

*Review*

## **A mini review on the associations of matrix metalloproteinases (MMPs) -1, -8, -13 with periodontal disease**

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**Abstract:** Matrix metalloproteinases (MMPs) are one of the most important endopeptidases in periodontal disease that generally degrade extracellular matrix (ECM) components of periodontal supporting tissues, leading to tooth loss. Among the MMP family, MMP-1, -8 and -13, which are also known as the collagenase group, play a vital role in the degradation of ECM collagen and non-collagen substances. Elevated levels of MMP -1, -8 and -13 are markedly significant within tissue, gingival crevicular fluid (GCF), and saliva of patients with periodontitis, which help to explain the progression pattern of the disease. This review provides an overview of MMP -1, -8, and -13 on their structures, functions and their critical role in periodontitis.

**Keywords:** collagenase; matrix metalloproteinase; MMPs; periodontitis; periodontal disease

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## 1. Introduction

Periodontal disease is a pathological condition that affects gingiva, periodontal ligament, radicular cementum and the alveolar bone surrounding teeth [1]. The disease is initiated by bacterial-host interactions at the biofilm-periodontium interface and is associated with chronic inflammation. Gingivitis is an inflammatory process of gingiva, developed when connective tissue is infiltrated by inflammatory cells and characterized by red and swollen gingiva with spontaneous bleeding [2]. As the disease progresses, there is an immune-mediated loss of periodontal tissue involving destruction of the periodontal ligament and alveolar bone, which is known as periodontitis. Oral biofilm or dental plaque is the main etiological factor for periodontal disease initiation. *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia* and *Aggregatibacter actinomycetemcomitans* are the most common periodontopathogens found in dental plaque that are responsible for periodontal disease [3]. Extracellular matrix (ECM) protein degradation by proteinases, which is the key feature of the periodontal pathological condition, could be derived from both micro-organisms in dental plaque and also from human cellular sources [4]. Previous studies have reported that proteases derived from both microbial and cellular sources could contribute to MMPs activation cascade, leading to the periodontal destruction process [3]. Moreover, microbial proteases activate proteolytic activators of latent human proMMPs and could increase their secretion by gingival resident cells, which results in collagen degradation of the ECM. Periodontitis affects up to 15% of the population globally [5]. The prevalence of the disease is highest among the older population (82%), followed by the adult population (73%) and the young population (59%) [6]. Men are more likely to be affected by the disease (57%) in contrast to women (39%). Smoking, diabetes, aging, presence of periodontal pathogenic microorganisms, physiological stress, host response and several systemic diseases are the established risk factors for periodontal disease [7].

Matrix metalloproteinases (MMPs) or matrixins are an important family of calcium-dependent zinc-containing endopeptidases. MMPs play an important role in tissue remodeling and degradation of various proteins in ECM components [8]. Moreover, MMPs are also able to break non-matrix proteins, including cytokines [9,10]. Overexpression of MMPs has been significantly identified in periodontal disease and is also associated with several pathological conditions such as cardiovascular disease, arthritis, cancer, chronic kidney disease, respiratory tract disorders, liver fibrosis, chronic obstructive pulmonary disease, inflammatory bowel disease and other pathological conditions [11,12]. MMPs and their endogenous tissue inhibitor of metalloproteinases (TIMP) must be well-balanced for physiological periodontal tissue remodeling [13]. Any abnormal alterations between MMPs and TIMPs will initiate an uncontrolled tissue breakdown, which leads to periodontal disease. Research has shown that during the pathological process of periodontal disease, elevated levels of MMPs are associated with the degradation of ECM such as collagen, which are the most abundant proteins that provide structural support for cells [14]. The aim of this review is to discuss about the pathophysiology; provide a detailed description of the collagenase group's (MMP - 1, -8, -13) structure, function, role on periodontal disease; and to discuss the current information on their association with other diseases and the response in their expression after appropriate periodontal therapy to better understand their impact on periodontal disease.

