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REVIEW

The role of matrix metalloproteinases in the oral environment

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Abstract

This review focuses specifically on matrix metalloproteinases (MMPs) and their role in physiological and pathological extracellular matrix (ECM) remodeling and degradation processes in the oral environment. A group of enzymes capable of degrading almost all ECM proteins, MMPs contribute to both normal and pathological tissue remodeling. The expression of different MMPs may be upregulated in pathological conditions such as inflammation and tumor invasion. The balance between activated MMPs and tissue inhibitors of metalloproteinases (TIMPs) controls the extent of ECM remodeling. Prior to mineralization, MMPs may participate in the organization of enamel and dentin organic matrix, or they may regulate mineralization by controlling the proteoglycan turnover. There is evidence indicating that MMPs could be involved in the etiology of enamel fluorosis and amelogenesis imperfecta. They seem to play a part in dentinal caries progression, since they have a crucial role in dentin collagen breakdown in caries lesions. MMPs have been identified in pulpal and periapical inflammation and are strongly correlated with periodontal diseases, since they are the major players in collagen breakdown during periodontal tissue destruction. The use of MMP inhibitors could help the prevention and treatment of many MMP-related oral diseases.

Key Words: *Dentin-pulp complex, endopeptidases, enzymes, MMP, periodontium*

Introduction

Matrix metalloproteinases (MMPs), collectively known as matrixins, form a multigene family within the metalloproteinase class of endopeptidases that mediate the degradation of practically all extracellular matrix (ECM) molecules, including native and denatured collagen [1–3]. To date, 24 different MMPs have been cloned, of which 23 are found in humans [3–5].

Much of the early literature suggests that each MMP has its own particular substrate. This concept has led to the use of substrate-focused nomenclature for MMPs, such that the collagenases break down intact fibrillar collagens, gelatinases degrade denatured collagen (gelatin), and metalloelastase attacks elastin. It is now recognized that MMPs usually degrade multiple substrates, with considerable substrate overlap between individual MMPs. Still, the substrate-based classification is widely used, and MMPs are usually divided into six subgroups: collagenases, gelatinases or type IV collagenases, stromelysins, matrilysins, membrane-type metalloproteinase, and others (see Table I for details).

Even though this classification is helpful, MMPs have a broad specificity [3]. Collectively, these zinc-dependent endopeptidases can degrade all proteinaceous components of the ECM, thereby facilitating tissue remodeling and cell migration. MMPs are also tightly linked to biological activation of many physiological processes in the tissues, either via the liberation of ECM-bound growth factors and other biologically active molecules, or by directly activating these molecules by directed proteolysis [6,7].

This review focuses specifically on MMP characteristics and their role in physiological and pathological ECM remodeling and degradation processes in the oral environment. Internet-based searches were conducted to identify published data using

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Table I. MMP description according to the traditional classification (modified from VISSE & NAGASE) [3]. MMP-4, -5 or -6 have been abandoned, as their activities could not be ascribed to a specific gene product, and MMP-18 is known only as a *xenopus* enzyme [2]

Group	Enzyme	Nomenclature
Collagenases	MMP-1	Collagenase 1, fibroblast collagenase
	MMP-8	Collagenase 2, neutrophil collagenase
	MMP-13	Collagenase 3
	MMP-18	Collagenase 4 (*Xenopus* collagenase)
Gelatinases	MMP-2	Gelatinase A
	MMP-9	Gelatinase B
Stromelysins	MMP-3	Stromelysin 1
	MMP-10	Stromelysin 2
	MMP-11	Stromelysin 3
Matrilysins	MMP-7	Matrilysin 1, Pump-1
	MMP-26	Matrilysin 2
Membrane-type MMPs	MMP-14	MT1-MMP
	MMP-15	MT2-MMP
	MMP-16	MT3-MMP
	MMP-17	MT4-MMP
	MMP-24	MT5-MMP
	MMP-25	MT6-MMP
Others	MMP-12	Macrophage elastase
	MMP-19	(No trivial name)
	MMP-20	Enamelysin
	MMP-21	Xenopus MMP
	MMP-23	CA-MMP
	MMP-27	CMMP (Gallus)
	MMP-28	Epilysin

representative terms for each area. The database searched was MEDLINE (1966 to 2005).

MMP structure

MMPs share extensive sequence homology, but differ in terms of substrate specificity and transcriptional regulation. All MMPs are regarded as derivatives of a 5-domain prototype structure formed by either the addition or deletion of regulatory domains [3] (Figure 1). MMPs comprise a subgroup of the much larger metalloproteinase superfamily, which includes astacin and ADAM proteinases [9], among others. The classical MMP must have two domains, namely the prodomain (around 80 amino acids containing the consensus sequence PRC*XX*PD) and the catalytic domain, containing an active site Zn^{2+} that binds three conserved histidines in the sequence HEXXHXXGXXH(S/T)XXXXXXM and a conserved methionine to the carboxyl side of the zinc-binding site (metzincins) [9].

