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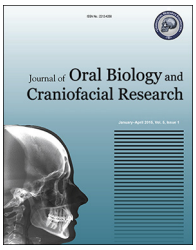

Review Article

Role of matrix metalloproteinases in dental caries, pulp and periapical inflammation: An overview

Atul Jain [a,*], *Rachana Bahuguna* [b]

[a] *Professor & HOD, Department of Conservative Dentistry & Endodontics, Rungta College of Dental Sciences & Research, Bhilai, India*

[b] *Professor & HOD, Department of Pedodontics, Rungta College of Dental Sciences & Research, Bhilai, India*

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ABSTRACT

Matrix metalloproteinases (MMPs) are a group of more than 25 secreted and membrane bound enzymes that represent class of enzymes responsible for degradation of pericellular substrates. They have been isolated from dentine, odontoblasts, pulp and periapical tissue. They play an important role in dentine matrix formation, modulating caries progression and secondary dentine formation. Earlier microbial proteolytic enzymes were believed to be responsible for degradation of dentine organic matrix, but lately the accumulated body of evidence suggests that MMPs have an important role in the process. During normal tissue modelling, differentiation during development, in modulating the cell behaviour, maintaining homeostasis and in numerous extracellular pathologic conditions, MMPs tends to be an equally important participant. Odontoblasts secrete some of the essential MMPs for both physiologic and pathologic conditions. MMPs also appear to be a participant in the process of reversible and irreversible pulpitis. Although they tend to have low expression and activity in adult tissues but at the onset of any destructive pathologic process, their production shoots up. They appear to have a significant presence during times of inflammation in the periapical region as well. We take a look at the various factors and evidence pointing towards the role of MMPs in the progression of caries, pulpal and periapical inflammation.

1. Introduction

The regulation of extracellular matrix (ECM) in both physiologic and pathologic conditions is carried out by different protease systems, viz. cysteine proteinase, aspartic proteinase, serine proteinase and metalloproteinase. Amongst the metalloproteinases, which comprise of several superfamilies, metzincin superfamily is the most important. The hallmark of matrix metalloproteinases, which belong to metzincin superfamily being, binding to zinc at the catalytic site and have a conserved 'Met-turn' motif.[1,2]

Matrix metalloproteinases are a group of more than 25 secreted and membrane bound enzymes that represent, a class of enzymes, responsible for degradation of pericellular substrates, including proteinase, clotting factors, chemotactic molecules, latent growth factors, cell surface receptors, cell–cell adhesion molecules and almost all structural ECM

* *Corresponding author.*
E-mail address: jaindratul@yahoo.co.in (A. Jain).

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proteins. As a consequence, they are an important player in normal tissue modelling, differentiation during development and in modulating the cell behaviour. They play an essential role in homeostasis and are also involved in numerous ECM pathologic conditions, viz. inflammation and degradation of bone, autoimmune disease and invasion, migration of cancer cells across the basement membrane as in tumour metastasis. Thus MMP family proteins elicit dual roles in the pathogenesis of inflammation, stimulating protective innate and/or adaptive immune functions, as well as tissue destruction.[3]

On the basis of their putative substrate specificity and internal homologies, MMPs are classified into five main classes – collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs and others (Table 1). Their role in tissue destructional pathological conditions is evident but still however not completely clear. Their expression is regulated by proinflammatory cytokines and growth factors, as well as ECM components. The collagenases include MMP-1 (collagenase-1), MMP-8 (collagenase-2) and MMP-13 (collagenase-3). The gelatinases (type IV collagenase) include MMP-2 (gelatinase A) and MMP-9 (gelatinase B). Collagenases and gelatinases, which tend to break collagens and laminins, are considered to be the key MMPs responsible for ECM and BM destruction in many pathological conditions.[4]

Although MMPs are activated extracellularly or at the cell surface, some of them can be activated intracellularly as well. The activity of MMPs is highly controlled so as to confine them to the specific area. Proteolysis of plasminogen initiates an activation cascade leading to cleaving pro MMPs, and every step is controlled by specific activator or inhibitor called tissue inhibitors of metalloproteinases (TIMPs). Any imbalance in the expression or activity of MMP can have grave consequences in disease. Controlled degradation of ECM is essential in various physiological situations, including developmental tissue remodelling, tissue repair, angiogenesis, bone remodelling, nerve growth, immune response, apoptosis, etc. On the contrary, their unregulated activity has been implicated in numerous disease processes.

