

Original Research Article

Matrix Metalloproteinases and Matrix Metalloproteinase- Inhibitors: Role in Oral Environment

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Abstract: MMPs hydrolyze components of the extracellular matrix and have been suggested to play an important role in the destruction of dentin organic matrix. The acidic environment, resulting from adhesive systems or the biological carious process, activates different dentinal MMPs which degrade the unprotected collagen fibrils within the hybrid layer. Host-derived MMPs can originate both from saliva and from dentin. However, MMP inhibition by several inhibitors, could provide a potential therapeutic way to limit degradation in dentin. This paper reviews the effect of MMPs on dentin and employment of various potential MMP inhibitors to pretreat the demineralized dentin interface.

Keywords: chlorhexidine, Dentin, galardin, matrix metalloproteinases (MMPs), MMP inhibitors

INTRODUCTION:

When compared to other restoratives, the major drawback of adhesive restoratives is their limited durability in vivo, where the most cited reasons of failure of adhesive restorations are loss of retention and marginal adaptation [1]. Degradation of these bonds occurs via the interaction of the components above the adhesive interface manifested by occlusal loading, thermo-cycling, moisture and PH fluctuation [2]. These extrinsic degradation mechanisms of the resin– dentine interface that originate in the adhesive above the hybrid layers are accompanied by intrinsic degradation mechanisms that originate from beneath dentine hybrid layers represented by dentinal fluid and intrapulpal pressure [3]. Several authors have shown the hydrolytic degradation of collagen matrices in aged dentin– resin bonds, even in the absence of bacterial enzymes [2, 4]. The recent reports of collagen lytic and gelatinolytic activities in partially demineralized dentine collagen matrices are indirect proofs of the existence of matrix metalloproteinases (MMPs) in human dentine [5]. The release and activation of these endogenous enzymes during dentine bonding are thought to be responsible for the in vitro manifestation of thinning and disappearance of collagen fibrils from incompletely infiltrated hybrid layers in aged, bonded dentine [5], resulting in hydrolytic degradation and reduction of bond strengths.

MATRIX METALLOPROTEINASES

Matrix metalloproteinases represent a family of dependent metal ions endopeptidases that are capable

of degrading all extracellular matrix components, including several types of collagen and basement membrane components [6, 7]. MMPs are classified into six groups based on their structural homology and their substrate specificity: collagenases (MMP-1, MMP-8, MMP-13, and MMP-18), gelatinases (MMP2 and MMP-9), stromelysins (MMP-3, MMP-10, and MMP-11), transmembrane MMPs (MT-MMPs) (MMP-14, MMP-15, MMP-16, MMP-17, MMP-24, and MMP-25), matrilysins (MMP-7 and MMP-26), and "other" (MMP-12, MMP-19, MMP20, MMP-21, MMP-22, MMP-23, MMP-27, and MMP-28). MMPs are metal-dependent since all members of this family have a zinc and a calcium-binding catalytic domain. They are secreted as inactive proenzymes (zymogens) and their activation occur in the tissue by cleavage of the N-terminal propeptide domain by other proteinases [8]. Presently, twenty-two MMPs have been identified in human tissues [9, 10]. They are classified into different groups according to similarity in structure, gene encoded and substrate affinity [11, 12]. MMP-2 seems to have a helicase activity and to cleave fibrillar type I collagen [13, 14]. Although stromelysins (MMP-3) and membrane-type MMPs do not have helicase activity, these enzymes play a crucial role during activation of other MMPs, promoting the cleavage of the propeptide region, which that maintains enzyme latency. MMPs are a family of zinc-dependent proteolytic enzymes that are capable of degrading the dentin organic matrix after demineralization [15]. Enzymes with gelatinolytic (MMP-2 and MMP-20) activities are present within

intact dentinal matrix and in carious dentin [15]. They may be inhibited in situ by tissue inhibitors of metalloproteinases such as TIMP-1 [16], or they may be released from mineralized dentin matrix from which they can be activated by low pH [15] and may cause degradation of the demineralized dentin matrix under different physiological and pathological conditions [15].

The cells of connective tissue—e.g., fibroblasts, osteoblasts, and odontoblasts—synthesize and secrete MMPs into the ECM. Under normal physiological conditions, these MMPs are expressed only when needed for tissue remodeling. These endopeptidases contain zinc methionine in their active site [17]. They cut the extracellular matrix (ECM)/core matrix proteins into various small peptides by hydrolyzing inner peptide bonds. Their activity depends upon Ca^{++} ions. MMPs are different from other endopeptidases because they do not function in the absence of metal ions [18].

