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

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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.

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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-

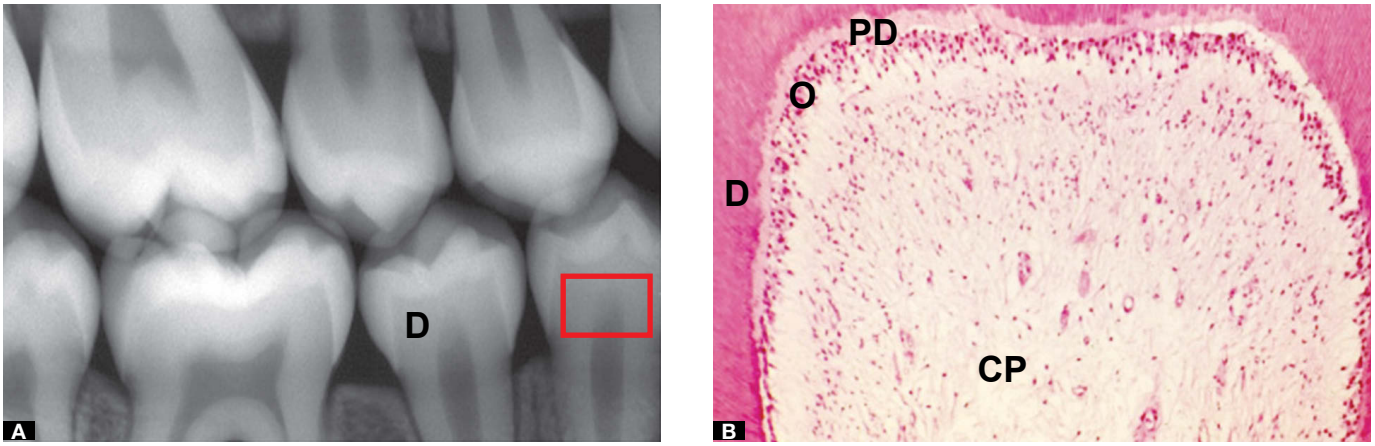

Figure 1. Dentin-pulp complex and its components: dentin (D), predentin (PD), odontoblastic layer (O) and central portion of the pulp (CP). (**B** = H.E., original mag. = 160X).

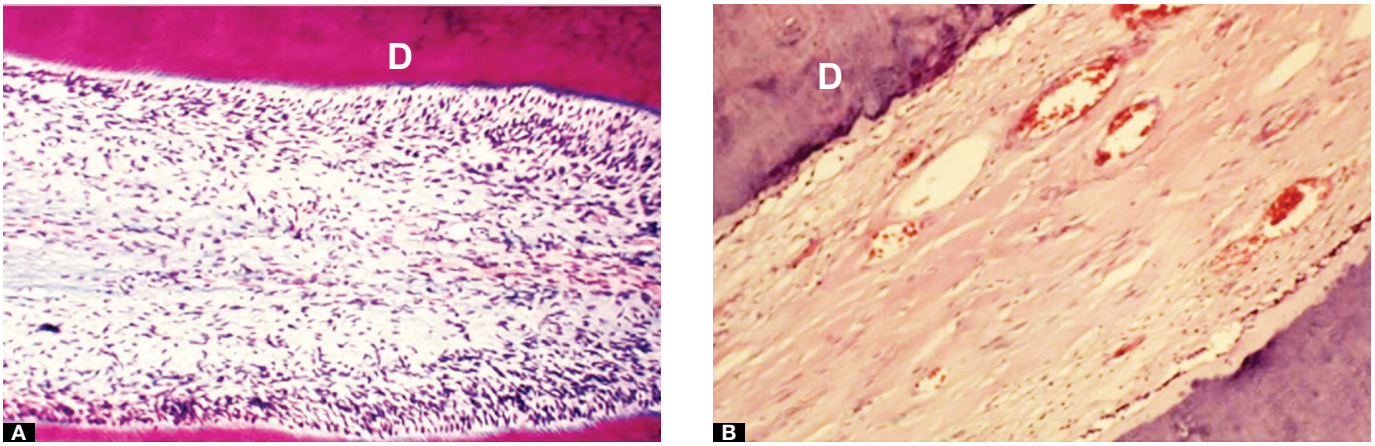

Figure 2. Young dental pulp, well cellularized, in **A**, and, aged pulp, in **B**, with exuberant fibrosis. D = Dentin (**A** and **B** = H.E., original mag. = 160X).

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.

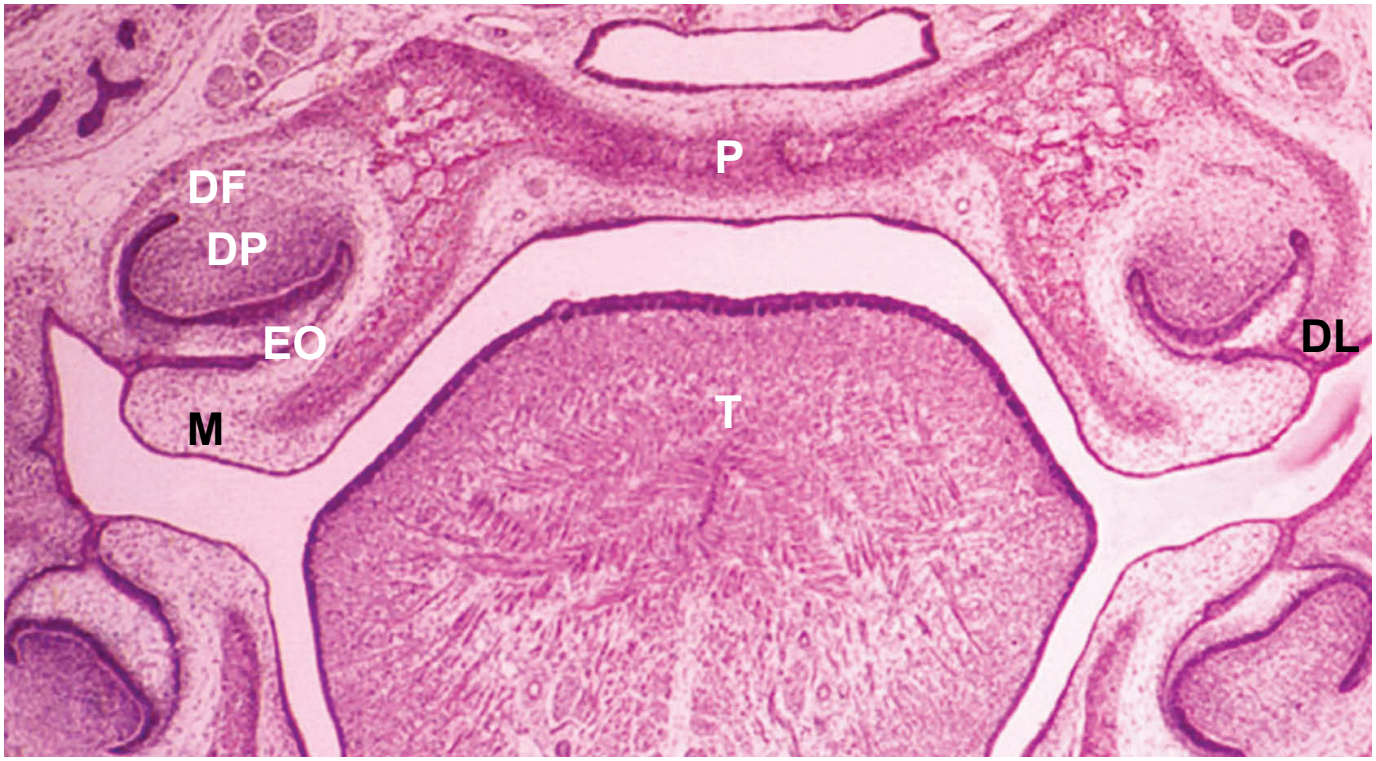

Figure 3. Frontal section of human embryo in oral and maxillofacial region, with four dental germs at bell stage. Dental lamina (DL) and primitive mesenchyme (M), enamel organ (EO), dental papilla (DP) and the dental follicle (DF) can be observed. T = tongue; P = palate. Covering the oral cavity, it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

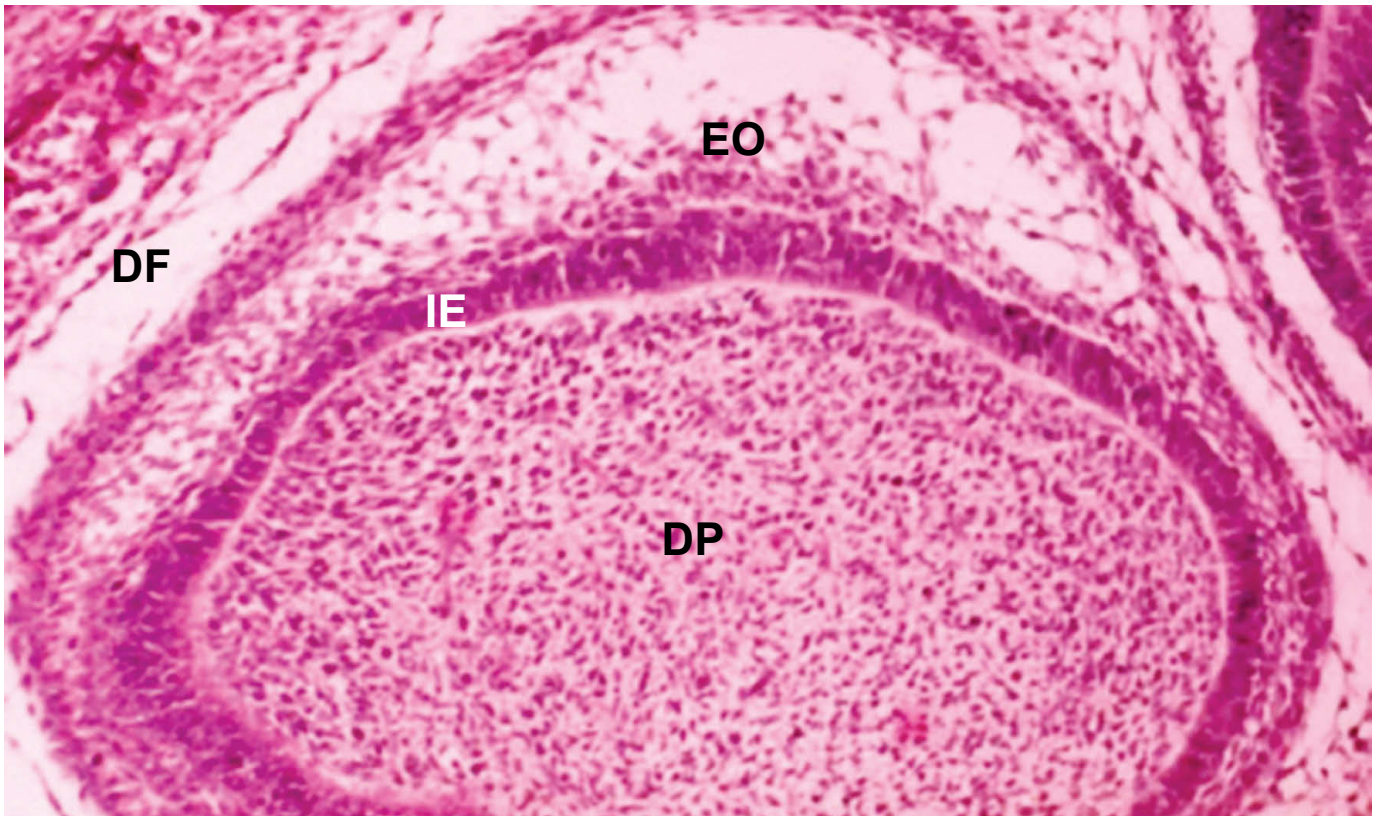

Figure 4. Dental germ at bell stage: EN = enamel organ; IE = Inner epithelium; DP = dental papilla; DF = dental follicle. (H.E., original mag. = 400X).

