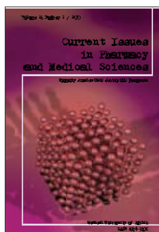

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Dentin adhesion and matrix metalloproteinases

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ABSTRACT

One of the challenges of contemporary conservative dentistry is prevention of secondary caries. Despite the growing bond strength values, which is obtained by chemical and technical improvements, rapid solubility and low durability still make a problem. The main reason of failure in adhesion is degradation of the collagens fibrils – the component of hybrid layer. The ground of this action can be matrix metalloproteinases (MMPs) – host-derived enzymes, which play a role in many physiological but also pathological processes. Basing on literature the authors divide them into groups and describe their structure and substrates.

Keywords: metalloproteinases, bonding, adhesive resins, hybrid layer

INTRODUCTION

Increase in the attractiveness of aesthetic restorations, as well as decreasing acceptance among patients of amalgam fillings forces dentists to broaden the knowledge of composite materials. Ability of bonding systems to ensure proper adhesion and stability of adhesion to the tooth tissues is one of the most important requirements addressed to them to make the filling permanent. Present restorative techniques are based on the adhesive properties of materials based on resins. Following the pioneering approach of Buonocore to increase adhesion forces (1955) [4], researchers and producers have perfected both adhesion (sealing) to the tooth tissues as well as the cement properties (bonding) of binding systems. Despite significant improvements, the surface adhesion of the composite to the tooth is still the weakest area of the filling. If the surface adhesion of the adhesive system to dentin is exposed to the oral environment, there are often marginal discolorations, poor marginal adaptation, and thus the loss of filling retention [17]. Although studies have shown almost immediate, short-term superior efficiency of bonding systems, the long-term durability of some of them is questionable [28]. In fact, recent studies have pointed out that the immediate binding properties of the bond do not always correlate with the long-term stability due to the degradation that occurs quite rapidly and encompasses the entire surface of the dentin covered with bond [2,9].

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Adhesion to dentin

Inherent part of filling of the cavity is "smear layer" – a kind of natural background that by blocking the open dentinal tubules protects them against the ingress of microorganisms and minimizes postoperative sensitivity. Sources give different values of the smear layer thickness – they range from 1 to 2 μm. In the tubular dentine the layer may be up to a depth of 6 μm. The smear layer consists of dentin-derived ingredients (hydroxyapatite, collagen, denatured dentin components, proteoglycans, glycosaminoglycans, derived elements inside the pulp and bacteria) and outer components (saliva, bacteria, blood, epithelial cells). The contents of the individual components of the smear layer are different depending on the depth at which it originated. The closer to the pulp, the more organic material in it, odontoblasts tabs, proteoglycans and glycosaminoglycans [15,25,26].

Currently, there are two strategies of bonding systems connection with tissues of the tooth, i.e., by removing the smear layer generated during cavity preparation (etch & rinse technique) or by leaving it as a substrate to create a connection between dentin and bonding system (self-etch technique). The difference between the two techniques lies in the preliminary and separate use of etching in systems "etch & rinse" (usually 35-37% orthophosphoric acid), which is then rinsed, however in the technique of "self-etch", the primer is only dried with air from the blower, and therefore remains in a modified

smear layer (this technique could be called “etch & dry”). The next step in the two adhesive systems is the primer and the bond, which may be separate or interconnected, depending on the type of system. The present classification of adhesives is based on the number of steps constituting the given system. The “etch & rinse” systems can be divided into three- or two-component, depending on whether the primer and bond are in a separate or in the same bottle. Similarly, the self-etching techniques can be two- or one-component, depending on whether the etch/primer and bond are in separate or in the same bottle [2,27,30].

“Bonding” is dentin impregnation with mixture of monomers [2]. It has been proved that certain monomers (in applying adhesive system according to the manufacturer’s instructions) cannot penetrate completely and homogeneously the dentin collagen network – especially those of high molecular weight. It turns out that about 98% of the resin monomers penetrate at 1 μm, 89% at 2 μm, 71% at 3 μm and the bottom of demineralized dentin (i.e. to a depth of 8 μm) is reached only by 18% of the resin monomers BisGMA, HEMA. This leads to retaining of many collagen fiber scaffolds exposed [21,33]. In addition, elution (evaporation) of the monomers and polymers of low molecular weight – probably by an incomplete polymerization – gives a similar result, increasing the porosity of the hybrid layer.