MMPs differ in the geography of the active site groove, allowing for different substrate and inhibitor specificities. Their common features include: a requirement that zinc be bound at their catalytic site; a family-specific zinc-binding motif; and a propeptide domain located at the N-terminal end of the catalytic domain, maintaining the enzyme as inactive zymogen until it is removed by limited proteolysis. For gelatinases, the hemopexin/vitronectin-like domain is thought to mediate enzyme-TIMP interactions and association with cell receptors (Figure 1). A cysteine-rich domain with homology to the collagen-binding region of fibronectin splits the catalytic domain of MMP-2 and -9 and is required for gelatin binding and cleavage [2].

MMPs are usually expressed as inactive zymogens, and the "pro" domain must be dissociated from the catalytic one before the enzyme is activated. This dissociation can be achieved by autocatalysis or by the action of proteolytic enzymes, such as furin, plasmin, or even other MMPs [10]. Besides being activated by proteinases, MMPs can be activated *in vitro* by chemical agents such as thiol-modifying agents, oxidized glutathione, SDS, chaotropic agents, and reactive oxygens. These agents probably work through disturbance of the cysteine–zinc interaction of the cysteine switch [3]. Changes in pH have also been demonstrated *in vitro* and *in vivo* to activate gelatinases; for example, when saliva pH is reduced [11,12]. Heat treatment can also lead to their activation.

MMP functional activity is regulated by four mechanisms: by positive and negative transcriptional controls of MMP genes, by activation from latent state, by differences in substrate specificity, and modulation by serum inhibitors or tissue inhibitors of metalloproteinases (TIMPs) [1]. TIMPs are specific inhibitors that bind to MMPs in a 1:1 stoichiometry. So far, four TIMP members have been described (TIMP-1 to -4) in vertebrates and their expression is regulated during development and tissue remodeling [3]. They are small multi-functional proteins. The overall shape of the TIMP molecule is like a wedge. It slots into the active-site cleft of an MMP in a manner similar to that of the substrate [3]. By forming complexes with MMPs, they may block activation of latent pro-MMP or they may influence their ability to hydrolyze a particular substrate [1,13].

Recently, it has been demonstrated that RECK (reversion-inducing-cysteine-rich protein with Kazal motifs) encodes a membrane-anchored glycoprotein that suppresses tumor invasion and angiogenesis by regulating MMP-2, MMP-9, and MT1-MMP activities and by proMMP-9 release from the cell [14,15]. The unique feature of RECK among MMP-inhibiting proteins is its localization in cell membrane [16]. Membrane localization seems to be important in RECK inhibitory activity, since soluble recombinant RECK is only moderately inhibitory against MMPs [17]. When the RECK gene was first identified, it was observed to be expressed in a wide variety of human tissues. Nuttall et al. [5] have shown that its transcripts are also present in a wide variety of mouse tissues, though at most sites RECK is expressed at a lower level than any of the four

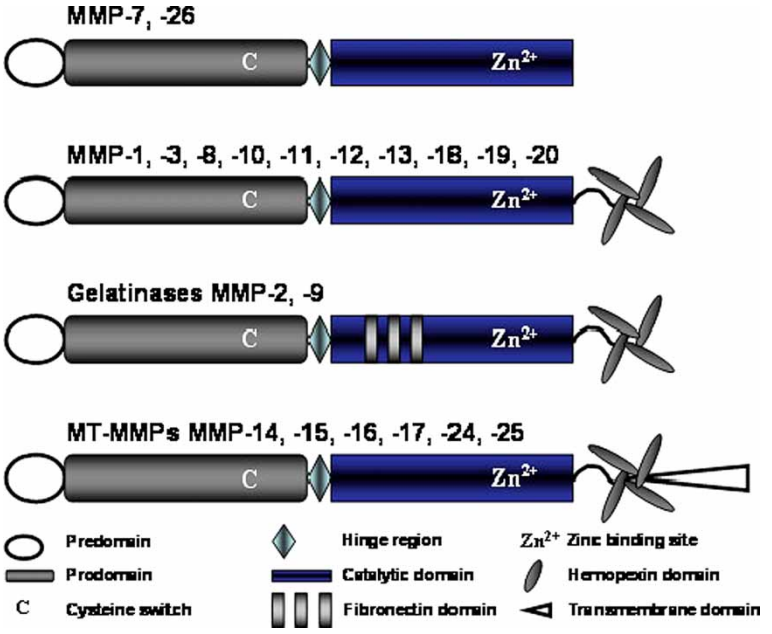

Figure 1. Schematic presentation of the basic MMP structure. All MMPs consist of a predomain, a prodomain holding a cysteine switch, a hinge region, and a catalytic domain with Zn^{2+} binding site. All except matrilysins (MMP-7, -26) contain the hemopexin domain. The MT-MMPs also contain the transmembrane domain, and gelatinases MMP-2 and -9 contain the fibronectin domain with strong affinity to gelatin. Modified from Vu & Werb [6] and Nagase et al. [8].

