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Chapter 2

ROLES OF MATRIX METALLOPROTEINASES IN PERIODONTAL DISEASES AND DENTAL CARIES

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ABSTRACT

This chapter summarizes the relationship between matrix metalloproteinases (MMPs) and the two most common oral pathologies (i.e., periodontal diseases and dental caries). MMPs play central roles in the physiological and pathological remodeling and destruction of oral tissues. Periodontal diseases result from host inflammatory reactions initiated by bacterial plaque accumulation leading to clinical periodontal attachment loss, alveolar bone resorption and ultimately tooth loss. Dental caries is a bacterially induced disease that progresses when acids produced by the bacteria causes dental hard tissue demineralization. A carious lesion starts either in the enamel or cementum and spread to the underlying dentin. MMPs in the saliva or stored in the dentin are

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activated in the acidic conditions produced by the bacteria and then cleave the collagen in the dentin, thus enhancing the lesion. MMPs may also play similar roles in composite resin restoration failures (i.e., secondary caries). Since collagen is a major component in both dentin and periodontal tissues, members of the MMP family clearly play major roles in the tissue remodeling and destruction associated with both diseases. The understanding of the roles played by MMPs and their inhibitors in both diseases is critical for exploring therapeutic options that may potentially inhibit the tissue destruction progression seen in both conditions.

MATRIX METALLOPROTEINASES AND PERIODONTAL DISEASES

Periodontal diseases are defined as chronic inflammatory diseases of the tooth attachment structures. They are considered the most common causes of tooth loss in adults and one of the most prevalent forms of bone pathologies in humans [1]. The bacterial biofilm attached to teeth surfaces trigger an intense inflammatory reaction that results in irreversible loss of periodontal tissue attachment and alveolar bone resorption [2]. Matrix metalloproteinases (MMPs) are believed to play key roles in the tissue destruction associated with chronic inflammatory conditions such as periodontal diseases. In the oral cavity, proteolytic enzymes (i.e., MMPs) are mainly released by the host cells such as fibroblasts, endothelial cells, polymorphonuclear leukocytes (PNLs), macrophages, osteoblasts, and epithelial cells [3-6]. MMPs are responsible for degrading most of the extracellular matrix (ECM) components and are thus implicated in the tissue destruction and loss of the supporting connective tissue. Reports have suggested that MMP-1, -2, -3, -8, -9, and -13 are among the most important mediators of periodontal tissue destruction [3-6].

Chronic periodontitis is the most common form of periodontal diseases and it affects more than 30% of the adult population [7]. It is considered a multifactorial disease, in which the degree of susceptibility, progression rates, and treatment outcome depends on various environmental and genetic factors. Multiple etiological factors are thought to contribute to the pathogenesis of periodontitis including poor oral hygiene, systemic diseases, genetic predisposition, smoking, and the composition of the oral microflora [8, 9]. However, the inflammatory reactions initiated in response to the bacterial insult in the subgingival area and the connective tissue destruction associated with these processes is the hallmark of chronic periodontitis [10].

The evidence for the roles of MMPs in periodontal tissue destruction has accumulated over the years and it is now well accepted [3-6]. Dental plaque and the bacterial biofilm, as well as the toxins and virulence factors produced by the periodontopathogenic bacteria, induce host cells to *de novo* express MMPs. In contrast to healthy gingival tissues, extracts of diseased gingival tissue and gingival crevicular fluid (GCF) from periodontitis patients contain significantly elevated levels of MMPs. Reports have also confirmed that the amount of MMP level elevation was directly related to the rate of disease progression and that different forms of periodontal treatment were capable of reducing their levels dramatically, which strongly suggest direct association [11-17].

