Review Article

Matrix metalloproteinase inhibitors in restorative dentistry

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Abstract

Matrix metalloproteinases (MMPs) have been identified as agents that disintegrate the collagen structures of dental hybrid layers, resulting in reduced restorative bond strength. Multiple MMP inhibitors (MMPIs) are known to counteract this degenerative mechanism, thereby preserving bond strength and promoting the longevity of resin-based restorations. Additionally, literature suggests that certain MMPI materials possess antimicrobial/anticariogenic properties, potentially reducing the risk of secondary caries development. Therefore, this review article aims to narrate on the integration of matrix metalloproteinase inhibitors into adhesive systems and their impact on bond strength.

Keywords: Matrix metalloproteinase inhibitors and bond strength; matrix metalloproteinases; matrix metalloproteinase inhibitors

INTRODUCTION

Dentistry, particularly restorative dentistry, has undergone multidimensional changes over the last 100 years. It has become apparent that the esthetic concerns within the patient community have increased significantly.[1,2] Tooth-colored restorations, coupled with modern adhesive strategies, have transfigured our perspectives on dental restorations. Introduction of acid-etching technique by Michael Buonocore in 1955 marked the embarkation of a new era of restorative dentistry. Subsequently, Masuhara *et al*. reported a major breakthrough in research. They discovered that when tri-n-butylborane was used along with methyl methacrylate, it produced a commendable bond in a collagen-added wet ivory model. Later, in 1969, the very first dental adhesive was made available in the commercial market by the name "direct bonding system," which was used for orthodontic bonding procedures.[3]

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Dental composites are among the most commonly used restorative materials for their biomimetic capacity. A resin adhesive is used to achieve adequate bonding of composite resins to the tooth structure.

Matrix metalloproteinases (MMPs), a set of closely related extracellular proteolytic enzymes, are crucial for diverse physiological functions such as embryonic development, tissue repair, and bone restructuring. In addition, they contribute significantly to pathological situations such as inflammation and arthritis.[4] Many cell types are known to produce MMPs. These include fibroblasts, endothelial cells, cementoblast-like cells, and epithelial cells.[5]

Ensuing the formation and mineralization of the collagen matrix during tooth development, MMPs enter an inactive state and become trapped within the calcified matrix. There is the potential for these dormant MMPs to be reactivated when exposed to an acidic environment.[6] This rise in acidity is attributed to the increased activity of caries causing bacteria or through the application of acidic agents during dental procedures. In case of etch-and-rinse adhesive, an acid etchant is utilized [Figure 1].[7] In Nakabayashi's own words, who proposed the concept of the hybrid layer,

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"the dentinal peptides (including collagen) must not be denatured when the dentin is decalcified. Furthermore, if the acid is too aggressive, it may expose collagen below the hybrid layer leaving a zone of weak dentin that is susceptible to long-term degradation."[8] Thus, this signifies the need to prevent collagen degradation to preserve adequate bond strength. Recent studies have pointed out that the degradation of collagen fibers within the hybrid layer and the consequential compromise of bond strength and integrity at the tooth–restoration interface are attributed to the activity of MMPs as a significant factor.[9] This suggests that the inhibition of MMPs is advantageous for the resin–hybrid layer complex, ultimately enhancing bond stability. Various materials have been shown to hinder or decrease the activity of MMPs.

Chlorhexidine (CHX) is one of the most commonly studied MMP inhibitors (MMPIs) in dentistry.[10] Other than the effects caused by the MMPs at the hybrid layer, it is also noteworthy to mention that MMPs play a key role in the progression of periodontal diseases. Numerous studies have been carried out so far to identify modalities to inhibit this pathological process.[11]

The aim of this article was to perform a literature review on the integration of MMPIs into adhesive systems and their impact on bond strength.

MATERIALS AND METHODS

The review article conducted a comprehensive evaluation of the existing literature concerning various MMPs and their intricate roles in dental adhesive bonding mechanisms. Scientific databases such as PubMed and Google Scholar were used to pool relevant articles. Terms such as "MMP," "Matrix metalloproteinase," "MMP inhibitor," "MMP inhibitor and dental," and "MMP inhibitor and bond strength" were used to retrieve articles from the databases. We included studies that addressed the mechanism of MMP and MMPIs, their uses in dental restorations, advantages, and limitations. Studies with insufficient data were excluded. Ultimately, this review focused on 63 articles concerning MMPIs and their applications in dentistry.