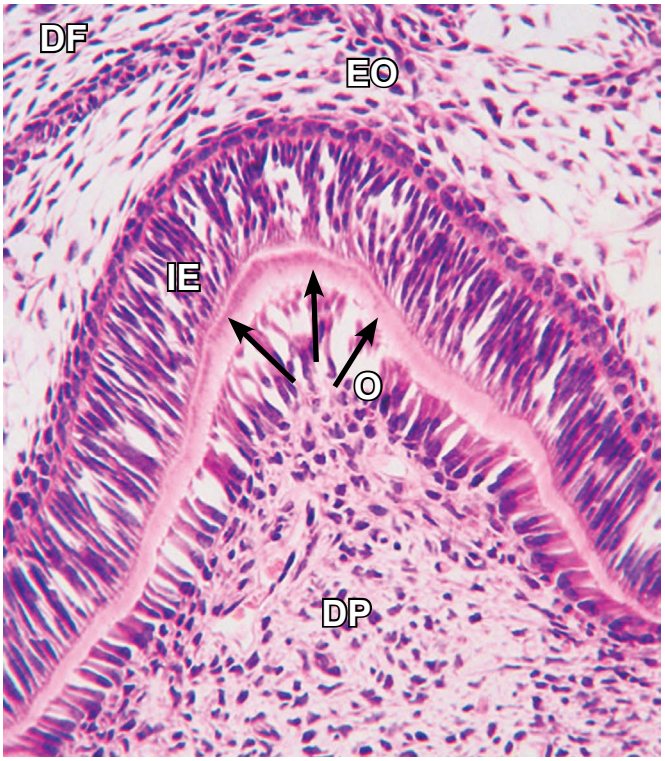

Figure 5. Dental germ with initial dentinogenesis (arrows). EO = enamel organ; IE = Inner epithelium; DP = dental papilla; DF = dental follicle; O = odontoblasts. (H.E., original mag. = 400X).

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

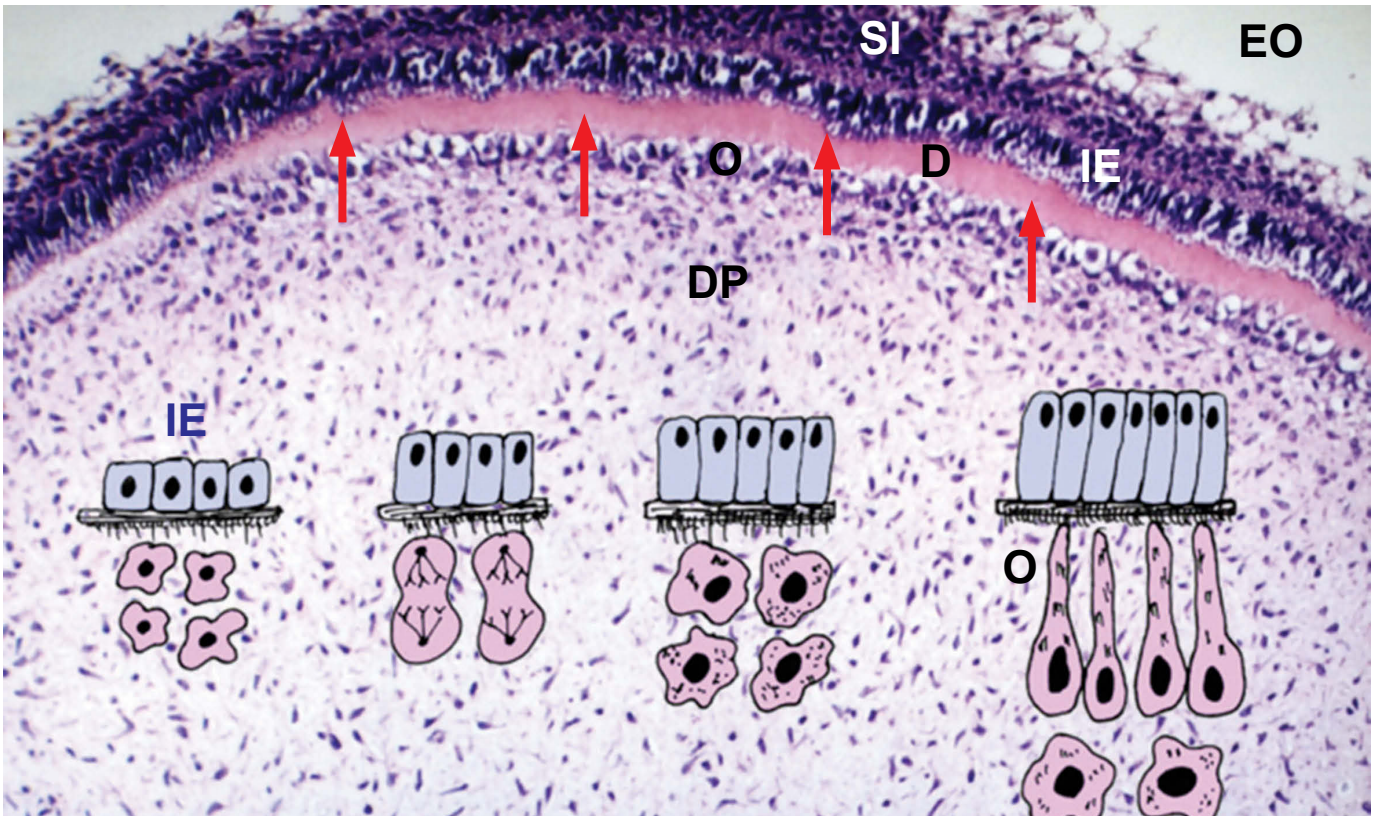

Figure 6. Dental germ with initial dentinogenesis (arrows). EO = enamel organ; IE = Inner epithelium; SI = stratum intermedium; DP = dental papilla; O = odontoblasts. In the scheme, the papillary cells get attached to the fibronectin layer next to the inner epithelium cells and assume the odontoblasts 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

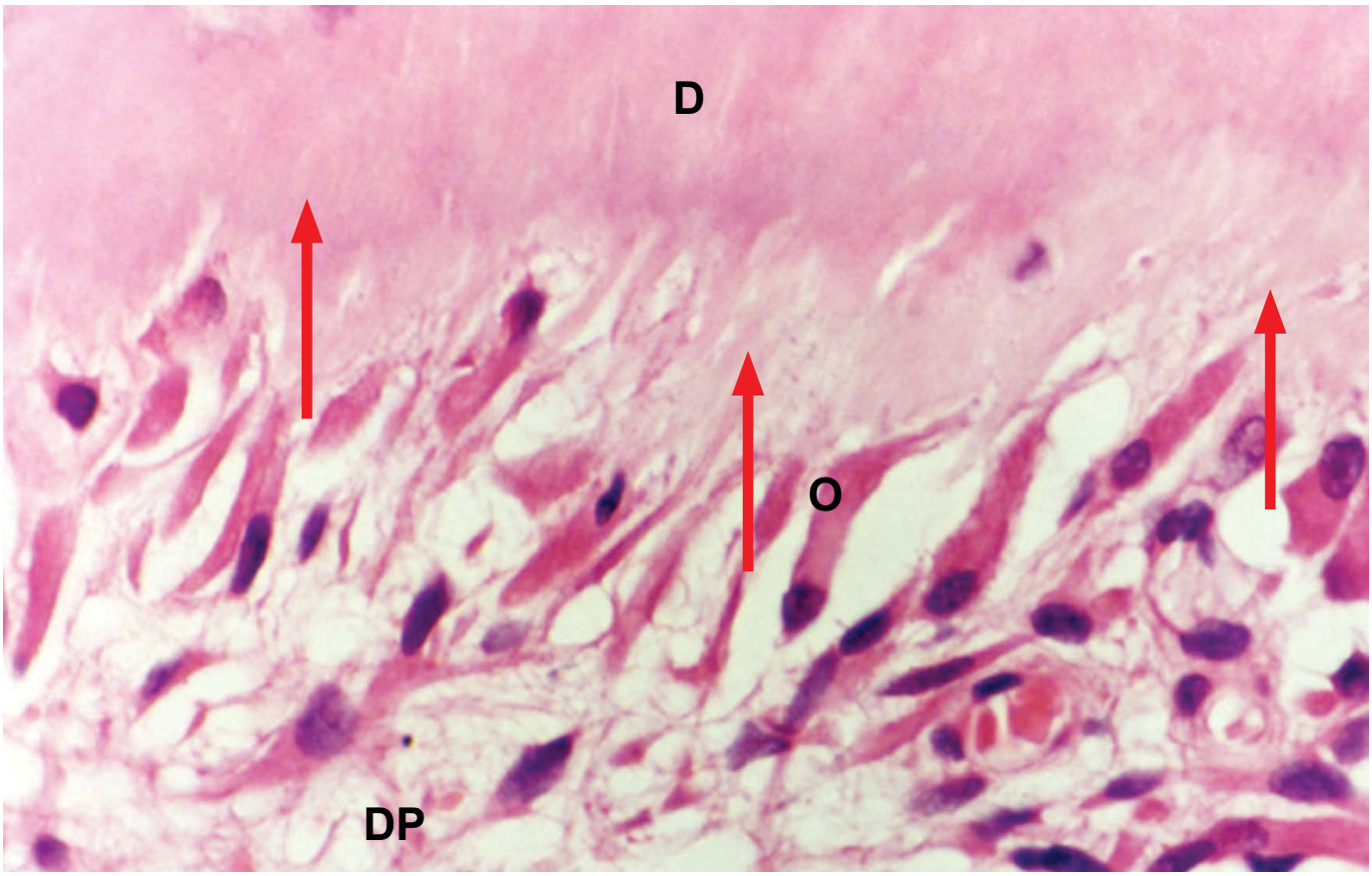

Figure 7. Odontoblasts (O) in dentinogenesis, forming a mineralized tissue barrier in pulpotomy with calcium hydroxide (arrows). D = dentin; DP = dental pulp. (H.E., original mag. = 400X).

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

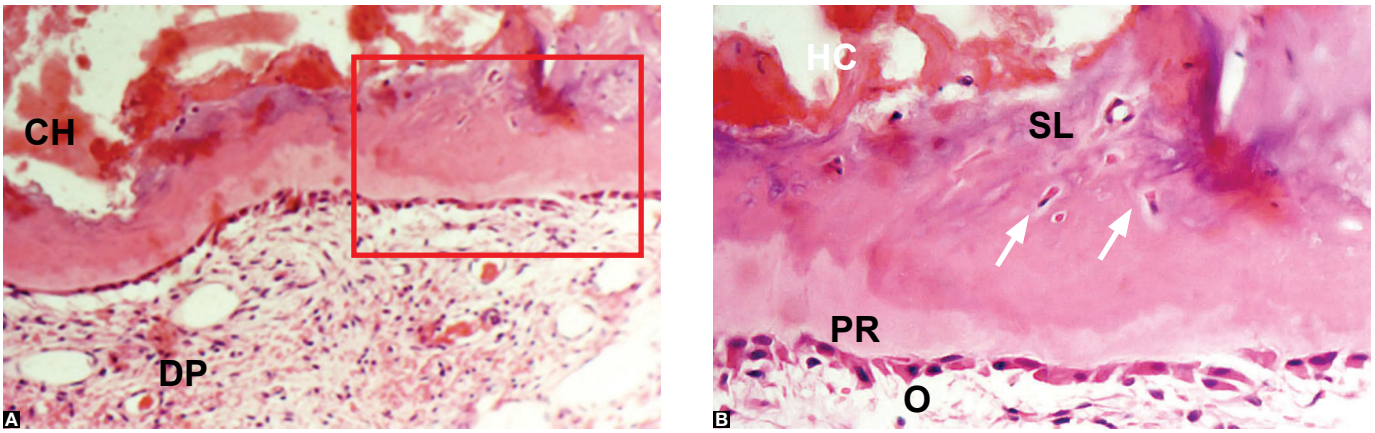

Figure 8. Mineralized tissue barrier formed after 45 days of direct application of calcium hydroxide to human dental pulp (DP). A disorganized superficial layer, basophilic (SL), is observed and reveals eventual cell inclusions (arrows). The deepest layer, facing the pulp, is eosinophilic . O = odontoblasts; 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).