## 2. Classification of MMPs

**Table 1.** MMPs classification with substrates.

| Group       | MMP    | Nomenclature  | Substrate  | Non-Collagen Substrate  | References |
|-------------|--------|---|--|---|------------|
| Collagenase | MMP-1  | Collagenase 1, Interstitial collagenase, Fibroblast collagenase               | Collagen type-I, II, III, VII, VIII, X, XI, Gelatin  | Entactin, fibronectin, aggrecan, tenascin C, nidogen, perlecan, ovostatin, casein, myelin basic protein, vitronectin, serpins                               | [14,18]    |
|             | MMP-8  | Collagenase 2, Neutrophil collagenase   | Collagen type-I, II, III, V, VII, VIII, X, Gelatin   | Aggrecan, elastin, fibronectin, laminin, nidogen, ovostatin   |            |
|             | MMP-13 | Collagenase 3   | Collagen type-I, II, III, IV, IX, X, XIV, Gelatin    | Fibronectin, aggrecan, laminin, perlecan, tenascin, plasminogen, osteonectin, casein, fibrillin 1, serine proteinases inhibitor, core protein               |            |
|             | MMP-18 | Collagenase 4   | Collagen type-I, II, III, Gelatin                    |   |            |
| Gelatinase  | MMP-2  | Gelatinase A, 72 kDa<br>Gelatinase/type IV Collagenase                        | Collagen type-I, II, III, IV, V, VII, X, XI, Gelatin | Aggrecan, elastin, fibronectin, laminin, nidogen, proteoglycan, versican, tenascin, myelin basic protein  | [19,20]    |
|             | MMP-9  | Gelatinase B, 92kDa Gelatinase, type IV Collagenase/type V Collagenase        | Collagen type-IV, V, VII, X, XII, XIV, Gelatin       | Aggrecan, fibronectin, elastin, nidogen, proteoglycan link protein, versican, casein, chemokines  |            |
| Stromelysin | MMP-3  | Stromelysin-1, Transin, Proteoglycanase, Collagenase Activating Protein (CAP) | Collagen type-I, II, III, IV, V, IX, X, XI, Gelatin  | Fibronectin, laminin, aggrecan, elastin, nidogen, perlecan, proteoglycans, entactin, tenascin, ovostatin, casein, osteonectin elastin, vitronectin, decorin | [21,22]    |
|             | MMP-10 | Stromelysin-2, Transin-2  | Collagen type-III, IV, V, IX, X, elastin             | Aggrecan, elastin, fibronectin, laminin, nidogen, casein, fibrillin-10  |            |
|             | MMP-11 | Stromelysin-  | -  | Aggrecan, fibronectin, laminin  |            |
|             | MMP-12 | Macrophage elastase, Metalloelastase  | -  |   |            |

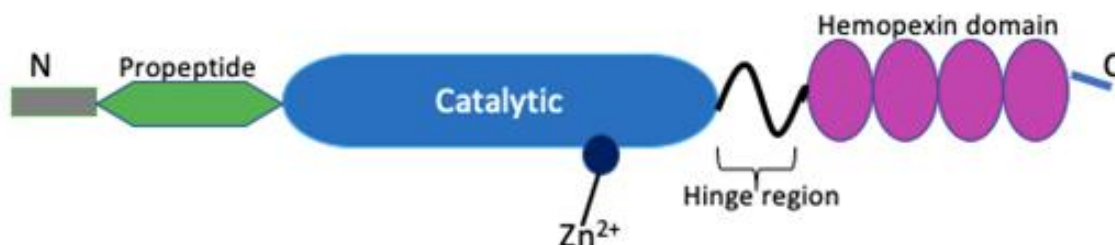
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| Group   | MMP    | Nomenclature  | Substrate                         | Non-Collagen Substrate   | References |
|---|--------|---|-----------------------------------|--|------------|
| Matrilysins   | MMP-7  | Matrilysin-1,<br>Putative metalloprotease (PUMP-1),<br>Matrin | Collagen type-IV, X, Gelatin      | Fibronectin, laminin, aggrecan, elastin, proteoglycan link protein | [14,22]    |
|   | MMP-26 | Matrilysin-2  | Collagen type-IV, Gelatin         | Fibrinogen, fibronectin, vitronectin                               |            |
| Transmembrane type (MT)-1 MMPs                            | MMP-14 | MT-1 MMP  | Collagen type-I, II, III, Gelatin | Aggrecan, elastin, laminin, fibronectin, proteoglycan, nidogen     | [15,16,19] |
|   | MMP-15 | MT-2 MMP  | Collagen type-I, Gelatin          | Fibronectin, perlecan, aggrecan, laminin, vitronectin              |            |
|   | MMP-16 | MT-3 MMP  | Collagen type-I                   | Fibronectin, aggrecan, laminin, perlecan                           |            |
|   | MMP-24 | MT-5 MMP  | Gelatin                           | Fibrinogen, proteoglycan   |            |
| Transmembrane type-2 MMPs                                 | MMP-23 | CA MMP  | Gelatin                           |  | [16]       |
| Glycosylphosphatidylinositol-anchored (GPI-anchored) MMPs | MMP-17 | MT-4 MMP  | Gelatin                           | Fibrinogen, fibrin   |            |
|   | MMP-25 | MT-6 MMP, Leukolysin  | Collagen type-IV, Gelatin         | Fibronectin, proteoglycans   | [16]       |
| Other MMPs  | MMP-18 |   |                                   |  | [21]       |
|   | MMP-19 | MMP RASI-1  | Collagen type-I, IV, Gelatin      | Aggrecan, fibronectin, laminin, nidogen                            |            |
|   | MMP-20 | Enamelysin  | Collagen type-V                   | Aggrecan, ameloblast, amelogenin, odontoblast                      |            |
|   | MMP-21 | Cysteine array - CA   | -                                 |  |            |
|   | MMP-22 | Chicken MMP   | -                                 |  |            |
|   | MMP-27 |   | Gelatin                           |  |            |
|   | MMP-28 |   | Gelatin                           |  |            |

The classification of matrix metalloproteinases is based on their substrate specificity. Different members in the group have different specificities to degrade various types of matrix proteins (Table 1). It currently consists of 28 members and among them, 24 are found in humans, including two forms of MMP-23 (MMP-23A and MMP-23B) that are encoded by different genes and further divided into eight different groups [15,16]. Although they share a mostly similar sequence of structures, there are differences in substrate specificity [17].