The predominant periodontal ECM components are collagen types I and III, which are mainly produced by the gingival and periodontal ligament cells. The initial breakdown of gingival and periodontal ligament collagens is the fundamental feature of active and progressive periodontal lesions, pocket formation, and bone loss [3]. This initial cleavage is believed to be mainly carried out by the host cell-derived interstitial collagenases. Fibroblast-type collagenase (MMP-1) is a major proteolytic enzyme that can cleave native interstitial collagens type I and III [3, 4]. Many reports have indicated that MMP-1 plays a major role in the tissue destruction that occurs in periodontal diseases [18-22]. MMP-1 has been identified in inflamed gingival tissues and in the granulation tissues of chronic periodontitis patients [18]. A single nucleotide polymorphism in the promoter region of the human MMP-1 gene was shown to be associated with a risk for severe chronic periodontitis and was thus suggested as a genetic marker for the disease [20]. Studies have also shown that MMP-1 together with other MMPs, such as MMP-3, initiate the ECM destruction and is responsible for most of the proteolytic activities expressed by cultured gingival fibroblasts. MMP-3 plays a role in activating pro-MMP-1, as well as pro-MMP-8 and pro-MMP-9. Significantly higher MMP-1 and MMP-3 levels have been reported in gingival fibroblasts after stimulation with interlukin-1 (IL-1β) and tumor necrosis factor (TNF-α). Evidence has shown that MMP-1 and MMP-3 in periodontitis tissues are locally produced by the host fibroblasts and then activated, thus participating in periodontal tissue destruction [18-22].

Gelatinases, also called type IV collagenases (gelatinase A or MMP-2 and gelatinase B or MMP-9), are known for their high proteolytic activity against denatured collagens. A number of studies have aimed at correlating MMP-2 and MMP-9 activities with severity of periodontal diseases [23-26]. They have been shown to be expressed by different cells in the oral cavity and some

reports have demonstrated that the amount of gelatinases increases during active disease periods and that their levels were significantly reduced after conventional periodontal treatment. Further, substantial increases of their active forms with time were observed only at sites with progressive periodontal tissue destruction. A study indicated that periodontal tissue breakdown was associated with the conversion of proMMP-9 to its activated form in the GCF [26]. MMP-9 was also detected in the junctional and pocket epithelium of inflamed gingival tissues and was thus suggested that, in addition to neutrophils that are the main cellular origin of MMP-9, oral keratinocytes contribute to the gingival responses to periodontal infection most probably mediated, in part, by the activity of MMP-9 [23].

Collagenase-3 or MMP-13, another member of the interstitial collagenase subfamily of MMPs, has also been reported to be expressed by periodontitis-affected human gingival sulcular epithelium and gingival fibroblasts [27-31]. Gingival fibroblasts responded to TNFα stimulation by an increase in MMP-13 gene expression [29]. MMP-13 and MMP-14 were detected in the bony resorption lacunae and on ruffled border of osteoclasts suggesting their involvement in control of osteoclast attachment to the bone surface, as well as to the osteoclast–matrix interactions that control the bone remodeling process. These findings lead some investigators to propose that due to its important role in bone remodeling, MMP-13 levels could reflect the amount of alveolar bone loss as a result of periodontitis and peri-implantitis [30, 31]. It was also recently shown that MMP-13 degrades fibrinogen and Factor XII of the plasma clotting system. These properties of MMP-13 were directly linked to bleeding, which is considered a significant clinical feature of periodontal diseases [32, 33].

Increased mRNA expression for MMP-8 has also been reported in inflamed gingival tissues [27, 32]. Associations between increased MMP-8 levels and activity with increasing periodontal diseases severity and tissue destruction have also been demonstrated [34-36]. It was shown that clinical improvement obtained by periodontal treatment was directly correlated to significant reductions in MMP-8 levels. The mean MMP-8 concentration in the saliva and GCF of the healthy individuals was 10-fold less than that of the periodontally compromised patients. This suggested that MMP-8 might be a potential marker of periodontal disease status and that measuring its level could possibly provide a practical periodontal diagnostic tool. A rapid chair-side test based on the immunological detection of levels of MMP-8 was thus developed for the purpose of monitoring the course of periodontitis, as well as to monitor the effects of various treatment modalities [34-36].