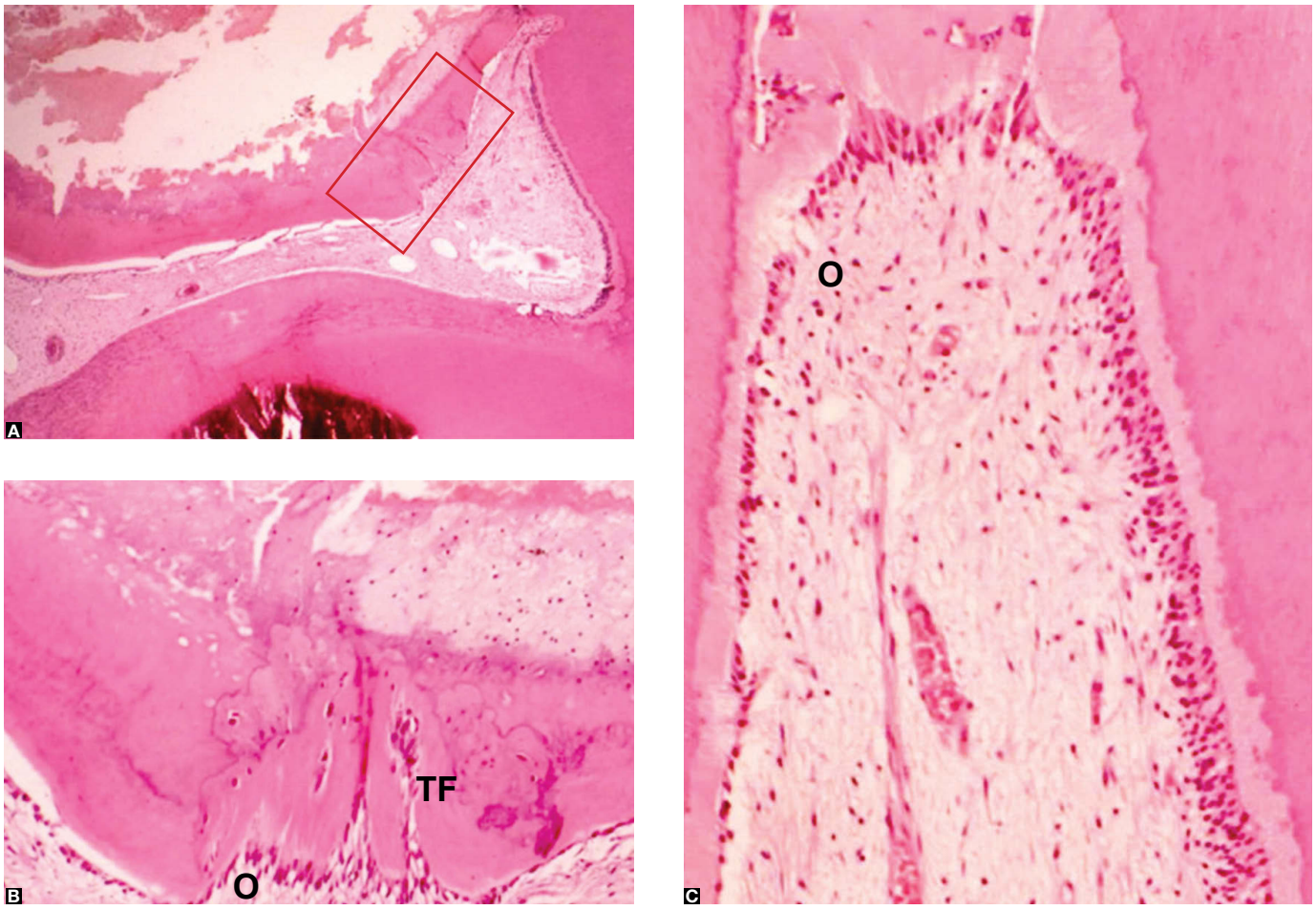

Figure 9. Organizational patterns of the odontoblastic layer in human dental pulps subjected to pulpotomies. The odontoblastic cells develop to a regular pattern in refined palisade, in **C**. O = odontoblasts; TF = tunnel formation (Source: Lanza[17]). (H.E. original mag. = 160X).

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.

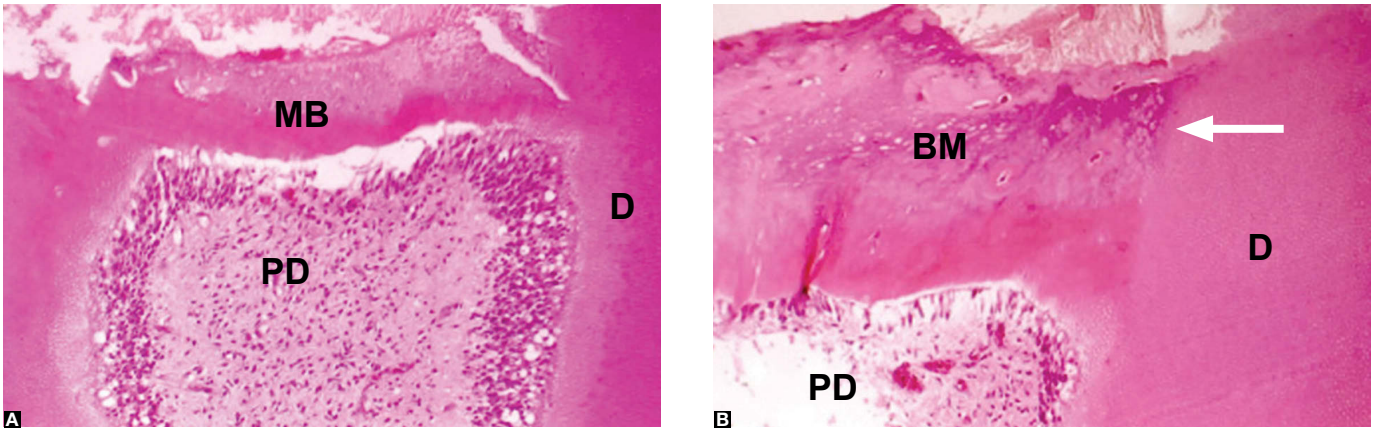

Figure 10. Relationship between the mineralized tissue barrier (MB) and the normal lateral dentinal walls in human dental pulps after direct application of calcium hydroxide, 90 and 120 days after pulpotomy. Juxtaposition is emphasized with linear or irregular interface on the superficial portion of 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

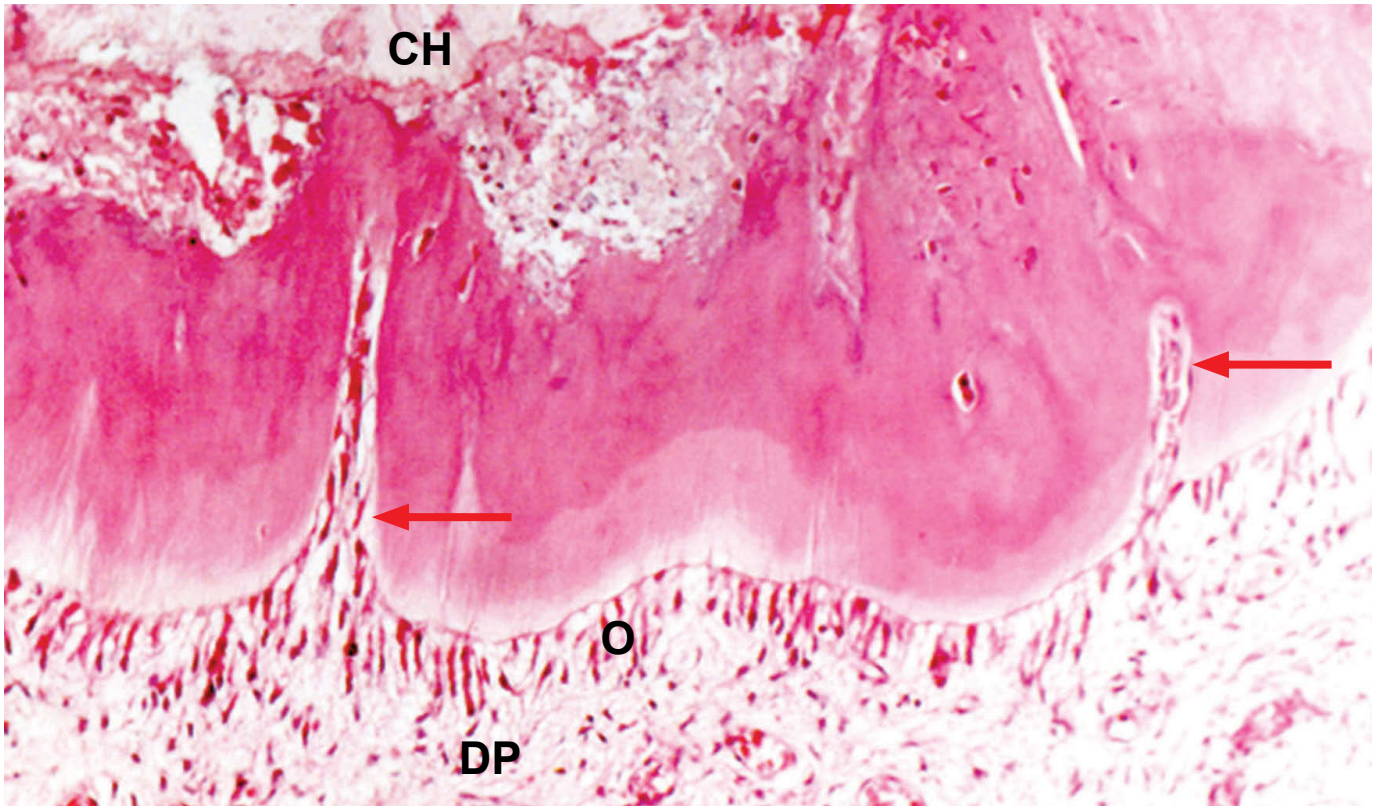

Figure 11. Mineralized tissue barrier showing canal or "tunnel" formations (arrows) filled with living pulp tissue, well cellularized and vascularized in human teeth, 90 and 120 days after pulpotomy with direct application of calcium hydroxide. DP = Dental pulp; O = odontoblasts; CH = calcium hydroxide.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

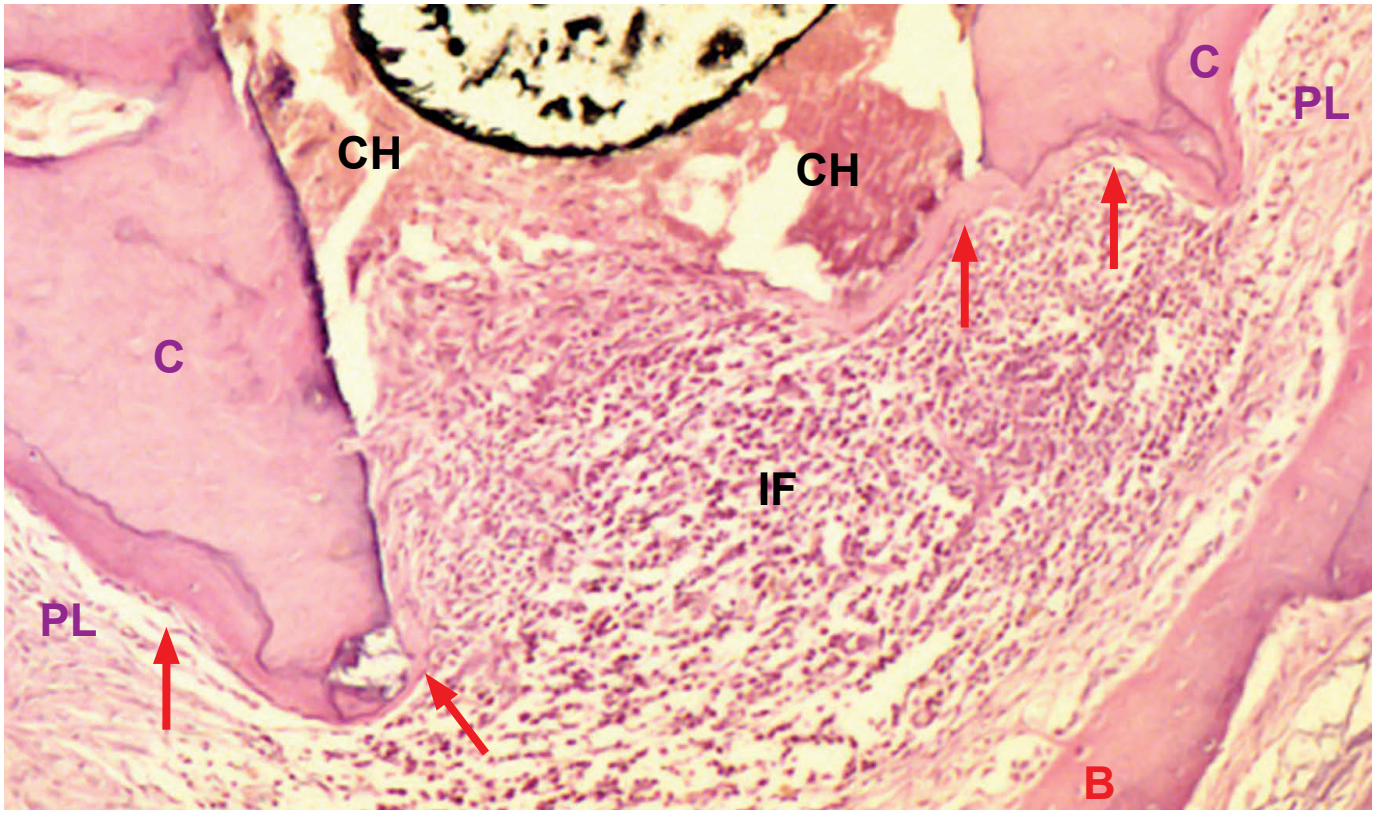

Figure 12. Mineralized tissue barrier (arrows) being formed over calcium hydroxide-based (CH) filling material. C = cementum; IF = inflammation; B = Bone; PL= periodontal ligament. (H.E., original mag.: 160X).