Most members of the MMPs family share a common structure. As shown in Figure 1, the structure comprises of a pro-peptide of about 80 amino acids, a N-terminal signaling peptide with variable length, a catalytic domain containing a zinc ion (170 amino acids), a hemopexin domain (200 amino acids) and a linker peptide of variable length that connects the catalytic domain to the

hemopexin domain in the hinge region [12]. MMP -14, -15, -16 and -24 have an additional C-terminal domain. Meanwhile, MMP -7, -23, -26 lack a linker peptide and hemopexin domain. MMP-23 has a cysteine array and an Ig-like domain instead of the linker and hemopexin domain.



**Figure 1.** MMPs structure showing signaling N-terminal, propeptide, catalytic domain with zinc ion, hemopexin domain and additional C-terminal.

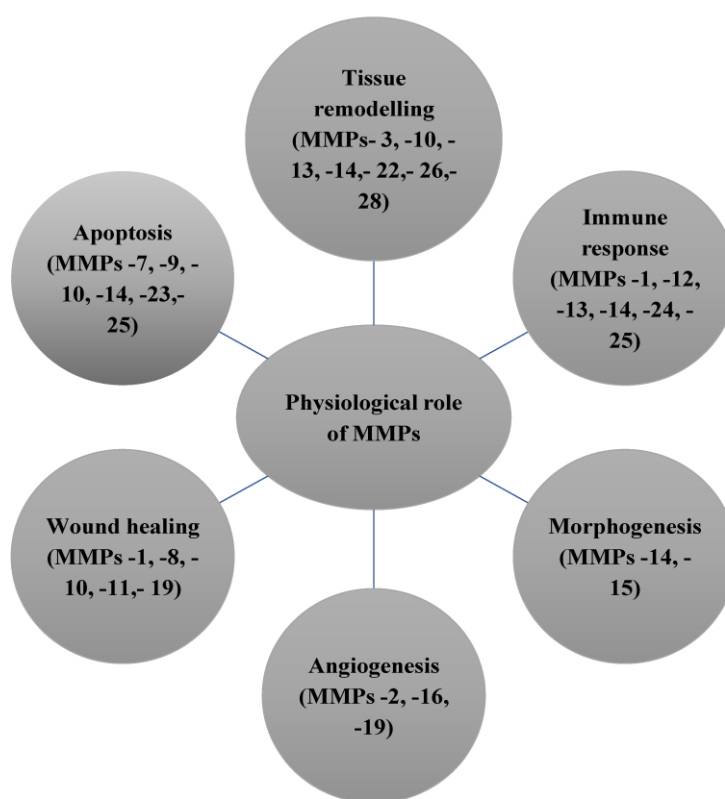
### 3. Pathophysiology of MMPs

Matrix metalloproteinases are responsible for the degradation of almost all ECM components, including fibers (collagen, elastin, laminin, fibronectin), proteoglycans, polysaccharides and thus, play an important role in various physiological and pathological processes [15,18]. The fibers are largely glycoproteins that contain collagen, which is considered as the main ECM protein. Generally, MMPs activities are tightly regulated by maintaining a balance between MMPs and TIMPs levels. Any abnormal alterations of those levels can lead to disease progression [23]. There are four types of endogenous inhibitors, namely TIMP-1, TIMP-2, TIMP-3, TIMP-4 that have been found in humans. They share 40% of structural similarity but differ in their substrate specificities. These inhibitors are involved in ECM turnover, tissue remodeling and cellular behavior which are mediated by MMPs [24,25]. Although Alpha-2 macroglobulin is the major inhibitor in circulating fluid, TIMPs are the more specific inhibitors because they form a tight non-covalent, 1:1 stoichiometric complex when their N-terminal domain chelates the Zn<sup>2+</sup> from the MMPs' catalytic domain, hence regulating the MMPs' enzymatic activity. This complex is not affected by heat denaturation or proteolytic degradation [23,26]. MMPs are responsible for several biologic processes that includes tissue repair, cell proliferation, differentiation, migration, wound healing, morphogenesis, angiogenesis, apoptosis and several other physiological conditions, as shown in Figure 2.

