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Review Article

The Dental Pulp: Composition, Properties and Functions

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Abstract

Derived from neural crests, the mature pulp is due to the proliferation and condensation of apical cells implicated in root lengthening. Adult cells are bound together by intercellular junctions (desmosomes and gap junctions), forming a network. They are further transported to the crown. Root lengthening is associated to tooth eruption and to vertical sliding. Composed by type I and III collagens, fibronectin, tenascin, and other non-collagenous proteins that include a series of proteoglycans, the extracellular matrix is favoring the sliding of pulp cells. A micro-vascular network differing in the root and crown, supply the blood flow. Endothelial cells, pericytes and lymphatic vessels are contributing to direct the vascular implication to pulp development. Inflammatory and immunocompetent cells are present with in the pulp, namely dendritic cells, macrophages, lymphocytes and endothelial cells. They add to the different forms of the immune response of the dental pulp implicated in the different types of programed cell death. Nerves play role in the neutrophin family, identified within the pulp. Neuromediators are released near the nerves. Stem cells issued from the pulp of permanent and deciduous teeth, from the apical papilla and from the dental follicle, contribute to pulp healing and regeneration. Sliding of the pulp cells from the apical to the coronal pulp, and later the lateral sliding due to the effects of Wnt visualized after BIO implantation, are factors implicated in the formation of reparative and reactionary dentin, and consequently to pulp healing.

INTRODUCTION

The dental pulp is enclosed in a rigid environment comprising three mineralized tissues: dentine, enamel and cementum. This strong mechanical support protects the pulp from the microbial-rich oral environment. During the initial carious attack, the pulp turn out to be inflamed and gradually, the evolution of the lesion leads to pulp necrosis and infection. Afterward, the inflammation expands to the surrounding alveolar bone [1].

Three stages of tooth formation are well identified *in vivo* 1) the crown should be completed, 2) root formation and eruption was achieved, and 3) the whole length of the root was complete. Attempts were carried out for culturing pulp cells, even a mixture of cells at immature and mature stages. Cultured *in vitro* for 15 days, pulp cells became ALP-positive and produced a calcified matrix, as judged from the von Kossa staining. However, the success for obtaining primary pulp cultures displays significant variability. Unpredictability was found during the different evaluations performed to establish cell lines. The proliferative activity was unrelated to donor age, individuals and passage number [2]. Altogether, these studies highlight the difficulties in establishing homogeneous and reliable cell lines from dental pulps.

Embryologically, the dental pulp derives from neural crest cells. Proliferation and condensation of the cells lead to the formation of a dental papilla, from which the mature pulp is derived (Figure 1). The peripheral odontoblasts and sub-odontoblastic Hoehl's cell layer are located in the lateral parts of the pulp, with different origins and differentiation programs. In addition to pulp fibroblasts, the dental pulp has high incidence on sensory nerves and on the capillary network. Immunocompetent cells are also implicated in pulp defense. Inflammatory processes lead to pulp wounding and degeneration. Regeneration of the pulp is mainly under the control of stem cells, cells side population, and growth and transcription factors (Figures 2, 3).

Adult pulp cells are bound by intercellular junctions, mostly desmosome-like and gap junctions [3,4]. Tight junctions were not identified. Intercellular junctions bind together pulp cells, which form a syncytium. This implies that pulp cells are transported from the central part of the root to the crown, from the apical pulp to the outer limits of the crown. Pulp cells are located just beneath the sub-odontoblastic, the so-called Hoehl's layer. Obviously, the pulp cells are not transported individually or separately toward the outer part of the pulp, despite the presence of processes acting independently. They are sliding and/or transported altogether from the apical papilla to the coronal pulp.

Pulp cells display cilia. Their motility influence aggregation and/or disintegration of the intercellular matrix. Type I and type III collagen fibrils are well identified in the intercellular matrix [5,6]. They form a stable network despite the presence of MMPs (matrix metalloproteases) implicated in extracellular

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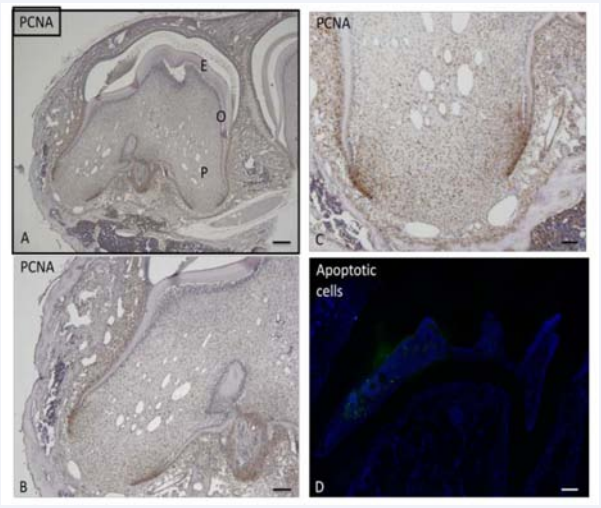

Figure 1 A, B, and C: Proliferation visualized by the cell nuclear antibody (PCNA). PCNA positive cells are present in the apical zone (in the apical cell-rich zone). D: Apoptotic cells are stained in the apical zone of the root (TUNEL staining). Roots are in the process of lengthening. P= pulp, O= odontoblasts, E= enamel. Bars: A,C=300micrometers; B,D = 75micrometers

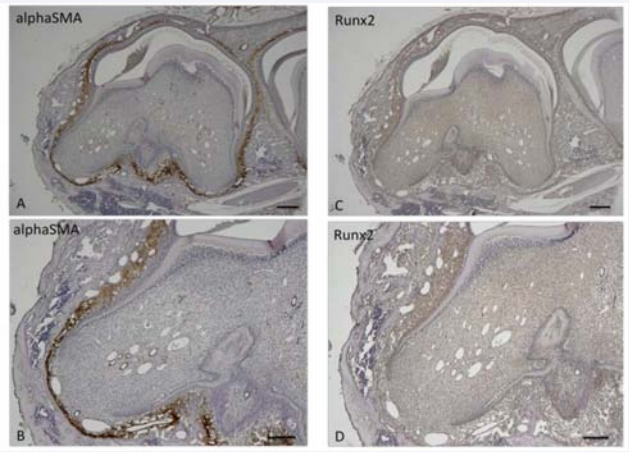

Figure 2 A & B: Immunolabeling of the periodontal ligament by alpha Smooth Muscle Antibody (SMA). C & D: tooth germ labeling by Runx2 antibody. Bars: A, C = 200 µm, B, D =300 µm.

matrix remodeling [7]. Pulp cells migrate and they slide together with extracellular matrix renovation. Small aggregates of proteoglycans (PGs) punctuate the thin collagen fibrils. There are also some indications that as an amorphous gel, PGs facilitate the migration of pulp fibroblasts within the dental pulp [4].

