Extracellular matrix degradation by host matrix metalloproteinases in restorative dentistry and endodontics: An overview

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Abstract

Matrix metalloproteinases (MMPs) are a group of over 25 secreted and membrane-bound enzymes responsible for pericellular substrate degeneration. In response to injury, they play key roles in morphogenesis, wound healing, tissue repair and remodeling. They have been isolated from dentin, odontoblasts, pulp and periapical tissue. They play a major role in the formation of dentin matrix and secondary and tertiary dentin. These are also responsible for releasing dentinal growth factors. MMP family proteins elicit a dual role in the pathogenesis of inflammation, stimulating protective innate and/or adaptive immune functions, as well as tissue destruction. The main organic component of tooth structure is collagen, and MMPs that degrade collagen and the extracellular matrix have been implicated in the progression of dental caries, dental erosion as well as degradation of the hybrid layer. MMPs have also been shown to be active in pulpitis, and studies have shown that they can be used as diagnostic markers of pulpal and periapical inflammation. This review describes the role of MMPs in dental caries, dental erosion, bond stability as well as in pulpal and periapical inflammation.

Keywords: Extracellular matrix, matrix metalloproteins, tissue inhibitors of metalloproteinases

INTRODUCTION

Matrix metalloproteinases (MMPs) were described initially in 1962, by Jerome Gross and Charles Lapiere, while observing enzymatic activity (collagen triple helix degradation) during tadpole tail metamorphosis (by placing a tadpole tail in a collagen matrix plate). They showed that the anuran tadpole had strong collagenolytic activity in the skin, gut and gills, tissues that underwent the most radical remodeling during metamorphosis.[1] Later, it was purified from human skin (1968)[2] and was recognized to be synthesized as a zymogen.[3] The first evidence of collagenolytic activity in dentin was reported in the early 1980s both in carious and intact dentin.[4]

The term MMP was coined by Okada et al. in 1987.[5]

MMPs are endogenous Zn2+- and Ca2+-dependent enzymes, known to play a key role in the catabolic...
turnover of extracellular matrix (ECM) components. They belong to a larger family of proteases known as the metzincin superfamily with a functional hallmark of binding to zinc at the catalytic site and having a conserved “Met-turn” motif. MMPs are produced by a wide variety of cell types which include epithelial cells, fibroblasts, endothelial cells, inflammatory cells and even cementoblast-like cells. According to the research studies conducted till date, MMPs also regulate the activity of several non-ECM bioactive substrates including growth factors, cytokines, chemokines and cell receptors, which determine the tissue microenvironment.

Synonym-matrixins.

STRUCTURE OF MATRIX METALLOPROTEINASE

The MMPs have a common domain structure consisting of:
- Pro-peptide/prodomain,
- Catalytic domain
- Hemopexin-like C-terminal domain, which is linked to the catalytic domain by a flexible hinge region
- At the end, a transmembrane domain, glycosyl phosphatidylinositol anchor or cytoplasmic tail.

The hemopexin domain contributes to substrate recognition, enzyme activation and protease localization.

ACTIVATION OF MATRIX METALLOPROTEINASES

MMPs are synthesized and secreted as inactive zymogens. Activation of MMPs requires the pro-peptide domain to be removed from the structure via a step-wise mechanism. The prodomain contains a conserved cysteine residue that interacts with the zinc ion in the active site and prevents binding and cleavage of the substrate, keeping the enzyme in an inactive form (cysteine switch).

The prodomain of these MMP enzymes is folded over to block the active catalyst site, so Zn or Ca ions cannot bind to activate MMPs. This prodomain part of the molecule can be cleaved off the pro-MMP by specific ECM proteins, also released from the dentin or present in saliva, to form an active MMP that can bind Zn and Ca ions from the extracellular environment.

MMPs can be activated by:
- Proteinases
- In vitro by chemical agents, such as thiol-modifying agents, oxidized glutathione, chaotropic agents and reactive oxygen species
- Low pH
- Heat treatment.

These agents and procedures probably work through disruption of the cysteine–zinc binding [Figure 2].

CLASSIFICATION

On the basis of substrate specificity, sequence similarity and domain organization, vertebrate MMPs can be divided into six groups (Visse and Nagase, 2003) [Figure 3].

MATRIX METALLOPROTEINASES IN DENTAL CARIES

Dentin is a collagen-based mineralized tissue consisting of inorganic apatite crystallites embedded in an ECM. The main component of the ECM is Type I collagen, which represents 90% of organic material and 10% of noncollagenous proteins.

In dental caries, microbial acids induce demineralization, and microbial proteolytic enzymes were thought to cause degradation of the dentinal organic matrix. The proteases developed by the cariogenic bacteria, which were thought to be responsible for the degradation of the organic dentinal matrix, are highly sensitive to pH and are not capable of resisting acidic pH (4.3) during the demineralization phase, thus having limited contribution to the process. The potential role of the host-derived proteases and particularly MMPs in the degradation of the dentin matrix is now clear. The lactic acid released by bacteria in a carious lesion can be involved in the activation...
of pro-MMPs that break down the collagen matrix during the caries cycle.[28]

Low pH contributes to prodomain cleavage and thus promotes the functional activity of MMPs.[19,20] Although active MMPs are stable in acid pH, they work best at neutral pH. Dentinal buffering mechanisms or salivary buffer systems allow neutralization of acids in the carious lesion, allowing the pH-activated MMPs to cleave matrix components.[19,21] Also, phosphorylated proteins that get released by microbial acids from the collagen matrix interact with TIMP-inhibited MMPs within the carious lesion and reactivate them, which ultimately enhance the degradation process.[22] The possible sources of MMPs and the studies conducted is shown in Table 1.