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

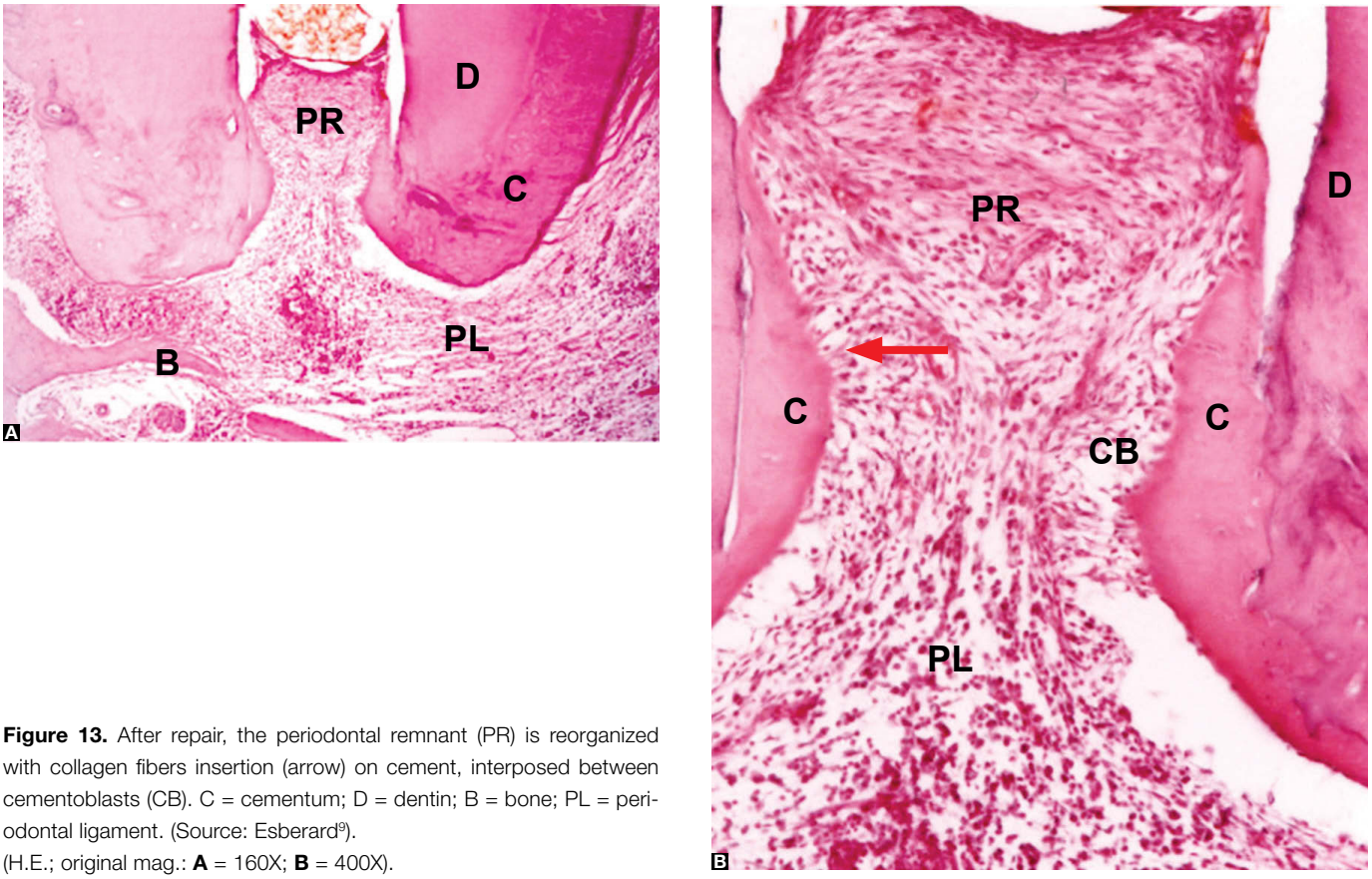

Figure 13. After repair, the periodontal remnant (PR) is reorganized with collagen fibers insertion (arrow) on cement, interposed between cementoblasts (CB). C = cementum; D = dentin; B = bone; PL = periodontal ligament. (Source: Esberard[9]).
(H.E.; original mag.: **A** = 160X; **B** = 400X).

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”

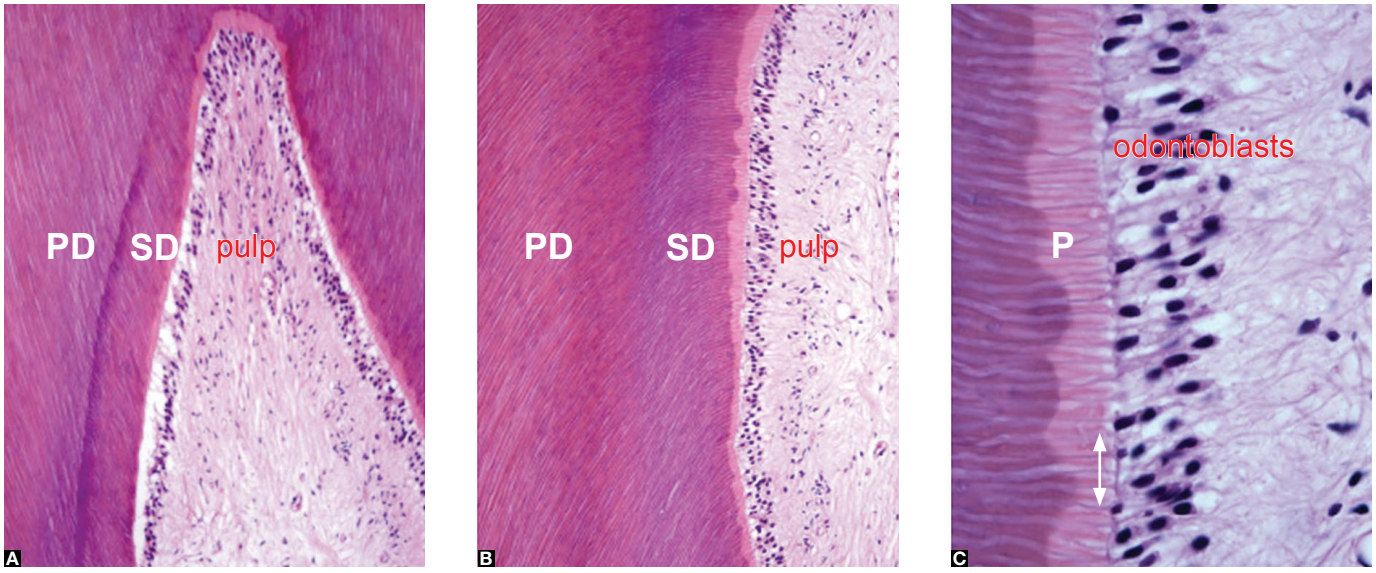

Figure 14. Dentin-pulp complex with primary dentin (PD) deposited before eruption and secondary dentin (SD), after eruption. The limits are only marked by a line with color alteration, but they are structural and functionally continuous. In **C** are shown the predentin (P) and the extensions in continuity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

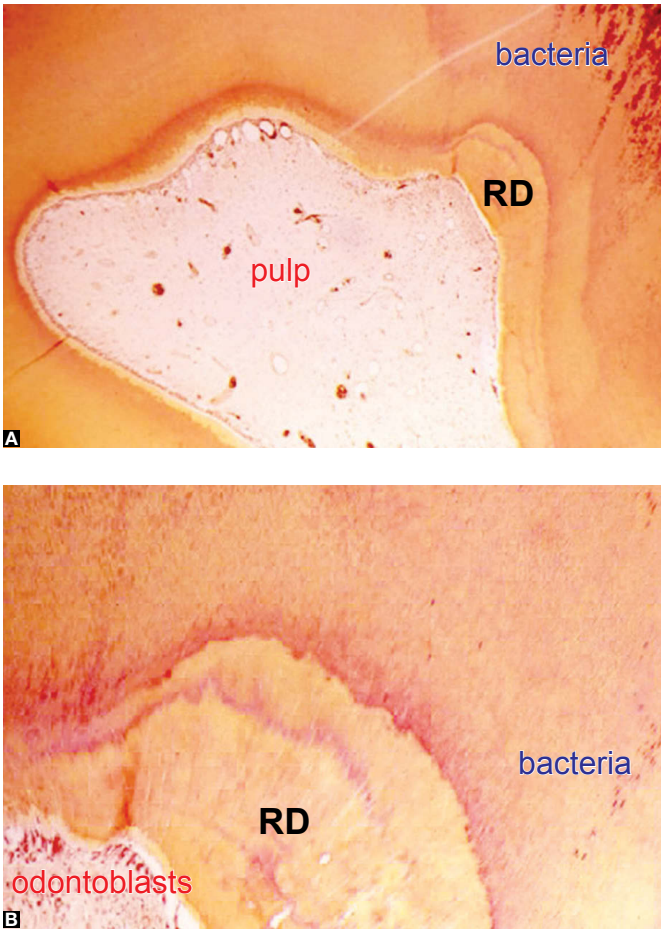

Figure 15. Dentin-pulp complex with reactive dentin (RD) subjacent to the cavity and its bacteria. Reactive dentin has lower number of tubules and less organized and mineralized structure, being originated by second generation odontoblasts (BB; original mag.: **A** = 40X; **B** = 160X).

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.

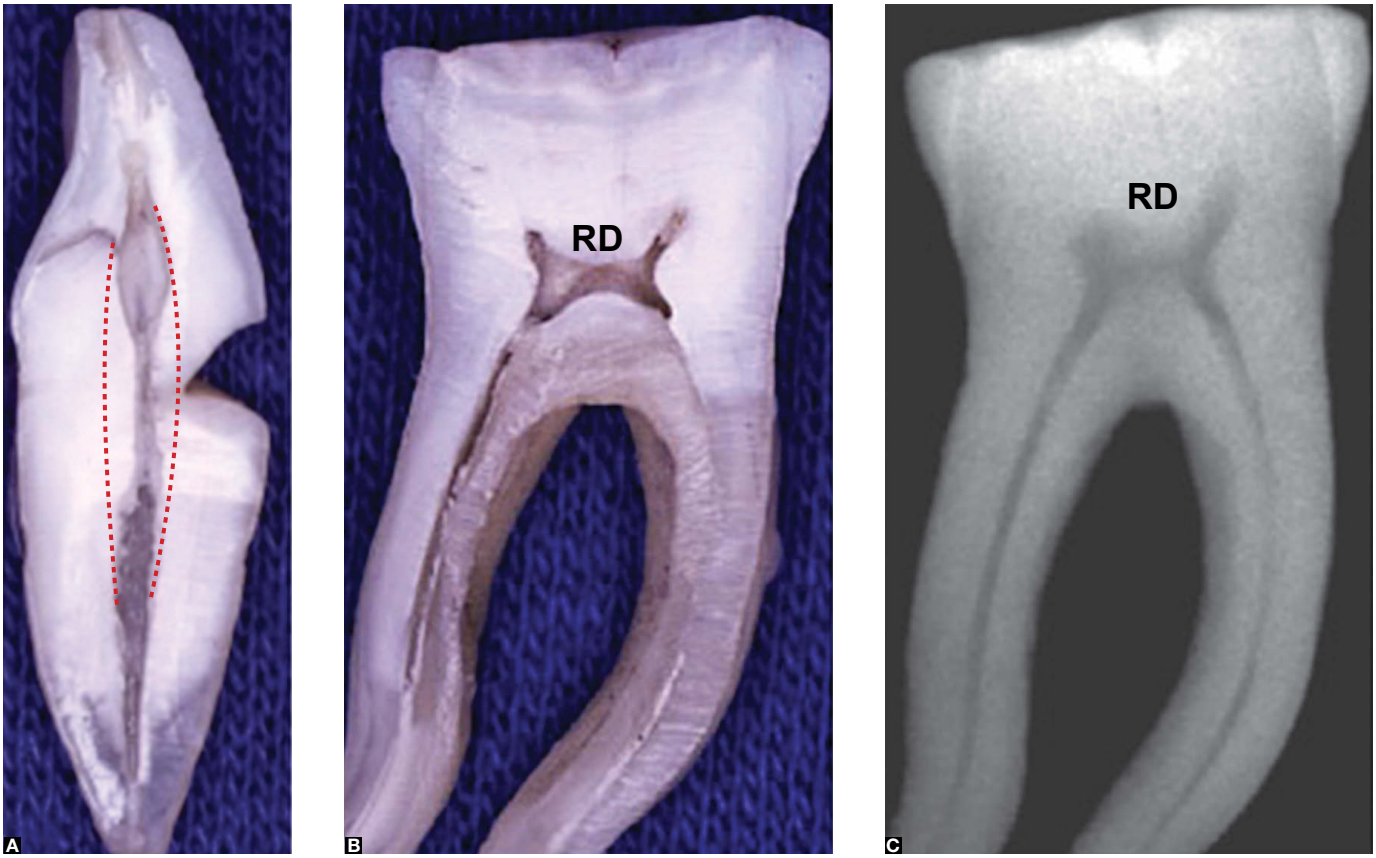

Figure 16. Pulp space reduction by excessive deposition of reactive dentin (RD) induced by abrasion in **A**, and by attrition in **B** and **C**.