Root lengthening

Three zones have been identified in molars after beads implantation (Figures 4-6). Zone I and zone II are located in the crown, namely they occupy the isthmus between the mesial and central pulp. In contrast, zone III is located in the apical root [8,9]. PCNA labeling is important in the root, and more restricted in the crown. It is also in zone III that a few cells underwent apoptosis. Apoptotic cells are rare and only detectable near the apex (Figures 1,4-8). They are disseminated in the apical zone and in the pulp located in the root. No labeling appears in the coronal parts, at least 7 days after beads implantation. Cell proliferation implies that root lengthening occurs essentially in the forming root. In the surrounding apical zone, an apical cell-rich zone is seen, followed by the apical papilla mesenchyme. The radicular dental pulp is located in the root, and this is the place where pulp cells differentiate into odontoblasts and in the sub-odontoblastic layer (the so-called Hoehl's layer) (Figures 9,10,13-14). The cells slide from the apical cell-rich zone to the apical papilla mesenchyme. Odontoblasts and Hoehl's cells migrate toward the dental pulp beneath the lateral margins. They start to synthetize and secrete the mantle dentin, a process followed by the formation of circumpulpal dentine. After a limited lengthening associated with tooth eruption, the coronal part forms and the tooth emerge in the oral cavity.

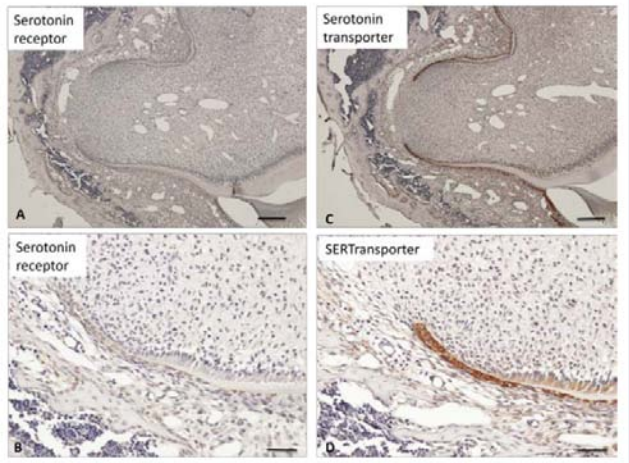

Figure 3 Root apical zone of the forming tooth. A & B: Immunolabeling for serotonin receptor. Bar= 100µm. C & D: immunolabeling for serotonin transporter. Bars: A, C = 300 µm, B, D =75 µm.

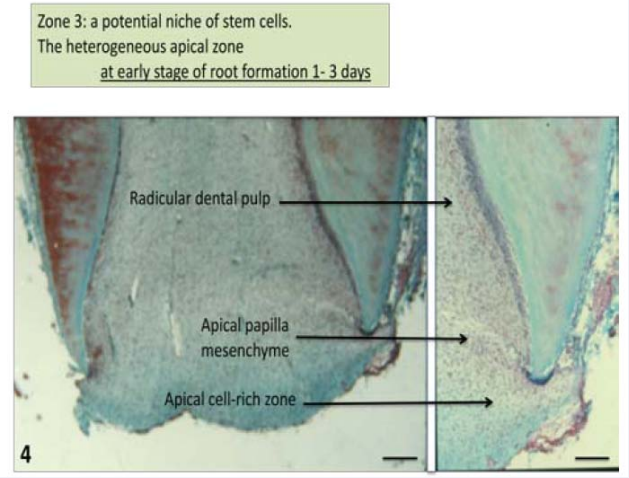

Figure 4 Respective location of the apical cell-rich zone, the apical papilla mesenchyme and radicular dental pulp. These are niches for stem cells at early stages of root formation. (at 1 to 3 days).

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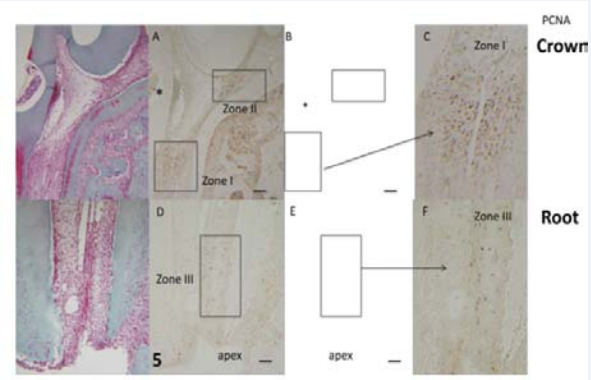

Figure 5 A & D: After the preparation of a cavity (asterisk) three zones are identified in the rat's molar. In B & C: Zones I is labeled with PCNA. In E and F: Labeling of root (zone III) with PCNA. Bar= 100 µm.

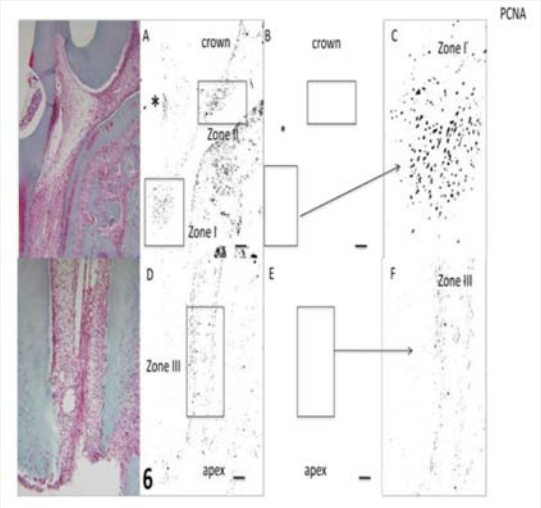

Figure 6 A & D: Zones I, II and III in the molar after the preparation of a cavity (asterisk). Coronal zone labeled with PCNA (zone I); E & F: Zone III: apical zone of the molar.

