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Metalloproteinases and their role in the degradation of bonding systems. Part 2

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Summary

The stability of the hybrid layer is crucial for ensuring the durability of fillings made of composite materials. Factors which weaken the bond strength are related to, among others, the presence of bacteria and their enzymes in the structure of the bacterial biofilm. Chronic damage the hybrid layer is also a result of hydrolysis and leaching of adhesive monomers which infiltrated the demineralised dentin matrix. Nanoleakage is also among the factors contributing to degradation. Many studies examine the effect of endogenous proteases on the degradation of the hybrid layer. Endogenous collagenolytic enzymes: metalloproteinases (MMPs) and cysteine cathepsins, are responsible for the degradation of the collagen matrix in the hybrid layer. Inhibition of endogenous proteases is therefore necessary to slow the degradation of fillings. The enzyme activity in dentine and in the hybrid layer can be regulated by endo- and exogenous inhibitors.
The paper is a review of the available literature published in the PubMed medical database, as well as in Polish dental journals in the years 2002-2017. Its aim is to assess the role of metalloproteinases and cysteine cathepsins in the degradation of the hybrid layer and to review the compounds with inhibitory properties in relation to these enzyme groups.

Performing the reconstruction of tooth hard tissues with a long period of clinical use is the measure of success in restorative dentistry. Currently, particular attention is given to the surface of adhesion between the tooth tissue and the filling. This is due to the fact that a gradual degradation of this zone is observed in the course of the functioning of a reconstruction. The exposure of collagen due to dentine etching or the application of self-etching primers removes or modifies the mineral phase of dentine. The exposed matrix is then impregnated with adhesive monomers, leading to the creation of the hybrid layer. The stability of resin polymers and the exposed collagen is crucial to the durability of the connection between dentine and the bonding material. The degradation of both these elements impairs adhesion and leads to the formation of a micro-gap between the tooth and the filling material, which can be penetrated by the bacterial flora (1).

Factors impairing the strength of the bond are connected with, among others, the presence of bacteria and their enzymes in the structure of the bacterial biofilm. It has been demonstrated that *S. mutans* has the capacity to release specific enzymes, esterases, which contribute to the degradation of resins in composite materials and bonding systems (2).

Chronic damage the hybrid layer is also a result of hydrolysis and leaching of adhesive monomers which infiltrated the demineralised dentin matrix. Leaching is facilitated by water sorption and its penetration through the zone of loose collagen fibres or the hydrophilic domains of bonding systems. Mechanical wear of the filling can further accelerate the degradation of this bond by abrading its surface and enlarging the enzyme and water penetration area. Hydrolytic degradation is considered to be the main cause of damage to the hybrid layer, leading to a reduction in bonding strength over time (3).

Nanoleakage is regarded as another factor contributing to the degradation of the hybrid layer, which is the penetration of small ions and molecules into the hybrid layer with no visible damage zones. Nanoleakage is formed as a result of a disturbed balance between the rate of dentine demineralisation and the level of its infiltration with bonding systems. This phenomenon is observed both in the case of systems in which tissues are etched in the course of

a separate procedure and in the case of self-etching ones. Neither of the options provide sufficient collagen network saturation, giving rise to a hybrid layer of heterogeneous, diverse thickness (4).

Lack of saturation of the collagen network with the bonding system contributes to the phenomenon of collagen fibres degradation. Fibres which have not been infiltrated, are destroyed as a result of the removal of resin from the space between them and their disorganisation. The degree of degradation may differ depending on the bonding system (3).

More and more research examines the effect of endogenous proteases on the degradation of the hybrid layer. These reports have important clinical implications. Endogenous metalloproteinases, among others, MMP-9, are involved in the process of degradation. The presence of water is necessary to obtain the hydrolytic activity of MMPs. In humid conditions, MMPs hydrolyse peptide bonds, causing the degradation of the bond between dentine and resin (5-8).

