CHAPTER A

Patho-biology of the dental pulp

«...Alfred Ogilivie has said "The pulp lives for the dentin and dentin lives by the grace of the pulp. Few marriages in nature are marked by greater affinity". I may add "and few marriages have been marked by greater obscurity"...»

Harald Loe 1985*

1. General structural aspects

The dental pulp is a well vascularized, specialised connective tissue derived from oral ectomesenchyme, which is entirely enclosed by dentin in the pulp chamber and root canal(s) of the tooth. It contains cells which belong to different groups that provide odontogenic, defensive, nutritive and sensory functions (odontoblasts, fibroblasts, undifferentiated mesenchymal and defence cells), extracellular matrix (collagen and reticular fibres), blood vessels and nerves. Pulp structure is not uniform, consisting of the odontogenic pulp periphery and the pulp core. Pulp structure and functions have been described by numerous excellent textbooks (Orban 1962, Siskin 1973, Baume 1980, Linde 1984, Seltzer and Bender 1984, Schroeder 1991, Avery 1994).

Three border zones can be distinguished in the coronal and the substantial portion of the radicular tissue, forming the odontogenic pulp region:

i) Odontoblast layer. It is a pseudostratified layer of highly differentiated cells responsible for the formation of the dentin during embryonic tooth development and its repair during the life span of the pulp organ. Odontoblasts have columnar cell bodies and odontoblastic procesess that extend into the adjacent dentinal tubules (see Sasaki and Garant 1996).

ii) Cell-free zone. It is an approximately 40 μm subodontoblastic zone, more distinct in the coronal pulp, which contains numerous branching cytoplasmic processes from cells located in the adjacent cell-rich zone, the major portion of the subodontoblastic capillary plexus and the terminal branches of the sensory and autonomic nerve fibres (plexus of Rashkow).

iii) Cell-rich zone. It contains bipolar cells (fibroblasts and undifferentiated cells) with spindle-shaped nuclei arranged with their cytoplasmic processes perpendicular or parallel to the dentin in the coronal and radicular pulp respectively. On the inner side, the cell-rich zone is continuous with the central pulp parenchyma.

The central pulp core consists mainly of fibroblasts and undifferentiated mesenchymal cells,

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extracellular matrix, large blood vessels and nerves fibres.

Pulp tissue is rich in collagenous fibrils, fibers and thin fiber bundles. Approximately 30-45 % of the pulpal collagen fibres consist of type III collagen, whith type I collagen constituting the remainder. Collagens type V and VI forming a meshwork of thin microfibrils throughout the stroma of the pulp core were also detected (Hillmann and Geurtsen 1997). All of the pulp elements are embedded in a gel-like ground substance with a high water content containing chondroitin sulfate, hyaluronic acid, dermatan sulfate, proteoglycans and glycoproteins.

Vessels enter and exit the pulp through the apical foramen. Narrow arteries and thin-walled arterioles run through the centre of the radicular pulp and show extensive branching in the periphery of the coronal pulp, forming the sub-odontoblastic capillary plexus. Venules and larger veins, run alongside the arteries in a spiralling course. Arteriovenous anastomoses exist in the coronal and the radicular pulp, independent of the peripheral pulp capillaries. Myelinated and unmyelinated nerve fibres enter the pulp through the apical foramen, running in close proximity to the blood vessels. Myelinated fibres form several branchings in the pulp periphery where they finally lose their myelin sheaths. (For more data concerning the structure of dental pulp see the above mentioned textbooks).

The dental pulp originates from the oral ectomesenchyme. During tooth development a cluster of ectomesenchymal cells, named the dental papilla, is formed beneath the developing dental epithelium (enamel organ). The boundary between the enamel organ and dental papilla is delineated by a basement membrane. When the enamel organ reaches the late bell stage of its development deposition of hard tissue begins. Cells on the epithelial side of the basement membrane become ameloblasts forming tooth enamel, while the rest of the organ is progressively reduced. Cells on the mesenchymal side of the basement membrane become odontoblasts forming primary dentin. The layer of odontoblasts and the remaining mass of the dental papilla consisting of spindle-shaped undifferentiated cells and extracellular matrix is transformed into dental pulp, surrounded now by dentin. This transformation is characterised by i) the differentiation of most of the undifferentiated cells into active fibroblasts and ii) the gradually increasing volume of collagen fibres. Some of the original undifferentiated mesenchymal cells remain in the dental pulp as a potential reservoir of progenitor cells assuming at a later time odontogenic or defensive functions (see Ruch et al. 1995 and Chapter B in this issue).