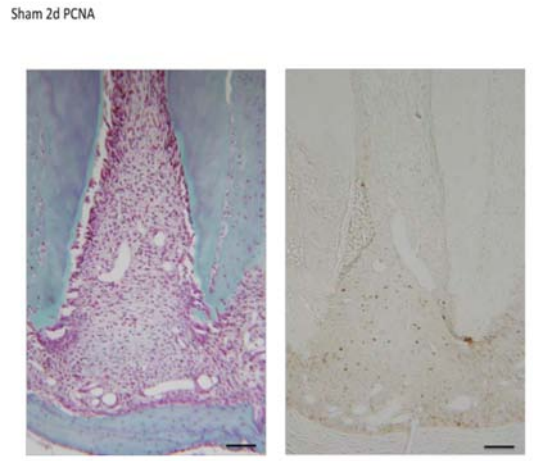

Figure 7 A: H&E staining of a sham rat. B: PCNA labeling. Molar day 2. Bar= 100µm.

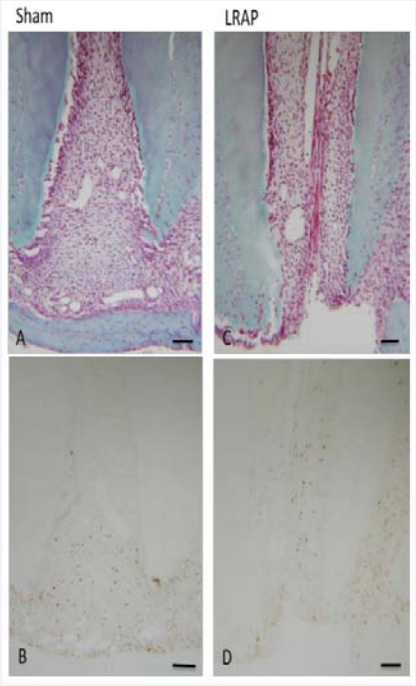

Figure 8 A & B: Sham rat. The apical cell-rich zone is labeled. C & D: Labeling after 2 days with leucine-rich amelogenin peptide (LRAP). The radicular dental pulp is labeled. Bar= 100µm.

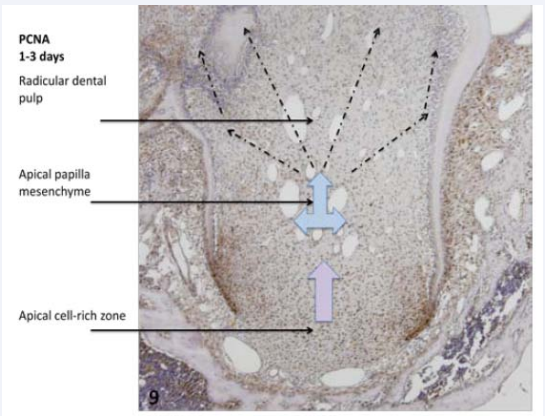

Figure 9 Schematic sliding of stem cells from the apical cell-rich zone to the apical papilla mesenchyme, followed by migration of the cells toward the radicular dental pulp. PCNA labeling of pulp cells after 1-3 days.

Pulp-derived fibroblasts: composition

The tooth pulp is composed by a syncytium or stellate-appearing fibroblasts. Gap junctions link cells. Desmosomes also contribute to establish intercellular junctions. The width of the cell body varies in shape, displaying several cell processes. The width of the cells ranged from 9 to 15µm, with some cell processes extending up to 22µm in length [10].

They synthetize type I collagen, fibronectin, osteonectin and bone sialoprotein. Dipeptidyl peptidase I, cathepsins B and D, may also contribute to the degradation of native type I collagen

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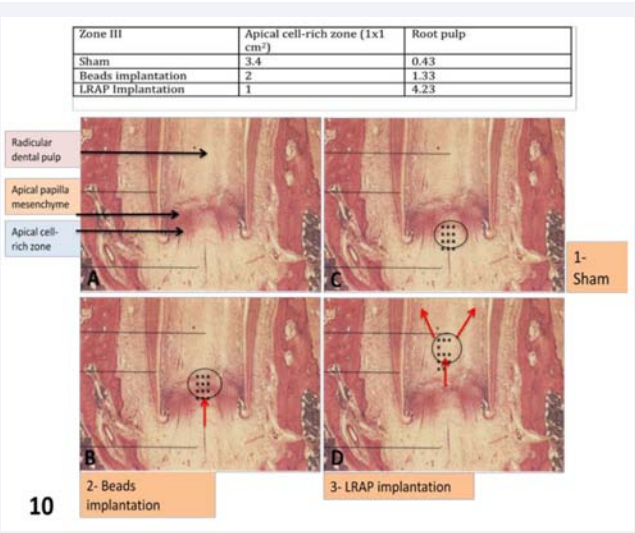

Figure 10 A: Localization in the apical cell-rich zone. B: After beads implantation, cell migration starts. C: Sham molar. Labeled cells are present in the apical cell-rich zone. D: After implantation of beads loaded with LRAP labeled cells are present in the radicular dental pulp.

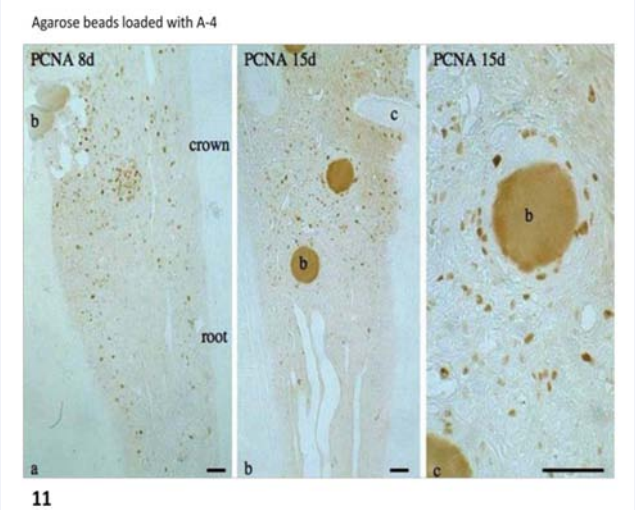

Figure 11 a: Implantation of agarose beads loaded with A-4 (an amelogenin peptide with exon 4 deletion), after 8 days. PCNA labeled cells are located beneath the odontoblast layer and in the crown. b & c: At 15 days, no labeling is detected in the root and labeled cells are seen in the crown around the beads. Bar= 100µm.

in vitro [7,11].

