Biomarkers in the Dentin-Pulp Complex: Role in Health and Disease

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Biomarkers are functional elements at the cellular or molecular level, playing important roles in health and disease. The dentin-pulp complex of the tooth houses several biomarkers at different stages of development, and a lack of these biomarkers results in developmental disorders. Furthermore, biomarkers play a very important role in the pathogenesis of dental caries, pulpal and periapical pathoses in two ways - they are essential elements in the pathological process and their detection helps in accurate diagnosis of the pathological condition. The aim of this paper is to review the literature regarding the important biomarkers involved in the development of the dentin-pulp complex and in the pathological conditions involving the dentin-pulp complex.

Key words: biomarker, dentin-pulp complex, diagnosis, immunohistochemistry, **ELISA, matrix metalloproteinase, cathepsin, bone morphogenetic proteins**

INTRODUCTION

The term "biomarker" was coined from the portmanteau of "biological marker" which pertains to an extensive subcategory of medical signs which can be measured accurately and reproducibly [1,2]. The National Institutes of Health Biomarkers Definitions Working Group has defined a biomarker as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention [3].

Essentially, biomarkers could be considered as responses that are functional, psychological, biochemical at the cellular level or a molecular interaction. Biomarkers are being extensively used in research and it has shown to be a boon in the medical field to aid in diagnosis and treatment. This article gives a comprehensive review on the various biomarkers present in the dentin-pulp complex. This will enable understanding of the role of biomarkers in health and disease with specific reference to restorative dentistry and endodontics.

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Biomarkers in the dentin-pulp complex in health: developmental stages

The dentin and pulp together form the dentin-pulp complex. The outermost cell layer at the boundary of pulp tissue and predentin consists of the odontoblasts, the cells that form and mineralize the dentin matrix [4]. This section reviews the role of the most important biomarkers involved in the development of the dentin-pulp complex.

Osteocalcin (OCN)

Odontoblasts express a glycoprotein called osteocalcin (OCN) in the dentin matrix [5]. It is known to be one of the reparative molecules and its expression occurs in reaction to dental pulp injury. It is believed to be colligated with collagen fibers and is also found embedded in the tertiary dentin, hence its expression occurs in response to cavity preparation [6]. In addition, it is also considered as a marker for mature osteoblast and has been utilized as mineralization markers for odontoblast/osteoblast-like differentiation on dental pulp stem cells [7].

OCN is expressed before the commencement of mineralization and that it is the highest non-collagenous protein in the bone extracellular matrix. It is suggested to be essential in interceding osteoclastic differentiation [7,8]. OCN has been found to be associated with macrophage proteins such as granulocyte macrophage colony-stimulating factor (GM-CSF) in bone forming cells. Expression of OCN occurs in the terminal differentiation of macrophages to osteoblasts. It is expressed in response to cavity preparation being associated with collagen fibers and is also found in tertiary dentin [9].

Osteonectin (ON)

It is found to be a major non-collagenous matrix protein in bone and dentin [10]. Bovine odontoblasts and, odontoblasts and predentin in human prenatal and postnatal samples have exhibited ON. Its various roles in initiation of mineralization in has been shown [11]. As a result of their study, ON is indicated as a protein associated with collagen formation in mineralized tissues like bone and human dentin.

Dentin Sialophosphoprotein (DSPP)

DSPP is the initial translational product of DSPP messenger RNA (mRNA) which is then cleaved to dental phosphoprotein and dentin sialoprotein (DSP) [12-14]. It was primitively regarded to be dentin-specific until it was detected in bone[15]. However, its expression dentin is about 400 times that of bone. Being one of the fundamental non-collagenous proteins required in tooth development and mineralization, it is chiefly expressed in odontoblasts.

The role of DSPP in dentinogenesis has been well substantiated [15] (Qin *et al*. 2002), thus it remains to be a substantial marker for odontoblast differentiation. DSPP, osteocalcin and matrix extracellular phosphoglycoprotein (MEPE) expression raised in time dependently in the dental pulp cells (DPC) induced cultures [13,16,17]. DSPP expression is also associated with several events like tooth terminal epithelial-mesenchymal interaction events, amelogenesis and dentinogenesis. Presecretory ameloblasts manifested transient DSPP expression while continuous expression was exhibited in the odontoblast [18].