Increased presence of MMPs along the dentin–enamel junction may lead to the widening of cavities as it progresses through the degradation of the collagen matrix along this junction.[23] MMP activity in both active and chronic carious lesions has been shown to decrease with age.[24]

Cysteine cathepsin present in dentin has the ability to activate latent MMPs,[25] which further increases with the extent of carious lesion. Cathepsin B and K have been shown to be more concentrated in caries-affected dentin relative to intact dentin.[26,27]

**MATRIX METALLOPROTEINASES IN DENTAL EROSION**

Dentin erosion is a complex process initially characterized by mineral dissolution, which in turn exposes the organic matrix to breakdown by bacteria and host-derived enzymes such as MMPs.[28]

The mechanism of erosion process is not fully understood in exposed dentin surfaces. When dentin is exposed to acids, the minerals from the peritubular/intertubular dentin junction are initially extracted. Next, the peritubular dentin is degraded and the dentinal tubuli becomes wider. Finally, a superficial layer of demineralized collagenous matrix (DOM) can be detected which is followed by a partially demineralized zone until the sound inner dentin is reached.[29] This DOM is resistant to mechanic removal by brushing forces up to 4 Newtons, and it is plausible that this outermost layer of collagenous matrix could shield the remaining dentin from mechanical forces, such as abrasion from the toothbrush.[30,31] It could also restrict ionic diffusion in and out of the demineralized surface,[32,33] which also occurs in root caries.[34] It has also been speculated that this organic layer may present buffering capacity. During an erosive challenge from the outer surface, it may adsorb H+, therefore protecting the inner dentin from pH decrease.[33,35] The rate of loss in dentin reduces with time, if the DOM is not removed.

However, the extent to which this protective effect is relevant in clinical reality is unknown because the DOM can be degraded by proteases.[36] Considering that erosive demineralization occurs in the absence of bacteria, it is plausible that host-derived enzymes are responsible for the degradation of the DOM, in contrast to caries lesions, where the impact of bacteria-derived enzymes might be higher. The proteolytic host-derived enzymes originate from either saliva or dentin.[19,25] The mechanism by which MMPs are activated by low pH is relevant for the comprehension of dentin erosion. Purified and human salivary MMPs (−2, −8 and −9) are activated in low pH (4.5).

Application of MMP inhibitors could reduce dentin loss during the subsequent erosive challenges, due to the preservation of the DOM.[28] Rinsing with green tea (10 mL, 1 min) after the erosive challenges significantly

| Group | MMPs | Other names | Substrates | Possible method of analysis |
|-------|------|-------------|------------|---------------------------|
| Collagenases | MMP-1 | collagenase-1 | collagen I, II, III, VII, VIII, X, aggrecan, gelatin, laminin | Immunoblot assay, immunocyto assay |
| | MMP-8 | collagenase-2 | collagen I, II, III, V, VII, VIII, X, aggrecan, elastin, fibronectin, gelatin | Immunoblot assay, fluorometric assay, activity-based profiling assay |
| | MMP-13 | collagenase-3 | collagen I, II, III, IV, aggrecan, gelatin | Fluorometric assay, activity-based profiling assay, immunoblot assay, immunocyto assay |
| Gelatinases | MMP-2 | gelatinase-A | collagen I, II, III, IV, V, VII, X, XI, aggrecan, elastin, fibronectin, gelatin, laminin, protocollagen, MMP-9, -13 | Bioassay, immunoblot assay, immunocyto assay, fluorometric assay, radioisotopic assay, multiple enzymes/multiple reagent assay |
| | MMP-9 | gelatinase-B | collagen IV, V, VII, X, XIV, aggrecan, elastin, fibronectin, gelatin | Bioassay, immunoblot assay, fluorometric assay, radiometric assay |
| Stromelysins | MMP-3 | stromelysin-1 | collagen I, II, III, IV, V, XI, aggrecan, elastin, fibronectin, gelatin, laminin, protocollagen, MMP-7, -8, -13 | Bioassay, immunoblot assay, fluorometric assay, phage-displayed assay |
| | MMP-10 | stromelysin-2 | collagen I, II, III, IV, V, aggrecan, elastin, fibronectin, gelatin, laminin, MMP-1, 4 | Bioassay, immunoblot assay, fluorometric assay, phage-displayed assay |
| Matrilysins | MMP-7 | matrilysin-1 | collagen IV, X, X, aggrecan, elastin, fibronectin, gelatin, laminin, protocollagen, MMP-7, -8, -13 | Bioassay, immunoblot assay, fluorometric assay, phage-displayed assay |
| | MMP-26 | matrilysin-2, -2 | collagen IV, gelatin, fibronectin, gelatin | Immunoblot assay, immunocyto assay, fluorometric assay, phage-displayed assay |
| Membrane-type MMPs | MMP-14 | MT1-MMP | collagen I, II, III, aggrecan, elastin, fibronectin, gelatin, laminin, MMP-2, 13 | Bioassay, immunoblot assay, immunocyto assay, fluorometric assay, phage-displayed assay |
| | MMP-15 | MT2-MMP | collagen I, II, III, aggrecan, elastin, fibronectin, gelatin, laminin, MMP-2 | Fluorometric assay, activity-based profiling assay |
| | MMP-16 | MT3-MMP | collagen I, MMP-2 | Fluorometric assay, activity-based profiling assay |
| | MMP-24 | MTS-MMP | fibron, gelatin | Fluorometric assay, activity-based profiling assay |
| Other MMPs | MMP-11 | stromelysin-1 | collagen IV, V, VI, aggrecan, elastin, fibronectin, gelatin, laminin | Immunoblot assay, immunocyto assay, fluorometric assay, phage-displayed assay |
| | MMP-12 | stromelysin-2 | collagen I, II, III, IV, V, aggrecan, elastin, fibronectin, gelatin, laminin | Immunoblot assay, immunocyto assay, fluorometric assay, phage-displayed assay |
| | MMP-19 | RAGE | collagen IV, fibronectin, aggrecan, COMP, laminin, gelatin | Immunoblot assay, fluorometric assay, activity-based profiling assay |
| | MMP-20 | membrane | collagen V, aggrecan, amebagin, COMP | Fluorometric assay, activity-based profiling assay |
| | MMP-21 | al-anti-trypsin | unknown | Fluorometric assay, activity-based profiling assay |
| | MMP-23 | CA-MMP | unknown | Fluorometric assay, activity-based profiling assay |
| | MMP-25 | MT6-MMP | collagen IV, gelatin, fibronectin, laminin, fibron | Fluorometric assay, activity-based profiling assay |
| | MMP-28 | epilysin | unknown | Fluorometric assay, activity-based profiling assay |