It is also known that the force of cohesion of the binding systems is correlated with the degree of evaporation of the solvent [14]. Prolonged application facilitates the removal (by evaporation) of water and solvent, which enhances the conversion process and the formation of the polymers cross-binding, thereby improving the mechanical properties of the resin in the hybrid layer. It is worth noting that systems based on water or ethanol (Single Bond, 3M ESPE) require a longer application time than those based on acetone (One Step, Bisco). This is probably due to differences in the solvents evaporation pressure of both systems. Acetone is a liquid with a high evaporation pressure (about 233 mbar) compared to water and ethanol (respectively 23 and 44 mbar) [1,19,21].

Other possibilities to reduce micro leakage in everyday practice are brought by procedures increasing the adhesion of the composite to the tooth (enamel shear at an angle, the use of micro filler bonding systems) and procedures to minimize polymerization shrinkage (composite layer condensation, the use of curing lights of increasing light intensity, re-bonding) [11,26].

In conservative dentistry, adhesion to dentin is currently achieved through the creation of a micromechanical connection between the joining resin and the demineralized dentin. Etching of dentin broadens dentinal tubules to a depth of up to 30 μm resulting from removal of peri-tubular dentin. Because of intra-tubular dentin demineralization, so called diffusion channels are formed with a length of 1-10 μm, exposing the network of collagen fibers. Micro retention with primer-soaked mesh of collagen fibers and adhesive resin determine the strength of adhesion. Hybrid layer and its quality play a major role in the adhesion because micro protrusions, as noted in 1982 by Nakabayashi, only slightly enhance retention. Hybrid layer and adhesive resin – which is an intermediate layer – constitute appropriate bond, i.e. *tight sealing film*. Besides this action, a hybrid layer also serves other, no less important functions, such as absorption and compensation of stresses generated during the polymerization, the changes of temperature and the chewing process. It should also be resistant to proteolytic enzymes and acidic environment [21,26,31]. As the hybrid layer is formed by an organic matrix of dentin, remaining hydroxyapatite crystals, resin monomers and a solvent, the “aging” may refer to individually each component or occur by a synergistic combination of degradation phenomena which occur in the hybrid layer [2].

Contemporary dental adhesives show favorable immediate results in terms of bonding effectiveness. However, the durability of resin-dentin bonds is their major problem. It appears that simplification of adhesive techniques is rather detrimental to the long-term stability of resin-tooth interface. The hydrostatic pulpal pressure, the dentinal fluid flow and the increased dentinal wetness in vital dentin can affect the close interaction of certain dentin adhesives with dentinal tissue. Bond degradation occurs via water sorption, hydrolysis of ester linkages of methacrylate resins, and activation of endogenous dentin matrix metalloproteinases [8].

There are many ways to strengthen the bond strength between bond and dentin. Repeated rubbing of successive layers of bond is one of them. Hashimoto et al. [12] showed that the bond strength increases and micro leakage decreases with each additional layer of adhesive up to the fourth layer. Moreover, a greater number of layers does not affect the quality of the bond strength and adhesion [2,12]. Another method of improving the adhesion is related to the evaporation of solvent in order to avoid phase separation in the layer adhesive system [2]. The air flow from the blower directed to the applied bond helps to remove the water, thereby improving the efficiency of binding [29]. Cadenaro et al. [5] proposed extension of polymerization time by 20 seconds than recommended by the manufacturers. Studies have shown that the permeability of the resin and loss of monomers result from insufficient polymerization of adhesive system, which reduces its efficiency [2].