LITERATURE REVIEW

The discovery of MMPs can be traced back to the early 1960s. The initial breakthrough occurred when collagenase activity was observed in an enzyme found in amphibian species.[12] Subsequently, this group of collagenases was termed "Matrix Metalloproteinase" by Harris *et al*.[13] Since then, the scientific literature has been filled with a vast array of studies on MMPs and their inhibitors. It is now well established that MMPs are involved in a multitude of metabolic processes, including tissue repair, bone remodeling, cell proliferation, wound healing, programmed cell death, and gonadal tissue reproduction. MMPs are classified broadly into six major groups that include (a) collagenases, (b) gelatinases, (c) stromelysins, (d) matrilysins, (e) membrane-bound MMPs, and (f) other MMPs or miscellaneous MMPs [Figure 2].[14] The relevance of MMPs in dentistry is manifold. Numerous MMPs are identified to be of dental tissue origin. In this regard, odontoblasts are known to produce MMP-2, MMP-9 belonging to the gelatinases,[15] MMP-3 (stromelysin),[16] MMP-8 (collagenase),[17] and membrane type 1 MMP.[17] The exact role of MMPs in dental odontoblasts is well known to none, but it has been suggested that these MMPs might play a role in organization of the dentinal organic matrix ahead of the mineralization process.[18] As previously mentioned in the introduction section, MMPs undergo a process where they become inactive and are then trapped within the calcified matrix following tooth development. Caron *et al*.[19] pointed to the presence of MMP-2 in secretory odontoblastic cells which are involved in dentin formation during tooth development. Later, Martin De Las Heras *et al*.[20] discovered the presence of MMP-2, a gelatinase, in human dentin. Sulkala *et al*.[21] proved the presence of MMP-20 in

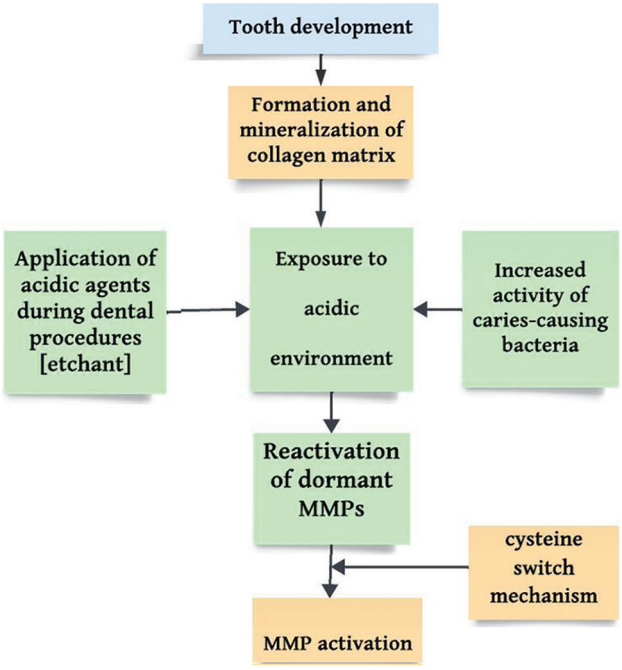

Figure 1: Mechanism of activation of matrix metalloproteinases in human tooth

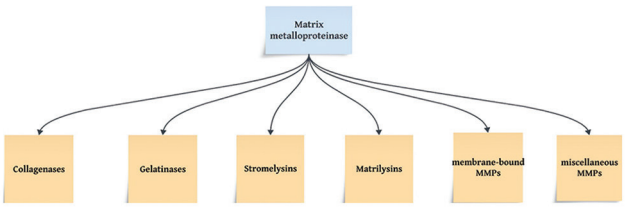

Figure 2: Classification of matrix metalloproteinases - the six major groups

the dentinal complex. Given this brief outlook on MMPs and its dental presence, it is crucial to understand its effects in dental restorations and the subsequent bond strength.