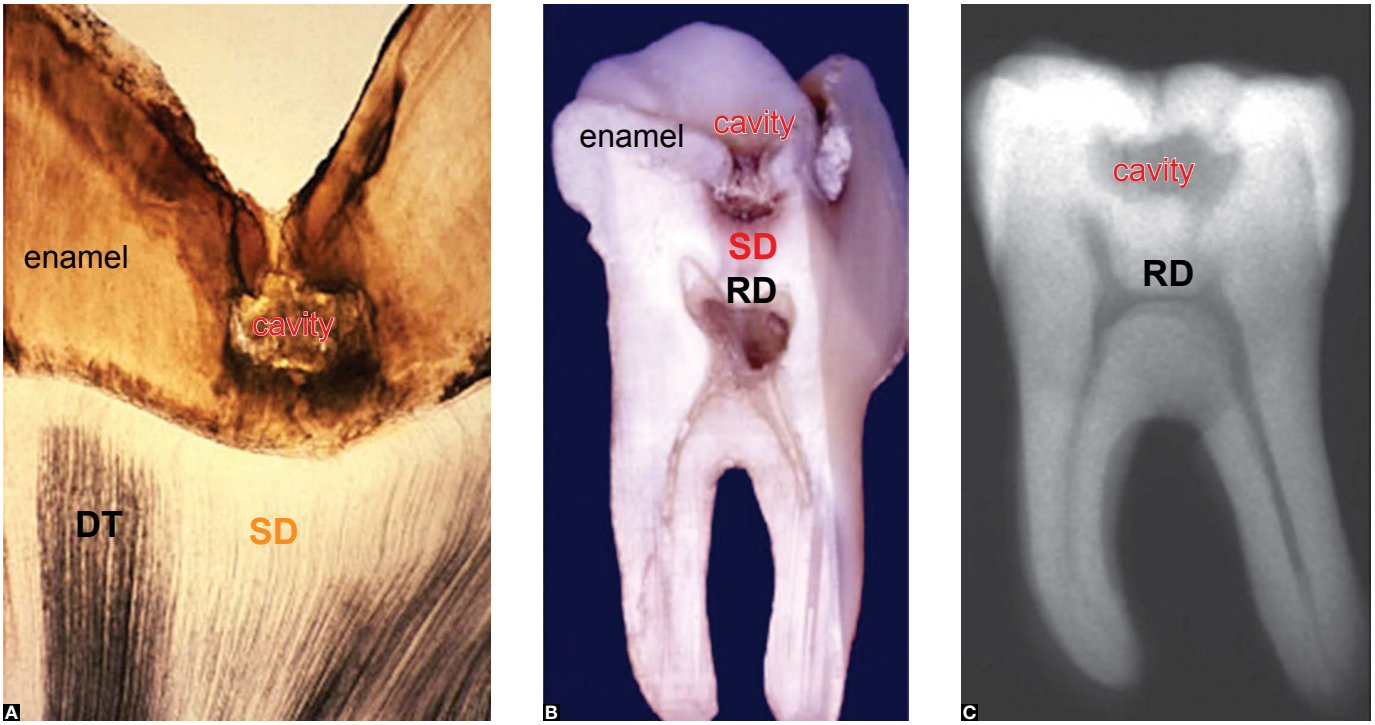

Figure 17. Subjacent to the cavity, sclerotic dentin (SD) is established with the closure of its tubules by peritubular dentin, and in the pulp walls reactive dentin (RD) is deposited, reducing the pulp space. The empty tubules are dark and named dead tracts of dentin (DT) (wearing, original mag.: **A**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

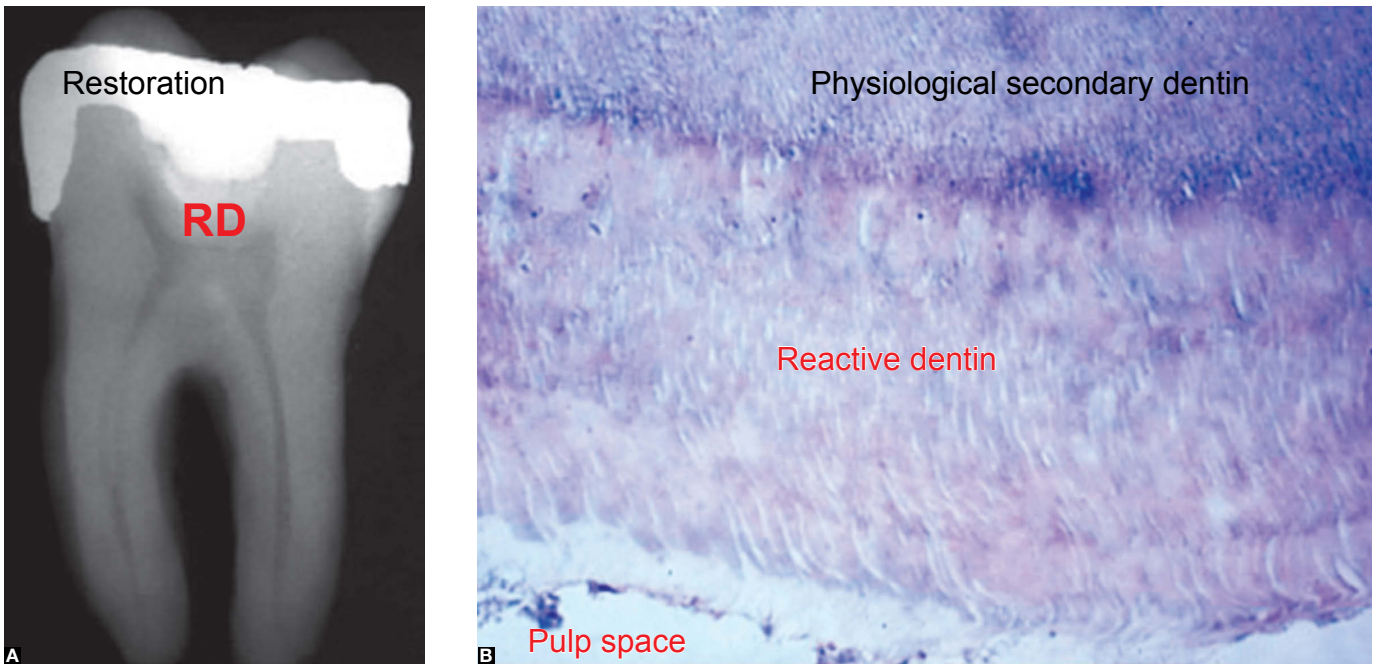

Figure 18. Subjacent to the amalgam restoration, pulp space reduction by reactive dentin (RD) can be observed. In **B**, the reactive dentin presents disorganized lower number of dentinal tubules, thus, a lower mineralized degree (H.E.: original mag.: **B** = 400X).

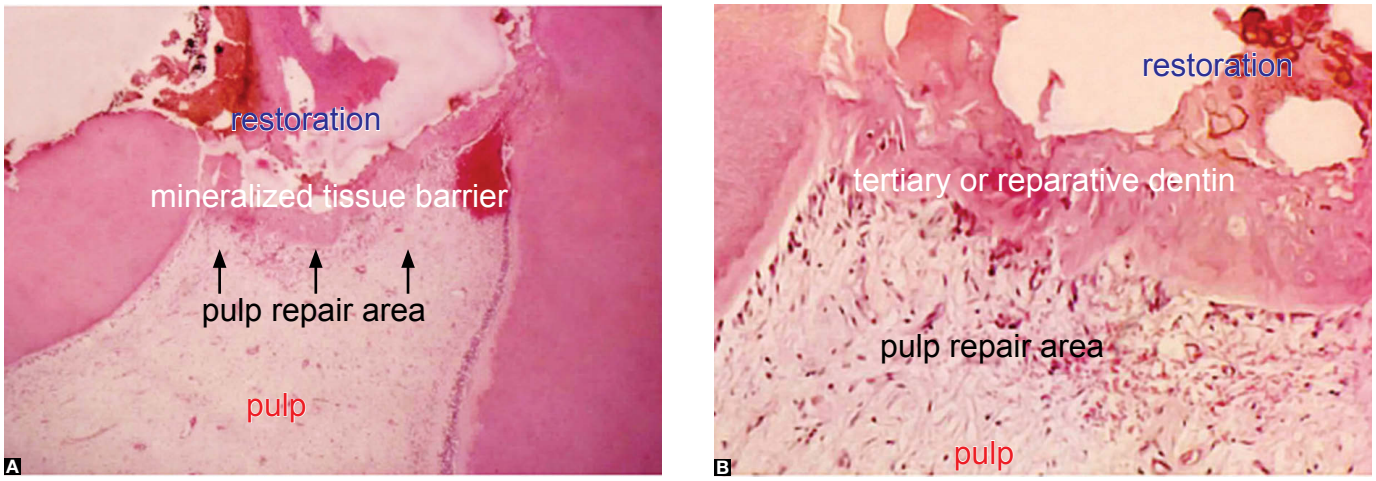

Figure 19. In the exposed human pulp area, at the interface with the calcium hydroxide-based material, pulp repair organized a new odontoblastic layer of third generation. A tertiary dentin was deposited, constituting a mineralized tissue barrier with cell inclusions and continuity with lateral walls (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

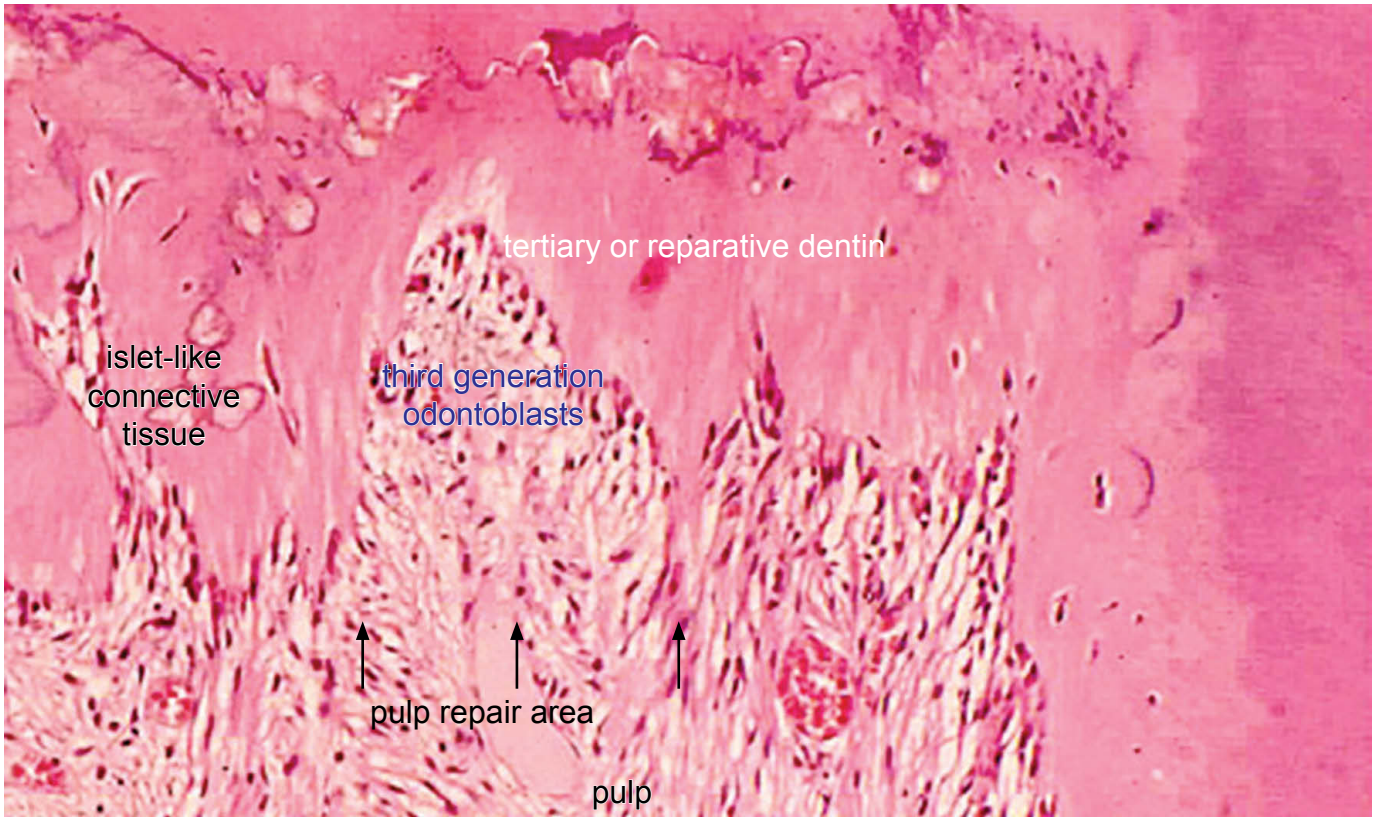

Figure 20. In the human exposed pulp, protected with calcium hydroxide-based material, pulp repair organized a new layer of third generation odontoblasts which deposited tertiary dentin, constituting a mineralized tissue barrier with cell inclusions and islet-like connective tissue in continuity with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