Matrix Metalloproteinases in Periodontal Disease

During inflammatory destruction of periodontal attachment apparatus the most important component of periodontium lost is the collagen type I. A wide range of evidences has indicated that the most important pathway is the one which involves matrix metalloproteinases (MMPs) [19]. Resident ligament cells such as fibroblasts, macrophages, osteoblasts, keratinocytes, and endothelial cells are activated in response to stimulus, contributing with the synthesis of cytokines and MMPs. MMPs are present in both active and latent forms in chronically inflamed gingival tissues and gingival crevicular fluid. Active collagenase and gelatinase are found in the crevicular gingival fluid of patients with periodontitis in much larger amounts than in control subjects [20]. In contrast, high concentrations of the natural tissue inhibitor of MMPs (TIMPs) are found in the gingival crevicular fluid of healthy gingiva [16]. The area occupied by collagen fibers in gingival tissue specimens with periodontitis is significantly decreased, and the presence of MMP-1, MMP-2, and MMP-9 is increased [21, 22].

MMPs in Caries

Dentin contains 18-20% of organic material and 11-12% of water, and provides a better substrate for degradation. Unlike enamel, by either bacteria or host proteinases. In general, collagens can be degraded by the human interstitial collagenases, which include MMP-1, MMP-8, and MMP-13, resulting in the release of 3/4- to 1/4-length peptides. These peptides lose the triple-helical conformation and can then be further degraded by the gelatinases MMP-2 and MMP-9. However, the specific cross-links (pyridinolines) between the collagen sub-units observed in dentin may provide collagen fibrils with extreme resistance to degradation [23]. Saliva penetrates the opened dentin lesion, and MMPs present in the saliva may have direct access to the demineralized dentin. It has been proposed

that these saliva-derived MMPs could be involved in the destruction of the organic matrix [15] the GCF appears to be the major source of the MMPs found in the saliva. GCF also contains α_2 macroglobulin, a non-specific inhibitor of MMP, which—in normal situations, where the concentration of MMPs is not elevated—would keep the MMPs in an inactive form²⁴ (Birkedal-Hansen, 1993). The GCF appears to be the major source of the MMPs found in the saliva. GCF also contains α_2 macroglobulin, a non-specific inhibitor of MMP, which—in normal situations, where the concentration of MMPs is not elevated—would keep the MMPs in an inactive form [24]. Saliva has also been shown to contain gelatinases [25], which appear to originate mainly from the GCF [26]. By in situ hybridization (ISH) or by immunohistochemistry, the collagenase MMP-1, the gelatinases MMP-2 and MMP-9, stromelysin-1 (MMP-3), the MMP-2 activator MT1-MMP, and enamelysin (MMP-20) have all been identified in either odontoblasts or in the predentin/dentin compartment [27, 28]. TIMPs were also detected, but their level was only slightly above background [29].

MMPs in dental Adhesion

Low pH and heat treatment may also directly lead to MMP activation [30]. The change in pH can alter the conformation of the propeptide and induce the cysteine switch, which represents a critical step in the activation process. Since Nano leakage can occur in the absence of frank gaps along resin-dentin interfaces created in vivo [31], the results of these studies suggest that degradation of incompletely infiltrated zones within the hybridized dentin by host-derived matrix metalloproteinases within the dentin matrix may precede in the absence of bacterial enzymes. In situ collagen degradation within incompletely infiltrated hybrid layers may also adversely affect the remineralization potential of the denuded collagen fibrils in vivo [32]. As the popularity of self-etching primers and all-in-one adhesives has increased, many all-in-one adhesives have been commercialized that have pH values of between 1 and 2. These acidic monomers may also demineralize dentin, but may not be sufficiently acidic to denature MMP activity. However, self-etching primers leave collagen fibrils partially covered with residual apatite crystals. These crystals, and possible chemical interactions of acidic monomers with residual dentin substrate, may provide more resistance to bond degradation than is possible in etch-and-rinse adhesives. In contrast to etch-and-rinse systems, self-conditioning systems usually contain more hydrophilic monomers, yielding an increased permeability of the hybrid layer for water and leading to an enhanced monomer elution [35]. Hence, also for self-etch systems there is exposed collagen which can be degraded hydrolytically by potentially activated MMPs. In the literature, inconsistent data exist regarding the question whether self-conditioning systems enhance the activity of MMPs in dentin (powder) [33]. Dentin over

etching results in deeper demineralization and exposure of collagen. Hence, the bonding agent used thereafter may infiltrate the deep, basal layer of the collagen network less efficiently, thus enhancing nano leakage [34]. In this deep, basal layer of collagen that is not infiltrated by the bonding agent, existing MMPs are activated both by the applied phosphoric acid (pH = 0.4) and by acidic monomers (pH = 2–2.8) contained in the bonding agent [35]. However, there is evidence that the activity of MMPs is initially decreased by phosphoric acid, but they are subsequently reactivated by the bonding agent. Consequently, exposed collagen is degraded by (re) activated MMPs at the bottom of the hybrid layer, which gradually disintegrates due to growing and merging nanometer sized porosities [36]. Clinically, this degradation results in loss of retention or of fillings, secondary caries and hypersensitivity [36].