TIMPs. Interestingly, mice with a RECK null mutation die *in utero* due to poor collagen formation and vascular defects [17], whereas mice lacking TIMP-1, -2, or -3 are viable. While the tertiary structure of TIMPs is very similar, with six cysteine residues in amino terminus being critical to MMP inhibition, RECK amino acid sequence and cysteine residue order is markedly different, leaving the biochemical mechanism for RECK inhibition to be evaluated [16]. These findings do not suggest a pattern of RECK expression distinct from the TIMPs to explain this embryonic lethality. Instead, the role of RECK in the regulation of angiogenesis may be of critical importance in tissue development and tumor growth [16–18]. Interestingly, part of TIMP-2 ability to inhibit endothelial cell migration seems to be delivered through increased expression of RECK [19] via dephosphorylation of paxillin tyrosine residues 31 and 118 [20]. However, inhibiting glycosylation at Asn86, Asn297, and Asn352 residues of RECK reversed RECK-suppressed tumor cell invasion, suggesting the role of glycosylation on RECK suppression of tumor cell invasion [21].

Ameloblastomas show decreased RECK expression compared to tooth germ tissues, suggesting that downregulation of this MMP inhibitor participates in tumor development and progression, probably controlling the activities of MMP-2, -9, and MT1-MMP [22]. While the role of RECK in inflammatory diseases is still widely unknown, at least in rheumatoid arthritis RECK expression is significantly decreased [23], indicating that RECK-delivered control of MMP activity may be important in inflammation-related tissue destruction.

MMP activity is also observed in a transmembrane protein containing both a disintegrin and metalloproteinase domain (ADAM) presenting both cell adhesion and protease activity. They are glycoproteins that share homology with snake venom metalloproteinase/disintegrins and sperm surface proteins. Structurally, ADAMs consist of a prodomain that blocks protease activity; a zinc-binding metalloproteinase domain; disintegrin and cysteine-rich domains with adhesion activity; an epidermal growth factor-like domain with cell fusion activity; a transmembrane domain; and a phosphorylated cytoplasmic regulatory domain. Among other biological functions, ADAMs are involved in the release of membrane-anchored proteins, such as tumor necrosis factor α (TNF-α), transforming growth factor α (TGF-α), and L-selectin, from the plasma membrane [24–27]. Despite ADAMs having diverse functions, their role in the oral environment is poorly understood. Among 30 known ADAMs, ADAM 28 has recently been implicated in tooth development and the regulation of odontogenic mesenchymal cells [28].

Role of MMPs in oral conditions

MMPs contribute to both normal and pathological tissue remodeling. Physiological roles for MMPs include cell migration, tissue remodeling during organogenesis and growth, wound healing, angiogenesis, enamel formation, and antigen processing and presentation. MMPs play a key role in the migration of normal and malignant cells and they act as regulatory molecules, by functioning in enzyme

Table II. Examples of MMP-related biological activities resulting from the degradation of substrate by named MMP (simplified from [3])

MMP group		Biological effect
Collagenases	MMP-1	Keratinocyte migration and re-epithelialization Platelet aggregation
	MMP-13	Osteoclast activation Release of βFGF
Gelatinases	MMP-2	Neurite outgrowth Mesenchymal cell differentiation with inflammatory phenotype Epithelial cell migration Increased bioavailability of MMP-9 Reduced IL-2 response Tumor cell resistance
Stromelysins	MMP-3	Mammary epithelial cell apoptosis Mammary epithelial alveolar formation Mammary epithelial cells epithelial-mesenchymal conversion Release of βFGF Increased bioavailability of TGF-β Disrupted cell aggregation, increased cell invasion
Matrilysins	MMP-7	Adipocyte differentiation Increased bioavailability of TGF-β Disrupted cell aggregation, increased cell invasion Fas receptor-mediated apoptosis
Membrane-type MMPs	MT1-MMP	Kidney tubulogenesis Epithelial cell migration
Several MMPs (3 or more)		Cell migration Generation of angiostatin-like fragment Generation of endostatin-like fragment Enhanced collagen affinity Increased bioavailability of IGF1 and cell proliferation Activation of VEGF Pro- or anti-inflammatory effects Reduced cell adhesion and spreading

cascades and by processing matrix proteins, generating fragments with enhanced or reduced biological effects [3,6–8]. MMPs are produced by tumor cells, so that they can metastasize and force their way into the surrounding stroma, penetrating the basement membrane until they reach the blood vessels [29].

MMPs are widely active on the biological processes (Table II). Collagenases have the ability to cleave interstitial collagen types I, II, and III, and they are capable of digesting other ECM and non-ECM molecules. Gelatinases degrade the denaturated collagens, gelatin. Stromelysin 1 (MMP-3) and stromelysin 2 (MMP-10) have similar substrate specificities, but MMP-3 has a higher proteolytic efficiency. Besides digesting ECM molecules, MMP-3 also activates proMMPs. Stromelysin 3, MMP-11, is also grouped as "other MMPs", since its sequence and substrate specificity differ from those of MMP-3. Matrilysins are characterized by the lack of a hemopexin domain (Figure 1). Matrilysin 1 (MMP-7) processes cell surface molecules, such as proTNF-α, pro-α-defensin, and Fas-ligand. Matrilysin 2 (MMP-26) also degrades ECM components. Membrane-type MMPs (MT-MMPs) are divided into type I transmembrane proteins (MMP-14, MMP-15, MMP-16, and MMP-24) and glycosylphosphatidylinositol (GPI) anchored proteins (MMP-17 and MMP-25). All MT-MMPs are capable of activating pro-MMP-2, except MT4-MMP. These enzymes can also digest ECM molecules. MT1-MMP is able to degrade collagen types I, II, and III and also plays an important role in angiogenesis [3].

