃES**[4]

ABSTRACT

Introduction: Dental trauma may be considered one of the main causes of permanent teeth loss, and root fractures are relatively uncommon in these situations. **Objective:** The aim of this study was to report a clinical case of horizontal root fracture promoted by a dental trauma and discuss their clinical implications. **Methods:** The horizontal root fracture occurred at the middle third of the maxillary central incisor with separation of the fragments. The tooth was diagnosed with pulp necrosis, and the endodontic treatment was performed. **Results:** After a follow-up period of two years with radiographic and tomographic images root complications were not observed, neither painful symptomatology, highlighting the importance of a correct diagnosis which results in a good prognosis, preserving the esthetic and psychological integrity of the patient.

Keywords: Endodontics. Dental trauma. Root fracture.

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[1]PhD Student in Dental Clinic – Endodontics, UNICAMP. Professor, Department of Endodontics – ABO and UNINGÁ.

[2]MSc and PhD in Dental Clinic – Endodontics, UNICAMP.

[3]Graduated in Dentistry, UNINGÁ.

[4]PhD in Dental Clinic – Endodontics, UNICAMP.

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Contact address: Jefferson José de Carvalho Marion
Rua Néo Alves Martins, 3176 – 6º andar – sala 64 – Centro
CEP: 87.013-060 – Maringá/PR – Brazil
Email: jefferson@jmarion.com.br / atendimento@jmarion.com.br

Introduction

One of the main causes of anterior permanent tooth loss is dental trauma, representing 24% of dental absence due to infection and mobility in traumatized teeth.[1,2] Upper central incisors are vulnerable to this type of injury, being affected in 80% of dental trauma, followed by upper lateral incisors and lower incisors.[3,4]

Considering the types of trauma, root fractures are less frequent, with prevalence of 0.5% to 7% in permanent dentition and 2 to 4% in deciduous teeth.[5] Similar to dental trauma in a general manner, these fractures occur mainly in central incisors (68%) and lateral incisors (27%), and only 5% in lower incisors.[6] It happens in consequence of an impact force at the top of the root and frontal forces in the compression zone directed to vestibular and lingual, dividing root in coronal and apical fragments. This division may cause harmful consequences to cement, dentin, pulp and periodontal tissues.[2]

Root stage development, displaced fragments reposition and all signals and symptoms associated, such as pain and mobility may influence repair process of root fracture.[7,8,9] Literature describes different positions where fracture may be located — such as those restrict to cervical third, oblique fracture involving cervical and middle third, in the middle third, and in the apical third — as well as its influence in the treatment success.[10] The treatment protocols related to cicatrization, reposition and contention reported in the literature are mainly determined empirically and without a relevant data base.[2]

The prognosis of root factures is favorable in 60-80% cases, however complications such as pulp necrosis, root resorption and calcification of root canal may emerge.[3]

According to pulp lesion, and considering the possibility of bacterial invasion in the fracture line, four types of tissue repair may occur: Fusion of fragments by means of hard tissue formation, interposition of connective tissue or a "fake union" due to the presence of chronic inflammatory tissue between the fragments.[11,12]

The present study has the purpose to report a clinical case of dental trauma with horizontal root fracture, pulp necrosis and endodontic treatment of fragments, as well as to discuss clinical implications.

Case report

A 16-year-old male patient attended a particular Endodontics clinic after he had suffered a dental trauma during football practice. He did not present systemic alteration. Clinically, it was observed soft tissue lacerations (lips, tongue and gingiva) as well as right central maxillary incisor commitment (tooth #11). In addition, gingival tissue at this tooth presented bleeding at the cervical region, with mobility and light extrusion. At the radiographic exam, it was observed that the right incisor presented horizontal root fracture at the medium third with separation of the fragments (Fig 1A).

The patient and his responsible were informed about the difficulties and complications of the treatment, agreeing to perform the protocol suggested by Gulinelli et al.[13] At first, lips lacerations were sutured, and then the fragments were repositioned through the application of a vertical force in the incisal portion of the tooth, with local anesthesia. It was performed splintage of the coronal fragment with composite resin. It was prescribed amoxicillin 500 mg for 7 days, nimesulide 100 mg for three days and sodium dypiron for three days too.

After 45 days, patient returned for clinical and radiographic follow-up, being possible to note root fragments separation and absence of pulp sensibility for thermal and cold tests (Endo-Frost, Roeko®, Germany) (Fig 1B).