The activation of the MMPs and their proteolytic activities are physiologically regulated at multiple levels including gene expression, conversion of proenzyme to active enzyme, and through their interactions with a family of endogenous proteins named the tissue inhibitors of metalloproteinases (TIMPs) [37]. TIMPs are known to be capable of inhibiting MMP activities. The health and integrity of the connective tissue matrix, as well as controlling the remodeling process, are maintained by a careful balance between activated MMPs and their endogenous inhibitors. The ECM breakdown associated with the majority of inflammatory diseases results from the loss of this balance and most probably in the form of elevated activated MMP levels that is uncompensated with a concomitant increase in TIMPs, thus resulting in tissue destruction [3, 37]. Among the TIMP family members, TIMP-1 and TIMP-2 have demonstrated inhibitory effects against fibroblasts and PNLs derived MMPs and have both been found in periodontal tissues excised from active lesions [38]. Reports have demonstrated that the MMPs and TIMPs are constitutively expressed at low levels in healthy periodontal tissues and significantly higher levels after exposure to periodontopathogenic bacteria. Some investigators have also confirmed that different levels of MMP/TIMP expression in the course of experimental periodontal disease are directly related to differences in the rate of disease progression [3, 38-40].

Since evidence for the roles of MMPs in periodontal destruction has grown over the years, they were frequently considered as potential targets for adjunctive periodontal treatment to inhibit or down-regulate periodontal tissue destruction and periodontal attachment loss. Both broad-spectrum and/or selective MMP inhibitors have been considered [41-43]. A number of synthetic inhibitors, including tetracycline analogues, have demonstrated promising therapeutic potential. The non-antimicrobial chemically modified tetracyclines reduced excessive periodontal collagenase activity, as well as prevented severe alveolar bone loss in animal models of periodontitis and in periodontitis-affected diabetic rats [41]. In human clinical studies, non-antimicrobial low doses of the commercially available semi-synthetic tetracycline (i.e., doxycycline) decreased MMP activity in the GCF and gingival tissues. These changes were associated with reductions in both pocket depth and attachment loss, as well as with reductions in the severity of the periodontal breakdown and bone resorption [41-43].

Several studies suggested the use of the sub-antimicrobial dose doxycycline (SDD) to control and suppress the progression of periodontal diseases [44-48]. More importantly, its use as a periodontal adjunctive therapy has been shown to be safe and tolerated since they have no antibiotic effects

against the pathogenic bacteria, thus ruling out the risk of developing any antibiotic resistance. The therapeutic action observed is mainly due to the modulation of the host responses and inhibition of the mediators of connective tissue breakdown, primarily MMP-8 and MMP-13. Currently, the tetracycline analog "doxycycline hyclate" is the only collagenase inhibitor approved by the United States Food and Drug Administration (FDA) and is available for use specifically in periodontal diseases [44-48].

Since doxycyclines have been reported to significantly down-regulate MMP activity, research has focused on exploring the mechanisms involved in their collagenolytic inhibition and their clinical effects [49-51]. Suggested mechanisms included their direct MMP inhibitory function through their calcium- and zinc-binding properties, their scavenger role and ability to reduce the PNL-derived reactive oxygen metabolites, and subsequently preventing the hypochlorous acid (HOCl) oxidation and inactivation of other MMP inhibitors such as the α_1-PI and α_2-macroglobulin. Taken together, tetracyclines and SDD through their direct ability to inhibit MMP activity and the reactive oxygen metabolites such as HOCl represents an important pathway for modulation of the destructive connective tissue events associated with chronic periodontitis and represent promising opportunity for introducing effective adjunctive treatment strategies in the management of patients with periodontal diseases [49-51].

In conclusion, MMPs released by the host cells are directly associated with the process of tissue destruction in periodontal diseases since they can degrade most of the ECM components as a sequel of inflammation. A body of evidence in the literature supports their key involvement in this destructive process as well as the roles of their inhibitors in distinct therapeutic options (Table 1).