MMPs have been isolated from dentine, pulp tissue and odontoblasts, where they play an important role in dentine matrix formation, modulating caries progression and secondary dentine formation. Several pieces of evidence support the fundamental role of MMPs during the development, remodelling and destruction of oral tissues.

Through a comprehensive literature review, this article aims to provide an overview of the role of MMPs in dental caries, pulp and periapical inflammation.

Table 1 – Classification of MMPs.

1. Collagenases
MMP-1 (collagenase-1, interstitial collagenase)
MMP-8 (collagenase-2, neutrophil collagenase)
MMP-13 (collagenase-3)
2. Gelatinases
MMP-2 (gelatinase A, 72-kDa gelatinase)
MMP-9 (gelatinase B, 92-kDa gelatinase)
3. Stromelysins
MMP-3 (stromelysin-1)
MMP-10 (stromelysin-2)
MMP-11 (stromelysin-3)
MMP-12 (metalloelastase)
4. Matrilysins
MMP-7 (matrilysin, PUMP-1)
MMP-26 (matrilysin-2)
5. MT-MMPs (Membrane type)
MMP-14 (MT1-MMP)
MMP-15 (MT2-MMP)
MMP-16 (MT3-MMP)
MMP-17 (MT4-MMP)
MMP-24 (MT5-MMP)
MMP-25 (MT6-MMP)
6. Other MMPs
MMP-18
MMP-19
MMP-20 (enamelysin)
MMP-21
MMP-23
MMP-27
MMP-28 (epilysin)

2. Search criteria

Inclusion criteria: The search was limited to experimental study articles, review articles and thesis. Restrictions were not placed regarding the study design and the language usage.

Exclusion criteria: Publications that did not meet the above inclusion criteria are excluded.

Search strategy: A literature review was performed in Pubmed Central, MEDLINE, the Cochrane Library, and the EBSCO host. The articles identified included those published between 1989 and December 2014 with the following Subject Headings terms and/or keywords in various combinations: Matrix metalloproteinase, dentine, dental caries, odontoblast, pulp inflammation and periapical inflammation. About 160 articles were found, out of which many were excluded based on the exclusion criteria mentioned above and 71 of the articles and 2 theses were used for this review.

3. Role of MMPs in dental caries

Dentine ECM is mainly constituted of Type I collagen fibrils (90%), Type III and V collagen fibrils (1–3%),[5,6] which undergo fibrillogenesis to form a template, which can be efficiently and effectively mineralized. Rest is made up of non-collagenous proteins.

ECM can be degraded by various different mechanisms. These mechanism comprise of – (i) release of enzymes by host and bacterial cells, (ii) phagocytosis of matrix components, (iii) release of reactive oxygen species and (iv) release of cytokines, inflammatory mediators and apoptotic proteins that influence enzymes connected with matrix components.

In dental caries, demineralization is caused by microbial acids, and degradation of dentinal organic matrix was thought to be carried out by microbial proteolytic enzymes. The proteases produced by the cariogenic bacteria, which were believed to be responsible for the degradation of dentine organic matrix, have been found to be highly pH sensitive. They are not able to resist the acidic pH fall (4.3) during the demineralization phase, thus suggesting their limited contribution to the

process.[7] Therefore the potential role of the host derived proteases and in particular MMPs in dentine matrix degradation has been introduced. The various possible sources of MMPs in the carious lesion are – saliva, gingival crevicular fluid and MMPs produced by the odontoblast.

Presence of both pre- and active forms of MMP-8, MMP-2 and MMP-9 in human dental carious lesion tends to suggest their active role in the carious process. MMPs are involved in matrix remodelling during dentinogenesis. MMP-8, MMP-2, MMP-9, MMP-3, MMP-2, MMP-14 and MMP-20 are the main MMPs that have been identified in pulp, odontoblasts and predentine/dentine.[8–13] MMP-13 has been found to be present in radicular dentine, which was expressed differently as caries progressed, but it was not found to have been expressed in the coronal dentine.[14] The immunoexpression of MMP-13 has been found to be weak and confined to the peritubular area in sound dentine and strong at the peritubular level and in Tome's process in dentine caries, where it decreased as the distance from the decay process increased.[15] The immunoexpression pattern with greater expression being found close to caries level is similar to the one described for other MMPs (MMP-20, -28 and -9) in crown and root lesions[9,16] despite often diverse expression levels.[16]