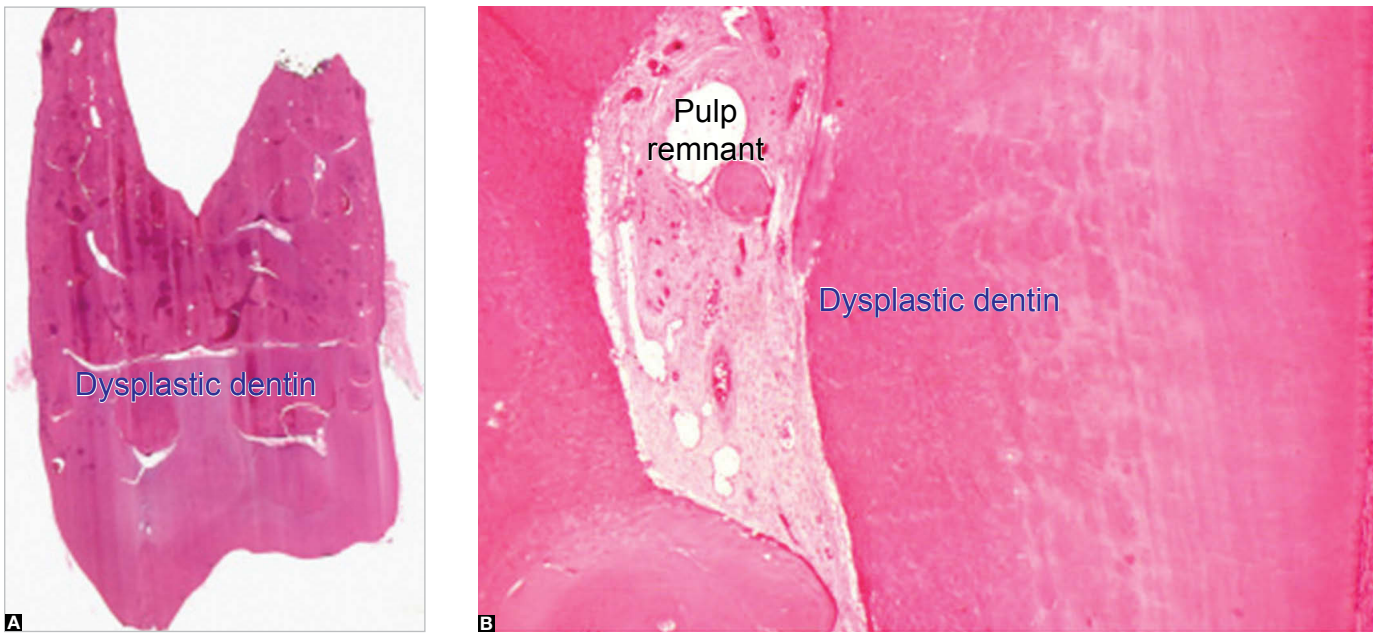

Figure 21. In human pulps, central cells may undergo metaplasia and assume odontoblasts morphology and functions, depositing randomly around it a disorganized and poorly mineralized dentin, also called dysplastic dentin. Dysplastic dentin occur in calcific metamorphosis, Induced by mild traumas, such 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).

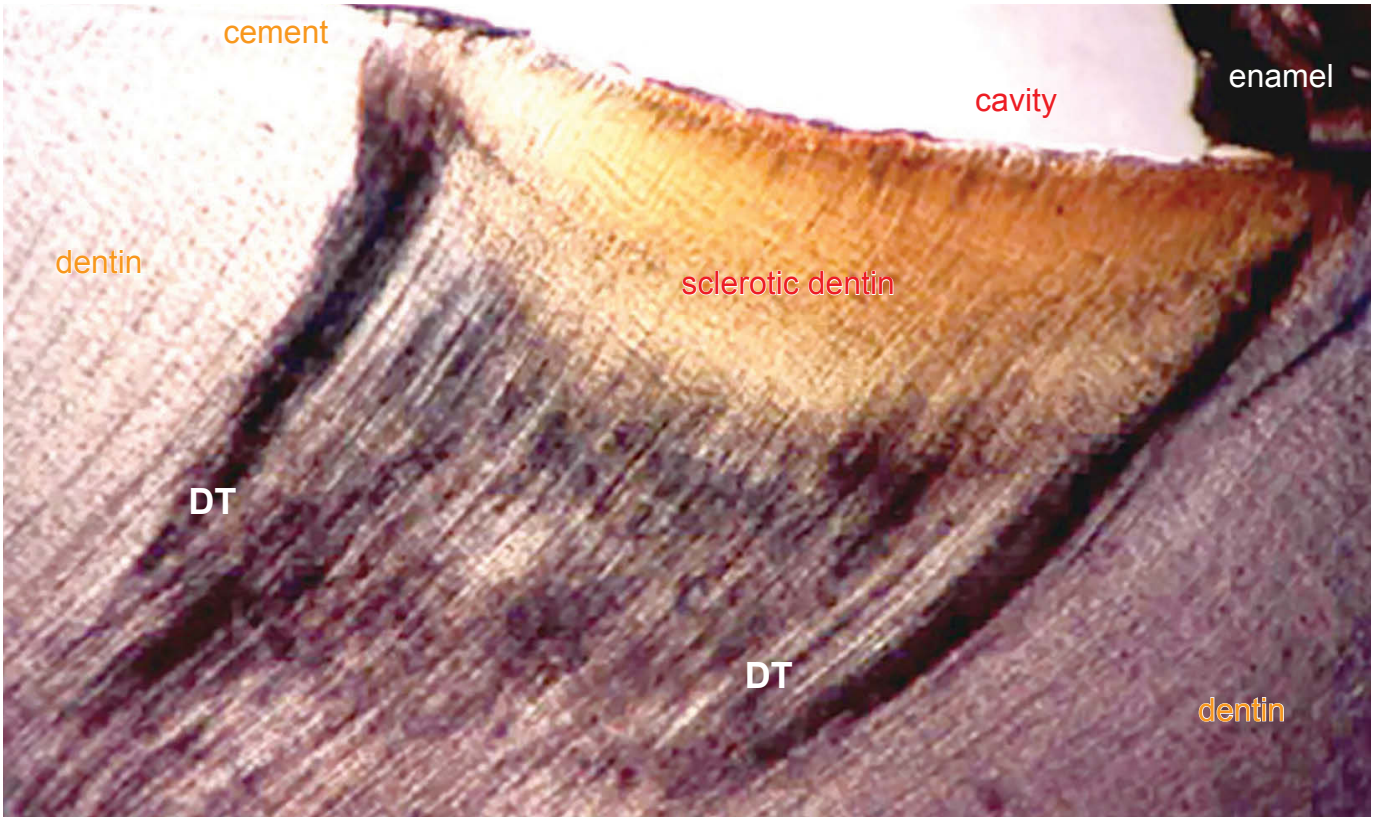

Figure 22. In cervical chronic cavity, sclerotic dentin is characterized by the closure of its tubules by peritubular dentin. The empty tubules are dark and named dead tracts of dentin (DT) (wearing, original mag.: 25X).

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.

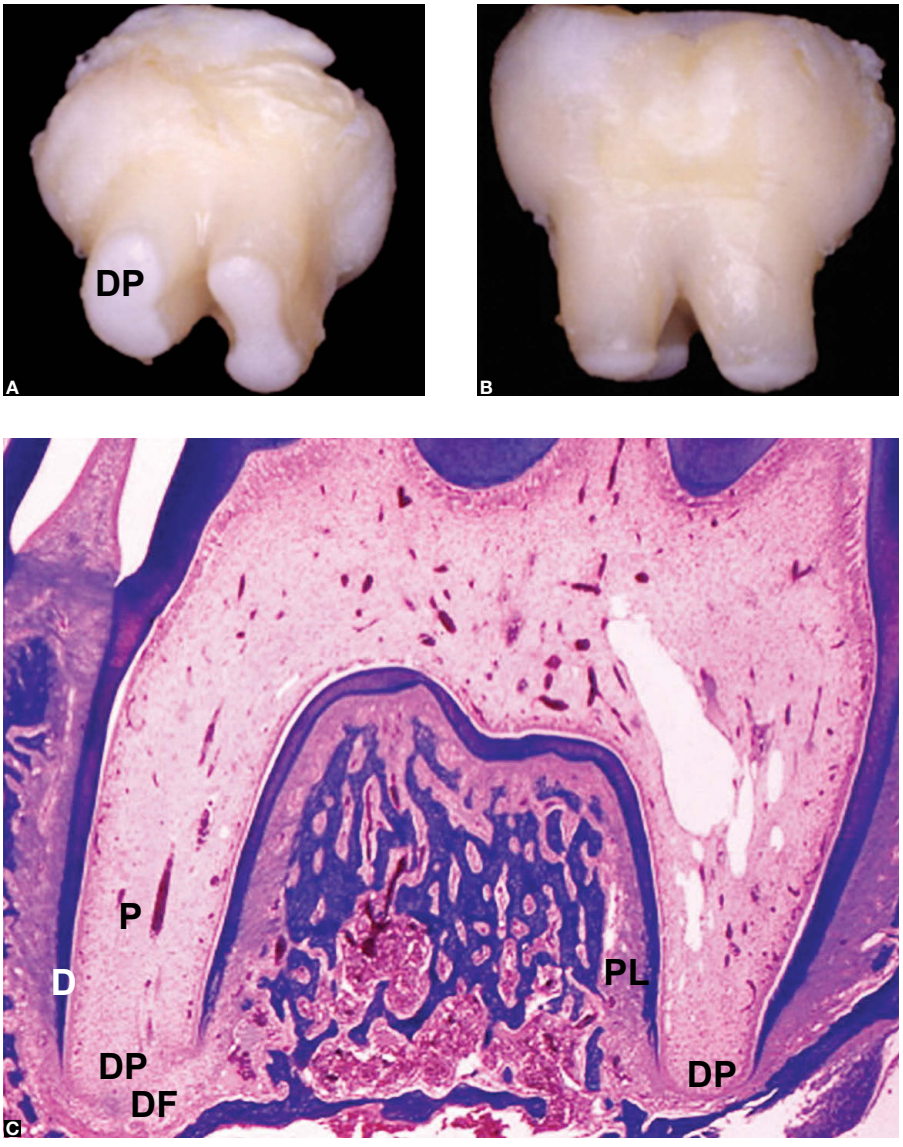

Figure 23. Tooth with incomplete root formation showing dental papilla (DP), dental follicle (DF), and also newly formed dentin (D) and dental pulp (P) (Masson trichrome: original mag.: in **C** = 40X).