Table 1. MMPs currently known to be involved in periodontal diseases

MMP (Alternative name)	References
MMP-1 (Collagenase-1)	[3-5, 13, 15, 17-22, 32, 33, 38]
MMP-2 and MMP-9 (Gelatinase A and Gelatinase B)	[3-6, 11-14, 23-26, 32, 33, 38]
MMP-3 (Stromolysin-1)	[3-5, 10, 11, 13, 17-19, 22, 28, 30]
MMP-8 (Collagenase-2)	[3-5, 10-14, 16, 17, 27, 29, 30, 32, 34-36]
MMP-13 (Collagenase-3)	[3-5, 12-14, 17, 27-33, 38]
TIMP-1 and TIMP-2	[3-5, 37-40]

MATRIX METALLOPROTEINASES AND DENTAL CARIES

The human tooth is a unique structure composed of enamel, dentin, cementum, and pulp. Enamel is a highly mineralized acellular tissue that forms the outer crystalline surface of teeth and is composed of 99% by weight of inorganic material [52]. The enamel formation is a complex process that can be divided into four stages: presecretory, secretory, transition, and maturation [53]. During the secretory stage, ameloblast cells secrete the enamel matrix proteins and crystallites are formed as well. The ameloblast cells also secrete structural proteins (amelogenin, ameloblastin, and enamelin) and MMP-20 (enamelysin) into the enamel matrix [53, 54]. During the transition and maturation stages, the ameloblasts control the degradation and reabsorption of the organic matrix proteins to create space for the enamel crystallites to expand in volume and thickness, a process that is followed by the mineral deposition [53, 54].

MMP-20 has been detected in ameloblasts and odontoblasts cells during the initial to mid-stages of the secretory phase of amelogenesis. After being secreted into the enamel matrix, enamelysin is capable of degrading amelogenin, which is predominantly organic dentin [55-57]. Studies have also shown that MMP-20 becomes entrapped in the dentin tubules and could be later released in carious dentin [55, 56].

The dentin is a protein-rich tissue that contains ~ 70% by weight inorganic material (mainly hydroxyapatite), ~ 20% by weight organic material (mainly collagen type I and a small amount of collagen type V and non-collagenous components) and ~ 10% water [58-60]. Reports have suggested that MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, and MMP-20 have been detected in different sites and concentrations in human dentin [59, 61-66].

A study investigated the different concentrations of MMPs in sound dentin, non-sclerotic dentin, and sclerotic dentin of attrited teeth using fluorescent microsphere immunoassays [59]. MMP- 1 was reported to be present at the same concentrations in all evaluated dentin [59]. In another study, MMP-2 and MMP-3 had higher concentrations in sound dentin than in non-sclerotic and sclerotic dentin [67]. MMP-8 and MMP-9 concentrations in sclerotic dentin were higher than in non-sclerotic dentin of attrited tooth and sound dentin [67].

MMP-2 is the predominant gelatinase in the human dentin. MMP-2 was reported to be highly concentrated in odontoblasts, predentin and dentin enamel-junction (DEJ) and was also detected in demineralized crown and root dentin as well [63, 64]. MMP-2 was linked with the dentin and DEJ

establishment [62]. MMP-3 activity was also detected in human dentin and odontoblast. It was localized at sound dentin and intertubular collagen fibrils [65]. This MMP releases dental components such as proteoglycans (decorin and biglycan) and four of the members of SIBLING family (small integrin-binding ligand N-linked glycoproteins): dentin sialoprotein, matrix extracellular phosphoglycoprotein, bone sialoprotein, and osteopontin [68].

MMP-8 has been reported to be evenly distributed among crown and root dentin [64]. A study which identified MMP-8 expression by human odontoblast and pulpal cells suggested that MMP-8 contributes to the organization of the dentin organic matrix and that the latent form of this MMP might be incorporated into the organic matrix during the steps of mineralization [66]. MMP-9 was also detected in non-mineralized fractions of crown and root dentin [64].

Enamel and under certain circumstances dentin/cementum are in direct contact with saliva. Saliva is 99% water and the remainder is mainly made up of sodium, potassium, calcium, magnesium, bicarbonate, phosphates, chloride, proteins, and enzymes [58, 69, 70]. The average flow of saliva distributed from the different salivary glands range approximately1 to 1.5 L/day and the variance is usually caused by an individual's general health [69]. A thin pellicle called "acquired enamel pellicle" grows on the teeth as a result of biopolymer adsorption at the interface between teeth and saliva [71, 72]. This pellicle contains salivary proteins, glycoproteins, bacterial polymers and lipids that act as a selective barrier by regulating the demineralization/ remineralization processes and helps determine the initial microbial colonization on the tooth [72, 73]. The roles that saliva plays in oral health includes the maintenance of tooth integrity (inhibiting the demineralization and enhancing the mineralization), controlling bacterial growth, neutralizing organic acids from the dental plaque and buffering activities [58, 69, 70, 74].