There are several studies indicating that MMPs have a fundamental role in oral tissue development and remodeling. MMPs are involved in the development of enamel and enamel fluorosis [30–32]. They are also associated with remodeling of the organic matrix of dentin [33–35]. The activation of MMP-2 and MMP-9 has been shown to have a crucial role in dentin collagen breakdown in caries lesions [11,36]. The inflammatory soft tissue breakdown has four recognized pathways: the plasminogen-dependent pathway, the phagocytic one, the osteoclastic pathway, and the MMP-dependent pathway [1]. MMPs have been identified in both pulpal and periapical inflammation [37,38]. They even more strongly correlate to periodontal diseases, since MMPs are the major players in collagen breakdown during periodontal tissue destruction

[1,39–42]. MMPs are essential components in the growth and invasion of oral tumors [43]. And finally, MMPs may be important in the time-dependent loss of composite restoration adhesion [44,45].

MMPs in periodontal disease

Collagen is the major extracellular component of gingiva. Periodontal disease, which is initiated by bacterial plaque, is characterized by inflammation, leading to the loss of periodontal attachment and bone destruction. It has been demonstrated that degradation of gingival tissue during active periodontitis is at least partly due to MMPs. They are expressed by inflammatory cells (monocytes, macrophages, lymphocytes, polymorphonuclear cells) and by resident cells (fibroblasts, epithelial cells, endothelial cells) [1].

Even though bone destruction is the hallmark of periodontal disease, the role and function of MMPs in periodontal bone loss is not very well described. However, basic research has gained insight into the MMP action in bone remodeling that most likely is applicable to periodontal bone destruction, too. Osteoclast express, along with cathepsin K, several MMPs, which together with periosteoblastic cell and osteoblast-derived MMPs contribute to bone resorption [46,47]. In osteoclastic bone resorption, cathepsin K is regarded as the key proteinase for matrix degradation after demineralization under osteoclasts, mainly because of cathepsin K's ability to cleave native triple helical collagen at multiple sites, a unique feature to mammalian collagenases [48]. There is good evidence, however, that MMPs are also important in bone resorption. MMPs are critical for osteoclast access to the resorption site, since MMP inhibition prevents cell migration [49,50] and MMP-9 and -14 seem to be the key proteinases in this respect [46,51]. MMP-14 is located in the ruffled border of osteoclasts, possibly contributing to the osteoclast–matrix interaction that controls osteoclast attachment and detachment to bone [52]. Osteoblast-lineage cells (periosteoclastic cells, osteoblasts, and osteocytes), but not osteoclasts, produce MMP-13 [47,53,54]. Still, MMP-13 is present in resorption lacunae, and it is essential for removal of collagen remnants left over by osteoclasts [55,56]. MMPs may also contribute to osteoclastic bone resorption by regulating osteoclast recruitment and activity by, for example, releasing cytokines and growth factors (such as TGF-β by MMP-9 or RANKL by MMP-14) from bone matrix, or by regulating the messenger binding to the receptors [46].

The importance of osteoblastic MMPs on bone development and formation has only recently been realized. At least MMP-2, -9, -13, and -14 are considered important in bone development and formation [57–60]. MMP-14 also participates in normal bone homeostasis: MMP-14-activated TGF-β inhibits apoptosis and maintains osteoblast survival during osteoblast trans-differentiation into osteocyte [61]. MMP-14 is also essential in maintaining osteocyte viability [62,63], thus significantly contributing to normal bone development and repair.

Mäkelä et al. [39] suggested that MMP-2 and MMP-9 could participate in tissue destruction in periodontitis. They located gelatinase production by various cells in the oral cavity and found that the amount of gelatinases increased during the periodontal disease, while conventional periodontal treatment effectively reduced the levels of these enzymes. There is also strong *in vivo* evidence for a direct role of active neutrophil collagenase in the pathological destruction of periodontal connective tissue. For example, it was demonstrated in a longitudinal cohort study that collagenase activity derived from neutrophils and not from bacteria or other host cells. Moreover, a significant increase of active collagenase with time was observed only at sites with progressive periodontal destruction. It is currently known that gelatinases (MMP-2, -9) and all collagenases derived from various cellular sources and expressed in various molecular species are involved in periodontitis [40,42,64–68].

The production of collagenase, as well as the secretion of physiological activators and inhibitors of enzyme, is modulated by cytokines. Procollagenase produced endogenously (MMP-1), stored in the ECM of periosteal tissue, can be activated and the derived enzyme degrades collagenous matrix until inhibited by TIMPs. A large amount of collagenous proteins (about 70% of the total amount of collagenous proteins) are degraded after procollagenase activation by plasmin in periosteum [41].