All MMP gene family members have a common characteristic: they are initially synthesized in an inert (latent), inactive form. At the molecular level, all MMPs possess just two domains: the propeptide domain, containing an essential cysteine residue, and the catalytic domain, which encompasses the zinc-binding site. Earlier studies on the latency in human fibroblast collagenase (HFC) stem from the creation of an intramolecular complex. This complex forms between a cysteine residue within its propeptide domain and the vital zinc ion located in the catalytic domain, effectively obstructing the active site. The activation of latent HFC can be induced through a variety of methods, all of which lead to the separation of the cysteine residue from the complex. This process is commonly referred to as the "cysteine switch" mechanism.[22]

The activation of MMP occurs in an acidic, low pH environment by triggering the cysteine switch. It has also been observed that MMPs activated by acids released by bacteria play a critical role in dentin destruction in caries.[23] The activity of endogenous collagenolytic and gelatinolytic enzymes in phosphoric acid-treated dentin can be regained through the use of etch-and-rinse adhesives. Furthermore, it was observed that the amount of regaining proteolytic activity in the tooth was proportional to the extent of acidity offered by the adhesive systems.[7] Lehmann *et al*. demonstrated that self-etching adhesives stimulate the release of MMP-2 from odontoblast cells.[24]

It is of no surprise that the scientific fraternity was intrigued to inhibit this mechanism, thus favoring better bond strength. Substances or materials that inhibit MMPs are termed as MMPIs. MMPIs can be broadly classified into two categories: endogenous inhibitors and synthetic inhibitors. Endogenous inhibitors are further subdivided into specific endogenous inhibitors and nonspecific endogenous inhibitors.[25] These endogenous inhibitors provide the necessary physiological counter balance between the extracellular matrix and MMPs. Tissue inhibitors of metalloproteinase are examples for specific endogenous inhibitors. Certain proteins have been identified as nonspecific endogenous inhibitors such as α2-macroglobulin and membrane-bound β-amyloid precursor protein.

It is, therefore, apparent that compounds studied for their MMP-inhibiting properties from the perspective of adhesive dentistry belong to the category of synthetic inhibitors.

Chlorhexidine

CHX is the most widely studied material for its MMP inhibition properties. In general, MMPIs are incorporated into adhesive, etchant, or primer. The proposed inhibitory mechanism involves cation chelation of calcium and zinc ions found in MMPs.[26] The bonding between CHX and the dentinal surfaces occurs due to electrostatic bonding.[27]

Distilled water or artificial saliva is the preferred aging solution.[10] The addition of 2% CHX to acid-etched dentin or its incorporation into the phosphoric acid conditioner reduces collagen degradation,[4,28] thus enhancing immediate bond strength. Long-term preservation of bond strength is noted in multiple studies.[29,30]

Studies have also pointed out that when CHX was incorporated into the etchant, the decrease in microtensile bond strength (μTBS) was less significant compared to the control group.[29] Loguercio *et al*.[29] observed the persistence of CHX molecules within the hybrid layer, as revealed by Raman spectroscopy, even after a span of 5 years.

Gendron *et al*. have pointed out that CHX has direct inhibition effects on MMP-2, MMP-9, and MMP-8, of which the former two belong to the gelatinase group, while the latter is of collagenase group.[26]

A study evaluated the effect of CHX in the prevention of secondary caries in experimental models and concluded that pretreatment of dentin with CHX slowed down the development of secondary caries.[31]

A study asserted that the considerable water solubility of large CHX molecules facilitates their leaching, resulting in a reduction of bond strength between interfaces.[32]

Literature lacks sufficient information to arrive at a conclusive decision on minimum MMP inhibition concentration.