**Figure 3:** Matrix metalloproteinases, extracellular matrix substrates and analysis (Maciejczyk et al.)
Table 1: Matrix metalloproteinases - source, conducted studies (developed by author)

| Source                          | MMP's/cathepsin            | Conducted studies                                                                 |
|--------------------------------|----------------------------|----------------------------------------------------------------------------------|
| Saliva                         | MMP-9, MMP-8, Cathepsin B  | (Tjäderhane et al., 1998a); (Shimada et al., 2009); (Hedenböjr-Lager et al., 2015); (van Strijp et al., 2003) |
| MMPS in pulp, odontoblasts and predentin | MMP-2, MMP-3, MMP-8, MMP-9, MMP-13 and MMP-20 | (Sulkala et al., 2004, 2007, Tjäderhane et al., 1998; Mazzoni et al., 2007; Boukessi et al., 2008; Lorenti et al., 2014; Toledano et al., 2010; Charadram et al., 2012) |
| Dentinal fluid                 | Cathepsin K and cathepsin B, MMP-2, MMP-9 | (Vidal et al., 2014; Vasudev ballal et al., 2017) |

MMPS: Matrix metalloproteinases

reduced dentin loss by ~30% as compared with water rinses. The use of gel as a vehicle to deliver MMP inhibitors to dentin has shown to prevent wear, completely giving protection against dentin erosion. The gels are applied for 1 min only. MMP inhibitors, such as 0.012% or 2% chlorhexidine, green tea polyphenols, 10 µM or 400 µM EGCG –30% epigallocatechin-gallate and 1 mM FeSO₄ in solutions or gels, reduce the dentin erosive wear.

MATRIX METALLOPROTEINASES IN PULPAL INFLAMMATION

MMPs elicit a dual role in the pathogenesis of inflammation, stimulating protective innate and/or adaptive immune functions, as well as tissue destruction. Inflammatory pulp destruction as seen in reversible and irreversible pulpitis is partially controlled by MMPs and MMP tissue inhibitors (TIMPs). In caries-affected teeth, it appears to be promising to assess molecules associated with the innate immune system, i.e., the polymorphonuclear neutrophil (PMN) response to the invading microorganisms. This is because PMN infiltration and associated soft-tissue breakdown are the key factors in histologically discerning reversible and irreversible pulpitis. PMNs that serve as the first cellular barrier to bacterial invasion, in addition to destroying bacteria, destroy the surrounding tissues by secreting MMPs, particularly MMP-8. Induced by bacterial products or toxins, PMN cells can release pro-inflammatory cytokines, which autocratically stimulates PMNs to release MMPs. PMNs migrate and recruit cells that are capable of penetrating dentinal tubules, as well.

MMP-9, obtained by cleaving MMP-14, is produced by PMN cells during inflammation. It allows inflammatory mediators to migrate through tissues, spreading inflammation through ECM that it has degraded. MMP-9 has been shown to be present in quantifiable quantities in the dentinal fluid of teeth with permanent pulpitis, whereas it is absent in the dentinal fluid of healthy teeth.

MMP-3 has been implicated in various physiological and pathological processes. It has a unique role in pulpitis that is not shared by other MMPs. MMP-3 degrade proteoglycans directly and indirectly stimulates other MMPs, such as MMP-1, -7 and -9. The concentration of MMP-3 in acute pulpitis was found to be significantly higher than that in normal pulp tissue. MMP-3 developed in pulpitis, stimulates degradation of surrounding collagen, leading to changes in the structure of ECM, inflammation, promoting angiogenesis and accelerating pulp wound healing through reparative dentin formation. MMP-3 was also found to mediate the healing of dental pulp as an anti-inflammatory and regenerative factor.

MMP-13 has the widest selection of substrates among interstitial collagenase and can cleave different components of basement membrane. MMP-13 cleaves Type II collagen more efficiently than Types I and III and among interstitial collagenases, it is most effective in cleaving gelatin. The level of expression of MMP-13 was found to be extremely high in pulp tissue relative to all other MMPs, leading to the conclusion that together with MMP-1, MMP-13 is the primary collagenase in pulp tissue.

MMP-1, MMP-2 and MMP-3 levels expressed predominantly by monocytes/macrophages and fibroblasts, are significantly higher in inflamed pulp compared to healthy pulp tissue. Interleukin (IL)-1 and TNF release will induce MMP-1, MMP-2 and the tissue inhibitor of metallo-proteinases-1 (TIMP-1) in pulp cells. Bacteroides increase the development of MMP-2, and their extracts cause pulp cells to excrete MMP-1 and MMP-22, as well as TIMP-1.