Recent studies have demonstrated the benefit of adding the electrical current to the application protocol of bonding system, which improves the infiltration of the monomers in etch & rinse technique as well as of self-etch

technique [3,20]. Electrical current is generated by the ElectroBond device, made by the Italian company Seti. This device consists of a tip with disposable sponge that is impregnated with bonding system and attached to the dentin in the cavity. Bond release is triggered by difference of electrical potentials between the tooth and bond. As in endometer, passive electrode is placed on patient's lip and connected via electrical circuit with the active electrode. Electrically-assisted application of bonding system improves the efficiency of binding, as evidenced by the increase in tensile strength of the test samples compared to control samples (with micro sponge without the use of electric current) [2].

Contemporary resin-dentin bonding is initiated by systems that use phosphoric acid or acidic resin monomers to remove mineral, however it exposes the superficial dentin collagen matrix. Collagen-associated proteins, including enzymes known as matrix metalloproteinases (MMPs), are also exposed. The collagen matrix is subsequently infiltrated with resins that are polymerized to establish an adhesive attachment to the dentin. Exposed collagen matrix that is not infiltrated with the adhesive, can be degraded by associated MMPs, which might result in deterioration of the adhesive-dentin bond over time [22,24].

Matrix metalloproteinases

Metalloproteinases (*MMPs*) are zinc-dependent membrane-bound cellular endopeptidases which are capable of degradation of all components of the extracellular matrix [2,25,26]. They are also involved in many physiological processes such as embryonic development, tissue formation, wound healing, angiogenesis, and in pathological processes such as cancer, ulcers, arthritis, periodontitis, fibrosis. Currently, there are 23 known human metalloproteinase [25]. The similarity in structure to MMP-1 (the first discovered metalloproteinase), dependence on zinc and calcium ions, inhibition by tissue inhibitors of metalloproteinases (TIMPs), are the qualities on which proteases are allocated to the appropriate family. Because of the substrate specificity and homology, metalloproteinases are divided into six groups: collagenases, gelatinases, stromelysins, matrilysins, metalproteinases of membrane type (*membrane-type MMPs*) and other metalloproteinases (Table 1) [8,16,22,24]. The structure of metalloproteinases is showed on Fig. 1. MMPs are multi-domain enzymes containing in their composition catalytic domain and the prodomain (with the exception of MMP-23) [16]. In addition, these proteins may also include hemopexine domain, linked to the catalytic domain by a flexible adapter composed of 15-65 amino acids. Prodomain comprises propeptide maintaining the enzyme in the form of a zymogen (propenzym, pro MMP) and is composed of three alpha-helices connected by flexible loops exposed to autoprotolysis. Catalytic domain, which is responsible for the proteolytic activity of the enzyme is composed of five beta-ribbons, three alpha-helices and loops connecting them [16]. This domain contains one catalytic (coordinated by three histidine residues, or – in the form of inactive – prodomain cysteines) and one structural zinc ion and predominantly 3 calcium ions necessary for enzyme activity and stability. In all isolated metaloproteinases (except MMP-7 and MMP-26), the catalytic domain is preceded by a C-terminal hemopexine domain responsible for connecting substrates and tissue inhibitors of metalloproteinases, the proteolytic activity and membrane activation. In the membrane-type metaloproteinases C-terminal domain attaches the molecule to the plasma membrane [23,32]. Hemopexine domain owes its name to the analogy of sequence to hemopexine – protein binding and transporting heme. It has a shape of an ellipsoidal disk comprised of four symmetrically arranged parts resulting from beta ribbons. It is important to identify properly the substrate, and in collagenases subfamily it is necessary in order that superhelix collagen etching could exist. Other important features of this domain include the ability to bind TIMPs by MMP-9, participation in the activation of proMMP-2, the stabilization of the enzyme [10,16].