Most clinical and histological textbooks describe the pulp and the dentin as a unit or a complex, on the basis that they form an embryological and functional entity. Recently, Goldberg and Lasfargues 1995 have suggested that this concept should be revisited.

2. Pathogenesis of disease and dynamics of repair

The pathology of dental pulp represents a network of inflammatory reactions involving pulpal cells, microcirculation and nerves whenever dentin and pulp is affected by caries, restorative procedures or trauma. Specific structural and functional characteristics, directly affect the outcome of the fundamental defensive mechanisms in the dental pulp. The most important of such characteristics are the function of odontoblast layer as a barrier, the enclosure of dental pulp in a hard tissue chamber, the organisation of neurovascular elements and the dynamics of the neural crest-derived cell elements of the dental pulp.

The barrier of the odontoblast layer. The peripheral odontoblast palisade act as a selective barrier regulating the transfer of ions, molecules or fluids from the pulp periphery to the pulp core (Bishop 1984). Adjacent odontoblasts are held together by numerons macula adherens junctions and a well developed distal junctional complex (Sasaki and Garant 1996). The tight junctions in the apical zone of the odontoblast cell bodies developing in early dentinogenesis (Aranachavez et al. 1997) might be the main factor responsible for this control function, allowing only a small portion of tissue products or serum molecules to affect the pulp (Turner et al. 1989, Ushiyama 1989).

The low compliance environment of the pulp chamber. The most significant difference between the pulp and other connective tissues in the pathophysiology of tissue disorder is the low compliance environment of the pulp organ. Pulp tissue comprises about 25% organic components and 75% water, which normally is under a pressure of 8-15 mm Hg regulated by vasoconstriction (Beveridge and Brown 1965, Van Hassel 1973, Bender 1978).

The initial vascular reactions during pulp inflammation (vasodilatation and increased vessel permeability) take place in the rigid enclosed pulp chamber and create conditions of increased hydrostatic tissue pressure (35 mmHg or higher). Despite the fact that the pressure increase is a local phenomenon (Van Hassel 1971) and the low compliance of the tissues are more favourable for the action of negative feedback mechanisms {stimulated by the increased hydrostatic pressure, the so-called hydrostating buffering and controlling changes in hydrostatic pressure by the oedema-preventing mechanisms (Heyeraas 1990)}, dental pulp pressure can quickly invote irreversible damage. In the low compliance environment of the pulp chamber, tissue responds to vasoconstriction in a similar way to that of other systems. In contrast the response of the pulp to vasodilatory agents, such as the mediators released during initial tissue insult by deep caries or mechanical trauma, is very different. In the pulpal system, vasodilatation resulting in a sharp, transient increase in pulpal blood flow can be followed by a dramatic decrease, a secondary vasoconstriction, (Kim and Dorscher-Kim 1990), which does not appear in other connective tissues. On the other hand, osmotic feedback mechanisms (in which increased filtration normally tends to dilute fluid proteins reducing colloid osmotic pressure) are not effective in the low-compliance systems because dilution is not possible owing to the relatively constant tissue volume (Heyeraas 1990). Dental pulp healing does not always follow the sequence of events taking place normally in other connective tissues. Since pulpal repair is strongly dependent on a number of factors, exacerbation of an initial trauma very often leads to generalised tissue necrosis.