Immunohistochemical staining showed MMP-8 protein in the odontoblasts, indicating that MMP-8 may participate in dentin matrix organization during dentin formation. In both pulpal and periapical lesions, MMP-8 staining was identified using immunohistochemistry in polymorphonuclear leukocytes (PMNs), macrophages and plasma cells.

MATRIX METALLOPROTEINASES IN PERiapICAL INFLAMMATION

In apical periodontitis, collagen degradation due to bacterial infection within the root canal system involves the activity of MMPs. MMPs are involved in a defensive reaction...
against pathogens in the dental pulp.\textsuperscript{[7]} MMP-1, MMP-8 and MMP-13 of collagenase subfamily are capable of initiating degradation of native fibrillar collagen Types I, II, III, V and IX.\textsuperscript{[62]}

MMP-1 (interstitial collagenase), which is most effective in cleaving collagen Type III, is synthesized and secreted by fibroblasts, macrophages and other cells, such as osteoblasts and odontoclasts.\textsuperscript{[63]} MMP-1 was found to be one of the key enzymes in the initiation of bone resorption of periapical lesion.\textsuperscript{[64,65]} It is the most common collagenase associated with normal tissue remodeling. MMP-2, MMP-3, MMP-8, MMP-9 and MMP-13 are the other MMPs that have been found in periapical lesions.\textsuperscript{[45,66‑69,73‑75]}

MMP-13 is expressed in many pathological conditions associated with severe ECM degradation. Plasma cells which enter the inflammatory tissue after PMNs, secrete immunoglobulins and also express MMP-8 and MMP-13. MMP-8 is effective in inducing degradation of Type I collagen, and is also found in root canal exudate and all odontogenic cysts. MMP-8 accumulates around the pulp abscess, suggesting that it also contributes to the abscess formation. MMP-8 levels in periapical exudate are significantly reduced during root canal treatments, making it a valuable tool as a biological marker to track inflammatory activity and root canal performance.\textsuperscript{[61]}

After calcium hydroxide dressing during root canal treatment (RCT), high levels of MMP-8 found in pulp abscess and root canal exudates drop. In teeth that were subjected to RCT using calcium hydroxide as root canal dressing, a lower inflammatory index and an increased percentage of fibroblasts were noted. In addition, the levels of MMP-2, MMP-8 and MMP-9 were found to be lower than those in teeth with nontreatment apical periodontitis or in teeth treated with single-visit RCT. These facts point to a reduced synthesis of MMP in an environment rich in calcium.\textsuperscript{[10]}

Macrophages, the major inflammatory cells in chronic apical periodontitis (CAP), are involved in PMN and lymphocyte activation. They are considered to be the major source of IL-1 and tumor necrosis factor-11, also expressing MMP-8 and MMP-13.\textsuperscript{[64]} MMP-1, -2 and -3 immunoreactivity has been detected in plasma cells, lymphocytes and macrophages present in the periapical lesions.\textsuperscript{[49]} These MMPs work both in intracellular (phagocytic process) and extracellular (tissue destruction) processes.

Osteoclast tends to remove bone at the periphery of CAP,\textsuperscript{[70]} which is partially due to MMPs, especially MMP-9, secreted by the former.\textsuperscript{[71,72]} MMPs secreted by cells other than osteoclast in CAP may, therefore, be responsible for ECM degradation and waste products that occur after osteoclast bone dissolution during CAP formation. In addition to the other bone destructive mechanism, MMPs exert a destructive role in CAP as well.

In addition to its antimicrobial properties, chlorhexidine, which is used as an adjunctive medication in the root canal treatment, exerts properties that directly inhibit MMPs and their oxidative activation.\textsuperscript{[73‑75]}

**MATRIX METALLOPROTEINASES ON BOND STABILITY**

Acid-etchants used in dentin bonding and weak acids released by cariogenic bacteria can uncover and activate matrix-bound MMPs, which are incorporated in the peripheral layer of dentin.\textsuperscript{[76,77]} These endogenous enzymes also remain entrapped within the hybrid layer during the resin infiltration process, and the acidic bonding agents themselves (irrespective of whether they are etch-and-rinse or self-etch) can activate these endogenous proteases.

Because resin impregnation is frequently incomplete, denuded collagen matrices associated with free water (which serves as a collagen cleavage reagent for these endogenous hydrolase enzymes) can be enzymatically disrupted, presenting the recognizable and available cleavage sites, making it more vulnerable to MMPs and cathepsins finally contributing to the degradation of the HL (hybrid layer).

Enzymatic degradation of the collagen matrix by host-derived enzymes plays a significant role in the destruction of the bonded interface.\textsuperscript{[78,79]} Evidence of collagenolytic and gelatinolytic activities in partially demineralized dentin treated with either etch-and-rinse or self-etch adhesives initially supported the potential involvement of these proteases in the disruption of incompletely resin-infiltrated collagen fibrils within HLs.\textsuperscript{[80,81]} These results were more recently confirmed with specific assays of MMP-2 and MMP-9 in dentin matrices treated with both etch-and-rinse and self-etch adhesives.\textsuperscript{[82,83]} In addition, a self-etch adhesive has been shown to increase MMP-2 synthesis in human odontoblasts,\textsuperscript{[84]} possibly increasing MMP-2 penetration into the HL via dentinal fluid.