MMPs are located throughout the dentine but they are located intensively along the enamel–dentine junction and in the predentine. Increased MMP presence along the dentine–enamel junction may contribute to the widening of caries along this junction, as it progresses into dentine.[17] MMP activity has been found to decrease with age in both active and chronic carious lesions.[18]

MMPs are secreted to the ECM as inactive proenzymes that require activation for carrying out degradation of matrix components. The pro-form of MMPs is converted into active form by the low pH, but the latter is active only for a short span of time. Low pH of the acids produced during caries tends to activate these endogenous MMPs,[19] but these activated MMPs are functional only in neutral pH. This neutralization of acids is achieved by dentinal buffering mechanism[20,21] through salivary buffer systems. Thus bringing about the change of matrix components through the pH activated MMPs.[19] Moreover, phosphorylated proteins released by bacterial acids from the collagen scaffold interact with TIMP-inhibited MMPs within the carious lesion and reactivate them, enhancing the degradation process.[22]

TIMPs as per current studies consist of four members – TIMP-1, -2, -3 and -4. They form complexes with the active forms of MMPs and under certain circumstances with their latent forms as well. TIMPs inhibit the activity of the fully competent MMP and also appear to block or retard MMP precursor activation. TIMP-1 and -2 seem to play a role in curbing hard dental tissue breakdown in the post- injury pathological process taking place in dental tissue (e.g. caries lesion),[23] even though the level of TIMPs found in active carious lesions is insufficient to block the progression of dental hard tissue destruction mediated by MMPs.[24]

Cysteine cathepsin found in dentine has the ability to activate latent MMPs.[25] The cathepsin activity increases with increasing depth of lesion. In carious lesions cathepsin tends to influence the process through activation of latent MMPs. Most recent studies have indicated role of cysteine cathepsins in dentine caries by degradation of collagen through activating MMPs.[26] Higher abundance of cathepsin B and K in caries-affected dentine as compared to intact dentine has been found in a recent study.[27]

Salivary MMPs also tend to have a significant contribution towards dentine matrix degradation during the carious process. Endogenous MMP-2 contained in sound dentine is activated during the carious process. Caries also tends to increase the level of endogenous MMP-2 synthesis.[28] Acidic pH during the carious process may both induce MMP production by the odontoblasts as well as their activation, thereby potentiating MMP proteolytic capacity, leading to enhanced dentine matrix degradation. MMP-2, MMP-20 and cathepsin B present in dentinal fluid further contributes to peritubular dentine degradation.[9,28,29]

4. MMP production by odontoblasts

Along with tissue building components, odontoblasts express tissue destructive enzymes, MMPs, which points towards the important role the latter play in dentine organic matrix remodelling.[19] Odontoblasts secrete at least gelatinase A (MMP-2), gelatinase B (MMP-9), collagenase 2 (MMP-8), collagenase-3 (MMP-13), enamelysin (MMP-20) and membrane type matrix metalloproteinase-1 (MT1-MMP).[8]

Possible role of MMPs in mature odontoblasts could be – (i) physiological secondary dentine formation and mineralization in intact and healthy teeth, (ii) matrix degradation during dental injury, (iii) role in tertiary dentinogenesis and (iv) role in pulp inflammation.[30]

Tumour growth factor (TGF) can upregulate MMP-9 expression and downregulate MMP-2 synthesis[31] but has no effect on collagen synthesis in odontoblasts.[32] TGF has a reducing effect on MMP-8 synthesis in mature odontoblasts.[33] TGF, a multi-growth factor found in dentine, tends to regulate tissue repair in teeth.[34] The downregulation of MMP-8 can be a factor leading to reparative dentine formation, since it is essential for modulating tissues during normal dentine formation. Thus the MMPs expressed by odontoblast may have a role in dentine and reparative dentine formation and the growth factors can act as MMP regulators, as well as collagen synthesis regulators.