CYSTEINE PROTEASES (CATHEPSINS)

Cysteine cathepsins hydrolytically degrade the extracellular matrix, in particular collagen, and, similar to MMPs, they seem to be involved in the degradation of exposed collagen at the bottom of the hybrid layer [37]. There are approximately 12 members of this family, which are distinguished by their structure, catalytic mechanism, and which proteins they target. Although most cathepsins are lysosomal enzymes that become activated in lysosomes by low pH, cathepsin K works extracellularly after secretion by osteoclasts in bone resorption [38]. This protease represents 98% of the total cysteine protease activity [39]. The physiologically relevant substrate of osteoclast-expressed cathepsin K is type I collagen which constitutes 95% of the organic bone matrix and 90% of the dentin matrix [40].

INHIBITORS OF PROTEOLYTIC/COLLAGENOLYTIC ACTIVITY

Chlorhexidine (CHX)

It has been added to the phosphoric acid etchant, used as an aqueous solution after acid-etching (etch-and-rinse adhesives) and incorporated into the adhesive system (etch-and-rinse or self-etch adhesive). Synthetic MMP inhibitors are being investigated as potential therapeutic agents in the treatment and/or prevention of oral diseases [42]. Chlorhexidine (CHX) has been shown to inhibit MMP-2, -8, and -9 activities directly at extremely low concentrations (i.e., 0.02% for MMP-8, 0.002% for MMP-9, and 0.0001% for MMP-2) [41].

Ethylene diamine tetra acetic Acid (EDTA)

Used as an aqueous solution of 2% EDTA on smear layer covered dentin (self-etch adhesives); or on demineralized dentin after acid-etching (etch-and-rinse adhesives). As EDTA is an effective Zn²⁺ and Ca²⁺ chelator, it might inhibit MMP activity. Infact, EDTA has inhibitory effect against human dentin MMP-2 and MMP-9 when applied for 1 to 5 minutes [43, 44].

Epigallocatechin-3-gallate

Very little is known about the potential utility of green tea polyphenol epigallocatechin-3-gallate (EGCG) in resin–dentin bonds. EGCG inhibits the activity of MMP-2 and MMP-9 by the degradation of the MMP molecule [45]. In dentistry, EGCG may inhibit the activity and expression of MMP-9 involved in the formation of osteoclasts in periodontal disease apart from its inhibitory effect on the growth of *Streptococcus mutans* when added to a bonding adhesive [46]. Green tea extract has also been reported to reduce dentin erosion-abrasion by inhibiting MMPs [47].

Galardin

Galardin is a potent and broad-spectrum hydroxamate-type synthetic MMP inhibitor designed as a molecular mimic of MMP substrates, which allows it to enter the active site of MMPs, where it binds the critical zinc atom [48]. Galardin is active against several MMPs [49].

Quaternary Ammonium Salts

One of the advantages of using quaternary ammonium methacrylates, such as 12-methacryloyloxydodecylpyridiniumbromide (MDPB) [50-52] is that they can copolymerize with adhesive monomers. Clearfil Protect Bond (Kuraray Noritake Dental Inc., Osaka, Japan) was the first commercial dentin adhesive to incorporate MDPB in its composition. Benzalkonium chloride (BAC) is also one antimicrobial substance containing a quaternary ammonium group in its molecule. This substance has been included in an acid phosphoric gel (i.e., ETCH-37w/BAC and ETCH-10w/BAC, Bisco Inc. Schaumburg, IL, USA) for several years. The use of these BAC-containing phosphoric acid gels did not affect the immediate bond strength to enamel and dentin. Recently, the anti-MMPs properties of BAC were tested against MMP-2,-8, and-9. The results showed potential for this substance to inhibit MMP-2,-8, and-9 [53].

Cross-linking Agents

Cross-linking is considered a potential method for improving the stability and resistance of collagen degradation within the demineralized dentin matrix [54, 55]. Chemically induced cross-linking has been tried in dentin adhesion since the 1980s by applying glutaraldehyde as a component of a priming solution [56]. Ultraviolet (UVA)-activated riboflavin has been shown to increase bond strength, stabilize the adhesive interface, and inhibit dentin MMPs [57]. Riboflavin has potential in adhesive dentistry because it is activated with a UVA blue light and is easy to apply, besides being biocompatible [58]. Proanthocyanidin is the plant flavonoid prevalent in pine bark, elm tree, somenuts, flowers, and grape seeds [59, 60] being known as a potent antioxidant and cross-linking agent with low toxicity.

Grape seed extract is one of the most used sources of proanthocyanidins [61]. In addition to its cross-linking effect, proanthocyanidin has also been shown to inhibit the synthesis of several MMPs from macrophages and inhibit the catalytic activity of MMP-1 and MMP-9 [62]. Carbodiimides have been used as alternative cross-linking agents to glutaraldehyde because they do not contain toxic components. Carbodiimides can inactivate dentin MMP-9 and other dentin proteases with only 1-minute application time [63].

CONCLUSION

For optimal durability of resin-dentin bonds, preservation of both resin and substrate components (i.e., dentin collagen) should be addressed. Literature indicates that the presence of MMPs in the dentin matrix is of more than academic interest. We need to understand the biochemistry of these enzymes and how they may respond to procedures and products used in adhesive dentistry.

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