During the application of acidic monomers,

1. The acidic resin monomers present in either etch-and-rinse or self-etch adhesives may, in fact, activate latent forms of MMPs (pro-MMPs) via the
cysteine-switch mechanism that exposes the catalytic domains of these enzymes that were blocked by pro-peptides[83]

2. Not only MMPs but also cysteine cathepsins are activated and might be involved in the degradation of HL over time[85,86]

3. Also activate MMPs by inhibiting tissue inhibitor of metalloproteinases-1 (TIMP-1; Ishiguro et al., 1994) in TIMP-MMP complexes, thereby producing active MMPs.[19,20]

MMP-8 is the major MMP-collagenase in dentin which is capable of cleaving Type I collagen into 3/4- and 1/4-length collagen fragments.[86] Type I collagen is an important component in the hybrid layer, thus its breakdown causes the failure of the resin–dentin bond.

Chlorhexidine,[87,88] tetracycline, galardin,[89] benzalkonium chloride,[90] and quaternary ammonium methacrylates[91] are just some of the tested MMP inhibitors showing positive effects on bond strength stability.

Application of chlorhexidine (0.2%–2%) preserves both the durability and bond strength of dental adhesives.[87,88] The inhibitory effect may last for 9–12 months. CHX, which effectively reduces the activity of MMP-2,-9 and -8[89] and cysteine cathepsins,[92] even at concentrations as low as 0.2%, demonstrated bond strength preservation and reduced interfacial degradation.[89]

**MATRIX METALLOPROTEINASE INHIBITORS**

MMP inhibitors can be classified into endogenous and exogenous inhibitors [Table 2].

**FUTURE PERSPECTIVE OF MATRIX METALLOPROTEINASES**

In recent years, much attention has been given to MMP-dependent breakdown pathway of ECM. MMP inhibitors that prevent collagen degradation during dentinal caries, should be recommended for use in the natural healing of carious dentin matrix to induce remineralization. It would be beneficial to apply MMP inhibitors during the dentin bonding process, which have the ability not only to inhibit the breakdown of dentin collagen in the hybrid layers, but also to improve the stability of dentin bonding, and to prevent secondary caries. New bonding systems should provide long-lasting MMP-inhibitory capabilities to maintain the integrity of the hybrid layer and to improve dentin bonding durability of adhesive restorations.

MMPs are currently considered as significant biomarkers for research under various specialized areas of dentistry. MMP-9 produced by PMN cells during inflammation helps to discern between reversible and irreversible pulptis.

**Table 2: Classification of matrix metalloproteinases inhibitors (developed by author)**

| Endogenous/natural | Exogenous/synthetic |
|--------------------|---------------------|
| TIMP-1,-2,-3,-4    | Bisphosphonates     |
|                    | Bisbiguanides-CHX   |
| Tetracycline-minocycline, doxycycline | CMT-1,-3,-8 |
| Hydroxamates-Marimastat, Batimastat (BB94), Glaradin (GM6001), CT116 | Quaternary ammonium compounds |
| Chelating agents like EDTA | PA |
| FeSO4, ZnCl2, ZnO | Grape seed extract, coco seed extract-PA |
| Oleic acid | Flavanoids |
| Carbodimide | Bilberry, cranberry, apple, black tea and green tea contain these flavonoids |
|                | flavonoids from Passiflora foetida |
|                | Green tea extract-EGCG |
|                | Morus nigra (black mulberry) and Morus alba (white mulberry) |
|                | Mulberry leaf extract |
|                | Barbadens Miller (Aloe vera) |
|                | Curcumin from Curcuma longa |
|                | Avacado |
|                | Soyabeen |
|                | Chitosan |

PA: Proanthocyanides, EGCG: Epigallocatechin-gallate, EDTA: Ethylene diamine tetraacetic acid, CHX: Chlorhexidine, CMT: Chemically modified tetracyclines
Measuring MMP-8 levels in periapical exudate can be used as a biological marker to track inflammatory activity and root canal treatment performance. Various assays such as gelatin zymography assay, specific ELISA, immunoassay, fluorometric assay, radioisotopic assay, and phage displayed assay can be used to detect MMPs in carious dentin, dental fluid, root canal exudates, etc.