5. Role of MMPs in pulpal inflammation

Pulp tissue destruction due to inflammation, as seen in reversible and irreversible pulpitis is regulated partially, by MMPs and tissue inhibitors of MMPs (TIMPs). The concentration of MMP-3 in acute pulpitis has been found to be significantly higher than in normal pulp tissue.[35] MMP-3 has a unique role in pulpitis that other MMPs do not share. MMP-3 activates other MMPs, such as MMP-1, -7 and -9 and has been implicated in various physiological and pathological process.[36–38] MMP-3 produced in pulpitis, brings about angiogenesis, fibroblast wound healing and reparative dentine formation.[39] MMP-3 has been found to mediate dental pulp healing as an anti-inflammatory and regenerative factor.[40] MMP-3 production during pulp tissue inflammation, stimulates degradation of surrounding collagen, leading to changes

in ECM structure, inflammation and promoting angiogenesis.[41]

MMP-13 (collagenase-3) has the widest substrate selection among the interstitial collagenase and it is able to cleave various BM components. MMP-13 cleaves type II collagen more efficiently than type I and III and among interstitial collagenases, it is most effective in cleaving gelatine. The expression level of MMP-13 has been found to be extremely high in pulp tissue in comparison to all other MMPs, thus leading to the conclusion that MMP-13 is the main collagenase in pulp tissue together with MMP-1.[30,42]

Endogenous and exogenous factors activate collagen degradation. The former include local variation in membrane thickness and reduction in collagen content. The latter include effects of bacterial metabolism and host inflammatory response. Infection-induced activation of MMPs has recently been shown to be related with excessive collagen turnover and membrane weakening, leading to tissue destruction.

Dental caries could lead to inflammation of pulp, resulting in aggregation of inflammatory cells that in turn release inflammatory cytokines.[43,44] MMP family proteins elicit dual role in the pathogenesis of inflammation, stimulating protective innate and/or adaptive immune functions, as well as tissue destruction.

The bacterial antigens and lipopolysaccharides (LPS) in the infected pulp increase the levels of immunoglobulins, prostaglandins and other proinflammatory mediators. In pulpal reaction, bacterial compounds and inflammatory factors can stimulate the neutrophil degranulation and secretion by monocytes/macrophages. The release of IL-1 and TNF is able to induce MMP-1, MMP-2 and the tissue inhibitor of metalloprotienases-1 (TIMP-1) in pulp cells.[45,46] Stimulation by bacteroides elevates MMP-2 production and anaerobic bacteria extracts provoke pulp cells to excrete MMP-1 and MMP-22, as well as TIMP-1.[32] Significantly higher levels of MMP-1, -2 and -3 have been found in acute pulpitis than normal pulp tissue.[47]

High levels of MMP-8 have been found in pulp abscess and root canal exudates. These high levels come down after Ca $(OH)_2$ dressing during RCT. In teeth subjected to RCT using Ca $(OH)_2$ as root canal dressing, a lower inflammatory index is noted along with an increased percentage of fibroblasts. Moreover MMP-2, MMP-8 and MMP-9 levels have been found to be lower than those in teeth with apical periodontitis without treatment or in teeth treated with single visit RCT. These facts point towards a reduced MMP synthesis in a calcium-rich environment.

Various studies have shown that bacteria and their products upregulate MMP-1 and MMP-2 in pulp cells but have no effect on MMP-9.[32,33] The levels of MMP-1, -2 and -3 expressed mainly by monocytes/macrophages and fibroblasts are significantly higher in acute pulpitis tissue than in healthy pulp tissue.[47] Bacterial products irritate PMNs to release MMP-8[48,49] and proinflammatory cytokines upregulate MMP-1 and MMP-2 in the pulp tissue.[45,50,51] Bacteria and their products tend to act in pulpal inflammation by upregulating cytokine production, via the cytokine pathway, increase MMP expression and directly irritate cells to produce MMPs. PMN cells are involved in pulp abscess formation and thus activated MMP-8 participate in the tissue destruction of pulp necrosis and abscess. Since PMNs are migrating and recruiting cells that are able to penetrate dentinal tubules, MMPs are helpful towards this end.[52]