In face of pulp necrosis, it was decided for endodontic treatment. During odontometry, it was possible to observe an access to apical fragment and working length was established at 25 mm including radiographic apex (Fig 1C). Chemomechanical prepare was performed in the whole extension of root canal fragments under the presence of 2.5% sodium hypochlorite (NaOCl).

After instrumentation, root canal was dried with sterile paper points (Konne®, Belo Horizonte, Brazil) and dressed with calcium hydroxide paste (Biodinâmica®, Ibiporã, Brazil) associated to propyleneglycol vehicle for a period of 30 days. After this period, intracanal medicament was removed with a manual file (Dentsply Maillefer, Ballaigues, Switzerland) and irrigant solution (2.5% NaOCl). In the sequence, root canal was irrigated with EDTA 17% (Biodinâmica®, Ibiporã, Brazil) for 3 minutes with mechanical agitation. EDTA

was removed by irrigating 2.5% NaOCl, with another insertion of paper points for drying the canal. Then, it was performed the main gutta-percha cone try out (Fig 1D), definitive obturation of root canal using gutta-percha cones and obturation cement with calcium hydroxide in its composition (Sealapex®- SybronEndo (SDS)/Glendora, USA). The obturation technique was the lateral condensation followed by vertical condensation. It was radiographically observed sealer overflow at the horizontal fracture region (Fig 1E).

Clinical and radiographic follow-up was performed after 30 days (Fig 2A), after 2 months (Fig 2B), 4 months (Fig 2C), 6 months (Fig 2D) and 1 year (Fig 2E). None of the follow-ups demonstrated pathological alteration such as: root resorption, radiolucency regions as well as painful symptomatology.

Two-year follow-up was performed with radiograph (Fig 3A) and tomograph (Figs 3B, C, D, E), being possible to note that the affected tooth presented characteristics compatibles with the normality.