Recent studies have indicated the importance of MMP-8 in periodontal destruction. It has been found that clinical improvement obtained by scaling and root planning (SRP) and the periodontal maintenance therapy is associated with significant reductions in MMP-8 levels. This suggests that MMP-8 is a potential discloser of periodontal disease. The reduction of MMP-8 levels in GCF indicates that this enzyme may be useful as an indicator of current disease status and as a predictor of future disease [67,68]. A chair-side test for MMP-8 was therefore developed [68]. This is an immunological dip-stick test that can be handled by the dentist without specific equipment, and that allows measurement of GCF MMP-8 level in 5 min. Measurement of GCF MMP-8 level may be a practical tool in the diagnosis and monitoring of periodontal diseases [43,67,68]. Not only are MMPs effectors of the inflammatory process, they are also modifiers of the host response. Therefore, levels of MMPs in GCF cannot be interpreted as indicating tissue degradation alone, but inflammation (protective and destructive) as a whole [69]. TIMPs seem not to be sufficient in

reducing the tissue destruction by pathologically elevated MMPs in periodontitis [70], but there are several studies examining the possibility of MMP inhibition by synthetic inhibitors being able to control the periodontal tissue destruction. Several studies have demonstrated that the MMPs considered important in periodontitis, including MMP-8, are sensitive to inhibition by doxycycline and chemically modified non-antimicrobial tetracycline (CMT) derivatives [71]. Pathologically elevated tissue-degrading MMP activity could be directly inhibited by pharmacological levels of doxycycline [72]. Low-dose doxycyline (LDD: Periostat®, CollaGenex Pharmaceuticals, Inc., Newtown, Pa., USA), with no demonstrable antimicrobial effect [73,74], has been approved as an adjunctive therapy in periodontitis in the USA and Europe [75–77]. LDD can inhibit pathologic collagenolysis by blocking mammalian collagenases and other MMPs and is efficient in reducing both the severity of periodontitis and MMP-8 levels and activity in GCF in periodontal patients. A 2-week regimen of low-dose doxycycline reduced metalloproteinase activity by approximately 60–80% [78]. The administration of this MMP inhibitor reduces both collagenase level and activity and connective tissue breakdown in humans [74,79,80].

While MMP inhibition is promising in the treatment of periodontal disease, further work that includes other approaches needs to be evaluated (reviewed by Reddy et al.) [77]. It must be kept in mind that the mere presence of MMPs in GCF and/or periodontal tissue does not automatically mean increased activity of the enzymes in tissue. Therefore, a functional clinical research approach is preferable to disclose the exact role of MMPs in periodontitis.

MMPs in human pulps

The human pulp, which has a mesenchymal origin, consists of an odontoblast layer adjacent to the dentin and of a connective tissue core with plenty of nerves, vascular tissue, and undifferentiated mesenchymal cells [81]. When toxins like those produced by bacteria reach the dentin, pulpal inflammation generally occurs. If the caries injury is not removed, this inflammatory process advances. Pulpitis is associated with tissue degradation.

When MMP levels and gelatinolytic activity in clinically healthy and inflamed human dental pulps were compared, the data indicated that MMP-9 may be important in the breakdown of inflamed human dental pulp tissue. Levels of MMP-1 and MMP-2 were significantly lower in symptomatic versus clinically healthy pulps. In contrast, levels of MMP-9 in inflamed pulps were significantly higher than those recorded in normal pulps. The overall gelatinolytic activity was elevated in inflamed pulps compared to healthy ones [38]. Another study demonstrated an abundant presence of MMP-8 in inflamed pulp tissue [37]. While MMP-13 (collagenase-3) is expressed in healthy pulp tissue in exceptionally high levels compared to most adult tissues, the effect of pulp inflammation on MMP-13 levels remains inconclusive [82]

MMPs are involved not only in inflamed pulps, but also in periapical inflammation. Examination of periapical exudates and granulomas demonstrated the presence of MMP-8 in periapical lesions indicating that MMP-8 plays a role in pulpal and periapical inflammation, most likely participating in tissue ECM degradation [37,83]. It was further indicated that MMP assay from periapical exudates could be used to determine and monitor inflammatory activity and the success of treatment in teeth with periapical lesions [37].

MMPs in enamel and dentin

MMPs have been implicated in tissue remodeling and in the regulation of cell-matrix interactions during tooth morphogenesis. To date, MMP-20 (enamelysin) is known to be almost exclusively expressed by tooth-forming cells. It has unique structural and enzymatic properties, being capable of degrading amelogenin, and may have an important role during enamel development [30–32]. It is expressed by mature human odontoblasts, which secrete it into the dentinal fluid. Being produced during primary dentinogenesis, MMP-20 is incorporated into dentin and may be released during caries progression [35].

Amelogenin is the major structural component protein of the enamel matrix. Another MMP related to enamel formation is MMP-2. Caron et al. [84] have demonstrated, through Northern blot and *in situ* hybridization analysis, that MMP-2 can cleave amelogenin into several fragments. MMP-2, along with its activator membrane type-1 matrix metalloproteinase (MT1-MMP), is expressed by ameloblasts and odontoblasts of the developing enamel and pulp organ. It may therefore play an important role during tooth development.

MMP-20, enamelysin, is even more important for proper enamel formation than MMP-2. MMP-20 knock-out (KO) mouse does not process amelogenin properly, resulting in altered enamel matrix and rod pattern. The resulting enamel is hypoplastic and delaminates from the dentin, greatly resembling the human amelogenesis imperfecta smooth type [30]. The overall enamel mineral content in MMP-20 KO animals is approximately one-half, and hardness about two-thirds, of that of the wild type mouse [32]. Collectively, these findings demonstrate that enamelysin activity is essential for proper enamel development.