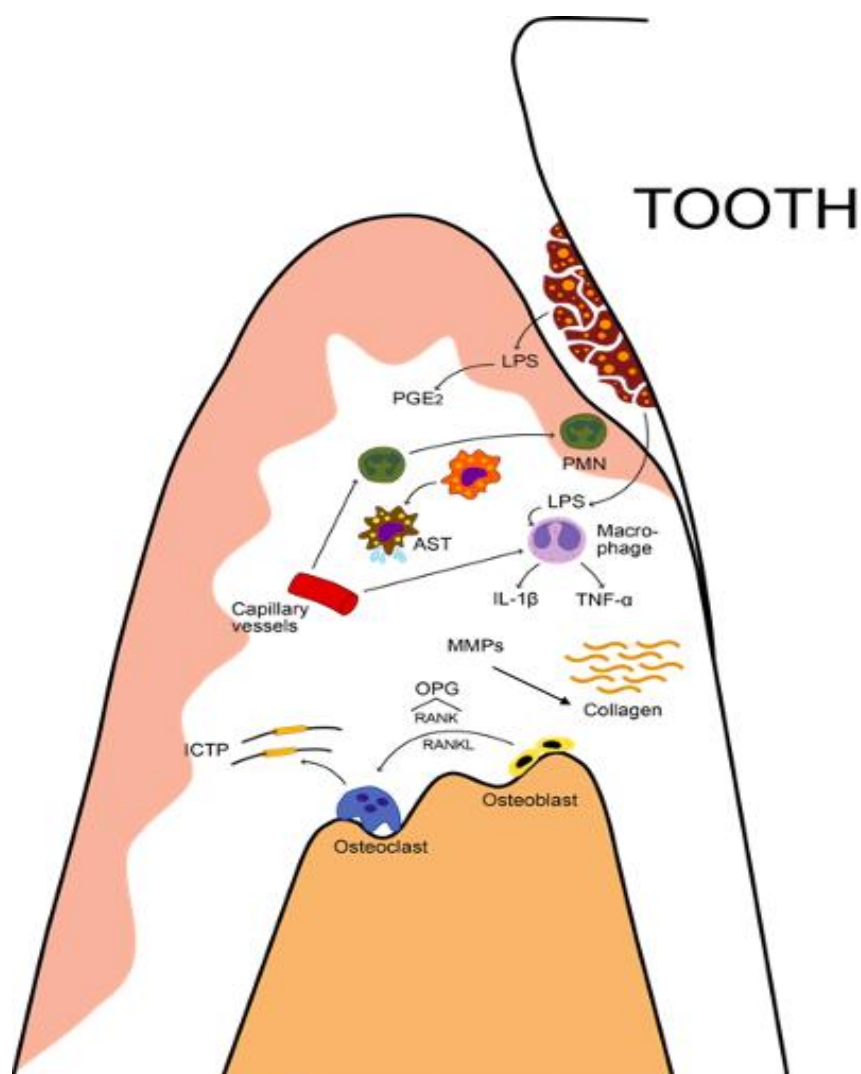
Abnormal regulation of MMPs activity can lead to tissue destruction, fibrosis and degradation of ECM as part of diseases development, including periodontal disease [14]. MMPs degrade ECM components, basement membrane and protective serpins, which also contribute to significant impacts on the cytokine alteration, osteoclastic activation, tissue turnover and loss of connective tissue attachment. Gingival resident cells and periodontal ligament fibroblasts play a role in producing collagenases (MMP -1, -8, -13). Neutrophils and macrophages also serve as a major source of collagenases and gelatinases (MMP -2, -9), which are responsible for the MMPs-mediated destructive periodontal disease. Increased levels of these MMPs are liberated from epithelial cells, which can influence the apical migration and lateral extension of junctional epithelium, thus leading

to the loss of connective tissue attachment [8]. Figure 3 shows the complex mechanism of periodontal disease, characterized by pathogen and host immune responses.

Although several MMPs have a significant contribution to the progression of periodontal disease (such as MMP -2, -7, -14), the most widely reported MMPs responsible for the periodontal pathological condition is the collagenase group (MMP -1, -8, -13). The collagenase group has a unique ability to degrade almost all types of collagen and non-collagen proteins present in the ECM. The ECM comprises of collagen fibrils as well as their related proteoglycans and fibronectin, which must all be eliminated by the collagenase in order to approach the collagen substrate. Previous studies showed that MMP-1 and -8 have stronger associations with cardiovascular disease and diabetes, alongside periodontitis [27]. Table 2 highlights the collagenase group MMPs (-1, -8, -13) substrate, production, physiological functions and association with other diseases.



**Figure 2.** Physiological roles of MMPs.



**Figure 3.** Production of MMPs and inflammatory cytokines in response to bacterial stimulation from macrophages and others gingival resident cells, leading to the collagen degradation of ECM and the loss of connective tissue attachment, osteoclastic activation.