Thyrotropin-Releasing Hormone (TRH)-Degrading Enzyme (DE)

It is otherwise known as pyroglutamyl peptidase II and it exhibits absolute functional specificity for its substrate, TRH [19,20]. It is oriented extracellularly, a membrane-associated peptidase (ectopeptidase) and it functions to cease peptide-mediated cell signaling. TRH-DE has been discovered in the dental pulp by microarray analysis and real time RT-PCR analysis [20]. The expression of TRH-DE mRNA in dental pulp stem/progenitor cells ($CD105^+$ and $CD31^-$ side population (SP) cells were enhanced by the induction of neural cells. Its presence in the neuronal processes in dental pulp was affirmed by immunohistochemical and in situ hybridization. In addition, TRH-DE mRNA was also expressed in the regenerated pulp 28 days following the transplantation of $CD31^-$ SP cells into root canals after pulpectomy.

Matrix metalloproteinase (MMP)

MMPs are synthesized by the cells of the connective tissue such as the fibroblasts, osteoblasts, and odontoblasts and it is secreted into the extracellular matrix. Several matrix metalloproteinases (MMP) have been identified in dentin and pulp by polymerase chain reaction (PCR) and immunohistochemistry [21-23]. MMPs have been classified into six groups based in their structural homology and their substrate specificity as collagenase (MMP-1, MMP-8, MMP-13 and MMP-18), gelatinases (MMP-2 and MMP-9), stromelysins (MMP-3, MMP-10, and MMP-11), transmembrane MMPs or MT-MMPs (MMP-14, MMP-15, MMP-16, MMP-17, MMP-24 and MMP-25), and others (MMP-12, MMP-19, MMP-20, MMP-21, MMP-22, MMP-23, MMP-27, and MMP-28) [22].

Several MMPs have been identified in mineralized human dentin, namely collagenase MMP-8 [24], gelatinases [25] and enamelysin MMP-20 [23]. The MMPs present in saliva and dentin are host-derived and are activated at acidic pH resulting from the release of lactate from cariogenic bacteria [26]. At a neutral pH, MMP effects in remodeling of the extracellular matrix. Tissue destruction is always colligated with aberrant expression of MMP.

The role of MMP in various physiologic process in dentin-pulp complex has been well understood.The roles include organization of matrix before mineralization, control of mineralization, peritubular dentin formation, and matrix alterations during aging [26-29]. MMPs are matrixins which has a function of hydrolyzing components of extracellular matrix [30]. The specific distribution of MMP-2 in human coronal dentin has been demonstrated through immunohistochemistry using monoclonal anti-MMP-2 antibody [28]. In this study, intense immunoreactives were distinguished in the zone adjacent to the predentin and dentino enamel junction (DEJ). Thus, this suffices to conclude that MMP-2 is involved in the organization of extracellular matrix and DEJ establishment. The high labeling of MMP-2 and MMP-9 electron immunostaining has been elucidated in the mantle dentin [31].

Transforming Growth Factor beta1 (TGF-β1)

Cells of the dentin-pulp complex react to growth factors and signaling molecules released by bacterial acids from the dentin extracellular matrix (ECM). Transforming growth factor beta1 (TGF-β1) and the other members of this growth factor family have been entailed in the development of tooth and repair of dental tissue [32]. TGF-β1 plays an important role in odontoblast secretory activity modulation during dental tissue repair. In dental matrix and pulpal cells, TGF- β1 presence renders a reservoir of bioactive molecules for the signaling of tertiary dentinogenesis [33].

Cathepsin

Cathepsins are the lysosomal cysteine proteinases which has the ability to degrade extracellular matrix proteins such as collagen, laminin, fibronectin, and proteoglycans [34,35]. Their expression and activity in dentin and odontoblasts are confined to cathepsin D in odontoblasts and predentin [36-39].

MMPs are co-expressed with bone cell cathepsins. Analysis of dentin cathepsin and MMP activities were done by degradation of specific fluorogenic substrates. A wide range of cysteine cathepsin expression that gave minor responses to TGF-β were exhibited in odontoblasts and pulp tissue. Both cathepsin and MMP activities were observed in all the dentin samples, with substantial negative correlations in their activities with tooth age [39].