Dental fluorosis was first reported in 1916 by Black & McKay [85]. It is referred to as a mineralization defect of enamel, characterized by increased porosity, due to exposure of the developing tooth organ to excessive amounts of fluoride [86]. Amelogenins are the most prevalent proteins in the developing enamel matrix. Fluoride exposure during enamel development delays the removal of the amelogenin, which is hydrolyzed by proteinases as the enamel matures [31]. One possible mechanism for the delay in protein hydrolysis could be through the extracellular proteinases needed to degrade enamel proteins. The effect of ingested fluoride on the relative activity of proteinases in the enamel matrix was examined by Denbesten et al. [31], especially its specific effect on MMP-20 activity. This *in vivo* study (in rats) indicated that fluoride ingestion could alter the relative amount of active MMP-20 in mature enamel. *In vitro* assays showed that micromolar concentrations of fluoride could alter metalloproteinase activity, and hydrolysis of amelogenin by recombinant MMP-20 (rMMP-20) was reduced in the presence of fluoride. These findings suggested that the effect of fluoride on MMP-20 secretion or activity could be involved in the etiology of fluorosis in enamel and other mineralizing tissues [31].

As early as 1983, Dayan et al. [87] demonstrated collagenolytic activity in intact dentin, but the source of the activity could not be determined. The presence of MMP-2 in human dentin was first identified by Martin de Las Heras et al. [34], providing indirect evidence of *in vivo* activity of dentin matrix remodeling after mineralization. This study emphasized the stability of the enzyme when embedded in a mineralized phase. Interestingly, extracts from older donors (aged between 21 and 40 years) presented significantly decreased amounts of functional MMP-2 [34]. While the presence and activity of collagenase in mineralized dentin, described by Dayan et al. [87], remains to be confirmed, the recent study demonstrates that MMP-8 (produced by the odontoblasts) is present in mineralized dentin matrix [88].

MMPs in caries lesions

It has been suggested that MMPs participate in the caries progression, but the exact origin and role of proteolytic enzymes in the caries process is still the subject of debate. *Streptococcus mutans* is the predominant organism involved in the development of caries lesions [89]. The ability of *S. mutans* to bind and to disrupt the synthetic peptide FALGPA, with the structure resembling collagen fibrils, indicates that collagenolytic activity by *S. mutans* may be an important virulence factor in dental decay [90]. However, the degradation of triple-helical collagen fibrils in mineralized tissues is much more complicated than the degradation of synthetic peptides [91]. Indeed, it has been demonstrated that dentin matrix degrading activity is not attributed to bacteria. *In vitro* experiments have shown that cariogenic bacteria could cause only demineralization of the dentin surface, but were not able to degrade the dentin matrix, which is necessary for cavity formation [92]. The bacteria collected from dentinal lesions created in an *in situ* model have failed to degrade collagen [93], and salivary cariogenic bacteria isolated from caries-active patients did not have any gelatinolytic activity [11]. Two studies have shown externally added collagenase to be necessary for the matrix degradation in *in vitro* dentin caries experiments [92,94]. The acid activation of salivary MMPs (lowering the pH for 30 min, followed by neutralization) (Figure 2), results in the degradation of demineralized dentin matrix [11].

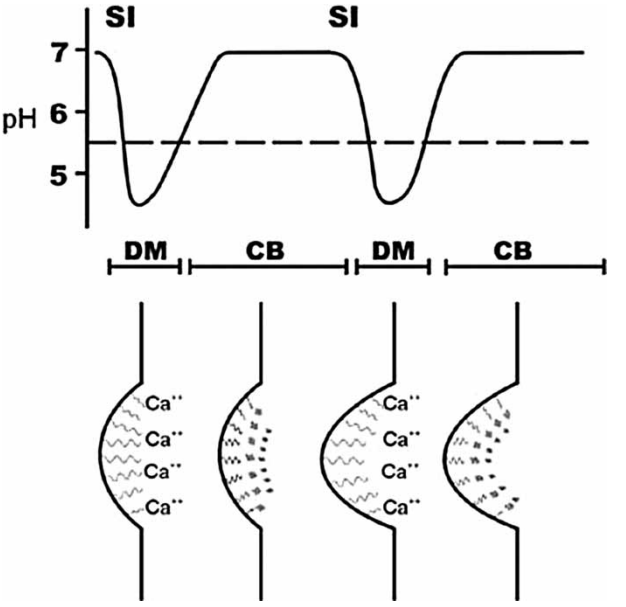

Figure 2. Schematic presentation of the altering sequences of demineralization and degradation of the organic matrix in a dentin caries lesion; changes in pH and corresponding changes in caries lesion are demonstrated. After sugar ingestion (SI), the pH decreases below the level in which demineralization occurs (pH 5.5). During demineralization (DM), the organic matrix collagen fibrils are exposed. The latent purified forms of MMPs are activated at low pH (4.5), but their activity is still low. After neutralization, their activity increases with a subsequent collagen breakdown (CB). Modified from [11].