Table 1. Classification of metalloproteinases

MMP (another name)	Enzyme group	Substrates (selected)
MMP-1 (collagenase-1)	Collagenases	Collagen type I-III, V, II, VIII, X, XI, gelatin, aggrecan, IL-1β, proteoglycans, entactin, ovostatin, laminin, vitronectin, L-selectin, perlecan, L-selectin, tenascin,α 2-macroglobulin, proTNF-α, proMMP-1, -2, -9
MMP-2 (gelatinase A)	Gelatinases	Gelatin, collagen type I-VII, IX-XI, elastin, fibronectin, laminin, aggrecan, decorin, versican, osteonectin, tenascin, vitronectin, α 2-macroglobulin, IL-1β, proTNF-α, TGF-β latent, proMMP- 1, -2, -9, -13
MMP-3 (stromelysin-1)	Stromelysins	Collagen type III-V, VII, IX-XI, telopeptides of collagen, gelatin, elastin, casein, osteonectin, ovostatin, entactin, fibronectin, laminin, aggrecan, perlecan, plasminogen, MBP (myelin basic protein), decorin, tenascin, laminin, entactin, versican, α 2-macroglobulin, IL-1β, proTNF-α, fibrinogen, proMMP-1, -3, -7, -8, -9, -13
MMP-7 (matrilysin-1)	Matrilysins	Collagen types I, IV, X, gelatin, elastin, fibronectin, laminin, aggrecan, decorin, casein, transferrin, osteonectin, tenascin, entactin, vitronectin, transferrin, plasminogen, MBP, α 2-macroglobulin, proTNF-α, $\beta_4$integrin, proMMP-1, -2, -7, -9
MMP-8 (collagenase-2)	Collagenases	Collagen Type I-III, V, VII, VIII, X, f gelatin, aggrecan, fibronectin, fibrinogen, bradykinin, α_2-macroglobulin
MMP-9 (gelatinase B)	Gelatinases	Collagen type IV, V, VII, X, XI, XIV, gelatin, entactin, decorin, versican, laminin, aggrecan, elastin, fibronectin, osteonectin, vitronectin, plasminogen, MBP, IL-1â, á$_2$-macroglobulin, proTNF-α, TGFβ_2
MMP-10 (stromelysin-2)	Stromelysins	Collagen type II, IV, V, f gelatin, casein, aggrecan, elastin, fibronectin, laminin, proMMP-1, -7, -8, -9
MMP-11 (stromelysin-3)	Stromelysins	Type IV collagen, gelatin, fibronectin, an inhibitor of á$_1$-proteinase, inhibitor α_2-proteinase, casein, α_2-macroglobulin
MMP-12 (Macrophage-elastase, metalloelastase)	Other enzymes	Collagen types I, IV, V, gelatin, elastin, casein, fibronectin,vitronectin, laminin, entactin, MBP, fibrinogen, fibrin, plasminogen, aggrecan, decorin, an inhibitor of α_1-proteinase, α_2-macroglobulin
MMP-13 (collagenase-3)	Collagenases	Collagen Type I-IV, VI, IX, X, XIV, telopeptides of collagen, gelatin, plasminogen, aggrecan, perlecan, fibronectin, tenascin, osteonectin, proMMP-9, α_2-macroglobulin

MMP-14 (MT1-MMP)	Membrane-type matrix metalloproteinases	Collagen type I-III, gelatin, casein, fibronectin, laminin, aggrecan, vitronectin, entactin, proteoglycans, tenscin, transglutaminase, α_2-macroglobulin, α-proTNF, fibrinogen inhibitor α_1-proteinase, proMMP-2, -13 , -20
MMP-15 (MT2-MMP)	Membrane-type matrix metalloproteinases	Fibronectin, entactin, laminin, aggrecan, perlecan, tenascin, transglutaminase, proTBF-α, proMMP-2
MMP-16 (MT3-MMP)	Membrane-type matrix metalloproteinases	Collagen type III, gelatin, fibronectin, casein, laminin, α2-macroglobulin, transglutaminase, proMMP-2
MMP-17 (MT4-MMP)	Membrane-type matrix metalloproteinases	Gelatin, fibrinogen, fibrin, proTNF-α, α_2-macroglobulin
MMP-18 (collagenase-4)	Collagenases	Collagen type I
MMP-19 (RASI1)	Other enzymes	Collagen types I, IV, gelatin, fibronectin, laminin, aggrecan, entactin, tenascin, fibrinogen, fibrin, COMP (oligomeric cartilage matrix protein)
MMP-20 (enamelisin)	Other enzymes	Amelogenin, collagen type XVIII, aggrecan, fibronectin, laminin, tenascin, COMP
MMP-21 (identified on chromosome I)	Other enzymes	α_1-antitrypsin (?)
MMP-22 (identified on chromosome I)	Other enzymes	Gelatine
MMP-23 (CA-MMP)	Other enzymes	Gelatine
MMP-24 (MT5-MMP)	Membrane-type matrix metalloproteinases	Collagen type I, gelatin, fibronectin, laminin
MMP-25 (MT6-MMP)	Membrane-type matrix metalloproteinases	Type IV collagen, gelatin, fibronectin
MMP-26 (matrilysin-2, endometaza)	Matrilysins	Type IV collagen, gelatin, fibronectin, an inhibitor of α_1-proteinase
MMP-27	Unidentified	Unidentified
MMP-28 (epilizyna)	Other enzymes	Casein
MMP-29	Unidentified	Unidentified