Benzalkonium chloride

Benzalkonium chloride (BAC) is categorized within the quaternary ammonium compound group. BAC is a cationic compound (positively charged) and thus exhibits affinity toward negative carboxylic groups in collagen.[33] According to Sabatini *et al*., specific experimental groups with BAC exhibited increased μTBS when compared to the control group at both the 24-h and 6-month time points. In addition, dentin matrices treated with BAC showed reduced mass loss and lower release of hydroxyproline (parameter used for the assessment of collagen solubilization) compared to the control.[31] Comba *et al*. conducted gelatin zymography assays and *in situ* zymography quantification analyses, revealing that formulations containing BAC led to a reduction in the expression of MMPs.[34]

Immediate micro-TBS (μTBS) values increased significantly at 24 h in an experimental BAC group,[35] whereas μTBS performance of 1% BAC experimental group was inferior than the control according to Comba *et al*.[34]

Sabatini *et al*. showed that BAC effectively deactivates dentin proteinases attached to the matrix in demineralized dentin structures, and this deactivation follows a dosage-dependent pattern. When BAC was applied at concentrations of 0.5% and 1.0% for a duration of 60 s, it resulted in a 31% and 54% reduction in total MMP activity, respectively.[36]

Adhesives containing BAC decrease endogenous enzymatic activity both immediately and over an extended period. Nevertheless, in certain experimental conditions with BAC, there was an observed rise in gelatinolytic activity over time, coupled with a decline in bond strength, irrespective of the adhesive employed.

Epigallocatechin-3-gallate (EGCG)

Epigallocatechin gallate (EGCG) is a polyphenolic compound abundantly found in tea leaves. It is also the major catechin found in green tea.[37] A previous research has noted that catechins found in green tea can impede the activities of MMPs and the activation of proMMP-2.[38] As per Gerhardt *et al*., the use of epigallocatechin gallate at a 2% concentration on dentin resulted in an enhancement of the bond strength of a self-etching adhesive to regular dentin.[39] Several research studies indicate that the addition of epigallocatechin gallate into the adhesive system resulted in consistent and stable bond strength over an extended period.[28,40,41] Yet, according to Amaral FL *et al*., there was a reduction in bond strength values after a 6-month duration, regardless of the dentin treatment.[42]

Proanthocyanidin

Proanthocyanidin (PA), a flavonoid classified among polyphenolic compounds, offers notable health advantages for humans. Typically derived from sources such as grape seeds and blueberries, this compound is commonly extracted.[43] PA is known to reduce oxidative stress.[44] Its antimicrobial potential is also widely studied.[45-47] The antimicrobial and anti-inflammatory properties of PA have attracted attention from the dental fraternity. Dias *et al*. ascertained the μTBS immediately and after a 12-month storage duration, along with the antimicrobial effectiveness of an adhesive containing various concentrations (%) of PA: 0%, 1%, 2%, 4.5%, and 6%. The incorporation of 2%, 4.5%, and 6% PA maintained the dentin μTBS even after a 12-month storage period, without affecting the solubility (Sp), sorption (Sl), or degree of conversion (DC%) of the adhesives.[48] Treating demineralized dentin with nanohydroxyapatite PA might serve as an alternative approach to enhance its strength by enhancing collagen stability and providing reinforcement.[49]

Zinc salts

Zinc ions act as competitive inhibitors for MMPs. This is due to the fact that the collagen contains four Zn binding sites located at the exact positions as the cleavage sites that collagenases target, thus preventing the action of collagenases and protects collagen degeneration.[50] Various salts of zinc were studied for its effects on dentin bonding, which includes zinc oxide (ZnO), Zn-methacrylate, ZnN_3, and zinc chloride ($ZnCl_2$). Of which, ZnCl2 demonstrated better inhibition properties.[51]

Barcellos *et al*. conducted a study about the lasting bond strength and cytotoxic impact of dentin adhesive infused with zinc over a 6-month period. The incorporation of zinc oxide nanoparticles (ZnO-n) effectively decreased the cytotoxicity associated with the adhesive. Furthermore, ZnO-n preserved the μTBS even after a 6-month storage period, preventing any decline.[52] These results align with the previous investigation conducted by Toledano *et al*.[53]

Incorporation of zinc ions either into experimental adhesive systems or commercially available adhesive systems had no notable distinctions in the tensile strength, flexural modulus, flexural strength compressive strength, and water sorption.[52]

The addition of 2 wt% of ZnCl2 was observed to inhibit both the reduction in resin–dentin bond and the increase in nanoleakage after 1 year of storage in water. According to Almeida *et al*., due to its high solubility, zinc chloride may undergo considerable leaching in the oral cavity, potentially influencing its efficacy over an extended period. It was further noted that additional studies would be required to investigate this issue.[51]