**Table 2.** Collagenase substrates, production, chromosomal location, physiological functions and associated diseases.

| MMP | Name   | Substrate  | Production   | MW KDa<br>Pro /Active | Chromosomal<br>location<br>(Human) | Physiological<br>Function   | Associated<br>Disease's  | References |
|-----|--|--|--|-----------------------|------------------------------------|---|--|------------|
| 1   | Collagenase1<br>/Interstitial<br>Collagenase/<br>Fibroblast<br>Collagenase | Collagen I, II, III,<br>VII, VIII, X, XI,<br>Gelatin,<br>Fibronectin,<br>Aggrecan,<br>Entactin, Tenascin,<br>Ovostatin, Casein | Fibroblast,<br>Keratinocytes,<br>Endothelial<br>cells,<br>Macrophages,<br>Osteoblast,<br>Chondrocytes,<br>Platelet | 55/45                 | 11q22-q23                          | Wound healing,<br>re-<br>epithelialization,<br>cell proliferation,<br>Keratinocyte<br>migration | Periodontitis<br>Rheumatoid<br>arthritis,<br>Atherosclerosis<br>, Fibrosis,<br>Autoimmune<br>disease, Cancer | [14,18,28] |
| 8   | Collagenase2<br>/Neutrophil<br>Collagenase                                 | Collagen I, II, III,<br>Fibronectin,<br>Aggrecan,<br>Ovostatin   | Chondrocytes,<br>Endothelial<br>cell,<br>Macrophages,<br>Smooth muscle<br>cell                                     | 75/64                 | 11q21-q22                          | Periodontal<br>tissue turnover,<br>Anti-<br>inflammatory<br>activity, Wound<br>healing          | Periodontitis,<br>Rheumatoid<br>arthritis,<br>Asthma,<br>Cancer  | [9,20,21]  |
| 13  | Collagenase3   | Collagen I, II, III,<br>IV, IX, X, XIV,<br>Fibronectin,<br>Laminin, Gelatin,<br>Aggrecan,<br>Plasminogen,<br>Osteonectin       | Epithelial cell,<br>Neuronal cell,<br>Connective<br>tissue<br>(Cartilage &<br>Bone)                                | 60/48                 | 11q22.3                            | Osteoclastic<br>activation, Anti-<br>inflammatory<br>activity                                   | Periodontitis,<br>Osteoarthritis,<br>Liver fibrosis,<br>Cancer   | [21,29,30] |



## 4. Collagenases structure and association with periodontal disease

### 4.1. MMP-1 (Collagenase 1)

MMP-1, which belongs to the collagenase group, is also known as collagenase 1, interstitial collagenase, and fibroblast collagenase. MMP-1 was first discovered by Jerome Gross and Charles Lapiere who isolated it from tadpole tail, fin and back skin in 1966 [31]. MMP-1 is liberated as a pro enzyme and synthesized as a single polypeptide. ProMMP-1 is likely activated through a two-advance proteolytic procedure, including either mast cell tryptase or urokinase [19]. Activated MMP-1 can be found in two distinct forms; a major unglycosylated form of 57 kDa and a minor glycosylated form of 61 kDa and it has a gene locus on chromosome 11q22.3 [32]. MMP-1 activity is inhibited by TIMP-1. MMP-1 contains a hemopexin domain that is able to cleave fibrillar collagen, leading to a loosening of the triple-helical structure that leaves collagen or gelatin exceptionally defenseless to degradation by other proteases [19].

MMP-1 plays a key role in several physiological activities, such as embryonic development, reproduction, tissue remodeling and also some pathological conditions such as arthritis, atherosclerosis, fibrosis, cancer, pulmonary emphysema, auto immune disease and also periodontal disease [14,17]. In addition, recent studies demonstrated that MMP-1 is a potential biomarker for the diagnosis and metastasis status of oral squamous cell carcinoma [33] that could have potential impacts on future oral cancer research. MMP-1 serves as a major protease that is responsible for fibrillar bone collagen degradation in localized aggressive periodontitis [34]. MMP-1 can degrade collagen type I, II, III present in the ECM and is usually liberated in inactive form as proMMP-1 [35]. In the periodontium fibrillar, collagen type I and III are predominant and resistant to most proteinases, though they could still be degraded by MMP-1 [36]. MMP-1 is the most abundant component in the periodontal tissue matrix and is usually expressed by the fibroblasts, keratinocytes, osteoblasts, endothelial cells, chondrocytes, macrophages, keratinocytes, platelets and tumor cells [37]. Thus, MMP-1 exerts a potential collagenolytic activity during periodontal inflammation. Studies have demonstrated that expression of MMP-1 by osteoblasts in reaction to resorptive signals can initiate osteoclastic activity, which leads to the degradation of the unmineralized collagenous osteoid layer that then leads to the degradation of collagen and bone resorption in severe periodontal conditions [38].