In culture, pulp fibroblasts synthetize type III collagen. *In vitro*, proteoglycans are synthetized and degraded by adult fibroblasts. Identified as chondroitin sulfate (CS), dermatan sulfate (DS) and heparan sulfate (HS), DS and CS are the principal GAGs associated with the cell layers. CS and DS correspond to decorin and biglycan. Versican and syndecan are also identified. After 10-15 days, cultured fibroblasts produce mineralized nodules. Pulp fibroblasts release predominantly glycoproteins with an apparent molecular weight of 230,000 but other species are also seen, the major fucosylated glycoprotein being fibronectin.

Fibroblasts (or pulpoblasts, according to Baume, 1980)[12] are involved in the mineralization of hard dental tissues. The main ECM molecules are type I and III collagens, and non-collagenous proteins such as fibronectin, tenascin, osteonectin and osteocalcin, and glycosaminoglycans including hyaluronic acid, chondroitin sulfate, heparan sulfate (CS, HS) and phospholipids.

The effects of DMP-1 on interleukin-6 (IL-6) and interleukin-8 (IL-8) are unclear [13]. Pulpoblasts are identified on the basis of immunoreactivity against vimentin and collagen 1α1 by immunofluorescence and negative staining to CD45, CD34 and

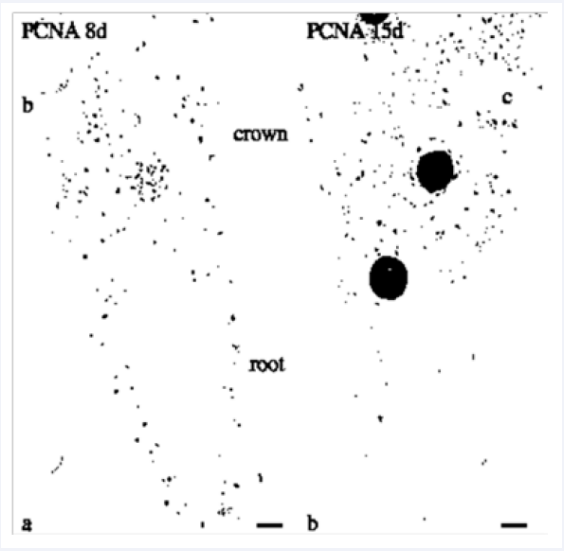

Figure 12 PCNA labeling after 8 (a) and 15 days (b). Cells have migrated from the apical part to the crown, around the agarose beads. Bar= 100µm.

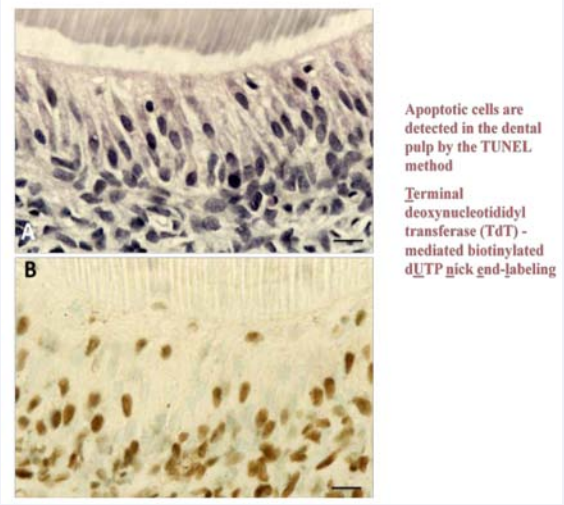

Figure 13 Detection of apoptotic cells in the odontoblast layer. A: H&E labeling. B: Apoptotic cells are labeled using the TUNEL method (terminal deoxynucleotididyl transferase (TdT)- mediated biotinylabeled dUTP nick end labeling). Bar= 100µm.

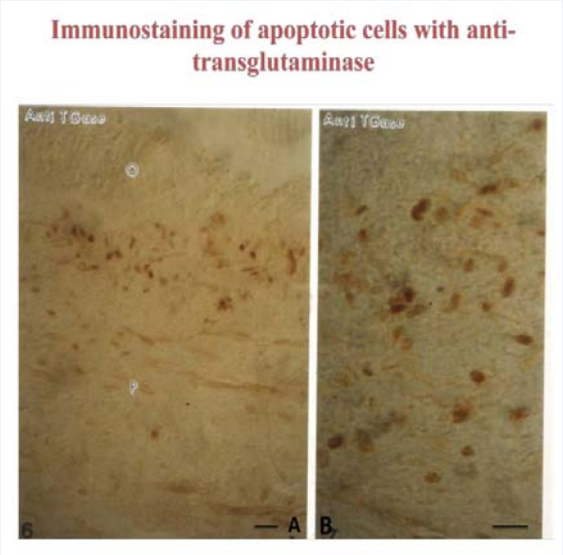

Figure 14 A & B Immunostaining of apoptotic cells with a transglutaminase antibody. Some sub-odontoblastic Hoehl's cells are labeled. No labeling is seen in the odontoblast layer (O), and in the dental pulp (P). Bar= 100µm.

cytokeratin. Inhibition of the p38 mitogen-activated protein kinase pathway blocked the proinflammatory effect of DMP-1 on dental pulp. The cells produce MMPs (collagenase-2, collagenase-9, also named gelatinases), and matrix metalloproteinase-3 (the "so-called" stromelysine). The tissue inhibitors of MMPs (TIMPs) play role in dental pulp destruction [11].

Collagen degradation was stimulated by IL-1α and inhibited by MMPs inhibitors. IL-α1 increased the secreted protein level of TIMP-1, and only affected the level of TIMP-2. It was concluded that IL-1α induces pulp destruction by different regulations of MMPs and TIMPs.

