Anatomical and Biological Complexity of the Root Canal

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Abstract
The anatomy and biology of dentin tissues vary according their different location in the teeth. Beneath the thin mantle dentin, distinct layers include a primary dentin (tubular or orthodentin), a secondary dentin (repairative osteodentin), and a tertiary dentin (reactionary dentin) [1-3]. Depending on the coronal and radicular parts of the tooth, substantial differences have been actually identified. In the dental pulp chamber, cell-free and cell-rich zones constitute two superficial layers located at the periphery of the central pulp. These outer layers are lining the roof, floor, mesial, distal, labial, and buccal surfaces. In the crown, fibrosis of the pulp, true and false dental stones and dystrophic calcifications contribute to pulp inflammation and repair. In the root canal, pulp cells (also called pulpoblasts) and fibers have structural incidences (e.g. Type I and Type III collagens) [1,2]. Adhesive molecules, including fibronectin, laminin, vitronectine and thrombospondin are determining factors implicated in the root canal composition. Elastase and cathepsin G contribute to serine proteases and metalloproteinase’s (MMP-2, MMP-9, MMP-3). Altogether, they are implicated in the biological parameters of the pulp horn. Proteolytically cleaved into DSP, DGP and DPP, DSPP is synthesized by secretory odontoblasts. Cbfa-1 is critical for the root canal biology. Proteoglycans such as HSP90, KS, CS are modulating the root canal response. Osteocalcin is a non-phosphorylated molecule contributing to the root canal condition. In addition, stem cells (DPSCs, SHED and SCAP) are involved in the recruitment and differentiation of cells located in the pulp root canal [4]. The anatomic complexity and the biology of the root canal have therapeutic occurrences.

INTRODUCTION
Two different types of dentin have been identified in temporary and permanent (including wisdom) teeth. Under a thin mantle dentin layer located at the enamel-dentin junction, the bulk of tubular orthodentin is found after tooth alteration, above the initiation of reparative dentin [5] (Figure 1,2). Osteodentin is a bone-like dentin, formed around osteoplasts that contain osteocytes. It was also identified as reactionary dentin beneath abroad dentin layer implicated in dentin thickening. A calcioatraumatic line divided the dentin into ortho- and osteo-dentin. Osteo-dentin fills the exposed pulp horn after drilling damages [3,5].

Coronal and radicular dentin includes 1) the pulp chamber, in the coronal portion of the tooth, and 2) a radicular portion, enclosing a complex network of root canals. A narrow passage connects the two structures separated by large canal orifices. In contrast, apical canal constrictions display minor diameter, whereas apical foramen with major diameter are associated at the cemento-dentinal junction. Accessory and lateral canal furcations built a complex ramification that branch out into several others [6] (Figure 1).

The cameral pulp implicates six parts. Firstly, the roof and floor of the pulp chamber constitute the superficial and deeper surfaces (Figure 1). Moreover, four distinct lateral surfaces are related to the mesial, distal, buccal and lingual uninterrupted pulp chamber characteristics (Figure 2). Pulp horns are distinguishable at the junction between the roof and lateral walls. In rats, accumulation of reparative dentin is the reverse of what is seen in human molars [7]. Reactionary dentin formation is occurring fast by reduction in height [8], whereas in the occlusal roof the speed of formation is decreased, and even slower in the furcation area of the sidewalls [9] (Figure 3). The pulp is reduced both in the mesio-distal diameter and in occlusal direction.

Young pulp chambers are growing and become older. Successive laminations have been identified near the roof covered by the occlusal layer. Lateral walls enlarge, become broader and thicker, reducing the pulp space after reparative dentin formation, namely after a pulp exposure. The floor of the pulp chamber is covered by new dentin formation during a long-term exposure, whereas reparative dentin forms faster in the furcation area. Pulp horns are maintained and stay stable at their actual location. Less dentin is formed along the lateral walls. Reactionary dentin forms quicker in humans. The speed of formation of the occlusal roof follows this construction, the slowest occurring at the furcation area. Once the fibrotic sub-odontoblastic layer is formed, the next and ultimate step leads to reactionary dentin mineralization.