MMP-1 is lowly expressed in physiological conditions. Various findings demonstrated that MMP-1 was highly elevated in GCF and gingival tissues of periodontitis patients as compared to periodontally healthy subjects [39]. Additionally, there was a remarkable reduction in MMP-1 levels after periodontal treatment, suggesting that these MMPs play a major role in the etiopathogenesis of periodontal diseases [40]. Over expression of MMP-1 mRNA can also be seen in swollen gingival tissue with chronic periodontitis as compared to healthy subjects [37]. Moreover, a significant reduction of MMP-1 levels was found in localized aggressive periodontitis patients after an adequate periodontal therapy [36]. Studies demonstrated increased levels of MMP-1 in the GCF of chronic periodontitis patients that was markedly reduced after receiving periodontal therapy [37,38].

#### 4.2. MMP-8 (Collagenase 2)

MMP-8, also known as collagenase 2 or neutrophilic collagenase, has a significant effect in the etiopathogenesis of periodontal disease. MMP-8 was first identified in 1990 from a patient with granulocytic leukemia [19]. MMP-8 is produced by chondrocytes, endothelial cells, macrophages and smooth and muscle cells. MMP-8 is the vital collagenase within periodontal tissue that regulates almost 90% of the collagenolytic activities within the GCF and saliva in periodontal disease [41]. It is the major host cell-derived collagenase obtained from neutrophils responsible for the degradation of collagen in gingival and periodontal ligaments and conducts periodontal tissue destruction [42]. Several studies have already evidenced MMP-8 as the most accurate diagnostic biomarker in the GCF for periodontitis. Also, MMP-8 has been established as the most needed biomarker for recent periodontal disease classification [43]. MMP-8 has remarkable similarities in structure and physiological function to MMP-1 and shows greater affinity to type I collagen in comparison to MMP-1. Studies have shown that MMP-8 plays a role in periodontal tissue turnover, degradation and wound healing and is highly associated with several pathological conditions such as rheumatoid arthritis and asthma [44]. Elevated MMP-8 concentrations have been reported in patient with diabetes and hypercholesterolemia and its expression was significantly reduced after treatment, which proved its association with the diseases [27].

Previously, MMP-8 was thought to be orchestrated distinctly by neutrophils, and consequently it was thus named neutrophil collagenase and polymorphonuclear leukocyte collagenase [9]. However, it is presently understood that other cells can also produce this protease [9]. The size of a mature MMP-8 enzyme is 64 kDa with glycosylation increasing the size to 75 kDa and it has a gene locus chromosome of 11q22.3 in humans [19]. Its expression is inducible and upregulated by several inflammatory cytokines (IL-1 $\beta$ , TNF- $\alpha$ , CD40 Ligand) [45]. The basic protein structure of MMP-8 comprises of a signal peptide, propeptide, catalytic domain, hinge region and hemopexin domain. Pro-peptide removal is induced by MMP-3 and -7, initiating the MMP-8 proteolytic activity. The common inhibitors for MMP-8 are TIMP-1 and -2 that restrict the overexpression of the enzyme during physiological conditions [9,19].

MMP-8 is the most promising biomarker for predicting, diagnosing, treatment prognosis and grading of periodontal disease [43]. Various commercially available ELISA kits or immunofluorometric assays (IMFA) kits are used to detect this enzyme [42]. Several studies have reported significant increases of MMP-8 levels in subjects with chronic periodontitis and periodontitis with diabetes [23]. In periodontitis patients, elevated levels of MMP-8 could be found in oral fluids [26]. Another study has reported elevation of MMP-8 levels in the GCF due to the presence of periodontal pathogens such as *T. denticola* and *T. forsythia*, which represent a cascade of host response induced by these organisms [46]. Effective periodontal treatment and adjunctive MMP-inhibitory drugs have been postulated to inhibit the progression of periodontal disease, which also lessen the level of MMP-8 in the GCF and saliva [41]. Subantimicrobial-doses of doxycycline (SDD) have been widely accepted as an important adjunctive therapy in the treatment of periodontitis and several studies have already shown its efficacy. Currently, the available collagenase inhibitors approved by the United States Food and Drug Administration are tetracycline analogues and doxycycline hyclate [47]. A combination therapy of scaling, root surface debridement along with

MMP inhibitors, significantly increased the clinical attachment level and probing depth in chronic periodontitis, which may be an effective approach to the treatment of periodontal disease [47]. Conventional hygiene-phase periodontal treatment and non-surgical periodontal treatment also help to reduce the elevation of MMP-8 level in oral fluids [41]. This was proven when MMP-8 expression was reduced in response to periodontal treatment, with improved periodontal pocket depth and clinical attachment level of the periodontal ligament [37,48]. Hence, MMP-8 can be considered as a potential biomarker for the chairside diagnosis of periodontitis and peri-implantitis patients [49].