Matrix metalloproteinase-8 (MMP-8) is expressed by pulp tissue. The effect of TGF-β1 down-regulated the expression, synthesis, and activation of MMP-8. Odontoblast-derived gelatinases participate in the organization of dentine organic matrix prior to mineralization [14].

Pulp vascularization and microvascular network - Angiogenesis: role of human pulp fibroblasts

The branches of artery entered the papilla by the apex. Resin casts [15] show in the root canal a peripheral fishnet arrangement, with central arterioles and venules. In the crown, a terminal sub-odontoblastic capillary network display veinous-veinous anastomosis, ending by capillaries and arterio-veinous anastomosis. Firstly, feeding arterioles (35-45 µm in diameter) are continuous with 2d feeding arterioles (24-34µm in diameter). Terminal arterioles (16-23µm), pre-capillaries (12-15µm), capillaries (>8µm), post-capillaries venules (12-23µm), collecting venules (24-50µm) and finally large venules (>50µm) form a continuous vascular bed within the dental pulp [16]. Shunts about 8-15µm in diameter are seen along the coronal pulp. Endothelial cells (intima and media, bearing a smooth muscle type) are covered by a continuous layer of flat pericytes. They are embedded in thin microstructures of the pericellular and extracellular matrix (adventice). They are limiting capillaries lumens where blood and blood cells are circulating [17].

Lymphatic microcirculation: substance P was seen to reduce the diastolic diameter by 56%, and the systolic diameter by 47%. When 0.2 to 0.3 cc of sterile colloidal carbon was injected in the pulp horn, and the teeth extracted 1 to 3h later, capillaries were characterized by a thin endothelium with large intercellular clefts, absence or incompleteness of basement membrane, absence of pericytes, absence of luminal red blood cells. The presence of a filamentous material and surrounding collagen fibrils were noted, displaying with some structural variations [18].

FGF-2 and VEGF are expressed in human dental pulp fibroblasts. The expression is increased after injury. Angiogenin factors are necessary for pulp healing, especially at the pulp injury site [19].

The dental pulp has a high blood flow, estimated to be 40-50 ml/min/100g of pulp tissue. Shunt vessels have also been observed. They can be arterio-veinous anastomoses, venous-venous anastomoses or U-turn loops. The dental pulp is composed by a gelatin-like material (proteoglycans and other glycoproteins), reinforced by irregularly arranged and interlaced collagen fibers. The primary function of pulp capillaries is to supply oxygen and nutrients to its constituent cells. It provides also an exit route for metabolic waste products from the tissue. Waste products are removed from the pulp by venules, collecting lymph in ganglia and the circulating blood in large vessels.

Perivascular niche of postnatal mesenchymal stem cells were identified in human bone marrow and dental pulp [20]. Human bone marrow stromal cells and dental pulp stem cells were isolated by immuno-electron using the antibody STRO-1 that recognizes an antigen on perivascular cells. Freshly isolated STRO-1 positive are expressed by von Willebrand factor, CD146, smooth muscle cells and pericytes (alpha-smooth muscle actin, CD146 and a pericyte associated antigen (3G5) by immunohistochemistry, FACS and immunomagnetic bead selection. This helps to understand the factors that regulate the formation of mineralized matrices. The exact location, developmental potential, and ontogeny of these stem cells are largely unknown. Identification of mesenchymal stem cell niches in bone marrow and dentin pulp help to elucidate the fundamental conditions necessary to selectively maintain and expand primitive multipotential populations *in vitro*, directing their developmental potentials *in vivo*.

Inflammatory cells & immunocompetent cells

Immune defense mechanisms of the dental pulp are divided into two major populations: B-lymphocytes and T-lymphocytes [21]. B-lymphocytes produce antibodies against specific antigens following blast formation, proliferation, and maturation into plasma cells. T-lymphocytes are divided into T helper cells (CD4+) and cytotoxic T-cells (CD8+). Upon activation, they secrete several cytokines, a group of biologically active molecules that regulate the intensity and /or duration of the immune response by stimulating or inhibiting the action of various target cells. CD4+ T-lymphocytes are further classified into Th1 and Th2 cells.

The Th1 cells produce interleukin (IL)-2 and interferon-gamma (IFN-g) and they are involved in activation of macrophages, whereas Th2 produce cytokines such as IL-4, IL-5 and IL-6 and stimulate proliferation and differentiation of B-lymphocytes (Table 1).

Dendritic cells (DCs) and macrophages belong to separate lineages. They share several properties in terms of morphology, phenotype and function. Macrophages predominantly act as scavenger cells. They can produce biologically active substances including (i) microbicidal enzymes and reactive oxygen species; (ii) several cytokines, such as IL-1; IL-6, and tumor necrosis factor (TNF); and (iii) growth factors for fibroblasts and endothelial cells that promote wound repair.

T-lymphocytes are now recognized as essential resident of the dental pulp. CD4+ and CD8+ lymphocytes were first detected in the normal human pulp. B-lymphocytes are rarely encountered in the normal dental pulp, and a significant role for B-lymphocytes is not obvious. Macrophages (histiocytes) show heterogeneity in terms of cytochemical markers expression. These cells express class II molecules with increased age of the animals.

Mast cells contain several bioactive substances (histamine, leukotrienes, and platelet-activating factor. They participate in neurogenic inflammation releasing histamine in response to neuropeptides.

Odontoblast-like cells differ from the pulp fibroblast response to TLR2-, TLR3- and TLR4-specific agonists. The respective roles of these cells in dental pulp innate immunity include the extent to which they influence immature DC migration [17]. Odontoblast-like cells stimulated with LTA initiate an immune response by producing chemokines and recruiting immature dendritic cells (DC).

Extensive cell death and tissue necrosis, also called coagulation necrosis, may also occur. More recently, a cascade of three stages was identified. There is actually a need for redefinitions of the physiopathological events, which might occur. Necrosis provokes a slight irritation and stimulates pulp repair. Cell migration and proliferation control mesenchymal and endothelial pulp cells, and also the formation of collagen. Necrosis affected pulp cells. Caspases exist in an active proform in the cytosol. Pyroptosis is a physiologically important form of cell death. Apoptosis or programed cell death is an active process characterized by cell shrinkage, membrane blebbing, formation of apoptotic cell bodies, chromatin condensation and genomic fragmentation [22].