Despite being critical for the maintenance of oral health during the carious process, saliva contacts the damaged dentin by penetrating the caries lesion (75). The saliva/dentin contact could be detrimental since several MMPs such as MMP- 8 [76, 77], MMP-2 and MMP-9 [23, 77] have been detected in saliva. Part of the MMP-8 detected could have origin in partially demineralized carious dentin [76]. Gingival keratinocytes can produce MMP-9, while MMP-2 could originate from the connective tissues and gingival fibroblasts (23). Patients with caries lesions were reported to have high levels of salivary MMP-8 when compared with healthy subjects [76].

The demineralization/remineralization process associated with dental caries development occurs several times every day. The demineralization

occurs after sugar ingestion when the pH in the plaque falls below 5.5 or as a result of the dental biofilm that can induce demineralization cycles due to the acids being produced by the bacteria [77, 78]. At this point, the bicarbonates, phosphates, and urea present in saliva modulate the pH and the buffering capacity of the saliva. In addition, the calcium, phosphate, and proteins modulate the demineralization/remineralization process [69]. The remineralization occurs when the cariogenic challenge is minimized [79]. However, the demineralization process can be followed by the destruction of the collagenous organic matrix of dentin and specific microbial colonization (77, 80). *Streptococcus mutans*, *Streptococcus sobrinus* and *Lactobacilli* are types of bacteria that are correlated with the caries process [81].

Dental caries is the result of the disproportion between the demineralization/remineralization process with significant mineral loss caused by the organic matrix exposure [75, 82]. A combination of factors results in localized dental destruction associated with a triad described by Keyes and Jordan that includes the presence of carbohydrates in the diet, the susceptibility of the involved teeth and bacteria from the dental plaque [83]. Recently, the teeth susceptibility factor has been questioned [84]. However, the dental bacteria are clearly involved with the caries process. Currently, it is known that the bacteria by itself is not responsible for the degradation of the dentin collagen [5, 75, 77, 85]. When the mineral portion of the tooth is dissolved, the organic matrix is exposed to enzymes derived from the bacteria and to host-derived enzymes, such as MMPs, from the dentin, pulp, and/or saliva [75].

Human caries start either in the enamel or cementum, which possibly leads to expansion to the underlying dentin. The process can result in a significant loss of the tooth mineral and degradation of the organic matrix. It was recently suggested that during the caries process, the bacterial damage results in demineralization (hydroxyapatite is solubilized), while MMPs mediate the degradation of the demineralized dentin organic matrix [77, 86].

The activation of inactive pro-MMPs can occur at different stages of the formation of carious lesions by acidic condition (pH 4.5) [75, 77, 87]. After the activation, the enzymes become stable by neutralization provided by the saliva. This results in an environment in which the exposed collagen fibers are susceptible to be degraded by the pH- activated MMPs [75, 77].

When considering caries affected dentin and normal dentin, distribution of MMP is variable [59]. Similar levels of MMP-2 were found in outer caries, inner caries, and normal dentin while MMP-8 and MMP-9 were detected in higher levels in outer caries when compared to inner caries. That could be

credited to the MMPs found in the saliva [77]. The effects of salivary MMPs on the outer caries were suggested as a contributing factor to additional breakdown of the dentin matrix. MMP-20 was also detected in higher levels in the normal dentin when compared to inner and outer caries [59].

Numerous studies have identified MMPs in the caries lesions in dentin (Table 2). The MMPs have been detected in different portions of the tooth structure. Recent studies have been focused on developing materials that could regulate the MMP activity after dental restorative treatment in order to improve the bond strength to dentin and to skip the possible deleterious effects caused by the acid activation of MMPs.