Cathepsin B and MMPs have at least partially independent pathways in cartilage proteoglycan breakdown [40]. However, cathepsin B from articular chondrocytes raises the levels of MMPs and induces angiogenesis by proteolytic inhibition of tissue inhibitors of MMPs. Moreover, active MMPS can activate procathepsin B and paradoxically, cathepsin B has been shown to be responsible for the activation of MMP-1 in gingival fibroblast cultures [41]. Cathepsin D and other lysosomal enzyme activities in odontoblasts have been shown to be increased in low calcium and vitamin D-deficient diet which effected in a disturbed dentin mineralization. This result indicates that cysteine cathepsins indeed contribute to the odontoblast response to metabolic disturbances.

Bone Morphogenic Protein (BMP-2)

Expression of BMP2 gene occurs in post-natal odontoblasts and ameloblasts from birth to approximately 20 days after birth during tooth cytodifferentiation [42]. A decrease in the quantity of dentin in crown occurs ensuing from the deletion of BMP2 gene in early odontoblasts, in addition to a more marked effect on root dentin. The dentin formed is defective with patchy unmineralized areas and dysmorphic dentinal tubules. There is no overt changes in the enamel quality although there is a delay in amelogenesis in the absence of odontoblast BMP-2. BMP-2 is believed to be important in the development of dental pulp as its absence results in reduced blood vessels and colligated pericytes [43].

Bone sialoprotein (BSP)

It is a important non collagenous protein in mineralized connective tissue. Its expression is majorly particular for mineralizing tissues which includes bone, mineralizing cartilage, dentin and cementum [44].

Biomarkers in the dentin-pulp complex in disease: Dental Caries

Dental caries is an irreversible disease of calcified tissues of teeth, characterized by demineralization of the inorganic portion and destruction of the organic substance of the tooth, which often leads to cavitation. Carious dentin has been described as comprising of two layers in relation to bacterial infection and degradation of dentine organic matrix. Dentin is infected by cariogenic bacteria and the organic matrix is entirely denatured in the outer layer while the dentin in the inner layer is partially demineralized and affected by the process of caries, but is not infected with bacteria [45].The bacterial invasion of dentino-enamel junction (DEJ) is the initial stage of dentinal caries followed by the enlargement of the gap between enamel and dentin, and the lesion propagates along the DEJ with superficial destruction of the mantle dentin. A cavity is formed when the fragments of enamel are no longer supported by the dentin and this cavity is filled with food debris and bathed in saliva[46].

The dentin-pulp complex is able to respond to a number of stimuli which ensues from carious disease progression. Studies suggest that bacterial collagenase is solely responsible for the organic matrix destruction. The collagensase enzymes are capable of cleaving collagenous and non-collagenous proteins of carious dentine at neutral pH [47,48]. *In vitro* research has shown that cariogenic bacteria induce surface mineralization, and weak protease activity has been demonstrated. However, the weak protease activity is unable to digest collagenous matrix subsequent to acid treatment [49]. The dental matrix degradation contributed by this enzyme may be less significant than was initially conceived, with the fact that the bacterial collagenase did not resist acidic challenge (pH 7.3) during the demineralization phase of a pH cycling model. Thus, host-derived proteolytic enzymes such as MMPs are implied to play a more substantial role in dentin organic matrix degradation.

Matrix Metalloproteinases (MMP)

Host cell-derived matrix metalloproteinases (MMPs) participate in dentin destruction following demineralization by bacterial acids based on their study. The activity of MMPs in the developmental and pathological process of dental caries has been substantiated by these authors. Regardless of the subject's periodontal status, both MMP-1 (interstitial collagenase) and MMP-8 (PMN- derived collagenase) can be quantified in the saliva [50]. Western blot analysis and gelatin zymography analyses have led to the identification of MMP-2, MMP-9, MMP-8. Latent and active forms of gelatinases have also been observed. MMP-20 has been demonstrated sin dilated dentinal tubuli of caries lesion by immunohistochemistry but Western blot showed no reaction. Hence, it may be speculated that the production of MMP-20 occurs in primary dentinogenesis by the dentin-pulp complex which was integrated in dentin. MMP-20 is then liberated and its possible activation occurs during the progression of caries.