Pashley et al. (9) studied the activity of proteolytic enzymes on demineralised dentine stored in water, artificial saliva and oil, and evaluated the role of proteolytic enzyme inhibitors in the protection of the demineralised collagen matrix. The results obtained suggest that the hydrolytic degradation of collagen occurs in the absence of bacteria. According to the authors, the metalloproteinases from the demineralised matrix may be activated during dentine etching or treatment and are probably responsible for the degradation of the hybrid layer in the aquatic environment.

Having confirmed the presence and action of endogenous metalloproteinases in dentine, the activity of these enzymes in the hybrid layer was assessed (10). It was demonstrated that active proteolytic enzymes are present inside the hybrid layer. Their activity was observed in the bottom zone of the hybrid layer, which correlates with the presence of areas of demineralised collagen, not saturated with the bonding system. Interestingly, exposed areas of collagen were found in the distribution area of nano pores in the hybrid layer, giving rise to progressing damage of the layer.

It has been proven that in dentine treated with an isolated etchant, as well as in one treated with self-etching systems, MMP-2 and MMP-9 enzymes are secreted. The effect of the metalloproteinases can be intensified as a result of the loss of the inhibitory properties of TIMP, the main natural MMPs inhibitors present in the tissues of the tooth. By combining with metalloproteinase molecules, these endogenous compounds inhibit their activity. Therefore, the balance between them and MMPs is crucial to ensuring the stability of healthy tooth tissues (10, 11). TIMPs control matrix metalloproteinases in two ways: by inhibiting the phase of proenzyme transformation into an enzyme, as well as by inhibiting the activity of MMPs.

So far, 4 types of TIMPs have been known (TIMP-1 to TIMP-4), which have the ability to create enzyme-inhibitor complexes. TIMPs are composed of two sub-units: the N-terminal and the C-terminal one. All TIMP inhibitors inhibit metalloproteinases. Under physiological conditions, at low concentrations of MMPs – usually inactive – the MMP-TIMP system works reliably and is involved in tissue modelling. In pathological conditions, however, the tissue inhibitors are unable to inhibit the activity of the increased titre of active MMPs (6, 12, 13).

In the process of bonding systems degradation, cathepsins also play a significant role. Lowered pH of the oral cavity environment, induced by the application of the procedures aimed at generating adhesion, creates conditions for the activation of these enzymes. Studies have shown the potential correlation between the activity of metalloproteinases and cathepsins, both in healthy and demineralised tissue (3, 14, 15). The application of acidic monomers activates both metalloproteinases and cathepsins. Cysteine cathepsins are an important group of proteolytic enzymes present in dentine. Their diversity is comparable with the number of existing metalloproteinases. Cathepsins demonstrate the ability to degrade dentine matrix in a physiological and pathological way. They also play a role in the course of tooth decay and gum and periodontium diseases, being responsible for the occurrence of a gradual loss of tightness of adhesive reconstructions. It has been observed that cathepsins have different degradation potential depending on the area of infected dentine, the location of a carious lesion and its activity. The levels of these enzymes increases significantly in very deep cavities located near the pulp, which indicates that cathepsins of dentine or pulp origin play a key role in the progress of active lesions and the hybrid layer degradation. They are self-activated at lowered pH. Under neutral conditions they remain inactive – in contrast to MMPs, which are the most active at neutral pH (3, 14-17).

In the light of the foregoing, it would seem to be the key to ensure the integrity of the collagen matrix by inhibiting endogenous proteases. This may increase the durability of the bond between dentine and the bonding system and ensure long-term sustenance of fillings from composite materials, thus inhibiting the development of secondary caries. The suspicion of secondary caries is the most common cause of tooth filling replacement. Therefore, in scientific considerations, much attention is given to the prevention of secondary caries formation and the possibility of carious dentine remineralisation. Modern strategies for carious lesions treatment are continuously sought, which would involve the strengthening of dentine structure and promote the use of metalloproteinase inhibitors. The activity of MMPs in dentine and the hybrid layer can be adjusted by endogenous and exogenous inhibitors. Endogenous compounds are obtained from human cells derived from other organs, while the exogenous ones are synthesised artificially (1).