GM1489

GM1489 is a human-made synthetic inhibitor of MMPs, meticulously designed to target MMP 1, 2, 3, 8, and 9.[15] Our literature search revealed limited studies available in dental literature on GM1489. Adhesive systems incorporating GM1489 exhibited superior material properties and maintained bond strength stability, even under certain experimental conditions which involved storage of samples in water for 12 months.[30] da Silva *et al*. also proposed that GM1489 could function as an MMPI in etchant and adhesive systems, with the potential to preserve bonding stability over an extended duration.[30]

Sodium fluoride

The positive effects of sodium fluoride (NaF) in preserving and sustaining adhesive dentin interface bond strength have been demonstrated in studies. Kato *et al*. reported a reduction in dentin degradation by MMPs when NaF gel was incorporated into demineralized dental matrices.[54] Furthermore, investigations have explored NaF's impact on the intrinsic activity of MMPs within dentin matrices.[55] An *in vitro* study by Alaghehmand *et al*., where fluoride was integrated into the adhesive system, concluded that fluoride-containing adhesive enhances the durability of resin–dentin bonds.[56]

Other matrix metalloproteinase inhibitors

Hesperidin (HPN), a citrus extract, is a natural flavonoid with various advantages, including antioxidant and anti-inflammatory properties, collagen cross-linking abilities, caries protection, remineralization, and antimicrobial effects.[57] Dental adhesive systems containing 0.5 wt% HPN demonstrated a favorable antibacterial property without adversely affecting adhesive properties. However, the μTBS was notably diminished after thermocycling.[57] In a research conducted by Islam *et al*., the introduction of HPN into the self-etching primer demonstrated a beneficial impact on both the immediate μTBS and the mechanical characteristics of the bonded interface.[58] Islam *et al*. noted that the inclusion of 2% HPN in the self-etching primer had a positive influence on both the immediate μTBS and the mechanical properties of the resin–dentin interfaces. In addition, in the 5% HPN group, the structure of collagen in the hybrid layer remained intact even after a storage period of 1 year in artificial saliva.[59]

Batimastat, a synthetic analog of the collagen substrate, functions as a zinc ion chelator. Initially, it was investigated for its ability to impede tumor progression and metastasis.[60] In a study by Almahdy *et al*., which infused two MMPIs (batimastat BB94 and galardin GM6001), it was found that the μTBSs of all adhesive systems remained unchanged compared to their respective control groups without MMPIs.[61]

Ilomastat, which is well known by its proprietary name galardin, is a broad-spectrum MMPI. In experimental models, galardin completely inhibited MMP-2 and MMP-9; however, incorporation of galardin as an added primer produced no effect on immediate bond strength. Nevertheless, it significantly decreased bond degradation after 1 year when stored in artificial saliva aging solution.[62] In a study conducted with galardin and its solvents in extracted third molars, significant results were observed, with no decrease in immediate bond strength.[63]

Limitations

1. The research studies found in literature search are limited to *in vitro*/experimental studies
2. In long-term studies, the study samples were primarily aged using distilled water or artificial saliva; thus, the oral environment could not be reproduced
3. The majority of the studies focused on noncarious dentin.

CONCLUSION

The incorporation of MMPIs yields a significant enhancement in dentin bond strength, with no discernible regression observed. This indicates promising prospects for advancing dental bonding procedures. However, further investigations are warranted to evaluate the biocompatibility of MMPI-incorporated dental adhesive systems and to conduct clinical trials to ascertain their ultimate effectiveness in clinical practice.

Clinical significance

Incorporation of MMPI at any step of the dental adhesive system reduces the rate of bond strength degradation. This enhancement contributes to the longevity of dental resin-based restorations. Some materials used as matrix metalloproteinase inhibitors also exhibited antimicrobial/anticariogenic properties. Thus, incorporation of matrix metalloproteinase inhibitors in adhesive systems could be a viable option, as the incidence of secondary caries development shall be reduced considerably. This underscores a compelling direction for the advancement of dental adhesive technologies.

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Conflicts of interest

There are no conflicts of interest.

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