The pulpal neurovascular organisation. The activation of sensory nerve fibres during various dental procedures does not only serve to signal pain but also to release vasoactive peptides (substance P, CGRP, NKA, VIP and NPY) lead to vascular reactions locally within the pulpal environment (Akai and Wakisaka 1990, Olgart 1990). Such local reflex reactions might be beneficial to the pulp organ under low-grade tissue irritations (Olgart 1990). The stimulation of periodontal nerve fibres can also increase blood flow to the pulp (Olgart 1992). The stimulation of sensory nerve fibres may contriibute to control of the function of arteriovenous anastomoses, regulating blood supply and subsequent tissue healing in the pulp (Heyeraas and Kvinnsland 1992). It seems from the findings on this much studied topic, that the neural supply of the mammalian dental pulp contributes importantly to hemodynamic reactions and subsequently to tissue repair.

The neural crest-derived cellular elements within the pulp tissue. During the last 3 decades extensive studies have shown that cells originating from the neural crest migrate and interact with putative dental epithelial cells of the oral epithelium in a stomatoderm (Jonhston and Lstgarten 1972, Lumsden 1988). During the early stages of tooth germ development, cells forming the dental papilla undergo mitosis. According to a tooth-specific temporo-spatial pattern, peripheral cells become post-mitotic cells differentiating into odontoblasts (see Ruch 1985). The highly differentiated and functional odontoblast layer in the pulp periphery represents a specific dentinogenic organ responsible for the formation and repair of the circumpulpal dentin (see Baume 1980, Linde and Goldberg 1993). The most numerous cells in the dental pulp are fibroblasts. Fibroblasts communicate with each other and with odontoblasts through gap-junctional processes (Harris and Griffin 1967, Sasaki and Garant 1996). They mainly secrete types I and III collagen, proteoglycans and glycosaminoglycans. It has been suggested that they can differentiate into odontoblast-like cells; perivascular cells whose origin has not been established yet, have also been suggested to act as odontoblast progenitor cells (Fitzgerald et al. 1990, Yamamura 1985). It is not known whether the dental pulp fibroblasts and perivascular cells originate only from neural crest or whether other mesenchymal cells can also contribute to the development of pulp tissue. In any case, the cells which can provide replacement cells of the odontoblast layer seem to originate from the neural crest-derived pulpal elements (Thesleff and Vaahtokari 1992).

The existence of a specific pulp environment, i.e. the pulpal tissue totally enclosed by dentinal walls which are lined by the odontoblast palisade, is one of the most important requirements for the survival of dental pulp tissue. Whenever the basic structure of the dentin-odontoblast layer is affected by exogenous stimuli, cells of the underlying pulpal mesenchyme display the ability to replace degenerated odontoblasts, or to differentiate into new hard tissue-forming cells (see Baume 1980, Yamamura 1985, Lesot et al. 1993, 1994, Tziafas 1994). It might be suggested that the expression of

the above mentioned specific potential of the pulp cells depends on dynamic interactions between type of trauma, reactions of the neurovascular system within the pulp microenvironment and the structural\fuctional state of the pulp tissue.

The applied pathology of the dental pulp, i.e. pulp responses to various environmental and iatrogenic stimuli, has been described in recent decades by numerous excellent textbooks and review papers (Langeland 1957, Seltzer et al. 1963, Langeland 1968, Hassel 1973, Seltzer 1973, Bender 1978, Avery 1981, Seltzer and Bender 1985, Chiego 1993, Ingle et al. 1994, Simon et al. 1994).

Pulp response to caries. During the carious process the dentinal matrix is demineralized and progressively invaded by micro-organisms. Bacterial metabolites and products released from hydrolysed and enzymatically digested dentin can affect the underlying pulp (Bergenholtz 1981, Trowbridge 1981, Larmas 1986). Under initial lesions, bacteria do not involve the dentinal tubules and only slight cytological modifications of the odontoblasts (reduction in size and number of intracellular organelles, swollen mitochondria, enlargement of the intercellular spaces between odontoblasts, etc.) can be detected (Brannstrom and Lind 1965, Baume 1970, Magloire et al. 1981, Yoshida and Massler 1984, Couve 1986, Silverstone and Mjor 1988). The increase in protein synthesis by odontoblasts in the affected area, leading to dentin sclerosis consitutes the main pulpal response to the superficial carious lesion. However, under chronic carious processes, irritating factors from bacteria and tissue breakdown affect odontoblasts for long periods and progressively destroy them. Capillaries leak plasma and inflammatory cells (neutrophils, histiocytes and monocytes) become extravasated, releasing various chemotactic factors and cytokines. Prolonged release of bacterial metabolites and products to the pulp core leads to chronic inflammatory reactions including plasma cells and B- and T-lymphocytes (Stanley et al. 1965, Bergenhotz 1977, Trowbridge 1981, Langeland 1987). Focal chronic inflammatory lesions lead to total pulp necrosis (see Ingle et al. 1994, Simon et al. 1994).