Acknowledgment
The authors are grateful to Dr N Meena, MDS, Professor and HOD, Department of Conservative Dentistry and Endodontics, Vokkaligara Sangha Dental College and Hospital, Bengaluru, for helping out in reviewing of this article.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Gross J, Lapiere CM. Collagenolytic activity in amphibian tissues: A tissue culture assay. Proc Natl Acad Sci U S A 1962;48:1014-22.
2. Eisen A, Jeffrey J, Gross J. Human skin collagenase. Isolation and mechanism of attack on the collagen molecule. Biochim Biophys Acta 1968;151:637-45.
3. Harper E, Bloch KJ, Gross J. The zymogen of tadpole collagenase. Biochemistry 1971;10:3035-41.
4. Dayan D, Binderman I, Mechanic GL. A preliminary study of activation of collagenase in carious human dentine matrix. Arch Oral Biol 1985;29:185-7.
5. Okada Y, Nagase H, Harris ED Jr. Matrix metalloproteinases 1, 2, and 3 from rheumatoid synovial cells are sufficient to destroy joints. J Rheumatol 1987;14:41-2.
6. Maciejczyk M, Pietrzykowska A, Zalewska A, Knaś M, Daniszewska I. The significance of matrix metalloproteinases in oral diseases. Adv Clin Exp Med 2016;25:383-90.
7. Kähäri VM, Saatkalho-Kere U. Matrix metalloproteinases in skin. Exp Dermatol 1997;6:199-213.
8. Nagase H, Woessner JF. Matrix metalloproteinases. J Biol Chem 1999;274:21491-4.
9. Du M, Wang Y, Liu Z, Wang I, Cao Z, Zhang C, et al. Effects of IL-1β on MMP-9 expression in cementoblast-derived cell line and MMP-mediated degradation of type I collagen. Inflammation 2019;42:413-25.
10. Page-McCaw A, Ewald AJ, Werb Z. Matrix metalloproteinases and the regulation of tissue remodelling. Nat Rev Mol Cell Biol 2007;8:221-33.
11. Van Wart HE, Birkedal-Hansen H. The cysteine switch: A principle of the progress of enamel and root breakdown. J Dent Res 1987;76:588-95.
12. Cotra LP, Cross JB, Shimura Y, Fridman R, Schlegel HB, Mobashery S. Insight into the complex and dynamic process of activation of matrix metalloproteinases. J Am Chem Soc 2001;123:3108-13.
13. Nagase H, Suzuki K, Morodomi T, Enghild JJ, Sulvesen G. Activation mechanisms of the precursors of matrix metalloproteinases 1, 2 and 3. Matrix Suppl 1992;1:237-44.
14. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: Structure, function, and biochemistry. Circ Res 2003;92:827-39.
15. Mazzoni A, Tjäderhane L, Checchi V, Di Lenarda R, Salo T, Tay FR, et al. Role of dentin MMPs in caries progression and bond stability. J Dent Res 2015;94:241-51.
16. Jain A, Bahuguna R. Role of matrix metalloproteinases in dental caries, pulp and periapical inflammation: An overview. J Oral Biol Craniofac Res 2015;5:212-8.
17. Kawasaki K, Featherstone JD. Effects of collagenase on root demineralization. J Dent Res 1997;76:858-95.
18. Chaussain-Miller C, Fioretti F, Goldberg M, Menashi S. The role of matrix metalloproteinases (MMPs) in human caries. J Dent Res 2006;85:22-32.
19. Tjäderhane L, Larjava H, Sorsa T, Uitto VJ, Larmas M, Salo T. The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions. J Dent Res 1998;77:622-9.
20. Sulkala M, Wahlgren J, Larmas M, Sorsa T, Teronen O, Salo T, et al. The effects of MMP inhibitors on human salivary MMP activity and caries progression in rats. J Dent Res 2001;80:1545-9.
21. Chaussain C, Boukessi T, Khaddam M, Tjäderhane L, George A, Menashi S. Dentin matrix degradation by host matrix metalloproteinases: Inhibition and clinical perspectives toward regeneration. Front Physiol 2013;4:308.
22. Fedarko NS, Jain A, Karadag A, Fisher IW. Three small integrin binding ligand N-linked glycoproteins (SIBLINGs) bind and activate specific matrix metalloproteinases. FASEB J 2004;18:734-6.
23. Osorio R, Yamauti M, Osorio E, Ruiz-Requena MF, Pasley D, Tay F, et al. Effect of dentin etching and chlorhexidine application on collagen degradation. Eur J Oral Sci 2011;119:79-85.
24. Nascimento FD, Minciozi CL, Geraldeli S, Carrilho MR, Pasley DH, Tay FR, et al. Cysteine cathepsins in human carious dentin. J Dent Res 2011;90:506-11.
25. van Strijp AJ, Jansen DC, Degroot J, Ten Cate JM, Everts V. Host-derived proteinases and degradation of dentin collagen in situ. Caries Res 2003;37:58-65.
26. Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol IL, Geraldeli S, et al. Optimizing dentin bond durability: Control of collagen degradation by matrix metalloproteinases and cysteine cathepsins. Dent Mater 2013;29:116-35.
27. Vidal CM, Tjäderhane L, Scaffa PM, Tersariol IL, Pasley D, Nader HB, et al. Abundance of MMPs and cysteine cathepsins in caries-affected dentin. J Dent Res 2014;93:269-74.
28. Buzalaf MA, Kato MT, Hannas AR. The role of matrix metalloproteinases in dental erosion. Adv Dent Res 2012;22:72-6.
29. Meurman JH, Drysdale T, Frank RM. Experimental erosion of dentin. Scand J Dent Res 1991;99:457-62.
30. Ganss C, Schlueter N, Hardt M, von Hinckele J, Klimek J. Effects of toothbrushing on eroded dentin. Eur J Oral Sci 2007;115:590-6.
31. Ganss C, Hardt M, Blazek D, Klimek J, Schlueter N. Effects of toothbrushing force on the mineral content and demineralized organic matrix of eroded dentin. Eur J Oral Sci 2009;117:255-60.
32. Klont B, ten Cate JM. Remineralization of bovine incisor root lesions in vitro: The role of the collagenous matrix. Caries Res 1991;25:39-45.
33. Klont B, ten Cate JM. The influence of enamel and root surface lesions under plaque as a function of time. Caries Res 1991;25:39-45.
34. Ogaard B, Rolla G, Arends J. In vivo progress of enamel and root surface lesions under plaque as a function of time. Caries Res 1988;22:302-5.
35. Buzalaf MA, Carone S, Tjäderhane L. Role of host-derived proteinases in dentine caries and erosion. Caries Res 2015;49 Suppl 1:30-7.
36. Hannas AR, Pereira JC, Granjeiro JM, Tjäderhane L. The role of matrix metalloproteinases in the oral environment. Acta Odontol Scand 2007;65:1-3.
37. Kato MT, Magalhães AC, Rios D, Hannas AR, Attin T, Buzalaf MA. Protective effect of green tea on dentin erosion and abrasion. J Appl Oral Sci 2009;17:560-4.
38. Kato MT, Leite AL, Hannas AR, Buzalaf MA. Gels containing MMP inhibitors prevent dental erosion in situ. J Dent Res 2010;89:468-72.
39. Kato MT, Leite AL, Hannas AR, Oliveira RC, Perreira JC, Tjaderhane L, et al. Effect of iron on matrix metalloproteinase inhibition and on the prevention of dentine erosion. Caries Res 2010;44:309-16.