Table 2. MMPs currently known to be involved in dental caries and restoration failures

MMP (Alternative name)	References
MMP-1 (Collagenase-1)	[61, 86, 120]
MMP-2 and MMP-9 (Gelatinase A and Gelatinase B)	[59, 62-64, 67, 77, 86, 88, 90-91, 106, 114]
MMP-3 (Stromolysin-1)	[65, 67-68, 86, 88, 91]
MMP-8 (Collagenase-2)	[59, 61, 64, 66-67, 76-77, 86, 88, 91]
MMP-20 (Collagenase-3)	[55-57, 59]

MATRIX METALLOPROTEINASES IN SECONDARY CARIES AND RESIN COMPOSITE RESTORATIONS FAILURE

Among the enzymes found in dentin, the MMPs have recently gained much attention due to their possible roles in several physiological and pathological processes in dentin (i.e., dental caries). Interest has been especially focused on the roles that MMPs play in secondary caries and the degradation of the demineralized dentin that is infiltrated by an adhesive resin at the hybrid layers/resin-dentin interface [88].

MMPs play crucial roles during tooth development and are trapped within the mineralized dentin matrix [89]. Studies have reported that MMP-2, -3, -9, -8, and -20 are expressed in human odontoblasts and pulp tissue. MMP-1, -2, -3, -8, -9, and -20 have all also been identified in dentin [65, 85, 86, 88, 90, 91].

MMPs are secreted as zymogens or inactive pro-MMPs. Pro-MMPs can be activated by proteases, other MMPs, heat, and low pH. The activation of MMPs occurs through a process called the "cysteine switch" where the bond between the cysteine's sulphydryl group of the pro-domain and Zn^{2+} ion of the catalytic domain is disrupted (Figure 1). As a result, active MMPs are able to digest type I collagen in dentin. The collagenolytic process of MMPs involves a three-step process. These include binding, unwinding and cleaving of the collagen. The first cleavage step of type I fibrillar collagen occurs at a specific cleavage site between Gly/Ile of the α1(I) chain and between the Gly/Leu bond α2(I). This generates ¾ N-terminal fragments and ¼ C-terminal fragments. These fragments are further digested by gelatinases and other proteinases [92].

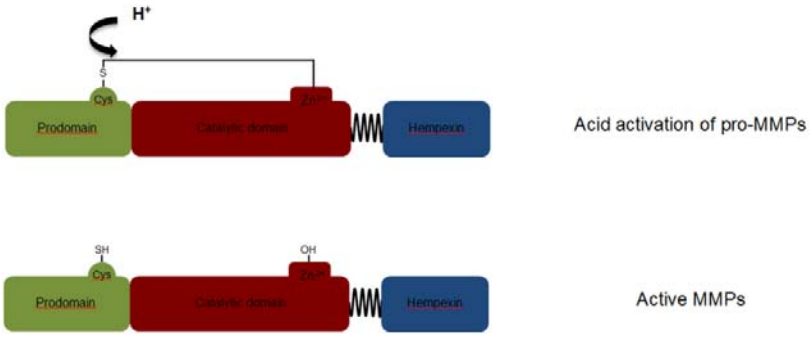

Figure 1. Activation of pro-MMPs by acid: acid (H^+) reduces the bond between cysteine in the prodomain and Zn^{2+} of the catalytic domain of MMP structure.

Resin composite is considered one of the favorable materials of choice for direct tooth restorations due to their superior esthetics and the minimally invasive preparations when compared to traditionally used amalgam restorations. However, the average lifetime of composite restorations is only 5.7 years and they have an average annual failure rate of 2.2% [93]. Secondary caries is defined as the carious lesion that develops at the margin of the restorations after the initial caries has been removed and replaced by a restoration. Secondary caries is recognized as the primary reason of restoration failures and the need for replacement. The reasons for this are attributed to the degradation of the hybrid layer. The hybrid layer is the zone that the resin monomers flow and polymerizes in the acid-etched dentin and creates a micromechanical interlocking for resin-dentin bonding. In the hybrid layer,

demineralized collagen matrix (type I collagen) serves as a scaffold for the infiltration of hydrophilic resin monomers [94].