Cellular components are necessary for the initial recognition and the subsequent processing of antigens that elicits an immune defense reaction. Immune cells in the dental pulp are peripheral T cells (helper/inducer and cytotoxic/suppressor). Cells presenting the major antigens are the DCs, histiocytes/macrophages, mega-karyocytes, hematopoietic precursor cells, and osteo/odontoblast progenitors present foreign antigens as HLA-DR antigens on the cell surface to CD4 T-lymphocytes. Other antigen-presenting cells are similar to macrophages, four times more common than the DCs.

Table 1: Properties of antigen-presenting cells.

Cell Type	Expression of Class II MHC molecules	Co-stimulatory molecules
Dendritic cells	Constitutive	Constitutive
Macrophages	Inducible	Inducible
B-lymphocytes	Constitutive	Inducible
Endothelial cells	Inducible	Constitutive
Various epithelial and mesenchymal cells	Inducible	?

In the intact pulp, two distinct DCs populations have been identified. CD11c+ are present at the pulp-dentin border, beneath occlusal fissures, whereas F4/80+ DCs are almost concentrated in the perivascular region of the inner pulp and in the subodontoblastic layer. CD11c+ DCs express Toll-like receptors 2 and 4 and are CD205 positive. F4/80+ migrate from the inner pulp, increase in size, and display CD86 expression. Anti-inflammatory agents, including steroids, interleukin-1 (IL- 1) receptor antagonist, soluble tumor necrosis factor (TNF) receptor, IL-10, nitric oxide (NO), heme oxygenase-1, and regulatory T lymphocytes (Tregs), are produced in order to limit tissue damage. Nemosis, necrosis, apoptosis are implicated in apical reparative dentinogenesis.

Odontoblasts, dental pulp fibroblasts and immature DCs are involved in dental pulp immune response to oral pathogens invading dentine during the carious process. Pro-inflammatory cytokines are produced during inflammation. As a response to tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-Iβ) and interleukin-8 (CXCL8) upon stimulation with lipoteichoic acid (LTA), and Toll-like receptor 2 are activated [23]. In inflamed pulp, the cell origin of TNF-α is unknown, whereas IL-1β is produced by macrophages, and CXCL8 by macrophages, lymphocytes, endothelial cells and odontoblasts. In response to TLR2 activation by LTA, TNF-α, IL-1β and CXCL8 are produced by odontoblasts, dental pulp fibroblasts and immature DCs.

Pulp innervation

During initiation, nerves are seen below the primordium and its presumptive area. During the bud formation, nerves are seen below the dental papilla. They form a plexus at early cap stage, nerve emerging from the basal plexus. Later, innervation is seen around the follicle, and later innervation of the papilla is seen inside the pulp papilla during tooth development. Penetration of the human dental papilla occurs at the same time dentinogenesis starts. At later stages, pulp contains substance P and somatostatins, which are neuropeptides associated with small diameter nociceptive fibers. Monoamines, vaso-active intestinal peptide (VIP) have been located in autonomic fibers in the dental pulp. Developmental studies of substance P-containing neurons and cholinergic and mono-aminergic neurons correlate with stages of innervation of tooth development [24].

The receptor tyrosine kinases ErbB3, ErbB4, and neuroregulin-1 mRNA are expressed locally in a different way during tooth development [25].

Antisera to S-100 protein, neurofilament protein, neurone-specific enolase and protein gene product 9.5 are markers of pulp innervation [26]. PGP 9.5 is the most superior marker.

Four members of the neurotrophin family have been identified in mammals: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5) [27]. Neuropeptides substance P (SP), neurokinin A (NKA), calcitonin gene-related peptide (CGRP), neuropeptide Y (NPY) and vasoactive intestinal polypeptide (VIP) have growth regulatory properties on pulpal cells *in vitro* [28]. Neuropeptides (CGRP and SP) may be involved in pulp development (proliferation) and in wound healing after pulpal injury [29,30].

Most nerve fibers in the dental pulp are unmyelinated. A-beta, A-delta and C-fibers have been also identified as non-myelinated sympathetic fibers. Although some larger fibers are present, myelinated fibers in the A-delta range are also recognized. Some cellular substances are associated with nociceptive nerve cells, namely substance P (SP), calcitonin gene related peptide (CGRP) and fluoride-resistant acid phosphatase (FRAP). Carbonic anhydrase (CA), GM1 ganglioside (choleragenoid binding receptor), and RT 97, a monoclonal antibody against neurofilament protein are expressed [31].

Pain sensitivity to thermal stimuli, thermal change, mechanical deformation or trauma induces pain. Careful regulation of blood flow is of critical importance, and alterations are the first to occur with the onset of pulp inflammation. The maxillary artery enters the tooth via arterioles feeding. Pulp vessels are organized in a hierarchical system. Central arterioles form a capillary network located at the periphery of the pulp and the blood drains into venules at the center of the pulp.

Perivascular sympathetic nerves liberate noradrenaline (adrenergic post-ganglionic fibers) and possible neuropeptide Y causing a reduction of pulp blood flow (SP or CGRP). Sensory nerves involved in pulp pain perception and transduction are branches of maxillary and mandibular divisions of the trigeminal nerve. Small branches enter the apical foramina and progress coronally and peripherally following the blood vessels and they branch extensively subjacent to the cell-rich zone, forming the plexus of Raschkow. The plexus contains both large myelinated A-δ and A-β fibers (2-5 μm in diameter) and the smaller C fibers (0.3 – 1.2 μm). Almost all the A-δ fibers are located in the coronal portion of the pulp, with the greater density in the pulp horn.

1,25-dihydroxyvitamin D3 and TGF-β [32] have examined the effects of 1,25-(OH)2 and TGF-β on the synthesis of SPARC and ALP activity in human pulp fibroblasts. The interaction of TGF-β and 1,25 $(OH)_2$ D_3 may influence the function and differentiation of dental pulp fibroblasts [32].