PULP FIBROSIS
Reparative dentin (fibrodentin) is extended beneath a calcio-
traumatic line isolating the already formed orthodentin from the newly formed reactionary dentin layer. Osteodentin is taking place after an initial orthodentin formation. It is also named reparative dentin. Fibrosis detected within the pulp result from fibrils accumulation between cells in a non-mineralized tissue. Mineralization is produced later, along and between the collagen fibers.

Pulp stones (or denticles) are developing when the dental pulp is transformed into older tissue, wrapping arterioles and venules. Laminations indicate a rhythmic activity. True or false denticles, adherent or embedded pulp stones are identified in old pulp chambers. Pulp stones are classified into three different structures: 1) true or 2) false denticles, located near the border or 3) in the central part of the pulp. They may also appear as widely spread or diffuse pulp mineralization’s (dystrophic calcification). With time, pulp stones add gradually to endodontic difficulties.

Based on their location, structurally they are either true or false pulp stones [10]. Calcification of the extracellular matrix occurs after accumulation of fibers (fibrodentin) within the dental pulp, around vessels and nerves. Diffuse mineralization may seal the pulp.

**CELL FREE AND CELL RICH ZONES**

In addition to the central pulp, the peripheral cell free zone (40 microns thick) encloses the nerves or a capillary plexus. In contrast, an outer cell rich zone is present in the sub odontoblastic layer (beneath the odontoblastic layer). Due to the migration of cells issue from pulp proper, fibroblasts and/or undifferentiated mesenchymal cells, undifferentiated cells, accounting for about 5 to 15%, defense cells (namely macrophages, reported by some authors as histiocytes located close to the blood vessels), nerves and lymph vessels, contain a large collection of heterogeneous cells [1,2]. They are phagocytes, involved in the engulfment and digestion of foreign material. Dendritic cells that are also members of the Langerhans cells family (1-3% of the pulp cells) are found within the pulp. Melanocytes may be responsible for pigmentation. Unpigmented Langerhans and Merkel cells are immune cells, implicated as defense cells in the protection of the dental pulp. These non-keratinocyte cells or macrophages constitute 8-9% of the pulpal cell population [2] (Figure 3).

Pulp inflammation is induced by lymphocytes, plasma cells and macrophages. Non-specific mediators such as histamine, bradykinin, serotonin, interleukins (IL-1 and IL-2) are numerous. Mast cells, the main source for histamine, are found in inflamed pulp. Four fold increases originates from pulp histamine levels within 30 minutes of thermal injury, supporting that histamine plays role in pulp inflammation. Plasma or tissue kallikrein leads to the production of bradykinin producing signs of inflammation. Phospholipase A2 causes the release of arachidonic acid from cell membranes, resulting in various prostaglandins, thromboxanes and leukotrienes. Platelets that aggregate in the vessels release serotonin [13].

**PULPOBLASTS IN THE ROOT CANAL**

Pulp cells comprise pulpoblasts and/or pulpocytes. In addition to inflammatory cells, odontoblasts and fibroblasts are located in the coronal dental pulp. These cells are also present in the root. Macrophages, dendritic cells and lymphocytes are implicated in pulp cell mitosis, specifically in the root canal. They are observed mainly when newly formed cells replace wounded or dead odontoblasts [14-17] (Figure 4,5).

Pulp cells are producing structural fibers such as type I and III collagens, precisely during the early steps of dentinogenesis. Adhesive molecules of the glycoproteins family (fibronectin, laminine, vitronectine and thrombospondin) are synthesized by pulp cells. Within the pulp, a few elastic fibers have been identified. Two serine proteases are released by pulpoblasts, precisely elastase and cathepsin G, cleaving ECM components such as Type 1 collagen.
Figure 3 In the apical zone containing many stem cells, at early stage of root formation, the apical cell-rich zone is located beneath the apical papilla mesenchyme and the radicular dental pulp.