Bone Sialoprotein (BSP)

BSP has been entailed in the mineralization of dentin and modulation of MMP-2. using pooled RNA isolated from the pulp tissue from both healthy and carious teeth, it has been observed that there is an 8-fold up-regulation in the genetic expression of BSP in pulps of teeth with active caries [51]. However, the pulp tissue expression of MMP-2 in response to the carious process remain unknown. Immunohistochemical analysis of extracted third molars and premolars did not reveal any detection of MMP-2 and BSP in the tubular lumen of healthy dentin. Hence, both BSP and MMP-2 might be involved in host defense mechanism which leads to calcification of regions affected by caries.

Alkaline Phosphatase (ALP)

It is involved in early mineral deposition and the calcification of different tissues. In addition, it is considered as a marker for odontoblast-like differentiation. Studies show that pulp cells exhibit high levels of ALP activity [52]. It is believed to be an essential constituent in the mechanism of repair and healing after pulpal injury.

Biomarkers in the dentin-pulp complex in disease: Pulpal Inflammation and Periapical pathoses

Infection, exposure, trauma and chemicals can result in loss of vitality of the pulp. As a result of this tooth injury, a signal to progenitor stem cells is sent to stimulate differentiation, proliferation, and migration as part of reparative dentinogenesis. Influx and recruitment of polymorphonuclear macrophages (PMNs) is an initial response to bacteria and its metabolites. In addition to the proliferation of fibroblasts and angiogenesis, the infiltration of macrophages, lymphocytes and plasma cells are characteristic features in a more chronic condition.

MMPs have also been identified both pulpal and periapical inflammation [53-55]. This group of enzymes is responsible for the degradation of extracellular matrix (ECM), as seen in inflammation of pulp and periapical region. The destruction of ECM elicited by the intracellular components of bacteria, bacterial metabolites, and other molecules, leads to the formation of periradicular lesions [55]. Essentially, MMPs constitute of a group of structurally linked but genetically distinguishable endopeptidases which are expressed in normal tissues at low levels, but is upregulated in inflammation. It is also interesting to note that the levels of MMP-8, which is usually higher in the case of periapical exudates, is significantly reduced after the first visit of root canal treatment [53]. IHC staining demonstrated the presence of MMP-8 in pulp and periapical granulomas with the PMNs being the preponderant cell type to express MMP-8.

Enzyme linked immunosorbent assays have demonstrated that the level of MMP-1 was below the detection limit in healthy and inflamed pulps, while the MMP-9 level was significantly elevated in inflamed human dental pulps. In contrast, the levels of MMP-2 and MMP-3 was reduced in symptomatic inflamed pulps in comparison with normal pulps [55]. This suggests the principal role of MMP-9 in the degradation of inflamed human dental pulp tissue. The role of MMP-9 in inflamed pulp is further supported by another report [54]. Another study also noted that the MMP-9 mRNA gene was increased in inflamed pulps [55]. Furthermore, the in-situ localization of MMP-9 expression in pulp specimens showed significantly higher expression of MMP-9 in inflamed pulps compared to clinically healthy pulps. Increased activity of MMP-9 and MMP-2 has also been shown in the gingival crevicular fluid of teeth with periapical lesions [56]. Thus, the use of MMP-9 and MMP-2 as biological markers can be substantiated. Furthermore, periapical granulomas demonstrated higher MMP-9 and MMP-13 activity compared with radicular cysts [55].

The role of Osteocalcin in pulpal pathoses was not clearly elucidated until recently, when it was shown that expression of osteocalcin was higher in reversible pulpitis compared to irreversible pulpitis [8]. Osteocalcin is a reparative molecule in the dental pulp and in the case of repair, it is localized in cells and matrix surrounding the areas of calcification and in cells around blood vessels. However it is absent in normal tissues. This finding positively correlates with angiogenic markers such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), which supports its role in pulp repair.

Substance P, neurokinin A, calcitonin gene-related peptide are present in the pulp and periodontium [57,58]. These peptides arise from the trigeminal ganglion and are expressed in unmyelinated C-fibres and some A-delta fibers. These peptides exhibit profound effects on blood flow, inflammatory and immune responses. Hence their role in pulpal pain could be substantial. In an investigation of pulp tissue from extracted or endodontically treated painful teeth, a significantly higher concentration of substance P (SP), neurokinin A (NKA) and calcitonin gene-related peptide (CGRP) was found in the painful teeth compared to non-painful teeth [59]. The elevation of substance P, a neuropeptide, in inflamed human periradicular tissue has also been shown by Tuncer *et al* [60].