Currently, dentin adhesives can be simplistically classified into two major categories based on distinct mechanisms: removing (etch-and-rinse adhesives) or modifying (self-etch adhesives) smear layer [95-98]. The etch-and-rinse adhesives involve simultaneous dentin etching for a predetermined period of time. Subsequently, the etched surface is rinsed and a primer followed by an adhesive resin is applied. In self-etch adhesives, the acidic primer diffuses into the smear layer to etch and prime the dentin simultaneously. The ultimate goal of resin-dentin bonding is to create a complete resin-infiltration into the demineralized dentin matrix. However, the incomplete infiltration of resin monomers into demineralized dentin normally occurs and possibly contributes to the less durable resin-dentin bonds compared to bonding to enamel. The incomplete resin infiltration in the hybrid layer is observed by nanoleakage [99].

Nanoleakage is defined as the diffusion of nanometer-sized molecules (i.e., silver nitrate) within the adhesive resin, demineralized dentin or hybrid layer in the absence of gap formation [99, 100]. The term nanoleakage was used because the spaces that allow the leakage was approximately as small as 20-200 nm compared to 10-20 μm for microleakage [101]. These spaces of nanoleakage were too small for bacterial penetration. However, they are large enough for the penetration of water and enzymes. Therefore, nanoleakage could be a channel of degradation of the hybrid layer [101]. There are two types of nanoleakage: reticular and spotted types [102]. The reticular type is related to incomplete resin infiltration and degradation of the hybrid layer, while the spotted type results from the adsorption of silver nitrate by hydrophilic and/or acidic functional groups of the adhesive resin [101-103]. As the degradation occurred at the hybrid layer, the reticular pattern of nanoleakage was observed especially at the bottom of the hybrid layers [94, 104-106].

The demineralized dentin that is not fully infiltrated by resin monomers is normally occupied by water. The water that remains in the demineralized dentin deteriorates the hybrid layer by two main mechanisms.

1. Degradation of Adhesive Resin by Esterases and Hydrolysis

Hydrolysis is a chemical reaction that involves the breaking down of chemical bonds using water. One molecule gains a hydrogen ion and the other

gains a hydroxyl ion. Hydrolysis can also be catalyzed by an enzyme (i.e., esterases) and is called "biodegradation." The enzymes can originate from salivary glands, microorganisms, or human cells (i.e., human gingival and pulp fibroblasts) [107, 108].

The adhesive resins used in dentistry are methacrylate-based resins that contain ester linkages (i.e., bisphenol A diglycidyl ether diemthacrylate (Bis-GMA); triethylene glycol dimethacrylate (TEGDMA); or 2-hydroxyethyl methacrylate (HEMA). The ester linkages are vulnerable to hydrolysis by esterases in the oral cavity. When the ester linkages of the adhesive resin are broken, the parent molecules are converted into carboxylic acid and alcohol [108].

The degradation products of adhesive resin include methacrylic acid (MA), triethylene glycol (TEG) and bishydroxy-propoxy-phenyl-propane (BisHPPP). These products induce human cells (gingival and pulp fibroblasts) to release hydrolytic enzymes and cytokines/growth factors that result in increased degradation of the adhesive resin [108, 109]. Furthermore, these degradation products (i.e., MA or TEG) also regulate the growth and the expression of glucosyltransferase B (gtfB) from *Streptococcus mutans* that are involved in biofilm formation and caries [110, 111].

As a consequence, this degradation process compromises the hybrid layer, promotes water absorption and more hydrolytic degradation, increases bacterial microleakage and finally contributes to recurrent caries, hypersensitivity, and inflammation [109, 112].

2. Degradation of Collagen Fibrils by MMPs and Other Proteases

The exposed collagen network resulting from incomplete resin infiltration is vulnerable to enzymatic degradation by the MMPs and other proteinases. MMPs are trapped within dentin during tooth development and contribute to the degradation of collagen fibrils. During the acid-etching procedure of dentin bonding, the phosphoric acid of etch-and-rinse adhesive system or the acidic primer of self-etch adhesive system not only demineralize the dentin, but the acids also activate the MMPs (Figure 1) [94, 113]. A study investigated the role of the two-step etch-and-rinse and the one-step self-etch adhesives on MMP-2 and MMP-9 and found that the acids could activate the endogenous MMP-2 and MMP-9 in the human dentin which supports the role of endogenous MMPs in the degradation of the hybrid layers created by these

adhesives (114). It was also demonstrated that there were significant increases MMP-2 and MMP-9, especially at the bottom of the hybrid layer where the most intense activity of the MMPs was observed [91].