Pulp STEM cells

Human dental pulp stem cells (DPSCs) are characterized by the self-renewal capability, multilineage differentiation capacity and clonogenic efficiency [33,34]. Like osteoblasts, pulp cells express bone markers such as bone sialoprotein, alkaline phosphatase, type I collagen and osteocalcin. They express members of the TGF-β superfamily and cytokines. Oil red O-positive lipid clusters showed a significant up-regulation of PPARγ2 and lipoprotein lipase. Multipotent pulp cells differentiate into osteogenic, chondrogenic, and adipogenic stable mesoblastic phenotypes. They express dentin sialoprotein (DSP), bone sialoprotein (BSP), dentin matrix protein 1 (DMP1), MEPE and type I collagen. DPSCs were positive for MSCs markers CD13, CD29, CD44, CD73, CD90, CD105, CD146, and negative for the hematopoietic markers, CD45 and HLA-DR.

In the apical papilla apexogenesis involve the proliferation of a population of mesenchymal stem cells residing near the end of the root. They are named stem cells from the apical papilla (SCAP), very similar to dental pulp stem cells (DPSCs). These cells express the cell-surface STRO-1. They are sources of odontoblasts, responsible for the formation of root dentin. Isolation and characterization of these stem cells favor their contribution to a root continuously in construction and maturation, and their potential utilization for pulp/dentin regeneration [35,36]. In addition to their dentinogenic differentiation, Alizarin Red-positive nodules formed during SCAP cultures after 4 weeks of induction. They exhibit adipogenic and neurogenic differentiation capabilities and express specific markers such as dentin sialophosphoprotein and CBFA1/Runx2 molecules. They may present an appropriate cell source for neural tissue regeneration. Mesenchymal-stem-cell-like (MSC) derived from bone marrow (BMMSCs) are promising alternative multipotent MSC sources [37-39]. These cells have the capacity for self-renewal and multilineage differentiation potential and clonogenic efficiency. They give rise to osteogenic, chondrogenic, adipogenic, myogenic, and neurogenic cells. They play role in regenerative medicine [35]. Human mesenchymal cells were evaluated by Pittenger et al. [39], as being about 0.001 to 0.01% of the grand total of pulp cells. According to Sloan and Waddington (2009), the subset of undifferentiated cells can represent in the dental pulp as little as 1% of the total cell population. Side population (SP) cells in human deciduous dental pulp were evaluated as 2% of the total cells [40]. According to Kenmotsu et al. [41], approximately 0.40% of the pulp cells may be stem cells or side population when they are found in young rats, whereas only 0.11% is found in old rats (Table 2).

Human dental pulp stem cells were mainly isolated from adults. Cells cultured in medium promoting differentiation toward cells of the osteo/odontoblastic lineage become alkaline-phosphatase–positive. They produce a calcified matrix and form dentin-like matrix on scaffold *in vivo*.

As a regenerative response to caries, stem/progenitor cells have the potential utility in induction of reparative dentine. A population of side population was isolated from dental pulp, based on the exclusion of the DNA binding dye Hoechst 33342 by flow cytometry and compared its cell-renewal capacity and multipotency with non-SP cells, suggesting longer proliferative lifespan and self-renewal capacity of SP cells. Expression of type II collagen and aggrecan confirmed chondrogenic conversion

Table 2: Five different human stem/progenitor cells have been isolated and characterized
-Dental pulp stem cells (DPSCs),
-Stem cells from exfoliated deciduous teeth (SHED),
-Periodontal ligament stem cells (PDLSCs),
-Stem cells from apical papilla (SCAP), and
-Dental follicle progenitor cells (DFPCs).

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of SP cells (30%). Adipogenic conversion was demonstrated by proliferator-activated receptor γ and adaptator protein 2 showing adipogenic conversion. Neurofilaments and neuromodulin confirmed neurogenic conversion (90%). Bmp2-stimulated the expression of Dspp and the expression of DSPP and Enamelysin in three-dimensional pellet cultures [42] (Figures 15-17).

The activation of Wnt signaling by 6-bromoindirubin-3'-oxime (BIO), a pharmacological GSK-3-specific inhibitor of glycogen synthase kinase-3, which inactivate efficient pathway activation in regulating human and mouse embryonic stem cells maintain the undifferentiated phenotype of the two embryonic stem cells sustains expression of the pluripotent state-specific transcription factors Oct-3/4, Rex-1 and Nanog. Wnt signaling is endogenously activated in undifferentiated mouse ESCs and down-differentiated upon differentiation. BIO-mediated Wnt activation is functionally reversible. This suggests that the use of GSK-3 specific inhibitors such as BIO may have practical applications in regenerative medicine [43]. The GSK-3 inhibitor BIO promotes proliferation of the terminally differentiated mammalian cells because it is believed that they are unable to divide and maintain self-renewal in embryonic stem cells. It is shown that the maintenance of self-renewal and the induction of proliferation share common molecular pathways [44] (Figures 18-20).

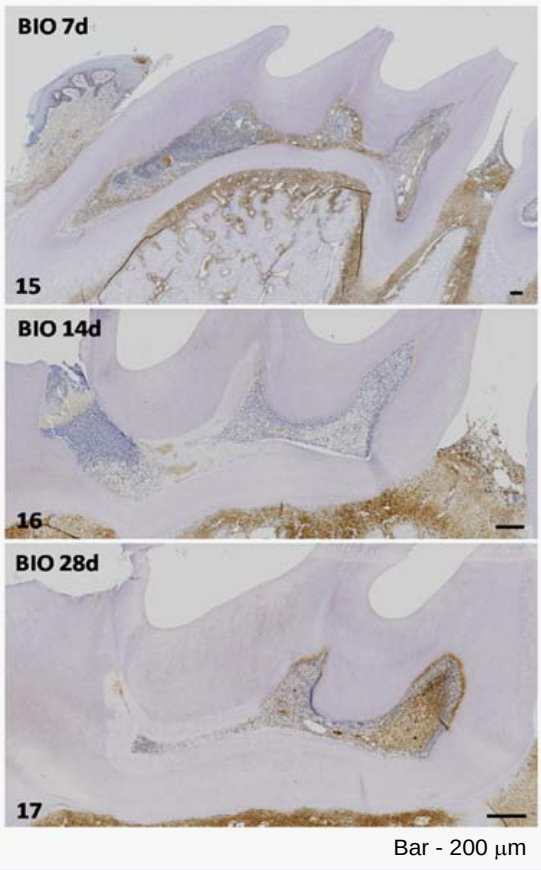

Figure 15-17 After 7 days the mesial pulp chamber is slightly inflamed (figure 15). After 14 days, the isthmus between the mesial and central pulp chamber is filled with a mineralized tissue (Figure 16). The central pulp chamber and the isthmus are totally mineralized (Figure 17). Bar= 200μm.