Pulp response to mechanical and chemical irritants. The mechanico-chemical irritation due to cavity preparation (Stanley and Swerdlow 1959, Brannstrom 1961, Zach and Cohen 1962, Zach 1972), dentin dehydration (Langeland 1959, Brannstrom 1963), acute or chronic physical trauma (Ingle 1960, Andreasen 1970, Hargraves 1972, Jacobsen and Zachrisson 1975, Stanley et al. 1978, Meister et al. 1980, Cooke 1982, Andreasen and Andreasen 1994) and chemical injury (Massler 1956, Langeland et al. 1971, Mjor 1977, Brannstrom and Olivera 1979, Leiskar and Helgeland 1981, Cox et al. 1987, Stanley 1993) can cause pulpal damage. The plasma membranes of the odontoblast cell bodies and odontoblast processes within the dentinal tubules are affected and junctional complexes between odontoblasts are destroyed (Cotton 1965, Avery 1975, Searls 1975, Heys et al. 1981, Ten Cate et al. 1985). Disruption of the odontoblasts results in concentration of potentiating inflammatory factors which initiate chemotactic reactions (Stanley 1977, Oshima 1990). Mechanical trauma further acts by activating nerve terminals releasing neuropeptides, substance P and VIP and causing alterations in pulpal microcirculation (Kim 1985, Avery and Chiego 1990). Generally, initial inflammatory tissue reactions are more extensive in the dental pulp due to chemotactic and vasoactive signals from odontoblast death and distortion of the peripheral nerve terminals. Subsequent vascular changes and inflammatory cell recruitment release histamine, seretonine and prostaglandins. These reactions are followed by the normal macrophage response, stimulating cell migration and fibroblastic activity from the adjacent fibroblasts or the perivascular cells, where prolonged irritation of the pulp is prevented (see Ten Cate 1992). Sufficient vascular function in the inflamed area and an appropriate environment favour rapid and complete tissue healing. Usually inter-odontoblastic spaces become filled with plasma leaking from the capillaries, where the clotting cascade takes place preventing further ingress of irritants to the pulp core. Odontoblasts re-establish their plasma membranes and junctional complexes and secrete extracellular matrix leading to a progressive restoration of normal tissue function (see Avery 1994). Prolonged mechanico-chemical irritation of the dental pulp and/or contamination of the injured area by oral bacteria are responsible for continuing pulpal inflammation and pulpal necrosis (Mjor 1977, Stanley 1978, Brannstrom and Olivera 1979).

3. Terminology for cells and matrices

It has been widely recognized that several forms of mineralized tissues are synthesized by hard tissue-forming pulpal cells which differentiate outside of the specific temporo-spatial pattern of tooth development, in the absence of dental epithelium and its basement membrane. Since a broad spectrum of mineralized tissues can be observed during the defensive pulpal reactions in various pathological or experimental conditions and particularly during the natural wound healing process, a variety of terms have been used to characterise the above matrices and the related formative cells (see Baume 1980).

From the developmental point of view the following types of calcified tissues can be distinguished on the basis of their formative cells (Fig. 1 and plates 1 and 2):

Primary odontoblasts. Ectomesenchymal cells differentiated during embryonic tooth development, which are responsible for the secretion of the extracellular matrix of predentin and biomineralization of the primary dentin up to the completion of tooth crown and root formation and the repair of this dentin matrix throughout the life of the dental pulp. They are also responsible for the production of physiological secondary dentin and are distinct from the pulp cells differentiated during the post-developmental tooth life which can also contribute to the formation of tertiary dentin, at specific foci in the tooth in response to external stimuli.