The collagenolytic and gelatinolytic activities may lead to the loss of the integrity of the hybrid layer due to the collagen fibril degradation, especially at the bottom of the hybrid layer where the incomplete resin infiltration normally occurs. This could lead to increases of dentinal fluid influx into the hybrid layer and hydrolytic degradation of both the adhesive and the collagen in the upper parts of the hybrid layer. Eventually, the destruction of the hybrid layer may contribute to the secondary caries or failure of the restorations [115].

Several approaches have been recently proposed to enhance the durability and overall clinical success of resin composite restorations. One strategy is to hinder the collagenolytic activity of the MMPs using inhibitors. These inhibitors have included galardin [106], chlorhexidine (CHX) [104, 116, 117], carbodiimide [118], epigallocatechin gallate (ECGC) [116, 119], and doxycycline (DOX) [120].

A study investigated the effects of galardin, a synthetic MMP inhibitor, on dentin powder treated with 1% phosphoric acid. The inhibitory effects of galardin on MMP-2 and MMP-9 activity were assayed by gelatin zymography. After 1 year of aging in artificial saliva, the microtensile bond strength significantly decreased in the control compared to the galardin-treated group. Interfacial nanoleakage revealed reduced silver deposits in galardin-treated specimens compared to the controls [106]. These results suggested that acid demineralization is able to activate trapped MMPs and that the use of a MMP inhibitor could provide more resistance to enzymatic degradation of the hybrid layer [106].

Chlorhexidine (CHX) is one of the most commonly investigated MMP inhibitors that have been used to prevent the degradation of the hybrid layers. The results from an in vitro study suggested that the use of 2% and 0.2% CHX decreased the reduction of bond strength to dentin after aging [117]. Another study investigated the use of 2% CHX as a dentin pretreatment after 37% phosphoric acid etching and showed significant higher microtensile bond strength compared with no treatment. These findings suggest that using MMP inhibitors could help prevent hybrid layer degradation. However, the inhibitory effects of CHX on dentin bond durability were not observed after a longer period of aging (> 18 months) [117, 121, 122].

A clinical study conducted on non-carious lesions revealed no improvement of the clinical durability of adhesive restorations when MMPs inhibitors specifically CHX was used prior to an application of the adhesive

[121]. The long-term clinical performance of a sample of non-carious cervical lesions with and without an application of 2% CHX to the demineralized dentin using a split mouth design was investigated. The restorations of the control group started de-bonding before 6 months of clinical function, while in the CHX treated group none of the restorations de-bonded until the 12-month evaluation. This suggested that CHX stabilized the hybrid layer for up to 12 months [121]. Therefore, a sustained release of MMP inhibitor could present a promising alternative option to provide long-term and more stable resin-dentin bonding.

DOX, an FDA-approved MMP inhibitor, was encapsulated into aluminosilicate clay nanotubes (Halloysite nanotubes, HNT) to provide sustained release [120]. Unlike CHX that was used as a dentin pretreatment, DOX was first encapsulated into HNTs that serves as a drug reservoir which is then incorporated into the adhesive resin to sustain the release of the DOX into the hybrid layer. The results showed that DOX significantly inhibited MMP-1 activity when compared to the control. This provided solid evidence for effectiveness of encapsulated MMP inhibitors and their promising application in therapeutic adhesives that may enhance the longevity of hybrid layers and the overall clinical performance of adhesively bonded resin composite restorations.

To conclude, it is clear that the MMPs play major roles in the development of periodontal diseases, dental caries, and restorations failure. Their levels of expression and activity are also directly associated with the lesion severity and disease progression. MMP inhibition by several inhibitors could thus provide possible therapeutic options to limit the tissue destruction associated with these conditions. Only a comprehensive understanding of the roles played by MMPs and their inhibitors will allow scientists to be able to explore more promising therapeutic and preventive pathways.

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