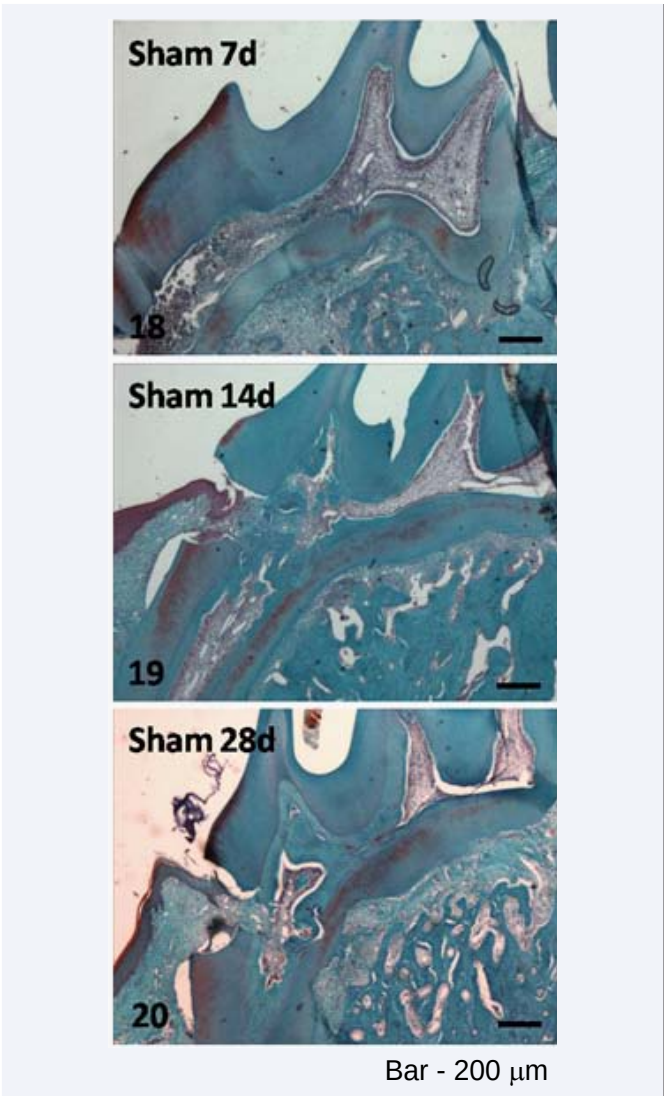

Figure 18-20 Sham 7days to 28 days: the mesial horn and the isthmus are filled by mineralized tissue. Bar= 200μm.

In the sham group mineralization was limited to the mesial part and seen at the junction between the mesial root and mesial pulp horn. Twenty eight days after implantation, reparative dentine was seen along the mesial pulp chamber and reactionary dentine filled the mesial pulp and the isthmus connecting the mesial and central pulp was totally mineralized.

Seven days after implantation of beads loaded with BIO in the mesial pulp chamber, no visible reaction was ever seen. After 14 days, the central part of the pulp chamber was filled by reactionary dentin formed along the walls of the central pulp. A calciotraumatic line was clearly detectable between the dentine formed before bead implantation. The central horn was largely filled by a heterogeneous mineralization. After 28 days, a large part of the mesial pulp chamber, the isthmus located between the mesial and central parts of the pulp chamber, and nearly all the central pulp were filled by a mineralized heterogeneous

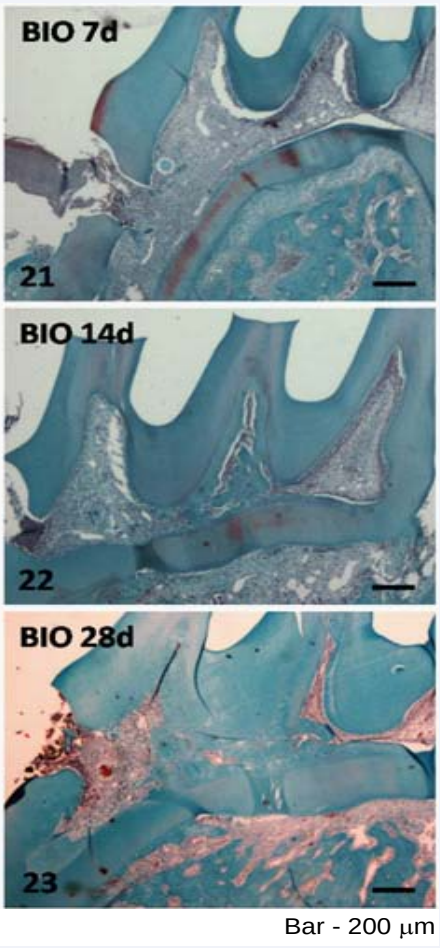

Figure 21-23 Bio implanted for 7-28 days induce the formation of a mineralized tissue in the isthmus between the mesial and central pulp chambers. Bar= 200µm.

structure. The distal pulp chamber was not mineralized, and the cell didn't display any evidence of proliferation. It was clearly seen that BIO implantation produced the shift of the mineralized tissue from the mesial part toward the central part of the pulp. Obviously, sliding of the mineralized pulp horn occurred after BIO implantation from the mesial to the central parts of the pulp chamber (Figures 21-23).

To conclude, firstly mesenchymal cells slide from the apical part to the sub-odontoblastic border (the so-called Hoehl's cell layer)(see root lengthening). Then, secondly, odontoblasts migrate toward the coronal pulp, and BIO influences the migration between the mesial to the central pulp chamber parts. The first and second cell sliding constitute crucial events leading to the formation of reactionary and reparative dentine, and subsequently to pulp mineralization (Figures 15-17).

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