Primary dentin. The hard tissue that constitutes the skeletal body of each tooth, produced only by primary post-mitotic odontoblasts, during the embryonic period of tooth development as mantle initially and circumpulpal dentin up to the completion of root formation. This tissue is secreted at a relatively high rate.

Physiological secondary dentin. Dentin which is formed at a very reduced rate post-developmentally by primary odontoblasts, throughout the life span of the dental pulp organ.

Reactionary dentin. A tertiary dentin which is formed at a relatively high rate post-developmentally by primary odontoblasts in response to appropriate environmental stimuli which stimulate but do not adversely affect the survival of primary odontoblasts (see Smith et al. 1995).

Odontoblast-like cells. Elongated polarized cells able to secrete tubular matrix in a polar predentin-like pattern in the absence of dental epithelium and/or basement membrane during the reparative process of the pulp and\or in response to specific inductive influences. They are responsible for the production of reparative dentin either in the odontogenic pulp periphery on the pulpal aspect of the primary and secondary dentin, or at ectopic pulp sites of the pulp core, onto physical or artificial substrata. They have also been referred to as new odontoblasts, 2nd generation odontoblasts, replacement odontoblasts, reparative pulp cells, etc.

Reparative dentin. A tubular calcifiable matrix produced by odontoblast-like cells in a polar predentin-like pattern, during the reparative process of the pulp and\or in response to specific inductive influences, either in the odontogenic pulp periphery, or at central pulp sites. This type of matrix has also been referred to as tertiary or replacement dentin, neodentin, etc.

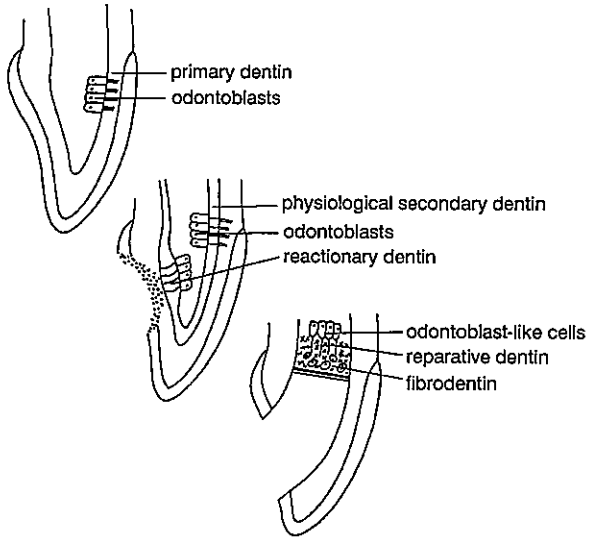

***Figure 1.** Schematic representation of the types of hard tissues and the related formative pulp/papilla cells.*

Fibrodentinoblasts (pulpoblasts). They are cuboidal or spindle-shaped pulp cells which produce atubular hard tissue. They thought to develop during incomplete odontoblast differentiation due to the effects of radiation or antimitotic agents, or

during the stereotypic processes of pulp wound healing before the appearance of odontoblast-like cells, or in a non-appropriate pulp environment. Phenotypically they are poorly characterized and it is still unclear as to whether they represent a distinct cell type.

Osteodentinoblasts. They are osteoblast-like cells in the pulp, forming an osteotypic form of fibrodentin. Cells surrounded by fibrodentinal matrix are often referred to as osteodentinocytes.

Fibrodentin. The atubular hard tissue formed by fibrodentinoblasts, either in the odontogenic pulp periphery, or in ectopic pulp sites.

Osteodentin. The osteotypic form of fibrodentin. It is also refer to as trabecular dentin.

4. Phenotype of formative cells

Only the dental pulp cells possess the ability to differentiate into odontoblasts. It is generally accepted that no other population of mesenchymal cells is able to differentiate into odontoblast or odontoblast-like cells and this specific ability seems to be acquired by exposure to morphogenetic influences during tooth development. Interactions between the oral ectoderm and the adjacent ectomesenchymal cells, during the initial stages of tooth formation, seem to determine the odontoblastic potential of dental papilla cells (Theslef et al. 1992).