Alkaline phosphatase (ALP) is vital in mineralized tissue formation and its activity was shown to be increased 8 fold compared to the normal pulp. However, in irreversible pulpitis, the values of ALP activity was approximately equivalent to the normal healthy pulp [61]. Thus, this could suggest the function of ALP in the initial pulp response after injury. Cellular proteases, including elastase (PMN-E) and cathepsin-G (PMN-G) are liberated by the lysosomal degranulation of Polymorphonuclear leucocytes and these bring about degradation of connective tissue. However, a naturally occurring serum protease inhibitor alpha 2-macroglobulin (A2-M) has been shown to be able to modify nonspecific pulpal tissue destruction. A significant correlation has also been shown between PMN-CG and A2-M in moderate to severely inflamed pulps [62].

Periapical lesions also release interleukins which are responsible for bone cell activity modulation. Interleukin-1 (IL-1) which is produced chiefly by monocytes and macrophages is found to be a potent stimulant of bone resorption in organ culture. The presence of IL-1β activity in human periapical lesions has been demonstrated by using IL-1β enzyme-linked immunosorbent assay. This further affirms the production of IL-1β and its local release in periapical lesions which mediate bone resorption [63].

Gingival crevicular fluid (GCF) has the potential of serving as biological markers in diagnosing pulpal pathologies. There is an increase in the levels of inflammatory markers in the GCF of teeth which were clinically diagnosed with irreversible pulpitis. An elevated level of IL-8 (CXCL8) has been found in the GCF of patients with irreversibly inflamed pulp with comparison of healthy pulps in contralateral teeth [64]. However, another study was unable to exhibit a significant higher level of IL-1β or DSP in the GCF of diseased teeth [5]

Biomarkers as a diagnostic tool

As there is significant expression of MMPs in pulpal inflammation and pathology, MMP analysis from periapical exudates can be an option to indicate and monitor inflammatory activity as well as an indicator of the success of root canal treated teeth that have prior periapical lesions. There is a strong denotation that MMP-8 arises from the periapical inflammation site as it is still exhibited in the second visit of root canal treatment in spite of removal of pulp tissue and cleaned canals during the first appointment which implies that active phase of periapical site inflammation is still present. The disappearance of inflammation and partial onset of healing is reflected by the absence of MMP-8 in the root canal exudate during the third visit. Therefore, level of MMP-8 could function as a biochemical indicator to assess the inflammatory status of the periapical tissue. MMP measurement from the root canal during treatment serves as a potential prospect as a diagnostic tool to appraise the condition of the periapical inflammation. MMP-9 also serve as a potentially reliable marker as its level significantly increases in pulpal inflammation [66].

The presence of differential expression of molecules within cyst or granuloma has the potential to provide information to differentiate between periapical cysts from granuloma before executing endodontic procedure as cysts have lower healing rates [55]. Zehnder *et al* were the first to provide a clinical relevance for the use of MMP-9 as a marker for pulpal pathoses [66]. They performed a study to improve pulpal diagnosis by assessing the levels of MMP-9 in the dentinal fluid of symptomatic teeth diagnosed with irreversible pulpitis and healthy counterparts. In order to perform a non-invasive assessment of the pulp, collection of the dentinal fluid from the dentin wound can be done after cavity preparation. After the access cavity was prepared, a folded sterile polyvinylidene difluoride (PVDF) filter membrane was used to collect dentinal fluid from the exposed dentin and this fluid was then subjected to a MMP-9 fluorescent assay. This particular assay has been claimed to have sensitivity which is more superior than that of conventional ELISAs as it functions based on substrate turnover which effects in fluorophore liberation. In conclusion, dentinal fluid samples of symptomatic teeth had significantly higher MMP-9 levels compared to healthy pulp.