The main direction of research related to the inhibition of metalloproteinases and improved sustainability of bonding systems is the use of chlorhexidine (CHX). It is typically a component of mouthwash liquids used in endodontics to eliminate micro-organisms, including *E. faecalis*. CHX also effectively neutralises the effects of MMP-2, -9 and -8 as well as cysteine cathepsins (3, 18, 19). Pashley et al. confirmed its effectiveness in inhibiting the collagenolytic activity of enzymes (9). It has also been shown that chlorhexidine can improve the stability of the hybrid layer structure and reduce the time-dependent decrease in the bonding strength of bonding systems in both in vivo and in vitro conditions (3, 18, 19).

MMPs are enzymes which depend on the presence of calcium or zinc ions. The degree of CHX inhibitory effect on MMPs can be modified by its chelating capacity, and calcium ions released by primers may be responsible for a decrease in its activity over time (3, 18).

The introduction of CHX in low concentrations (0.2-2.0%) to compounds containing methacrylates does not change the degree of water sorption, and even slightly increases resistance to bending and the level of elasticity modulus of the adhesive compounds involved in creating the hybrid layer (20). The degree of CHX release from polymerised bonding systems depends on its concentration and remains stable (21). The idea of incorporating inhibitors in bonding systems to slow down the degradation of collagen yields promising results. *In vitro* studies have been carried out to show that the application of CHX on dentine and enamel surface treated with phosphoric acid does not reduce the bonding strength of the bonding system after 24 hours (22). Carrilho et al. examined dentine samples after the application of E&R Single Bond, 3 m Espe two-stage system. In the control samples, the degradation at the bottom of the hybrid layer after 6 months increased significantly compared to samples treated with CHX. These reports confirm that the weakest element in the hybrid layer are areas of partially exposed collagen at the bottom of the hybrid layer or below, where collagen fibres are not provided adequate adhesive resin infiltration and protection against degradation. The use of CHX eliminates or slows down the rate of collagen degradation. However, there is a need to continue research in this area in order to develop clinical standards of conduct (23).

Galardin is another synthetic metalloproteinase inhibitor (3, 18). It displays powerful anti-MMP-1, -2, -8 and -9 properties. Its structure resembles collagen and it bonds with the active part of the enzyme. It has inhibitory effects on proteolytic activity at concentrations of 10-100 times lower than in the case of CHX. Including galardin in primer composition resulted in improving the strength of the bonding between the system and the tooth tissues directly after its application. However, after a three-month observation period, no differences were observed in the degree of degradation of the adhesive compounds as compared to the control samples (1, 3, 22).

There are also reports in the literature on the role of EDTA in the inhibition of metalloproteinase activity. Its activity consists in binding calcium and zinc ions. Additionally, as an acid, EDTA has an etching effect. It has been suggested that its use may result in the formation of a hybrid layer resistant to degradation. The time it takes to obtain the effect of etching, as well as the rapid leaching of EDTA from dentine confirmed in studies, which leads to arresting the inhibitory effect on MMPs, are factors limiting its use (3, 18).

Tetracyclines – a family of antibiotics with a broad spectrum of action – are another potential inhibitory compound demonstrating chelating properties. Tetracycline and its semi-synthetic analogues – doxycycline and minocycline belong to this group. In addition to binding the zinc and calcium ions, the chemotherapeutic agents may also influence the expression of metalloproteinase mRNA. Doxycycline demonstrates specific inhibitory properties with respect to MMPs. Among synthetic inhibitors, chemically modified tetracycline (CMTs) yields good results, but it does not demonstrate antimicrobial properties. The mechanism of action of this compound consists in the inhibition of the enzyme secretion and activity, as well as in binding calcium ions. However, the effect of this group of inhibitors on the durability of the hybrid layer requires further research. There is a risk that these potentially potent MMPs inhibitors will contribute to the discolouration of the tooth tissue. Inability to eliminate this problem may limit the possibility of the clinical application of tetracycline group molecules in aesthetic teeth reconstruction materials (3).