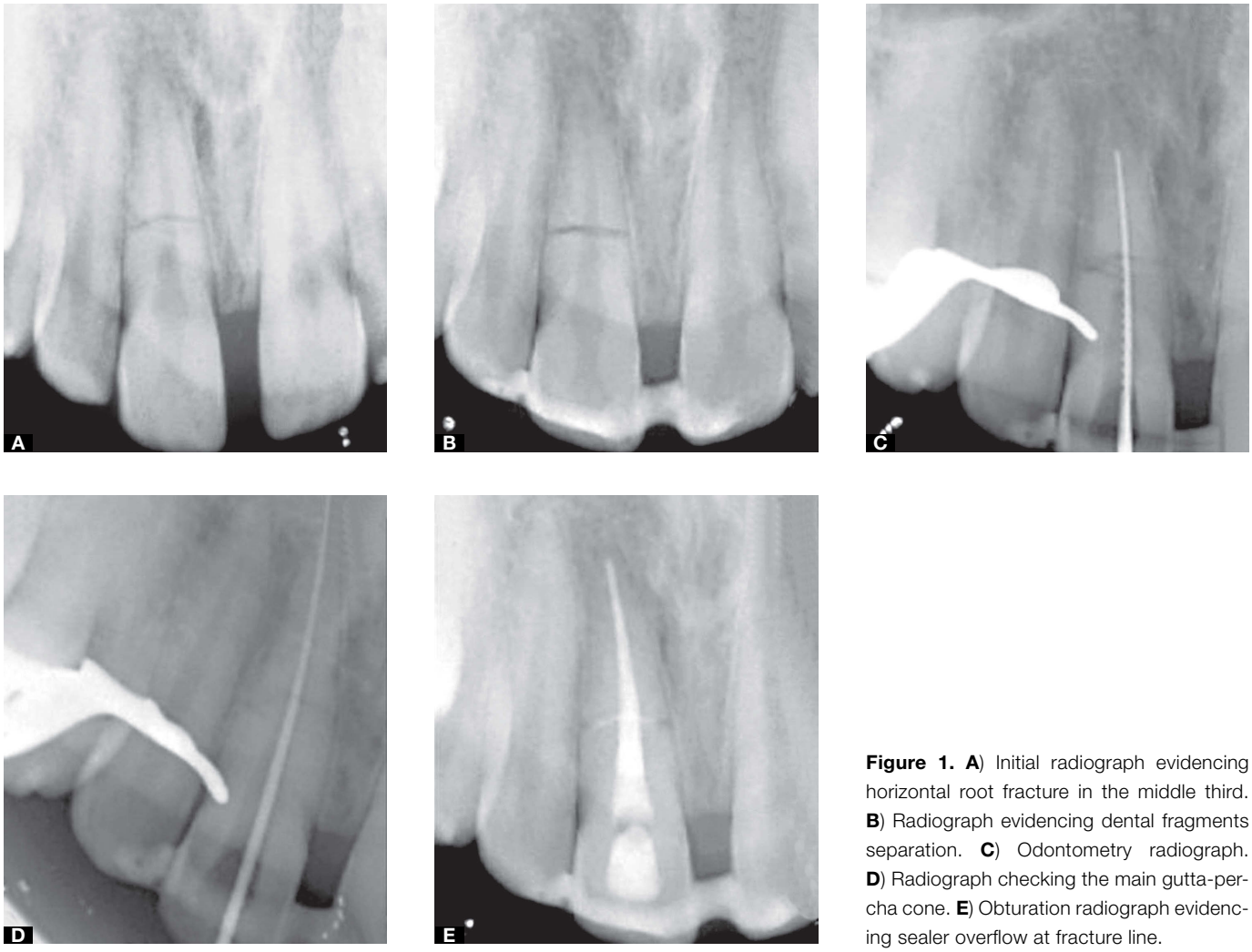

Figure 1. A) Initial radiograph evidencing horizontal root fracture in the middle third. **B**) Radiograph evidencing dental fragments separation. **C**) Odontometry radiograph. **D**) Radiograph checking the main gutta-percha cone. **E**) Obturation radiograph evidencing sealer overflow at fracture line.