REFERENCES

1. Scaros O, Fisler. Biomarker technology roundup: from discovery to clinical applications, a broad set of tools is required to translate from the lab to the clinic. Biotechniques; Suppl: 30-32.2005.
2. Strimbu K, Tavel JA. What are biomarkers? Curr Opin HIV AIDS 2010; 5: 463-466.
3. Biomarkers Definition Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Therapeutics;69: 89-95. 2001,
4. Ten Cate AR. Structure of the Oral Tissues. In : Ten Cate AR (ed.) Oral Histology : Development, Structure, Function. 5th St. Louis: Mosby, 45-57, 1994.
5. Abd-Elmeguid A, Abdeldayem M, Kline LW, et al. Osteocalcin expression in pulp inflammation. J Endod; 39: 865-872. 2013.
6. Hirata M, Yamaza T, Mei YF, et al. Expression of osteocalcin and Jun D in the early period during reactionary dentin formation after tooth preparation in rat molars. Cell Tissue Res; 319: 455-65. 2005.
7. Gortes V, Quartier E, Boute P, Wisse E. Immunocytochemical localization of osteocalcin in developing rat teeth. J Dent Res; 66: 784-90. 1987.
8. Ishida M, Amano S. Osteocalcin fragment in bone matrix enhances osteoclast maturation at a late stage of osteoclast differentiation. J Bone Miner Metab; 22: 415-29. 2004.
9. Evans DB, Bunning RA, Russell RG. The effects of recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) on human osteoblast-like cells. Biochem Biophys Res Commun; 60: 588-595. 1989.
10. Fujisawa R, Kuboki Y. Changes in the level of osteonectin in bovine dentin during tooth development. Arch Oral Biol; 34: 89-92. 1989.
11. Reichert T, Störkel S, Becker K, Fisher LW. The role of osteonectin in human tooth development: an immunohistological study. Calcif Tissue Int; 50: 468-72. 1992.
12. Butler WT. Dentin specific proteins. Methods Enzymol; 145: 290-303. 1987.
13. Butler WT, Ritchie H. The nature and functional significance of dentin extracellular matrix proteins. Int J Dev Biol; 39: 169-179. 1995.
14. Butler WT, Brunn JC, Qin C, McKee MD. Extracellular matrix proteins and the dynamics of dentin formation. Connect Tissue Res; 43: 301-307. 2002.
15. Qin C, Brunn JC, Cadena E, et al. The expression of dentin sialophosphoprotein gene in bone. J Dent Res; 81: 392-4. 2002.
16. Sreenath T, Thyagarajan T, Hall B, et al. Dentin sialophosphoprotein knockout mouse teeth display widened predentin zone and develop defective dentin mineralization similar to human dentinogenesis imperfect type III. J Biol Chem; 278: 24874-80. 2003.
17. Zhang X, Zhao J, Li C,et al. DSPP mutation in dentinogenesis imperfect Shields type II. Nat Genet; 27: 151-2. 2001.
18. Papagerakis P, Berdal A, Mesbah M, Peuchmaur M, Malaval L, Nydegger J, Simmer J, Macdougall M. Investigation of osteocalcin, osteonectin, and dentin sialophosphoprotein in developing human teeth. Bone; 30: 377-85. 2002.
19. Bauer K, Carmeliet P, Schulz M, Baes M, Denef C. Regulation and cellular localization of the membrane-bound thyrotropin-releasing hormone-degrading enzyme in primary cultures of neuronal, glial and adenohypophyseal cells. Endocrinology; 127:1224-1233. 1990.
20. Yamamoto T, Murukami M, Ishizaka R, Iohara K, Kurita K, Nakashima M. Identification of thyrotropin-releasing hormone (TRH)- degrading enzyme as a biomarker for dental pulp tissue. Dentistry; 2:114. 2012.
21. Palosaari H, Pennington CJ, Larmas M, Edwards DR, Tjäderhane L, Salo T. Expression profile of matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs in mature human odontoblasts and pulp tissue. Eur J Oral Sci; 111: 117-127. 2003.
22. Chaussain-Miller C, Fioretti F, Goldberg M, Menashi S. The role of matrix metalloproteinase (MMPs) in human caries. J Dent Res; 85: 22-32. 2006.
23. Sulkala M, Larmas M, Sorsa T, Salo T, Tjäderhane L. The localisation of matrix metalloproteinase-20 (MMP-20, Enamelysin) in mature human teeth. J Dent Res; 81: 603-607. 2002.
24. Sulkala M, Tervahartiala T, Sorsa T, et al. Matrix metalloproteinase-8 (MMP-8) is a major collagenase in human dentin. Arch Oral Biol; 52; 121-7. 2007.