Figure 4 Scoring the leucine-rich apical pulp cells implantation in the apical cell-rich zone (B), is much below the values obtained after beads implantation in the apical papilla mesenchyme (C) and implantation in the radicular dental pulp (D).

Figure 5 Apical stem cells migrate from the apical cell-rich zone toward the apical papilla mesenchyme. The cells migrate toward the radicular dental pulp, beneath odontoblasts and the sub-odontoblastic layer.
IV collagen, laminin, fibronectin and HSPG. Gelatinase 72kDa (MMP-2) and 92kDa (MMP-9) are involved in the degradation of the ground substance, including glycosaminoglycans and chondroitin sulfate proteoglycans. They constitute a transitional medium.

**REGULATION OF APOPTOSIS**

Dental pulp cells play important roles in the regulation of apoptosis. Bcl-2 displays pro- and anti-apoptotic immune reactivity. Bcl-2 inhibits apoptosis by increasing the time-to-death and other death signals. Bax and p53 are present in the inter phase nuclei of mammalian. They control the apoptotic pathway. Tumor necrosis factor is a cytoplasmic gene predominantly associated to the plasma membrane. Bax is a transcriptional target, implicated as tumor suppressor protein. The Bcl-2 family of proteins regulates apoptosis. They controls mitochondrial permeability. Growth factors and transcription factors are implicated in the generation of orphan diseases. Growth factors are naturally occurring substances, stimulating cellular growth, and cell proliferation. Curative, wound healing and cellular differentiation interfere with these molecules. They act as signaling molecules between cells. Nerve Growth Factor (NGF) contributes to the neurotrophin family. Fibroblast Growth Factor (FGF 23), Epidermal Growth Factor (EGF), Human Growth Hormone (HGH), Insulin-like Growth Factor 1 (IGF1), Insulin-like Growth Factor (IGF2), Platelet-derived Growth Factor (PDGF) contributes to the functions of growth factors. Altogether, growth factors and transcriptional factors are implicated in various pulp functions [18-22] (Figures 4,5).

Mature cells components are produced intra cellularly by resident cells and secreted into the ECM via exocytosis. Three major components are detected: 1) highly viscous proteoglycans (heparan sulfate, keratan sulfate and chondroitin sulfate); 2) insoluble collagen fibers, providing strength and resilience to the tissue, and 3) soluble multi-adhesive extracellular proteins (fibronectin, laminin). These components bind proteoglycans and collagen fibers to the receptors located on the cell surface. Cell adhesion molecules (CAMs) are members of the integrins, cadherins, selectins and the immunoglobulin super family [N-CAM, ICAM, V CAM]. In addition, cell adhesion molecules modulate signal transduction by interacting with receptor tyrosine kinases, Rho-GTPase and components of the Wnt signaling pathway. Matrix metalloproteinases MMP-2 (MMP-2), MMP-9 (MMP-9), Stromelysin (MMP-3). They involve also a series of inhibitors (TIMP-1, -2, -3, -4) and 3 stromelysines as well (SL-1, -2, -3). Calcitonin gene-related peptide receptor expression (CGRPα) and CD163+ are active in catabolic activities within the dental pulp [23].

Dentin sialophosphoprotein (DSP) is processed into dentin sialoprotein (DSP), dentin glycoprotein (DGP) and dentin phosphophrotein (DPP). DSP acts as a ligand and binds to integrin β6, promoting cell attachment, migration, differentiation and mineralization of dental mesenchymal cells. Dentin sialophosphoprotein (DSP) is proteolytically cleaved into dentin sialoprotein (DSP) and dentin phosphophrotein (DPP). Distinct roles are played by DSP and DPP in dentin mineralization, with DSP regulating initiation of dentin mineralization, and DPP being involved in the maturation of mineralized dentin.