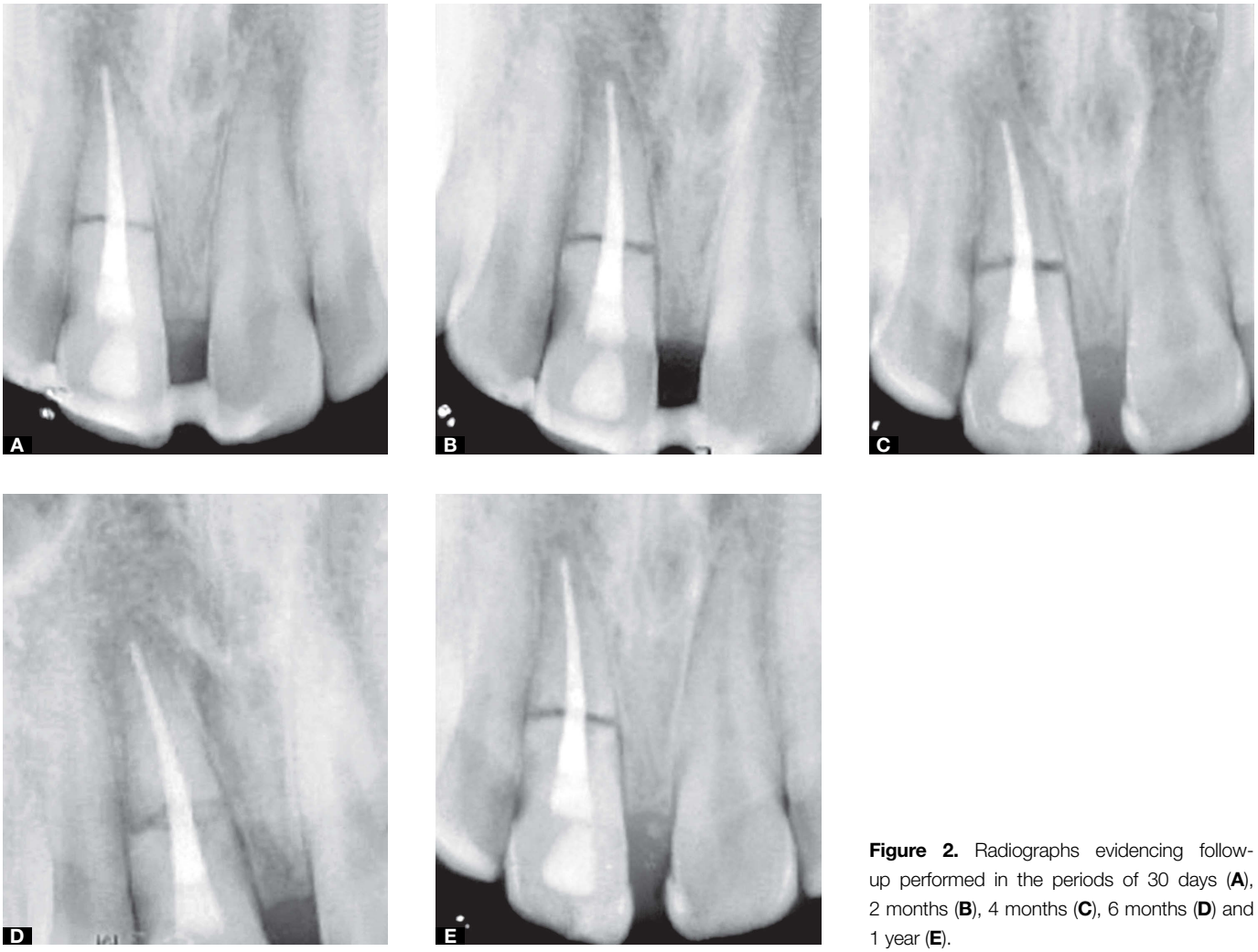

Figure 2. Radiographs evidencing follow-up performed in the periods of 30 days (**A**), 2 months (**B**), 4 months (**C**), 6 months (**D**) and 1 year (**E**).

Discussion

Dental trauma may be considered a multifactorial problem considering diagnosis, long-term treatment and follow-up.[14] These injuries may occur due to car accidents, sportive lesions, violence and malocclusion.[3,15] The present case report described a right central incisor trauma due to sportive activity, which is in accordance with other studies that show 80% of prevalence of dental trauma in upper central incisors.[3,4]

Root fractures in children and young people, and root fractures with minimum separation of the fragments should not preventively receive endodontic treatment since its prognosis is favorable.[16] In addition, patient age at the moment of the injury may be considered one of the main factors to pulp repair after root fracture.[17] In the present case report, after 45 days, it was observed pulp necrosis despite of being a young patient. This situation also may had occurred due to the rupture of neuro-vascular bundle of the dental pulp, or even because of bacterial invasion through apical foramen promoting bacterial infection and subsequent pulp ischemia in a manner that revascularization could not happen.[18]

Endodontic treatment of the tooth was performed considering that literature reports repair of root fractures after endodontic treatment.[19] In addition, studies describe four types of endodontic treatment for these situations: 1) chemomechanical preparation only in the coronal fragment and endodontic obturation of it; 2) chemomechanical preparation of both fragments with endodontic obturation of them; 3) chemomechanical preparation only of the coronal fragment with endodontic obturation of it and surgical removal of apical fragment; 4) chemomechanical preparation of coronal and apical fragments, filling the canal with calcium hydroxide dressing and posterior