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

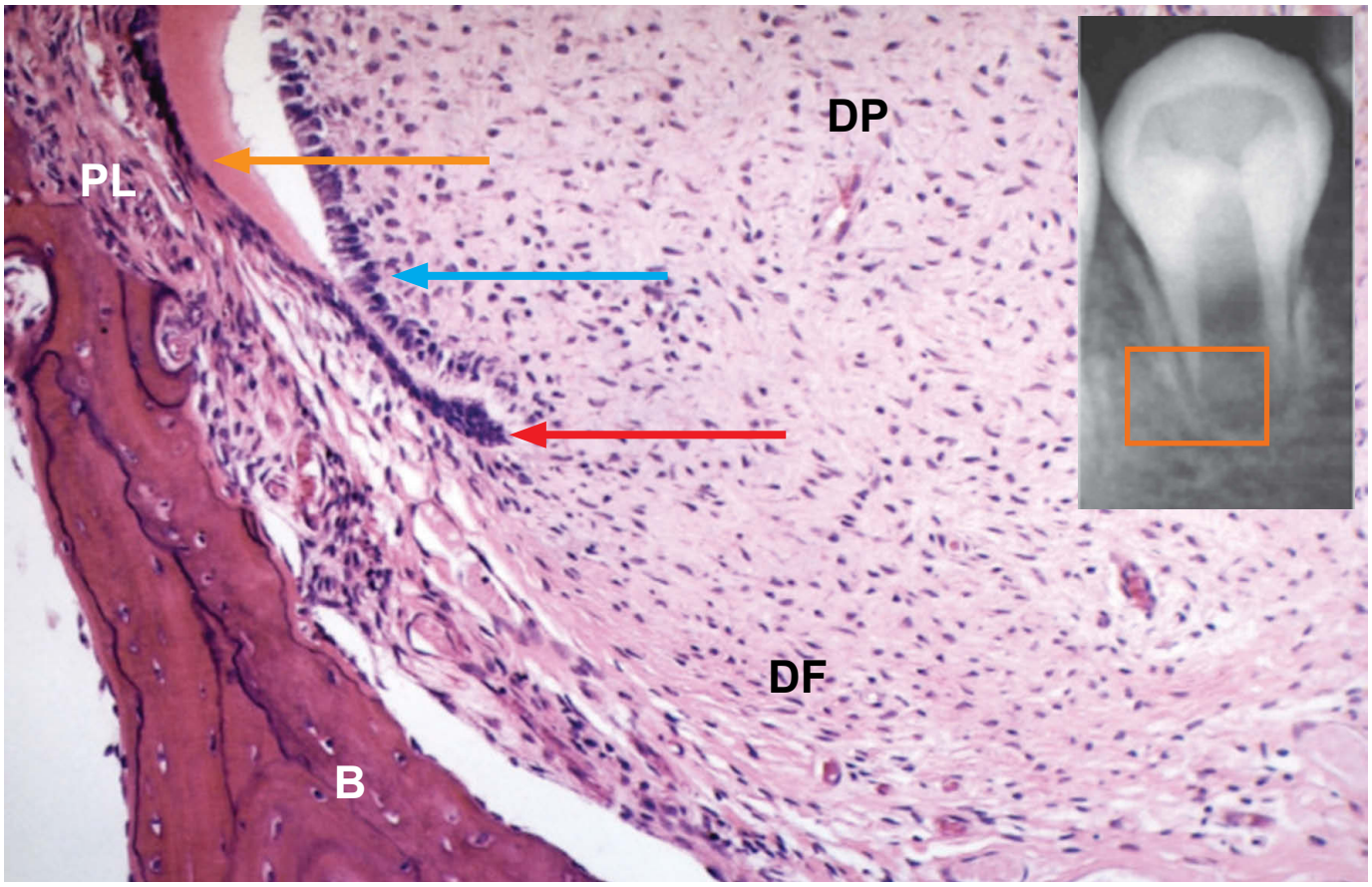

Figure 24. Hertwig's sheath cells (red arrow), odontoblasts (blue arrows) and dentin (orange arrow) in a tooth with incomplete root formation. B = bone; PL = periodontal ligament; DP = dental papilla; DF = dental follicle (H.E.: original mag. = 160X).

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

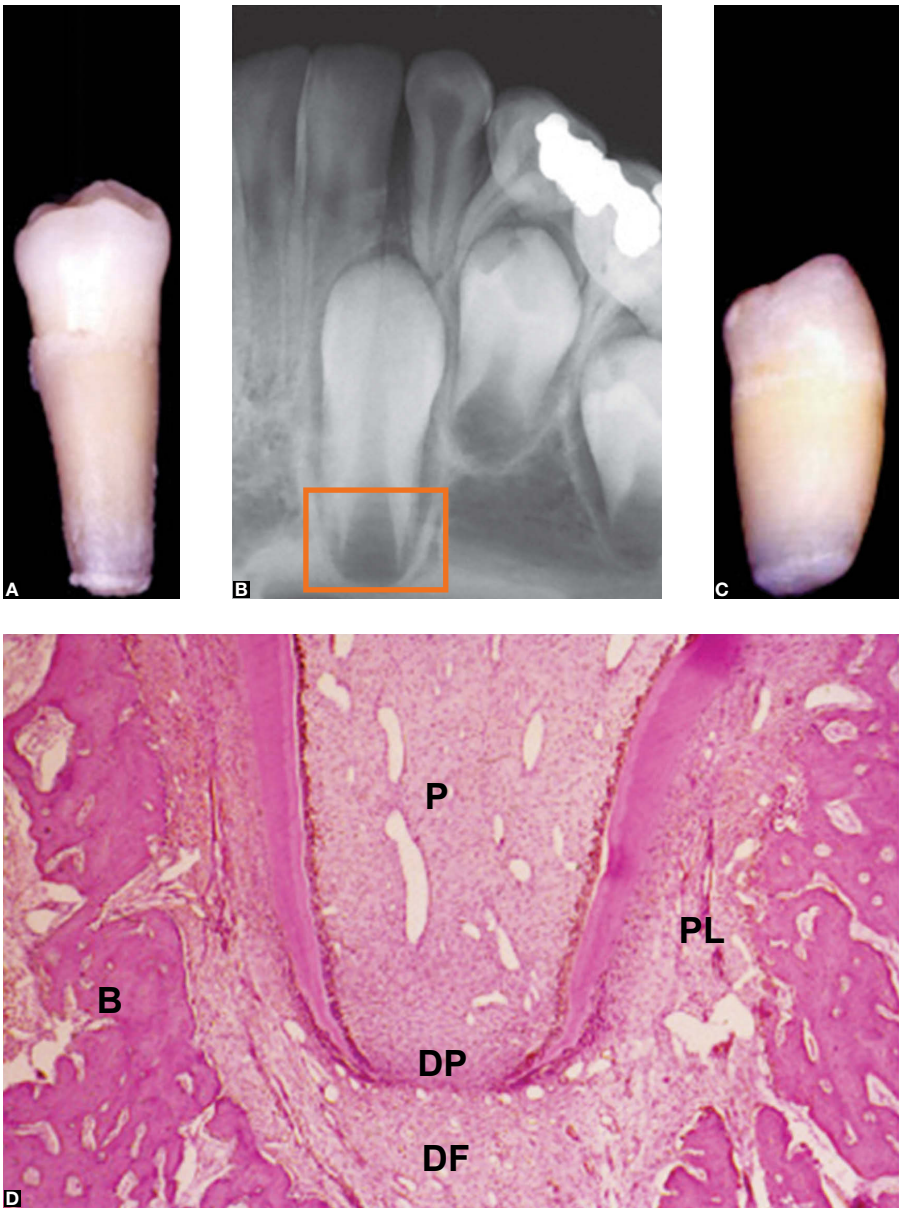

Figure 25. Teeth with incomplete root formation, specifying the root walls shape, the pulp space width and the apical area occupied by dental papilla.
DP = dental papilla; P = pulp; PL = periodontal ligament; B = bone (HE: original mag.: 40X).

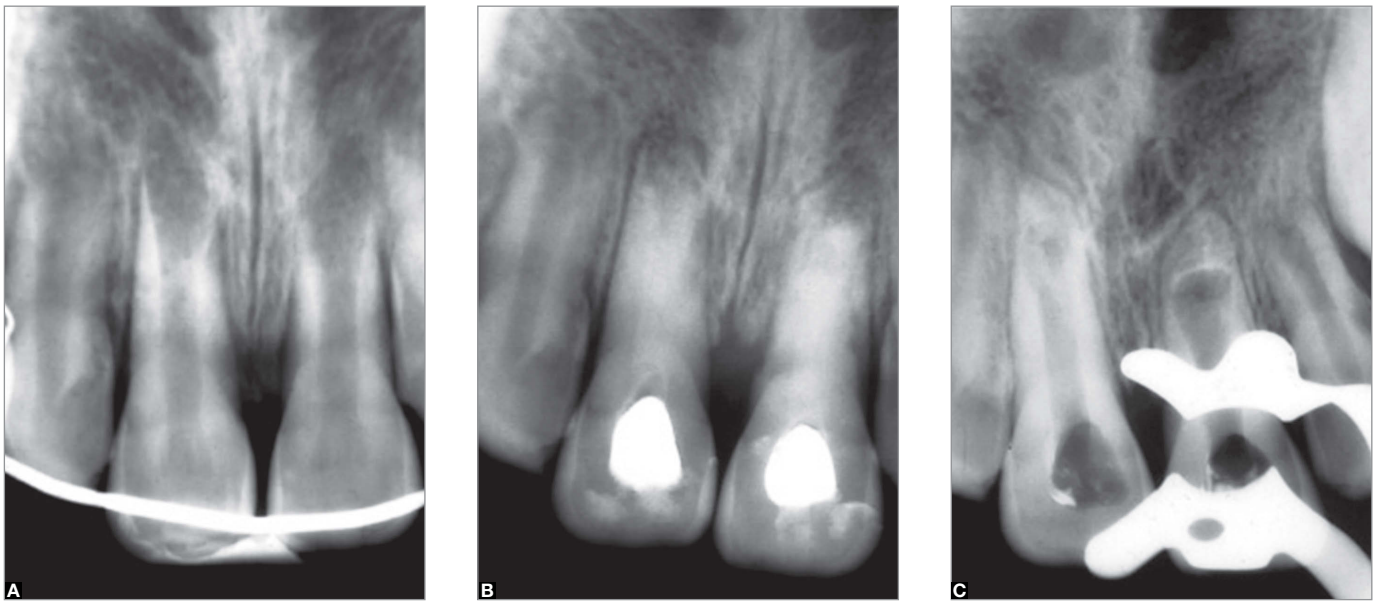

Figure 26. Clinical case with complete apical complementation of teeth with incomplete root formation, of which injury led to loss of root pulp vitality, without affecting the dental papilla and follicular tissues (from Dr. Júlio César Bento dos Santos, Limeira/SP).

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.

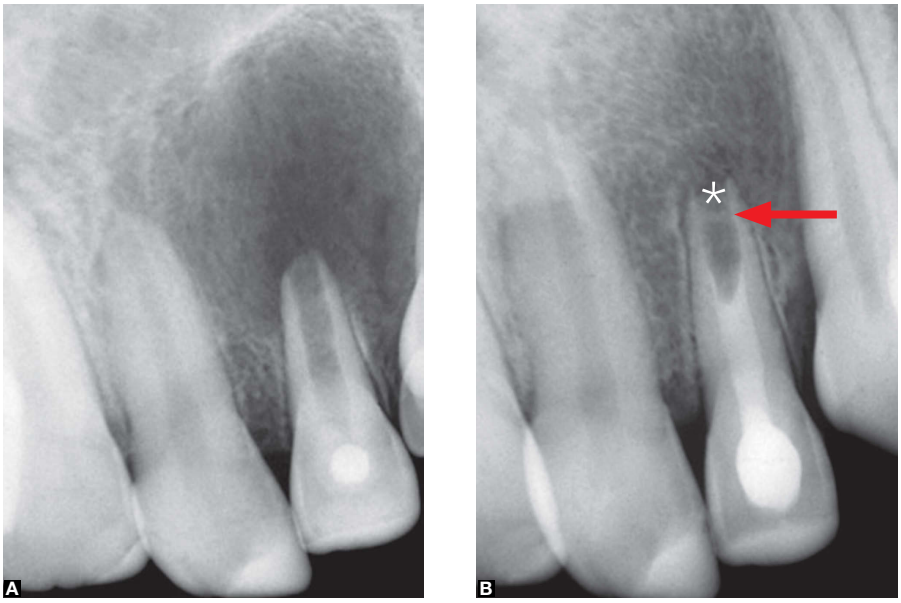

Figure 27. The apical complementation of upper lateral incisor with incomplete root formation, of which traumatic lesion led to loss of root pulp vitality, without affecting the dental papilla and follicular tissues, is being characterized by the likely pulp tissue formation (asterisk) from the surface 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).