Bisphosphonates are another group of protease inhibitors, effective owing to the phenomenon of calcium and zinc ions chelation. This group of inhibitors is currently least well known and requires more detailed study (3). Recent reports suggest that, similarly to chlorhexidine, biphosphonates form electrostatic bonds with collagen. Is believed that their use can be more beneficial than the application of CHX due to the phenomenon of "locking up" a bisphosphonate molecule in the collagen matrix (3, 24). This phenomenon could provide extended durability of the bond between dentine and the bonding system.

With time, the problem of active compounds and ions leaching from the hybrid layer prompted researchers to assess cross-factors associated with collagen. These are molecules containing one or more reactive termini capable of chemical addition to specific groups of proteins or other structures. Their potential is related to the possibility of mechanical strengthening of the collagen network structure, improving the resistance to enzymatic degradation and inactivating exposed metalloproteinases bound with collagen. The efficacy of these compounds in the chemical and physical modification of dentine collagen has been proven, but they still require further research before they are applied in clinical practice (3, 11, 14).

Quaternary ammonium compounds (QAMs) are yet another substances with inhibitory effect. Salts of these substances stabilise the pH and demonstrate antimicrobial properties. Similarly to CHX, they are cationic compounds soluble in water, however, unlike CHX, they are not washed out of adhesion surfaces. QAMs inhibit MMP-9 activity and are more efficient than galardin in inhibiting the degradation of demineralised collagen (25). Because of their antibacterial properties, quaternary ammonium compounds, and especially MDPB (12-methacryloyloxydodecylpyridinium bromide) are incorporated into the composition of self-etching bonding systems. They have the ability to form bonds with the molecules of the adhesive monomers. *In vitro* and *in vivo* studies indicate that QAMs compounds inhibit collagenolytic enzymes in the hybrid layer (26, 27). The inhibitory properties of MDPB with respect to metalloproteinases may contribute to the improvement of the durability of fillings. As a copolymer with resin methacrylates, MDPB retains its inhibitory effect on MMPs, despite the passage of time. The inhibition effect was used to block the release of collagen degradation products, including cross-linked telopeptides of type I collagen, C-terminal cross-linked molecules of type I collagen and cathepsins. Among the MMPs inhibitors so far evaluated, 12-methacryloyloxydodecylpyridinium bromide displays the greatest effectiveness (3). Although the exact mechanism of QAMs activity in MMPs inhibition is not completely understood, it is thought that the degradation of collagen is inhibited through the mechanism of electrostatic bonding (27, 28).

There are also attempts at dentine tissue regeneration, among others, biomimetic dentine remineralisation. This is an interesting concept which applies nanotechnology to achieve an effect similar to natural dentine remineralisation. In the areas where an insufficient amount of resin is replaced by water, it is displaced by apatite crystals. The size of the crystals is small enough to allow them to be incorporated both outside and inside collagen fibres. Despite the promising results, the concept still requires confirmation by continued studies (29, 30).

Conclusions

Metalloproteinases are a group of proteolytic enzymes capable of degrading almost all the components of dentine matrix. They participate both in physiological and pathological processes occurring in the environment of the oral cavity. Their presence was determined in saliva, gingival fluid, enamel and dentine. MMPs modify the course of both caries and periodontal diseases. They are identified in the hybrid layer and acid components of adhesive agents may contribute to their activation. Incomplete impregnation of collagen fibres with the bonding system may lead to the creation of nano damage allowing the penetration of water molecules, proteinases and pathogens. Many attempts are undertaken, with the use of various chemical compounds, at inhibiting MMPs activity. So far, quaternary ammonium compounds have given the best results in MMPs proteolytic activity inhibition. However, the search for the best inhibitors allowing for the stabilisation of the adhesion zones of composite materials to the tooth tissues is ongoing.

Conflict of interest

None

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