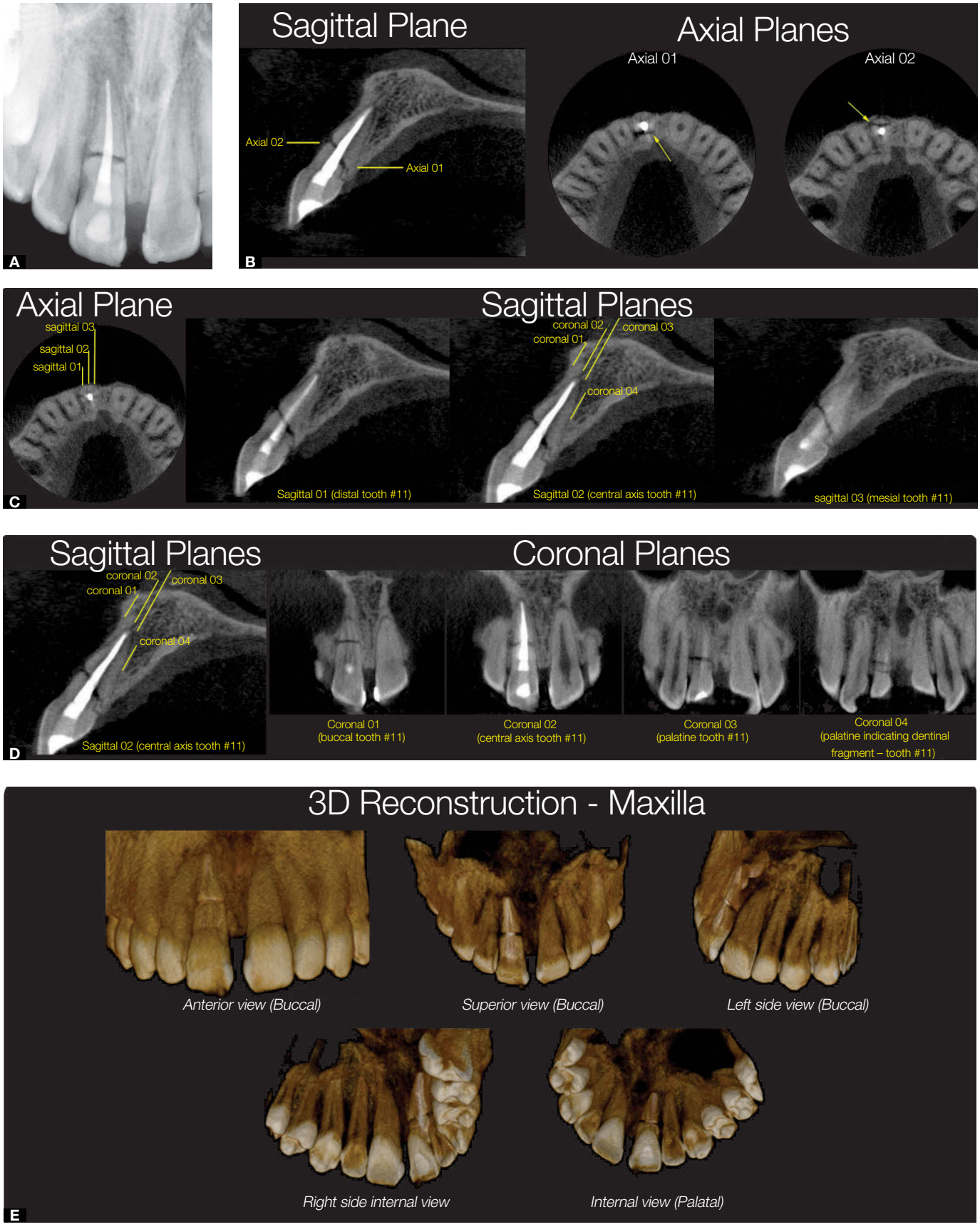

Figure 3. A) Periapical radiograph of 2-years follow-up; **B**) Sagittal tomographic slice indicating the axial tomographic slices with fracture in the middle third; **C**) Axial tomographic slice indicating the sagittal tomographic slices with fracture in the middle third. **D**) Sagittal tomographic slice indicating coronal tomographic slices with fracture in the middle third. **E**) Maxilla tomographic slice in tridimensional reconstruction.

endodontic obturation of them.[20,21] According to these studies, it was decided for the fourth treatment option aforementioned, considering that it was possible to access the apical fragment of the root and that calcium hydroxide antimicrobial and remineralizing actions should have positively contributed for the success.[22] However, there are other studies with follow-up periods ranging from 2 to 31 years,[3,23] that demonstrated 100% of repair in root fractures cases using only splintage.

Regarding tissue composition in the fracture line, Welbury et al[2] observed dentin and cementum formation, which is dependent of odontoblast and cementoblast activity. In the 2-year follow-up tomograph, it was not possible to observe root fracture repair with hard tissue deposition, which may be explained due to the extrusion and separation of coronary fragments, while connective tissue union is more observed in cases of pulp necrosis and coronary fragment separation.[2]

Another factor that should be considered is the importance of long-term follow-up, because there is the possibility of occurrence of pathological alterations several years after the lesion.[24]

Conclusions

It is important to remember that treatment success depends on the patient-professional relation, considering the realization of therapeutic attitudes immediately after the occurrence of complications. Pulp necrosis and absence of repair at the root fracture line through hard tissue deposition may occur in permanent teeth. A permanent tooth with root fracture that was endodontically treated may present a good prognosis, preserving esthetic and psychological integrity of the patient. Clinical and radiographic follow-up is essential for treatment success of a tooth with horizontal root fracture.

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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 Trials Registry), www.clinicaltrials.gov and http://isrctn.org (International Standard Randomized Controlled Trial Number Register (ISRCTN). The creation of national registers is underway and, as far as possible, the registered clinical trials will be forwarded to those recommended by WHO.

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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