6. Role of MMPs in periapical inflammation

Expression and activity of MMPs in adult tissues are normally quite low but increase significantly in many destructive pathological process, such as chronic inflammation and bone-destruction lesions.[53] MMP-1 (collagenase-1), MMP-8 (collagenase-2) and MMP-13 (collagenase-3) comprise a collagenase subfamily capable of initiating degradation of native fibrillar collagen types I, II, III, V, IX.[54] MMP-1, which is most efficient in cleaving collagen type III, is synthesized and secreted by fibroblasts and macrophages. It is the most often associated collagenase with normal tissue remodelling. It is also produced by other cells, such as osteoblasts and odontoclasts.[55] MMP-8 is most effective in initiating type I collagen degradation. Its main cellular source is PMNs, thus it plays a key role in tissue destruction during inflammatory diseases.[55] MMP-13 is expressed during many pathological conditions associated with excessive degradation of ECM during chronic inflammation.[56–58]

Under normal conditions, degradation and synthesis of ECM components is in balance, so that these MMPs are expressed at very low levels, if at all. Whenever active tissue remodelling is required, their production and activation increase dramatically. MMPs have a role in periapical lesion formation because MMP inhibition significantly increases the lesion level.[19] MMPs are involved in defensive reaction against microbes present in the dental pulp or periapical area and the increase of the lesion level might be due to more rapidly advanced pulp infection.

In apical periodontitis, collagen degradation due to bacterial infection within the root canal system is one of the processes that have been attributed to the presence of MMPs. In case of apical periodontitis, persistence of microorganism following root canal therapy has been associated with the presence of severe tissue disorganization and increased levels of MMPs in the periapical area. MMP expression in the apical periodontitis has been confirmed in various studies. Interstitial collagenase (MMP-1) has been found to be one of the key enzymes in the initiation of bone resorption of periapical lesion.[59,60] MMP-2, MMP-3, MMP-8, MMP-9 and MMP-13 are the other matrix metalloproteinases that have been found in periapical lesions.[44,61–64]

In chronic apical periodontitis, MMP-8 has been immunohistochemically detected in polymorphonuclear neutrophils, macrophages and plasma cells. It is a complex process involving bacteria and their products, immunoglobulins, proinflammatory cytokines and other inflammatory mediators, which tend to affect one another. PMNs which act as the first cellular barrier to bacterial invasion, in addition to destroying bacteria, destroy the surrounding tissues by secreting MMPs especially MMP-8. Induced by bacterial products or toxins, PMS cells can release proinflammatory cytokines,[65] which autocrinically stimulates PMS to release MMP. Plasma cells which enter the inflammatory tissue after PMNs, secrete immunoglobulins and also express MMP-8 and MMP-13. Macrophages, the major inflammatory cells in CAP,

take part in PMN and lymphocyte activation. Macrophages are considered to be the major source of IL-1//, IL-1/ and TNF-11.

MMP-1, -2, and -3 immunoreactivity has been detected in plasma cells, lymphocytes and macrophages present in the periapical lesions.[35] Monocytes/macrophages express MMP-8 and MMP-13.[66,67] These MMP work in both intra-cellular (phagocytic process) and extra-cellular (tissue destruction) process. Osteoclast tend to remove bone at the periphery of CAP,[68] which is partially due to MMPs, especially MMP-9, secreted by the former.[69,70] MMPs secreted by cells other than osteoclast in CAP may thus be responsible for the degradation of ECM and the waste products appearing after osteoclast bone dissolution during CAP formation.

The inflammatory process and tissue destruction in CAP, resembling to a large extent to the same phenomenon seen in periodontitis, are brought about by bacteria.[48,49] These oral bacteria trigger MMP release and activation by the PMN cells. In addition to the other bone destructive mechanism, MMPs exert a destructive role in CAP as well. The aim of root canal treatment in CAP is to remove bacteria, virulence factors and toxins, along with the inflammatory reaction in the apical area. Once the area is free of bacteria and their products, inflammatory process and inflammatory cells diminish. RCT thus decreases the active and latent form of MMP-8 in the root canal exudates and in turn the MMP-8 dependent inflammatory tissue destruction. Chlorhexidine used as a adjunctive medication in the periapical treatment in addition to its antimicrobial properties, exert properties directly to inhibit the MMPs and their oxidative activation.[71–73]

7. Conclusion

Taking into consideration the enhanced presence of MMPs at the site of carious lesion, inflamed pulp and periapical tissue, together with the fact that when this inflammatory response subsides, the level of MMPs diminishes, it can be concluded that MMPs do play an important role in the degradation of the collagenous structure and spread of the pathology. At the same time, they are an essential component of the tissue in the physiologic process of tissue remodelling.

Conflicts of interest

The authors have none to declare.

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