40. Demuele M, Brossard M, Pagé M, Gingras D, Béliveau R. Matrix metalloproteinase inhibition by green tea catechins. Biochim Biophys Acta 2000;1478:51-60.
41. Magalhães AC, Wiegard A, Rios D, Hannas A, Attin T, Buzalaf MA. Chlorhexidine and green tea extract reduce dentin erosion and abrasion in situ. J Dent 2009;37:994-8.
42. Le NT, Xue M, Castelnoble LA, Jackson CJ. The dual personalities of matrix metalloproteinases in inflammation. Front Biosci 2007;12:1475-87.
43. Rechenberg DK, Zehnder M. Molecular diagnostics in endodontics. Endodontic Topics 2014;30:51-65.
44. Ricucci D, Loghin S, Siqueira JF Jr. Correlation between clinical and histologic pulp diagnoses. J Endod 2014;40:1932-9.
45. Wahlgren J, Salo T, Teronen O, Luoto H, Sorsa T, Tjaderhane L. Matrix metalloproteinase-8 (MMP-8) in pulp and periapical inflammation and periapical root-canal exudates. Int Endod J 2002;35:897-904.
46. Prik K, Maisi P, Pirilä E. In vivo collagenase-2 (MMP-8) expression by human bronchial epithelial cells and monocytes/macrophages in bronchiectasis. Pediatr Pulmonol 2001;194:232-8.
47. Lin SK, Kok SH, Kuo MY. Sequential expressions of MMP-1, TIMP-1, IL-6 and COX-2 genes in induced periapical lesions in rats. J Eur Soc Odontol 2002;10:246-53.
48. Zehnder M, Wegehaupt EJ, Attin T. A first study on the usefulness of matrix metalloproteinase 9 from dental fluid to indicate pulp inflammation. J Endod 2011;37:17-20.
49. Suzuki K, Enghild JJ, Morodomi T, Salvesen G, Nagase H. Mechanisms of activation of tissue procollagenase by matrix metalloproteinase 3 (stromelysin). Biochemistry 1990;29:10261-70.
50. Zhou W, Liu S, Zhou X, Hannig M, Rupf S, Feng J. Modulation of host matrix metalloproteinases by bacterial virulence factors relevant in human periodontal diseases. Oral Dis 1995;1:279-86.
51. Hong CY, Lin SK, Kok SH, Cheng SJ, Lee MS, Wang TM, et al. The role of lipopolysaccharide in infectious bone resorption of periapical lesion. J Oral Pathol Med 2004;33:162-9.
52. Lin SK, Chiang CP, Hong CY, Lin CP, Lan WH, Hsieh CC, et al. Immunolocalization of interfibrillar collagenase (MMP-1) and tissue inhibitor of metalloproteinases-1 (TIMP-1) in radicular cysts. J Oral Pathol Med 1997;26:45-8.
53. Pashley DH, Tay FR, Breschi L, Tjaderhane L, Carvalho RM, Carrilho M. Effect of iron on matrix metalloproteinase inhibition and on the bonded interface. Dent Mater 2008;24:90-101.
54. Ashby DH, Tay FR, Breschi L, Tjaderhane L, Carvalho RM, Carrilho M, et al. State of the art etch-and-rinse adhesives. Dent Mater 2011;27:1-6.
55. Mazzoni A, Pashley DH, Nishitani Y, Breschi L, Mannello F. Matrix metalloproteinases-1 and tissue inhibitor of metalloproteinases-1 gene expression by interleukin-1β and tumor necrosis factor-α through a prostaglandin-dependent pathway. J Endod 2001;27:185-9.
56. Nakata K, Yamazaki M, Iwata T, Suzuki K, Nakane A, Nakamura H. Anaerobic bacterial extracts influence production of matrix metalloproteinases and their inhibitors by human dental pulp cells. J Endod 2000;26:410-3.
57. Wahlgren J, Salo T, Teronen O, Luoto H, Sorsa T, Tjaderhane L. Matrix metalloproteinases in pulpitis, chronic apical periodontitis and odontogenic jaw cysts. Int Endod J 2002;35:897-904.
58. Apajalahti S. Short Root Anomaly (SRA) prevalence and phenotypic features in families: With emphasis on matrix metalloproteinases in gingival crevicular fluid of SRA and orthodontic patients. Finland: PhD dissertation, University of Helsinki; 2004.
59. Ding Y, Uitto VJ, Firth J, Salo T, Haapasalo M, Kottinen YT, et al. The role of lipopolysaccharide in infectious bone resorption of periapical lesion. J Oral Pathol Med 2004;33:162-9.
60. Silva FW, D’Silva NJ, Silva LA, Kapila YL. High matrix metalloproteinase activity is a hallmark of periapical granulomas. J Endod 2009;35:1234-42.
61. Barkhordar RA, Hussain MZ, Hayashi C. Detection of interleukin-1 beta in human periapical lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;93:334-6.
62. Reponen P, Sahlberg C, Munuat C, Thesleff I, Tryggvason K. High expression of 92-1d type IV collagenase (gelatinase B) in the osteoclast lineage during mouse development. J Cell Biol 1994;124:1091-102.
63. Wucherpfennig AL, Li YP, Sterler-Stevenson WG, Rosenberg AE, Stetler-Stevenson WG. Expression of 92 Kd type IV collagenase/gelatinase B in human osteoclasts. J Bone and Mineral Res 1994;9:549-56.
64. Pezeli-Ribaric S, Anic I, Brekalo I, Miletić I, Hasan M, Simunovic-Soskic M. Detection of tumor necrosis factor alpha in normal and inflamed human dental pulps. Arch Med Res 2002;33:482-4.
65. Leonardi R, Caltabiano R, Loreto C. Collagenase-3 (MMP-13) is expressed in periapical lesions: An immunohistochemical study. Int Endod J 2005;38:297-301.
66. Silva FW, D’Silva NJ, Silva LA, Kapila YL. High matrix metalloproteinase activity is a hallmark of periapical granulomas. J Endod 2009;35:1234-42.
67. Barkhordar RA, Hussain MZ, Hayashi C. Detection of interleukin-1 beta in human periapical lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;93:334-6.
68. Azmak N, Atilla G, Luoto H, Sorsa T. The effect of subgingival controlled-release delivery of chlorhexidine chip on clinical parameters and matrix metalloproteinase-8 levels in gingival crevicular fluid. J Periodontol 2002;73:608-15.
69. Mäntylä P, Stenman M, Kinane D. Gingival crevicular fluid collagenase-2 (MMP-8) test stick for chair-side monitoring of periodontitis. J Periodontal Res 2003;38:436-9.
70. Zheng P, Chen H. Evaluate the effect of different mmps inhibitors on the expression of matrix metalloproteinases 2, 8, and 9 by chlorhexidine. Clin Diagn Lab Immunol 1999;6:437-9.
71. Le NT, Xue M, Castelnoble LA, Jackson CJ. The dual personalities of matrix metalloproteinase inhibition and on the prevention of dentine erosion. Caries Res 2010;44:309-16.
72. Pashley DH, Nishitani Y, Breschi L, Mannello F. Matrix metalloproteinases in pulpitis, chronic apical periodontitis and odontogenic jaw cysts. Int Endod J 2002;35:897-904.
73. Wahlgren J, Salo T, Teronen O, Luoto H, Sorsa T, Tjaderhane L. Matrix metalloproteinases in pulpitis, chronic apical periodontitis and odontogenic jaw cysts. Int Endod J 2002;35:897-904.
Tjäderhane L, et al. Reactivation of inactivated endogenous proteolytic activities in phosphoric acid-etched dentin by etch-and-rinse adhesives. Biomaterials 2006;27:4470-6.