25. Martin-de Las Heras S, Valenzuela A, Overall CM. The matrix metalloproteinase gelatinase A in human dentine. Arch Oral Biol; 45: 757-65. 2000.
26. Mazzoni A, Pashley DH, Nishitani Y et al. Reactivation of inactivated endogenous proteolytic activities in phosphoric acid-etched dentin by etch-and-rinse adhesives. Biomaterials; 27: 4470-6. 2006.
27. Mazzoni A, Pashley DH, Tay FR, et al. Immunohistochemical identification of MMP-2 and MMP-9 in human dentine: correlative FEI-SEM/TEM analysis. J Biomed Mater Res; 88: 697-703. A 2009.
28. Boushell LW, Kaku M, Mochida Y, Bagnell R, Yamauchi M. Immunohistochemical localization of matrixmetalloproteinase-2 in human coronal dentin. Arch Oral Biol; 53: 109-116. 2008.
29. Santos J, Carrilho M, Tervahartiala T, et al. Determination of matrix metalloproteinases in human radicular dentin. J Endod; 35: 686-9. 2009.
30. Brinckerhoff CE, Matrisian LM. Matrix metalloproteinases: a tail of a frog that became a prince. Nat Rev Mol Cell Biol; 2: 207-214. 2002.
31. Goldberg M, Septier D, Bourd K, Hall R, George A, Goldberg H, Menashi S. Immunohistochemical localization of MMP-2, MMP-9, TIMP-1, and TIMP-2 in the forming rat incisor. Connect Tissue Res; 44: 143-53. 2003.
32. Smith AJ, Lesot H. Induction and regulation of crown dentinogenesis: embryonic events as a template for dental tissue repair? Crit Rev Oral Biol Med; 12: 425-37. 2001.
33. Tjäderhane L, Palosaari H, Wahlgren J, et al. Human odontoblast culture method: the expression of collagen and matrix metalloproteinases (MMPs). Adv Dent Res; 15: 55-8. 2001.
34. Linde A, Persliden B, Rönnbäck L. Cathepsin D: Purification from rat liver and immunohistochemical demonstration in rat incisor. Acta Odontol Scand; 36: 117-26. 1978.
35. Dickinson DP. Cysteine peptidases of mammals: their biological roles and potential effects in the oral cavity and other tissues in health and disease. Crit Rev Oral Biol Med; 13: 238-75. 2002.
36. Linde A, Persliden B. Cathepsin D activity in isolated odontoblasts. Calcif Tissue Res; 23: 33-38. 1977.
37. Nygren H, Persliden B, Hansson HA, et al. Cathepsin D: ultra-immunohistochemical localization in dentinogenesis. Calcif Tissue Int; 29: 251-6. 1979.
38. Andujar MB, Hartmann DJ, Caillot G, et al. Immunolocalization of cathepsin D in dental tissues. Matrix; 9: 397-404. 1989.
39. Tersariol et al. Cysteine cathepsins in human dentin-pulp complex. J Endod; 36: 475-81. 2010.
40. Buttle DJ, Handley CJ, Ilic MZ, et al. Inhibition of cartilage proteoglycan release by a specific inactivator of cathepsin B and an inhibitor of matrix metalloproteinases: evidence for two converging pathways of chondrocyte-mediated proteoglycan degradation. Arthritis Rheum; 36: 1709-1717. 1993.
41. Kostoulas G, Lang A, Nagase H, et al. Stimulation of angiogenesis through cathepsin B inactivation of the tissue inhibitors of matrix metalloproteinases. FEBS Lett; 455: 286-90. 1999.
42. Aberg T, Wozney J, Thesleff I. Expression patterns of bone morphogenetic proteins (BMPs) in the developing mouse tooth suggest roles in morphogenesis and cell differentiation. Dev Dyn; 210: 383-396. 1997.
43. Yang W, Harris MA, Cui Y, Mishina Y, Harris SE, Gluhak-Heinrich J. BMP2 is required for odontoblast differentiation and pulp vasculogenesis. J Dent Res; 91: 58-64. 2012.
44. Boushell LW, Nagaoka H, Nagaoka H, Yamauchi M. Increased matrix metalloproteinase-2 and bone sialoprotein response to human coronal caries. Caries Res; 45: 453-459. 2011.
45. Fusayama T. Two layers of caries dentin: diagnosis and treatment. Oper Dent; 4: 63-70. 1979.
46. Erkstrand KR, Ricketts DN, Kidd EA. Occlusal caries: pathology, diagnosis and logical management. Dent Update: 28; 380-387. 2001.
47. Kawasaki K, Featherstone JD. Effects of collagenase on root demineralization. J Dent Res; 76: 588-595. 1997.
48. Dumas J, Hurion N, Weill R, Keil B. Collagenase in mineralized tissues of human teeth. FEBS Lett; 187: 51-55. 1985.