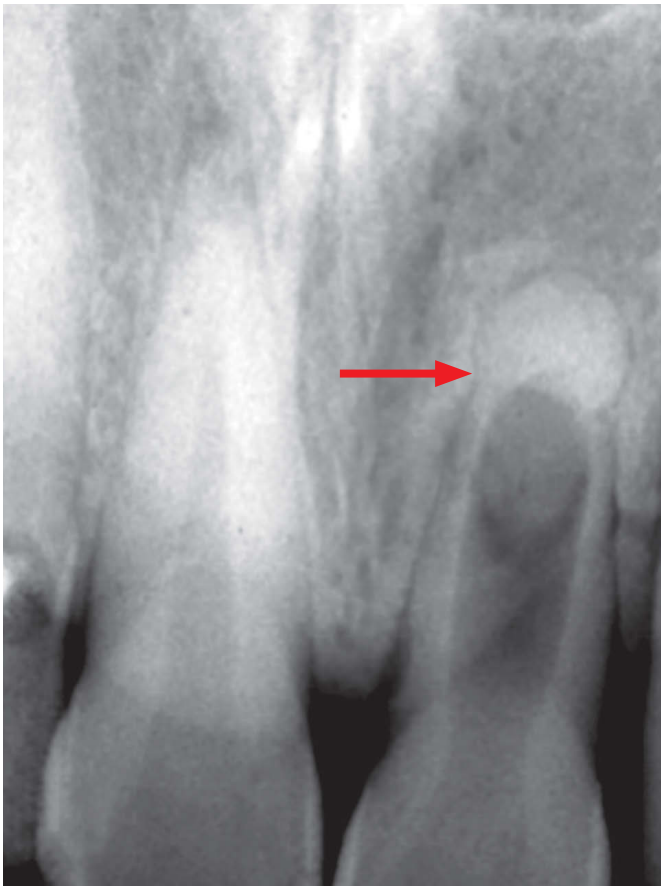

Figure 28. Periapical cap: Apical complementation of upper lateral incisor with incomplete root formation, of which injury led to loss of root pulp vitality disrupting and moving the dental papilla and follicular tissues from the root portion already formed, which continued originating dentin and cementum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

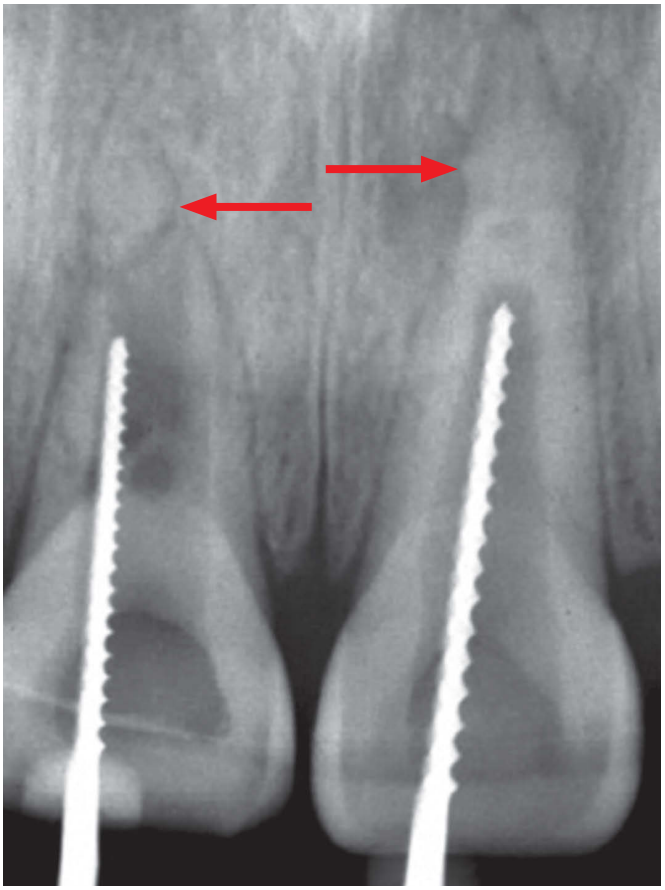

Figure 29. Both apical caps were formed after separation of the mineralized portion of the root, due to dental trauma. Over time, the periapical cap tends to merge with the formed portion, as observed in the upper left incisor and probably will happen on the right too, still apparently separated.

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).

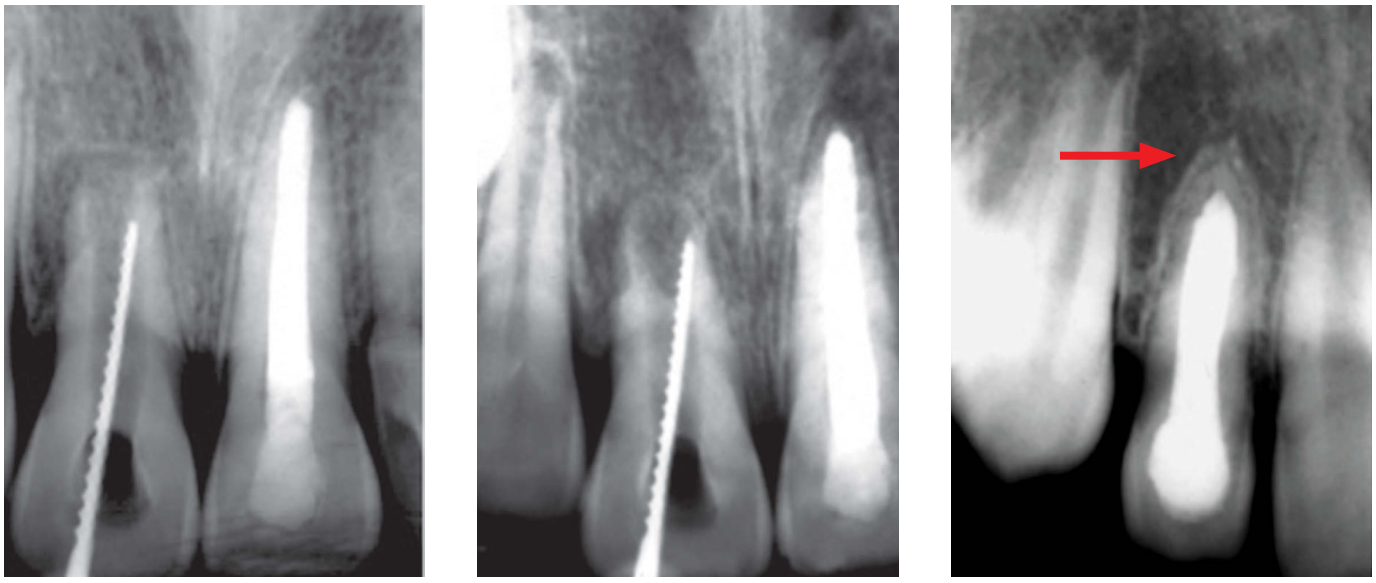

Figure 30. In some accidentally traumatized teeth, the apical complementation in teeth with incomplete root formation does not occur due to loss of root pulp vitality and dental papilla, only with the maintenance of follicular tissues, leading to the formation of cementum and ligament to form the apex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

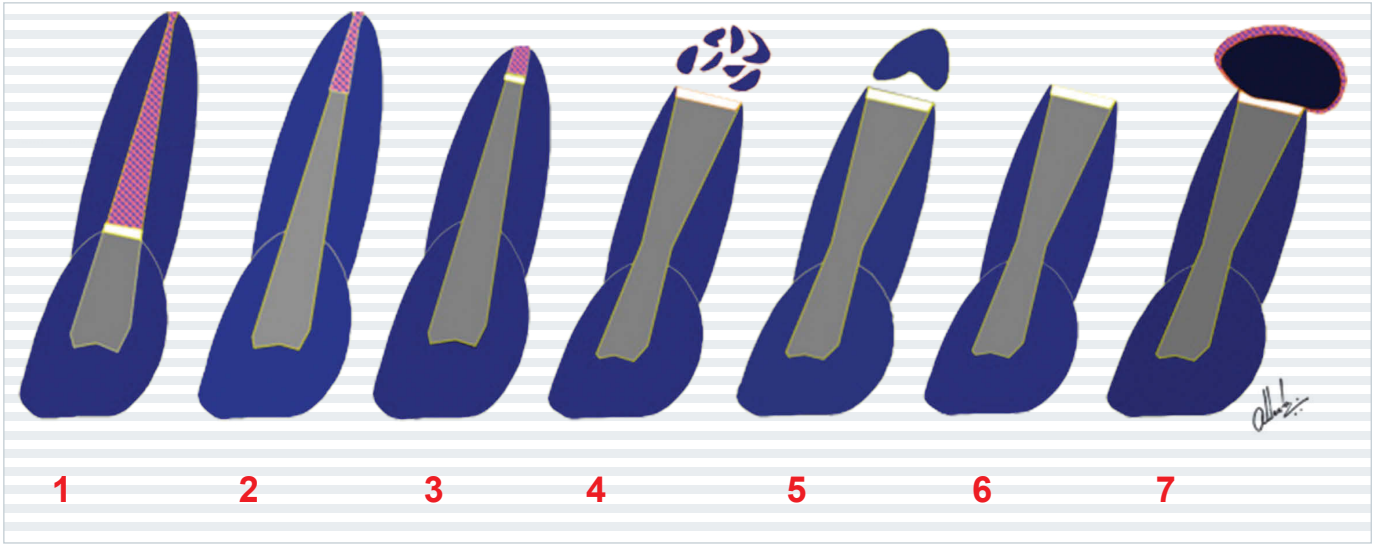

Figure 31. Different development of pulp repair in teeth with incomplete root formation injured by dental trauma (Source: Esberard and Consolaro[10]):
1 - apical complementation with impairment of coronary pulp, only;
2 - apical complementation with impairment of radicular pulp, only, but with dental 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,

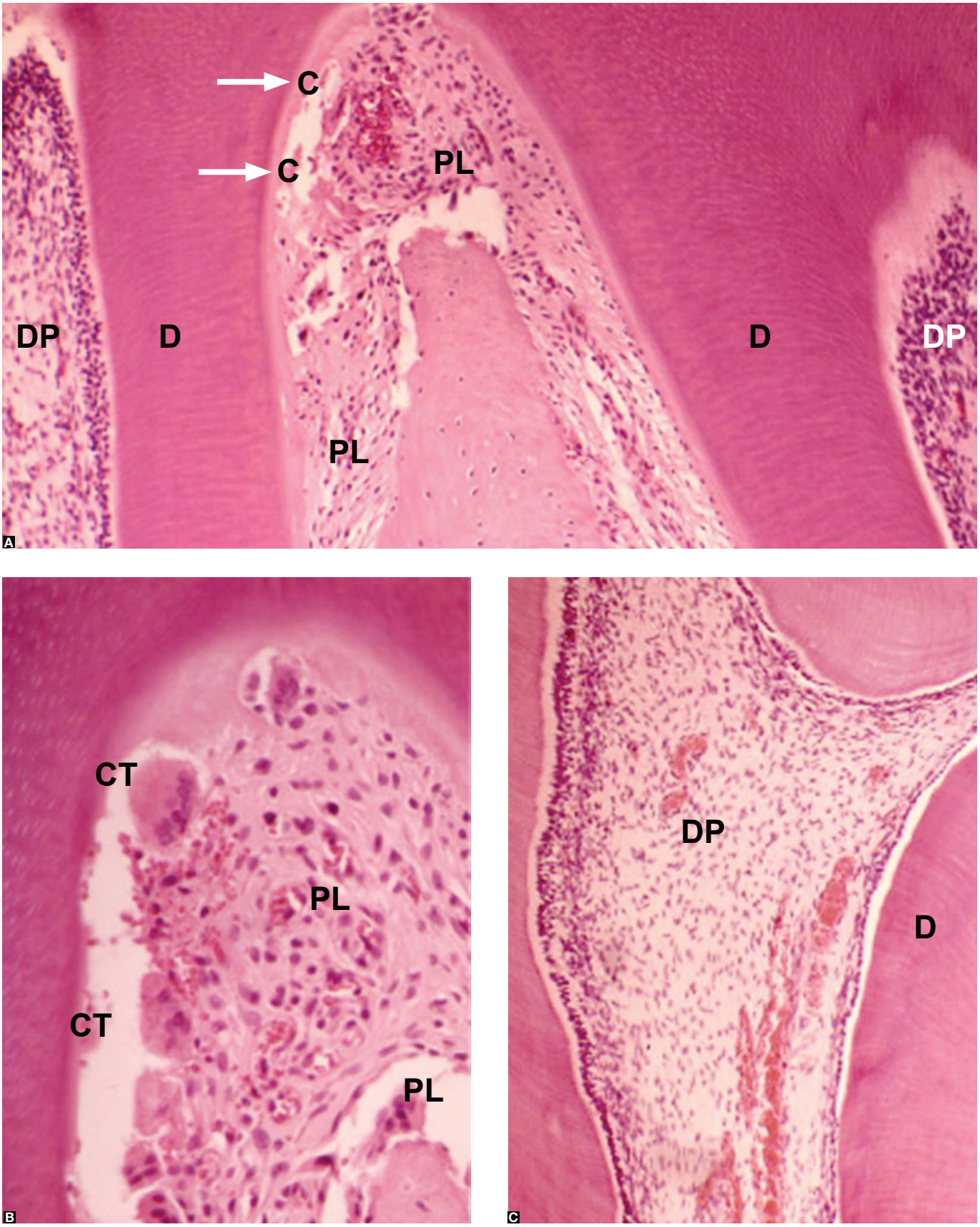

Figure 32. Orthodontically moved tooth, with cementoblasts death (arrows) in **A**, and root resorption with several clasts (CT), proving the effectiveness of the applied forces. The dental pulp (DP) kept its morphology indicating preserved vitality, despite the induced tooth movement. C = cementum; 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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