Odontoblasts. Odontoblasts are elongated polarized cells, aligned in a single layer at the periphery of the dental pulp; they are highly differentiated cells expressing unique functional characteristics and are incapable of further cell division (Stanley 1962, Chiba et al 1967, Robins 1967, Tiber 1971, Holz & Baume 1973). Odontoblasts originate from the neural crest and their differentiation results from continuous reciprocal interactions between epithelial and mesenchymal components of the developing tooth germ (Slavkin et al. 1981, Thesleff and Hurmerinta 1981, Kollar 1983, Ruch 1985). Expression of the odontoblast phenotype after withdrawal from the cell cycle is characterised by a sequence of cytological and functional changes which occur at each tooth cusp according to a specific temporo-spatial pattern (Moullec 1978, Ruch 1985).

Morphologically, odontoblasts are characterised as elongated cells having a basal nuclear position and one (or more) cytoplasmic processes extending into the primary dentin (Tominaga et al. 1984). Three cytological modifications, the development of the granular endoplasmic reticulum cisternae parallel to the long axis of the cells, a highly developed Golgi complex and the presence of specific secretion granules, are considered to be the more evident morphological characteristics of odontoblast differentiation (Takuma and Nagai 1971, Tominaga et al. 1984, Ruch 1985, Sasaki and Garant 1996). Functional odontoblast differentiation is characterized by the polarized secretion of a tubular matrix.

Odontoblast differentiation apart the above described cytological modifications, also implies transcriptional and traslational modifications enabling the cells to deposit dentin components. Fully differentiated odontoblasts synthesize and secrete mainly collagenous proteins and a unique group of non-collagenous proteins, such as the dentin-specific proteins (dentin phosphoproteins, dentin sialoprotein and dentin matrix protein), and the proteins frequently found in a variety of tissues, such osteocalcin, osteonectin, bone sialoprotein, phospholipids, growth factors and proteoglycans (see Veis 1985, Linde 1989, Bronckers et al. 1989, Robey 1989, D'Souza et al. 1992, Linde and Goldberg 1993, Veis 1993, George et al. 1994, Butler 1995, Goldberg 1995).

Collagens. The collagenous proteins consitute 90% of the total organic dentin matrix and they are composed mainly of type I (95%), type V (3%) and type VI (minor component variants), while the presence of type I trimer and type III is still controversial (Butler 1973, Volpin and Veis 1973, Scott and Veis 1976, Munksgaard et al. 1978, Cournil et al. 1979, Wright and Leblond 1980, Lesot 1981, Butler 1984). Collagen fibres are initially unmineralized in predentin matrix, but provide the framework for the deposition of the apatite crystals at a distance 20 μm from the body of odontoblasts. Mechanisms that promote or allow mineralization in dentin collagen fibrils which morphologically resemble to those of other non-mineralized tissues, such as the skin, have attracted interest during the last 2 decades. It seems that some unique features of the collagen fibrillar network give rise to structures conductive to mineral depo

sition, such as the fibrils composed from of I collagen with type V in the core (see Linde and Goldberg 1993, Butler 1995). Also, the nature of collagen - non collagenous protein complexes in mineralizing front during dentinogenesis, seems to promote the convertion of the unmineralized predentin into a substrate where apatite crystals can form and grow (Weinstock and Lemblond 1973, Jontel and Linde 1983).

Proteoglycans. These are large complex macromolecules consisting of glycosaminoglycan chains covalently bound to a core protein. Dentin proteoglycans contain mainly chondroitin sulfate while proteoglycans containing heparin sulfate and keratan sulfate have also been identified as ninor components of predentin (Pincus 1950, Linde 1973, Jones and Leaver 1974, Goldberg et al. 1978,1987, Jontel and Linde 1985, Goldberg and Takagi 1993). Two small proteoglycans named biglycan and decorin have been recently identified in dentin (Takagi et al. 1990, Cam et al. 1995).