The increasing body of evidence indicates that the MMPs present in dentin matrix [34,35,44,87,88] or in saliva [11,70] could be responsible for the dentin matrix degradation in dentinal caries lesions. Indeed, Dayan et al. [87] demonstrated increased collagenolytic activity in carious dentin, compared to intact dentin, suggesting the presence of collagenase activators or partial reduction of the collagenase-inhibitor complex secretion [87]. More recently, collagenase MMP-8 and gelatinases MMP-2 and -9 were all identified in human dentinal caries lesions

both in pro- and active forms, suggesting that the activation occurs *in situ* [11]. Increased MMP levels and activity have been found in the saliva of the patients suffering from Sjögren syndrome or post-radiation decrease of salivary flow [12,95], both conditions known to promote caries. Tetracycline derivates (doxycycline and chemically modified tetracyclines, CMTs; all potent synthetic MMP inhibitors) reduce salivary MMP activity both *in vivo* [96] and *in vitro* [36]. Finally, *in vivo* study with rat molars demonstrated a significant reduction in dentinal lesion progression with MMP inhibitors, along with reduced salivary MMP activity [36]. These findings are important, since they open a new pathway for caries prevention. Slowing down or preventing the irreversible destruction of organic matrix would allow for natural healing of the lesion, by remineralization of decalcified dentin. MMP inhibitors may prove to be useful in the prevention of caries progression. It is also noteworthy that chlorhexidine (CHX) directly inhibits MMP-2, -8, and -9 activities [97]. A chelating mechanism might be involved in the inhibition of these MMPs, since adding calcium chloride to the assay solution prevented the inhibition of these enzymes by CHX [97]. These findings indicate new beneficial antiproteolytic properties of this antimicrobial agent frequently used in caries treatment. However, the role of MMPs in caries progression is not clear-cut. In a study examining the presence and activities of MMP-1, -2, and -9 in saliva and in completely demineralized dentin specimens subjected to oral environment for 4 weeks, MMP activity was present both in saliva and in dentin collagen. Interestingly, practically all saliva samples contained proMMPs, while in dentin samples, only approximately 30% of samples contained proMMPs, even though MMP activity was present [98]. This supports previously noted activation of proMMPs in demineralized dentin *in situ* [11]. No correlation could be observed, however, between the level of enzyme activity and collagen loss of dentin specimens [98], failing to confirm the role of MMPs in matrix degradation. This may be due to the loss of MMPs during degradation, thus resulting in lower MMP levels in samples with the most pronounced matrix loss. It is also noteworthy that the same study protocol has previously failed to demonstrate the correlation between the oral bacteria and dentin matrix loss [99,100]. It can be concluded that the process of matrix degradation has large inter- and even intra-individual variations, and the process needs further research to be fully understood.

Possible roles of MMPs in dentin-pulp complex

There are several possible targets for MMP family members in the dentin-pulp complex metabolism. They have a role in dentin organic matrix organization prior to mineralization (Figure 3A, B). The significant expression of MMPs and TIMPs by mature human odontoblasts and pulp tissue suggests that they may participate in dentin matrix organization prior to mineralization [82,101,102]. MMPs

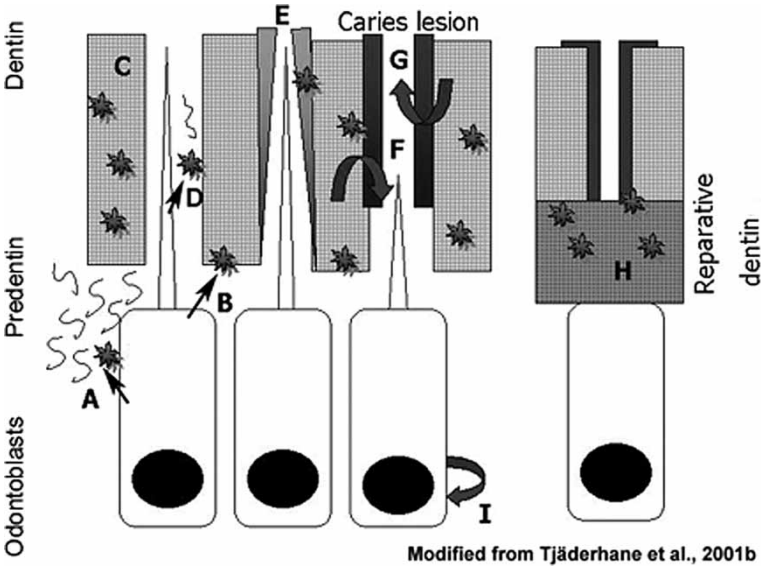

Figure 3. Possible sites of action for MMPs (marked with stars). MMPs may participate in the organization of the dentin organic matrix in predentin (A), or they may regulate mineralization by controlling the predentin proteoglycan turnover (B). MMPs are incorporated into mineralized dentin (C). They may degrade the intratubular collagen and other proteins (D) or participate in intratubular dentin formation and mineralization (E). In caries progression, MMPs may be involved in the release and activation of dentin-bound growth factors (F), which would then activate defense reactions in the pulp. As MMPs seem to have a role in dentinal caries progression, the matrix-bound MMPs may participate in caries pathogenesis or degradation of the restoration–dentin interface (G). During reparative dentinogenesis, MMPs may have a role in matrix degradation and contribute to the formation of atubular reparative dentin (H). Finally, MMPs may participate in the regulation of the ECM turnover in the pulp tissue (I) in physiological and pathological conditions.

may be needed to degrade type III collagen to facilitate its removal from the predentin [103]. Stromelysins (MMP-3) may control proteoglycan turnover in predentin [33]. MMPs seem also to be incorporated in mineralized dentin (Figure 3C) [34,35,88].