#### 4.3. MMP-13 (Collagenase 3)

MMP-13, also known as collagenase 3, was first discovered in 1994 in breast cancer tissue [19]. It has the ability to degrade almost all types of collagens, particularly fibrillar collagens present in the ECM, fibronectin, aggrecan, gelatin and it is an important element for new bone matrix formation [50]. MMP-13 is expressed by various cells of the periodontium, such as connective tissue and epithelial cells. Significant involvement of MMP-13 was found in periodontitis, rheumatoid arthritis and osteoarthritis [30,51].

MMP-13 is secreted by several cell types as a 60 kDa precursor form (proMMP-13) and the molecular weight of the mature enzyme is 48 kDa [29]. The basic component in the MMP dynamic site is a zinc particle facilitated by a tris (histidine) motif [52]. MMP-13 is structurally similar to other MMPs, with a catalytic domain, a linker region, and a hemopexin domain. The catalytic structure of the MMP-13 is the N-terminal that makes a salt extension with the Asp257 of helix 3, similar to those found in MMP-1 and MMP-8. This salt bridge plays a vital role in the MMP-13 collagenase activity [53].

MMP-13 is produced by leucocytes and resident cells and play a vital role in periodontal disease pathogenesis [54]. MMP-13 is responsible for osteoclastic activities that include the destruction and resorption of bone and cartilage [51]. Overexpression of MMP-13 presented in saliva has been documented in localized periodontitis compared to generalized periodontitis [55]. A previous study demonstrated the presence of MMP-13 in peri implant sulcular fluid level, which might play a vital role in the pathological bone destruction process [50]. Additionally, an elevated inflammation response increases production of MMP-13, which leads to periodontal tissue destruction [56]. Moreover, raised MMP-13 expression in the GCF was related with dynamic periodontal infection, supporting the significance of this enzyme in alveolar bone destruction [54]. Massive MMP-13 elevation in the GCF was also associated with expanded alveolar bone annihilation in chronic periodontitis cases [57]. However, the MMP-13 level in the GCF of chronic periodontitis patients was not reduced by standard therapeutic treatment, which might need additional host modulation [57].

## 5. Diagnostic importance

Currently, MMPs are known to be as one of the most important diagnostic biomarkers for periodontitis detection and several other diseases [24]. It is important to diagnose diseases earlier for prevention and better treatment planning. However, diagnosis of periodontal and peri-implant

diseases mostly depends on traditional diagnostic methods, which includes probing pocket depth, clinical attachment level and radiographic evaluation [43]. All of these methods are only able to demonstrate current disease severity rather than disease activity and its episodic progression pattern. Moreover, traditional diagnostic methods are invasive and uncontrolled probing force can lead to bleeding and infection [58]. Therefore, point of care (PoC) chairside testing is the current mode of diagnostic activity, which enables rapid tests that can be done outside clinical laboratories and have been validated by various countries around the world [58,59]. PoC tests can be categorized as microbial test kits, biochemical test kits and genetic test kits as shown in Table 3. These test kits are developed to detect potential periodontal pathogens, host-derived enzymes, inflammatory mediators, genetic polymorphism from dental plaque, GCF and saliva for the monitoring of periodontal disease severity and activity in a convenient way [60]. Activated MMP-8 (aMMP-8) is the major collagenase whose elevation and activation are reflected mostly within the oral fluids of periodontitis patients compared to healthy individuals [59]. Therefore, an aMMP-8 PoC oral mouth rinse test has been developed and it is non-invasive, inexpensive, rapid and safe, which makes it a promising chairside test to detect periodontal disease activity, severity and progression pattern [61]. The diagnostic sensitivity and specificity of the aMMP-8 PoC is 76–90% and 96% respectively [59]. In addition, this chairside test showed promising results as an early predictor for developing periodontitis in adolescents and adults as reported by several studies [58,62,63]. Furthermore, the aMMP-8 PoC mouth rinse test was integrated into the recent periodontitis classification that improved the diagnostic accuracy of periodontal disease [43]. In contrast, a recent study has reported that the sensitivity and specificity of aMMP-8 PoC were reduced with patients with impaired immune function such as Crohn's disease and rheumatoid arthritis [64]. Nevertheless, the development of PoC chairside tests has made a significant contribution to the management of periodontal disease at its early stage.

On the other hand, several other diagnostic methods such as enzyme linked immunosorbent assay (ELISA) and immunofluorometric assay (IFMA) are carried out to detect MMP -1, -8, -13 levels in the GCF, saliva and tissue samples as shown in Table 4. The ELISA test is mostly carried out to detect MMP-1/TIMP-1, MMP-13 levels in the GCF because of its efficacy, improved sensitivity, ease of use, flexibility, adaptability and simple but powerful instrumentation. Currently, commercially available ELISA kits are used for MMP -1, -13 for diagnostic purpose [37,66,67]. Hence IFMA is commonly used to detect MMP-8 level in oral fluids [68,69].