Glycoproteins/sialoproteins. This group includes dentin sialoprotein (DSP or 53-Kda dentin sialoprotein), osteonectin (ON), osteopontin (OPN) and bone sialoprotein (BSP), which were detected by immunohistochemical and in situ hybridization studies in odontoblasts and their cells processes (Fisher et al. 1983, Fugisawa and Kuboki 1989, Chen et al. 1991, D'Souza et al. 1992, Butler et al. 1992, Bronckers et al. 1994, Ritchie et al. 1994, 1995, Veis 1993). DSP is a sialic acid-rich acidic glycoprotein expressed primarily or exclusively by odontoblasts (but not by pre-odontoblasts) and related cells (odontoblast-like cells) (Butler et al. 1981, 1992, Ritchie et al. 1994, D' Souza et al. 1995). The cDNA sequence for DSP found in the odontoblast cDNA library, has not been detected in numerous other tissues and cells (Ritchie et al. 1995).

Phosphoproteins (phosphophoryns). The phosphoproteins (DPP) comprise over 50% of the total amount of the non-collagenous proteins in rodent teeth and include two highly phosphorylated molecules, one moderately and one weakly phosphorylated and a serine-rich phosphoprotein known as dentin matrix protein (DMP or AG1) (Veis and Perry 1967, Butler et al. 1972, Dimuzio and Veis 1978, Butler et al. 1983, Linde 1984, Fugisawa and Kuboki 1988, Sabsay et al. 1991). DMP is an acidic and highly phosphorylated molecule with one RGD sequence (an integrin-binding site) (George et al. 1993, 1994). The human DMP1 gene contains an open reading frame which predicts a highly acidic, serine-rich protein of 513 amino acids (Hirst et al. 1997). Phospoproteins have been identified in human crown and root dentin (crown DPP:root DPP = 1:2) but not in predentin, in the external zone of mantle dentin, in secondary and reparative dentin and in dentin matrix from dentinogenesis imperfecta I and II patients by phosphotungstic acid-chromic acid staining (Goldberg et al. 1978), by Stains All staining (Takagi et al. 1986, Takagi and Sasaki 1986, Takagi and Sasaki 1988), and by immunohistochemical analyses (Mac Dougal et al. 1985, Nakamura et al. 1985, Gorter de Vries et al. 1986, Rahima et al. 1988). These proteins comprise a smaller proportion of the non-collagenous matrix in human than rodent teeth and also, show some compositional differences. Further, Mac Dougal et al. (1997) suggested that both dentin sialoprotein and dentin phosphoprotein are expressed as a single cDNA transcript coding for a protein that is specifically cleaved into two smaller polypeptides.

Osteocalcin. The bone Gla-protein or osteocalcin is a small protein containing 50 amino acids (BGP) (Butler et al. 1981, Linde et al. 1982, Bronckers et al. 1985, Gorter de Vries et al. 1988). Immunohistochemical studies revealed intense staining in rat odontoblasts and their processes (Bronckers et al. 1987) but only weak staining in human and bovine predentin where odontoblasts did not react (Camarda et al. 1987, Gorter de Vries et al. 1988). Preliminary analyses in dog reparative dentin also showed a very weak staining reaction (Tziafas 1994).

Lipids. The lipids constitute 0.35% of the whole tissue (in rat dentin) and 1.75% of the organic matrix (Prout et al. 1973). They include cholesterol and cholesterol esters, mono-, di-, triglycerides, free fatty acids and phospholipids (Sharipo et al. 1966, Vogel et al. 1972). The main phospholipids prior to demineralization of the dentin that may be considered to be cellular components are phosphatidylcholine (66-77%), phosphatidyl ethanolamine (12-20%) and spingomyelin (9-11%), whereas those related to dentin extracellular matrix can be extracted after dentin demineralization and include phosphatidyl ethanolamine (28-37%), phospatidylserine (18-30%), phosphatidylinositol (10-11%), spingomyelin (14-18%)

and phosphatidylcholine (11-18%) (see Goldberg et al. 1995).