The presence of MMP-20 in dentinal fluid [35] promoted the suggestion that MMPs from odontoblast processes or dentinal fluid may be involved in the regulation of peritubular dentin formation, either by degrading the intratubular collagen (Figure 3D) or by influencing the mineralization process (Figure 3E) [104]. The presence of MMP-2 [34] and MMP-8 [88] in the dentin protein matrix prior to demineralization indicates that loosely bound gelatinase and collagenase are also present in dentinal tubules. The matrix-bound MMPs may be involved in the liberation of dentin matrix-bound bioactive factors (such as growth factors) that may further participate in the regulation of pulpal defensive reaction under carious dentin (Figure 3F). Dentinal MMPs may also participate in matrix degradation during caries progression (Figure 3G) [11,36].

Regulation of MMPs by dentin-derived growth factors may affect reparative dentin formation (Figure 3H) [105]. The mature human odontoblasts culture method showed that TGF-β1 had a negligible effect on collagen mRNA expression or protein synthesis in odontoblasts. This finding indicates that TGF-β1 may not induce dentin formation by up-regulating collagen synthesis *per se*, suggesting that the main function of TGF-β1 in the dentin-pulp complex may be to induce the differentiation of replacement odontoblasts [103]. However, growth factors may up-regulate dentin formation indirectly, having an important role in matrix maturation by controlling the MMP synthesized in the odontoblasts [106]. That may result in accelerated formation of mineralized dentin [107]. TGF-β1 seems to regulate odontoblast MMP expression differentially. It seems to strongly down-regulate MMP-8 expression, modestly down-regulate MMP-20 expression, and consistently up-regulate MMP-9 expression, with no apparent effect on MMP-2 [101,102,104–106]. A modification of MMP expression may result in altered ECM formation, which could contribute to the formation of atubular reparative dentin (Figure 3H) [105]. And finally, MMPs may be involved in pulp ECM remodeling and degradation in physiological and pathological conditions (Figure 3I) [104].

MMPs in demineralized collagen matrices

In adhesive dentistry, there is great concern about long-term stability of the adhesive interface, since it has been demonstrated that hydrophilic dentin adhesives deteriorate over time [108]. Exposed collagen matrices from acid-etched dentin, which have incompletely infiltrated collagen fibrils, are also susceptible to degradation [109]. The net effect of the deterioration of these structures is the loss of adhesion. The hydrolytic role of degradation was evident in an *in vitro* study carried out by Carrilho et al. [110]. Pashley et al. [44] investigated the effect of proteolytic enzymes in the demineralized dentin matrix stored in water, artificial saliva, or in oil. They also analyzed whether proteolytic enzyme inhibitors prevented demineralized collagen matrix from degrading. The results from this study demonstrated that hydrolytic degradation of collagen fibrils occurred in the absence of bacterial colonization. The authors speculated that MMPs from mineralized dentin matrix might have been activated during dentin acid etching and were probably responsible for collagen matrix degradation in aqueous environment. Therefore, they considered that preventing the degradation of incompletely resin-infiltrated collagen fibrils by MMPs in the hybrid layers would be an important procedure. They suggested that the application of chlorhexidine solution to acid-etched dentin could be useful, since chlorhexidine is a safe compound and widely used in dentistry. Later on, another study has corroborated this approach, showing that self-destruction of collagen matrices occurred rapidly in resin-infiltrated dentin *in vivo* but it is arrested with the use of chlorhexidine as an MMP inhibitor [111].

Conclusions

MMPs are a family of proteolytic enzymes that are capable of degrading almost all ECM proteins. It has been demonstrated that MMP family members are involved in normal physiology and in pathological events that occur in the oral environment. They have been identified in saliva, gingival crevicular fluid, in enamel and dentin structures, and also in periodontal diseases and carious lesions.

This review has shown the potential role of MMPs in the prevention and maintenance of oral health. There is still much to learn. Further investigations should elucidate the role of MMPs in the organization of the dentin-pulp complex, pulp pathology, caries pathogenesis, and also in degradation of the dentin–adhesive interface. Since MMP inhibitors, such as chlorhexidine, doxycycline, and minocycline, have proved to be efficient adjuncts in periodontal therapy, and tetracyclines and zoledronate have been shown to suppress the progression of dental caries, the use of MMP inhibitors in caries prevention and in the treatment of pulpitis and periodontitis is definitely worthy of further research. In cariology and pulp biology, *in vitro* experiments may lay down the groundwork for focused *in vivo* animal experiments before clinical studies can be justified. In periodontitis, the present clinical evidence needs

to be confirmed, preferably in prospective long-term clinical follow-up studies.

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