**Table 3.** Different PoC test kits importance and limitations.

| Test type                             | Importance  | Limitations  | Kits   | References |
|---------------------------------------|---|--|--|------------|
| Microbial test kits                   | Used to detect pathogens responsible for periodontal disease as well as monitor their suppression and eradication | Multistage testing, results could take a longer time.            | OMNIgene <sup>®</sup> (DNA Gentok Inc, Canada)<br>Evalusite <sup>™</sup> (Kodak, Eastman company, Switzerland)<br>My Periopath <sup>®</sup> (OralDNA Labs, USA)<br>Perioscan <sup>®</sup> (Oral B Laboratories, USA) | [65]       |
| Biochemical test kits                 | Used to evaluate host derived enzymes, inflammatory mediators in the GCF  | Multiple steps of testing, interproximal site cannot be sampled  | Prognostick (Dentsply)<br>Periocheck (Actech Inc. USA)<br>PerioGard <sup>™</sup><br>PocketWatch <sup>™</sup>   | [60]       |
| Genetic test kits                     | Identification of IL-1 $\alpha$ and IL-1 $\beta$ genes polymorphism to detect periodontal disease severity        | Limited to specific genes  | PST <sup>®</sup> Genetic susceptibility test<br>MyperioID <sup>®</sup> (OralDNA Labs, USA)   | [65]       |
| aMMP-8 PoC/Oral mouth rinse test kits | Used to detect aMMP-8 level in oral fluids.   | Results may be inaccurate with impaired immune function diseases | PerioSafe <sup>®</sup> PRO DRS (dentognostics GmbH, Germany)   | [59,64]    |

**Table 4.** Studies on collagenase diagnostic methods, sample types and clinical findings on periodontitis.

| MMP | Sample & Diagnostic method  | Clinical findings  | References |
|-----|---|--|------------|
| 1   | Tissue sample, ELISA (sandwich ELISA kit, Quantikine; R&D system) | Elevated amount of MMP-1 observed in periodontitis affected tissue compared to healthy tissue.   | [39]       |
|     | Gingival crevicular fluid, Immunofluorometric MMP kit             | Increased amount of MMP-1 in localized aggressive periodontitis patients, which reduced significantly after periodontal therapy (scaling, root planing, antibiotic treatment)  | [70]       |
|     | GCF, Ray Bio human MMP ELISA kit                                  | Increased MMP-1 level in chronic periodontitis patients compared to healthy group, which reduced after phase 1 periodontal therapy   | [38]       |
| 8   | GCF, Saliva, Serum. Time-resolved Immunofluorometric Assay (IFMA) | Study reflects aMMP-8 level in oral fluid and serum in healthy, gingivitis and stage 3 periodontitis subjects within the updated periodontal disease classification. Significant increase in MMP 8 level found in gingivitis and periodontitis patient. However, higher in periodontitis patient compared to gingivitis and healthy group. | [68]       |
|     | GCF, Saliva. Immunofluorometric Assay (IFMA)                      | Study reflects elevated MMP-8 level in saliva and GCF associated with periodontitis, increased periodontal pocket depth and bleeding on probing.   | [69]       |
|     | GCF, Immunofluorometric Assay (IFMA)                              | MMP-8 level increased with the severity of periodontitis and decreased after successful treatment.   | [71]       |
| 13  | Saliva, Human MMP-13 ELISA test kit                               | Salivary MMP-13 concentration increased in chronic periodontitis patients compared to healthy group. Higher periodontal parameters (PPD, CAL) demonstrated collagen fiber destruction.   | [66]       |
|     | Saliva, Human MMP ELISA kit                                       | Higher salivary MMP-13 level found in periodontitis patient compared to healthy group.   | [67]       |
|     | GCF, ELISA kit  | Higher MMP-13 level found in chronic periodontitis patients.   | [51]       |

## 6. Limitations of current studies and future consideration

MMPs are considered as potential diagnostic biomarkers in periodontitis and other diseases. Research has focused on the development of diagnostic kits as well as MMP inhibitory drugs as adjunctive periodontal therapy. However, some of the diagnostic methods such as aMMP-8 PoC chairside test results could be influenced by impaired immune system diseases and need further investigation. Moreover, further development in the MMP inhibitors is needed to suppress only disease related MMPs, while not inhibiting MMPs which are beneficial to the host physiological process. Also, it is important to understand MMPs and TIMPs structure, roles and functions for the development of better diagnosis and therapies in the future.

## 7. Conclusion

In conclusion, this review discussed up-to-date information regarding MMP -1, -8 -13 and their vital roles in periodontitis. There are numerous evidence showing the overexpression of these MMPs in the progression of periodontitis, which was reduced after appropriate periodontal therapies such as scaling and root surface debridement. The use of MMPs inhibitors as an adjunctive treatment could also have a potentially beneficial effect on the recovery of periodontal disease. MMP -1, -8, -13 can be strong predictors of the severity and activity of periodontitis. Also, their association with other diseases has been observed.

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## Conflict of interest

All authors declare no conflict of interest in this paper.

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