Growth factors. This polypeptide group, includes transforming growth factor-β molecules (TGF-βs) and insulin-like growth factor molecules/IGFs (Finkelman et al. 1991), bone mophogenetic proteins/BMPs (Bessho et al. 1991), and a rat incisor dentin polypeptide different from TGF-β and BMP, which shows in vitro chondrogenic activity (Amar et al. 1991). TGF-β 1, 2, 3 have been found in different extracellular matrix compartments in dentin (Smith et al. 1997). Some of these isoforms appear to be tightly associated with the collagen; they are not released from the dentin matrix by the demineralization process and require collagenase digestion for solubilization. Approximately half of the TGF-β1 in the EDTA fraction of dentin was found in active form, as the free molecule or in association with latency-associated polypeptide, soluble type III receptor and decorin, while odontoblasts showed reactivity for surface-bound type I and II receptors (Smith et al. 1997).

Serum-derived proteins. The serum derived proteins, include albumin, immunoglobulins IgG and a2 HS-glycoprotein (Kinoshita 1979, Butler et al 1981, Takagi et al. 1990).

Odontoblast-like cells. Different phenotypic characteristics have been described for odontoblast-like cells arising from various in vitro systems and in vivo experimental or clinical situations.

Odontoblast-like cells in vitro. Primary cell cultures retain more of the phenotypic properties of pulp tissue in vivo than subcultured cells and cloned cell lines (Nakashima 1991). Odontoblast-like cells in explant cultures synthesize type I collagen (Magloire et al. 1981). Pulp cells maintained in culture over a long period, showed formation of multi-layered structures (Mc Dougal et al. 1992, Tsukamoto et l. 1992, Andrews et al. 1993), expressing type I collagen, fibronectin, vimentin and dentin phosphoprotein (Mc Dougal et al. 1992). Odontoblast-like cells established from murine pulp-derived cells showed high expression of dentin phosphoprotein, type I collagen and alkaline phosphatase (Mc Dougal et al. 1995). Expression of c-jun and jun-B proto-oncogenes cound be a marker of differentiating cells to form new dentin matrix (Kitamura and Terashita 1997).

Odontoblast-like cells in vivo. The differentiated cells which secrete tubular dentin during pulp repair resemble morphologically the normally developed odontoblasts. They are elongated cells with clear nuclear, cytoplasmic and secretory polarity. Limited information on the biochemical profile of biosynthetic activity of odontoblast-like cells in vivo is presently available (Magloire et al. 1988, D'Souza et al. 1995, Tziafas 1994, Tziafas et al. 1995). Expression of collagen type III (Magloire et al. 1988), fibronectin (Magloire et al. 1988, Tziafas et al. 1995, Yoshiba et al. 1996), dentin sialoprotein (D'Souza et al. 1995) and osteocalcin (Tziafas 1994) has been identified in reparative dentin and associated odontoblast-like cells.

Criteria for assessment of differentiated odontoblast-like cells as reported in the literature are not well defined. Cells associated with the functional activity of odontoblasts, i.e. tubular matrix deposition in a polar pattern have often been identified as odontoblast-like cells, despite the absence of cell polarization or occasionally of cell elongation. Specific biochemical markers for odontoblastic activity may give clearer information. Veis (1985) reported that the ability of pulp cells to generate odontoblasts might be related to the fact that they are able to synthesize phosphophoryns. Since the secretion of the unique set of non collagenous proteins requires the fully expressed odontoblastic phenotype (Veis et al. 1984), the staining reaction of the matrix secreted by odontoblast-like cells with antibodies raised to dentin-specific proteins such as the dentin phosphophoryn (Laboux et al. 1994), may also recognize their odontoblastic specificity. It is important to recognize, however, that to fully characterize the odontoblast-like cell phenotype, a range are required including cell morphology, expression of cell-specific markers and the secretion of tubular matrix.

Fibrodentinoblasts. The non- specific formative cells which are related to the fibrous atubular dentin synthesis, have cuboid or polygonal shape. Ultrastructurally, they show a few organelles and numerus lipid droplets, while their calcified matrix exhibits numerus foci of highly mineralized ground substance with strong glycosaminoglycan reactivity and dispersed collagen fibrils (Baume, 1980). The functional phenotypic differences between odontoblast-like cells and fibrodentinoblasts cells are still totally unknown.