ECM displays labeling for dentin sialoprotein (DSP), Cbfa 1, proteoglycans [heparan sulfate proteoglycan 90 (HSP90), keratan sulfate (KS) and chondroitin sulfate (CS)]. Pulp cells express the three major proteins implicated in pulp/dentin healing/regeneration. The dentin sialoprotein Cbfa 1 is a member of Cbfa family of transcription factors first identified as nuclear protein binding to an osteoblast-specific cis-acting element activating the expression of osteocalcin, the most osteoblast-specific gene. Cbfa 1 have a role on development and differentiation, regulating the rate of bone matrix deposition by differentiated osteoblasts. Osteocalcin or bone-GLA-protein is a non-collagenous protein of the bone matrix. Osteocalcin is a small peptide formed by 49 amino acids. Initiated by the alkaline osseous phosphatase (PALO). It synthesis is also a function of vitamin K for the carboxylation of 3 carboxyglutamic acids. This structure provides a strong affinity for hydroxyl apatite. One major part is incorporated into bone; another febble part is released into blood circulation and therefore can be measured and quantified. The half-life of osteocalcin is short (about 5 minutes).

**STEM CELLS**

Stem cells contribute to pulp development, cell differentiation and dental mineralization. Osteocalcin expression is reduced in aged human dental pulp [16]. As STEM cells markers, chondrogenic and myogenic bearing differentiation potential. BrDU positive cells were observed in the central part of the pulp. Nestin, vimentin and Oct3/4 proteins are abundant while STRO-1 protein is restricted to perivascular niches [17].

At least, three groups of growth factors have been identified. They include respectively a group of cells and molecules such as the Dentin Pulp Stem Cells (DPSCs) [18-20]. SHED is implicated in pulp regeneration, whereas SCAP appear exclusively in the apical region. The cells slide along the root in the sub odontoblastic layer, and differentiate into functional odontoblasts. In addition, stem cells participate to the development of the periodontal ligament and also to the dental follicle of developing tooth. Other stromal cells containing stem cells will further develop and differentiate at later stages. Stem cells may be reprogrammed into iP5 (Induced pluripotent stem cells). iP5 are transitory cells. They may differentiate into stem cells (Figures 5).

**NON-KERATINOCYTE CELLS**

Pré mélanosomes, pigmented or non-pigmented, contain spiral coils [375 Å in diameter with a periodicity of 100Å]. Mélanoblasts are precursor cells of melanocytes, with electron-dense inclusions [0.7 x 0.3 microns]. In gingiva, they constitute about 7% of the basal cells. Mélanosomes are implicated in acid phosphatase activity. Langerhans cells contain Birbeck granules [400Å in diameter and 400 Å thick]. These cells are implicated in the immune reaction. Pulp root cells are also implicated in this cell collection.

**NEUROVASCULAR CHANGES**

Neuronal and vascular changes occur during aging. Pulp and its structural components (nerve and blood supply) become more fibrous and less cellular. There is an increase of the Weibel-Palade bodies in the lymphatic fibrotic pulps. Elastic fibers are lacking in the connective tissue surrounding the lymphatic vessels.
Arteries calcification began in the adventitia and progress into the media and intima. Protrusions in the blood vessel lumen contribute to neurovascular changes. Diffuse calcification was seen in 90% of the old teeth. Microcirculation: arterioles feeding individual microcirculation. Shunt vessels are observed in the pulp: arterio-venous anastomoses; venous-venous anastomoses, or/and U-turn loops bypassing the capillary bed are also important physiologically [15,23,24].

Reduction in sensitivity occurs during aging: Sensory nerves: branches of the maxillary and mandibular divisions of the trigeminal nerve are individualized. A nerve plexus contains both large myelinated A-δ and A-β fibers (2-5 µm in diameter) and the smaller unmyelinated C fibers (0.3-1.2 µm). These changes have high impact on the dental pulp. Neuro-vascular changes are detected in the roots of old pulp.

CONCLUSIONS

To conclude, the complexity of the biology and anatomy of the root canal have therapeutic incidences. In addition to potential pulp cells such as fibroblasts, pulpoblasts, inflammatory cells such as melanocytes, dendritic cells, Langerhans cells, pulp cells such as fibroblasts, pulpoblasts, inflammatory or/and U-turn loops bypassing the capillary bed are also important physiologically [15,23,24].

REFERENCES