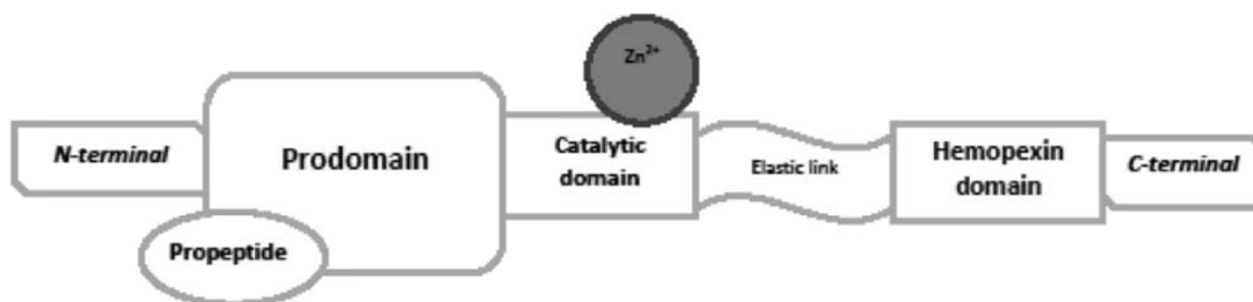

Fig. 1. General structure of metalloproteinases

Expression and activation of MMPs are regulated at the level of transcription, secretion, activation of the precursor in the form of a zymogen, interaction with the specific components of the extracellular matrix and by the inhibitory action of TIMPs. Transcription can be caused by various signals, such as cytokines, growth factors, hormones, mechanical factors, changes in the extracellular matrix leading to the modification of the interaction between the cells. MMPs activation is carried out by removing the prodomain, which results in exposure of the active site of the enzyme. MMPs may be activated by other proteases or factors, such as mercuric chloride, oxidized glutathione, sodium lauryl sulfate, low pH and high temperature. Most of these perturbents act by interfering with the interaction between cysteine and the catalytic zinc within the cysteine switch. Initially, there is initiation of activation of the hydrolysis in the propeptide, then the propeptide is removed exposing thus the active site. The active site largely determines the substrate specificity of MMPs [10,16,18].

The discovery that endogenous collagenolytic and gelatinolytic factors derived from etched dentin lead to degradation of the hybrid layer, suggested the use of metalloproteinase inhibitors for primers to slow or prevent this phenomenon [2,6]. Hebling et al. [13] demonstrated the structural integrity of the collagen network of the hybrid layer of the teeth impregnated with chlorhexidine as compared with the progressive disintegration of that network in control teeth.

CONCLUSIONS

In the process of the development of dental caries, the collagen matrix degradation occurs involving the enzymes of which metalloproteinases play an important role. This hypothesis was confirmed by studies in rats, in which a reduction in dental caries was observed after the use of metalloproteinases inhibitors: CMT-3 and zoledronat. Dentin metalloproteinases and their role in the formation of dental caries is a challenge for future research, as a selective effect on their activity is difficult to obtain. There seems to be hope in finding inhibitors of specific metalloproteinases and selective inhibition of these enzymes activity at the target site [7,24]. In summary, in a large family of metalloproteinases several enzymes are located in the dentin-pulp complex. In addition to their role in physiological processes during development and maintenance of the complex, their participation in the pathogenesis of carious process is emphasized.

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