49. Dung SZ, Gregory RL, Li Y, Stookey GK. Effect of lactic acid and proteolytic enzymes on the release of organic matrix components from human root dentin. Caries Res; 29: 483-489. 1995.
50. Wahlgren J, Salo T, Teronen O,et al. Matrix metalloproteinase-8 (MMP-8) in pulpal and periapical inflammation and periapical root canal exudates. Int Endod J; 35: 897-904. 2002.
51. McLachlan JL, Smith AJ, Bujalska IJ, Cooper PR. Gene expression profiling of pulpal tissue reveals the molecular complexity of dental caries. Biochim Biophys Acta; 1741: 271-281. 2005.
52. Murray PE, Garcia-Godoy F. Stem cell responses in tooth regeneration. Stem Cells Dev; 13: 255-62. 2004.
53. Gusman H, Santana RB, Zehnder M. Matrix metalloproteinase levels and gelatinolytic activity in clinically healthy and inflamed human dental pulps. Eur J Oral Sci; 110: 353-7. 2002.
54. Tsai CH, Chen YJ, Huang FM, Su YF, Chang YC. The upregulation of matrix metalloproteinase-9 in inflamed human dental pulps. J Endod; 31: 860-862. 2005.
55. de Paula-Silva FW, D'Silva NJ, da Silva LA, et al. High matrix metalloproteinase activity is a hallmark of periapical granulomas. J Endod; 35: 1234-1242. 2009.
56. Belmar MJ, Pabst C, Martínez B, Hernández M. Gelatinolytic activity in gingival crevicular fluid from teeth with periapical lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod;105: 801-806. 2008.
57. Gazelius B, Edwall B, Olgart L, Lundberg JM, Hökfelt T, Fischer JA. Vasodilatory effects and coexistence of calcitonin gene-related peptide (CGRP) and substance P in sensory nerves of cat dental pulp. Acta Physiologica Scand; 130: 33-40. 1987.
58. Ohkubo T, Shibata M, Yamata Y, Kaya H, Takahashi H. Role of substance P in neurogenic inflammation in the rat incisor pulp and the lower lip. Arch Oral Biol; 38: 151-8. 1993.
59. Awawdeh L, Lundy FT, Shaw C, Lamey PJ, Linden GJ, Kennedy JG. Quantitative analysis of substance P, neurokinin A and calcitonin gene-related peptide in pulp tissue from painful and healthy human teeth. Int Endod J; 35: 30-36. 2002.
60. Tuncer LI, Alaçam T, Oral B. Substance P expression is elevated in inflamed human periradicular tissue. J Endod; 30: 329-32. 2004.
61. Spoto G, Fioroni M, Rubini C, Tripodi D, Stilio MD, Piattelli A. Alkaline phosphatase activity in normal and inflamed pulps. J Endod; 27: 180-2. 2001.
62. Rauschenberger CR, McClanahan SB, Pederson ED, Turner DW, Kaminski EJ. Comparison of human polymorphonuclear neutrophil elastase, polymorphonuclear neutrophil cathepsin-G, and alpha 2-macroglobulin levels in healthy and inflamed dental pulps. J Endod; 20: 546-50. 1994.
63. Barkhordar RA, Hussain MZ, Hayashi C. Detection of interleukin-1 beta in human periapical lesions. Oral Surg Oral Med Oral Pathol; 73: 334-336. 1992.
64. Karapanou V, Kempuraj D, Theoharides TC. Interleukin-8 is increased in gingival crevicular fluid from patients from acute pulpitis. J Endod; 34: 148-51. 2008.
65. Brugener B, Ford AR, Situ H, Fayad MI, Hao JJ, Wenckus CS, Johnson BR, BeGole EA, George A. Biologic markers for odontogenic periradicular periodontitis. J Endod; 36: 1307-1310. 2010.
66. Zehnder M, Wegehaupt FJ, Attin T. A first study on the usefulness of matrix metalloproteinase 9 from dentinal fluid to indicate pulp inflammation. J Endod; 31: 17-20. 2011.