81. Nishitani Y, Yoshiyama M, Wadgaonkar B, Breschi L, Mannello F, Mazzoni A, et al. Activation of gelatinolytic/collagenolytic activity in dentin by self-etching adhesives. Europ J Oral Sci 2006;114:160-6.

82. Mazzoni A, Carrilho M, Papa V, Tjäderhane L, Gobbi P, Nucci C, et al. MMP-2 assay within the hybrid layer created by a two-step etch-and-rinse adhesive: Biochemical and immunohistochemical analysis. J Dent 2011;39:470-7.

83. Mazzoni A, Scaffa P, Carrilho M, Tjäderhane L, Di Lenarda R, Polimeni A, et al. Effects of etch-and-rinse and self-etch adhesives on dentin MMP-2 and MMP-9. J Dent Res 2011;39:82-6.

84. Lehmann N, Dehret R, Roméas A, Magloire H, Degrange M, Bleicher F, et al. Self-etching increases matrix metalloproteinase expression in the dentin-pulp complex. J Dent Res 2009;88:87-82.

85. Tersariol IL, Geraldeli S, Minciotti CL, Nascimento FD, Pääkkönen V, Martins MT, et al. Cysteine cathepsins in human dentin-pulp complex. J Endod 2010;36:475-81.

86. Tezvergil-Mutluay A, Agee KA, Hoshika T, Carrilho M, Breschi L, Tjäderhane L, et al. The requirement of zinc and calcium ions for functional MMP activity in demineralized dentin matrices. Dent Mater 2010;26:1059-67.

87. Carrilho MR, Geraldeli S, Tay F, de Goes MF, Carvalho RM, Tjäderhane L, et al. In vitro preservation of the hybrid layer by chlorhexidine. J Dent Res 2007;86:529-33.

88. Breschi L, Mazzoni A, Nato F, Carrilho M, Visintini E, Tjäderhane L, et al. Chlorhexidine stabilizes the adhesive interface: A 2-year in vitro study. Dent Mater 2010;26:320-5.

89. Breschi L, Martin P, Mazzoni A, Nato F, Carrilho M, Tjäderhane L, et al. Use of a specific MMP-inhibitor (galardin) for preservation of hybrid layer. Dent Mater 2010;26:571-8.

90. Tezvergil-Mutluay A, Mutluay MM, Gu LS, Zhang K, Agee KA, Carvalho RM, et al. The anti-MMP activity of benzalkonium chloride. J Dent 2011;39:57-64.

91. Tezvergil-Mutluay A, Agee KA, Uchiyama T, Imazato S, Mutluay MM, Cadenaro M, et al. The inhibitory effects of quaternary ammonium methacrylates on soluble and matrix-bound MMPs. J Dent Res 2011;90:535-40.

92. Scaffa PM, Vidal CM, Barros N, Gesteira TF, Carmona AK, Breschi L, et al. Chlorhexidine inhibits the activity of dental cysteine cathepsins. J Dent Res 2012;91:420-5.