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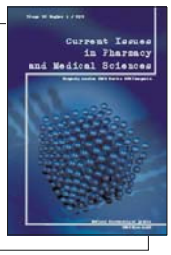

Role of chlorhexidine in adhesion to dentin

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ABSTRACT

The authors, basing on literature discuss the role of chlorhexidine (CHX) in adhesion to dentine in restorative dentistry. Many studies have shown that the chlorhexidine has an inhibitory effect on metalloproteinases (MMPs), by preventing the degradation of the connection between bonding system and a dentin. Despite the introduction of additional procedure in the form of disinfection of the cavity using chlorhexidine and thus the extension of the valuable chair-time, the fact of preventing microleakage and secondary caries seems to be convincing but further detailed studies must be carried out in this area.

Keywords: adhesion, dentin, chlorhexidine (CHX), metalloproteinase (MMP)

Despite significant progress in the quality of adhesion of materials placed into dentin, avoiding of microleakage – the effect of polymerization shrinkage, an inherent part accompanying composites – is still the number one problem of modern conservative dentistry. As a result of exposure of the dentin/adhesive interface to oral cavity, marginal discolorations, poor marginal adaptation and subsequent loss of retention of the restoration and finally recurrent caries, are found during clinical examination [1,5,11,12]. Among the reasons thereof we can include: leaving the infected dentin in prepared cavity, excessive etching and/or drying after etching of dentin and the associated hydrolysis of collagen by matrix metalloproteinases, temperature fluctuations or occlusal loads [5]. While the strength of adhesion to enamel binding systems is high, dentin-bonding adhesives are not as durable as was previously thought. Although the immediate microtensile bond strength is high, it gradually falls 30-40% in 6-12 months [15]. The reason for this is degradation of the hybrid layer formed by collagen fibrils and the organic matrix of dentin, remaining hydroxyapatite crystals, resin monomers and the solvent [1]. This process occurs with the involvement of matrix metalloproteinases (MMPs), endopeptidases involved in many physiological processes such as embryonic development, tissue formation, wound healing, angiogenesis, and also in pathological processes such as cancer, ulcers, arthritis, periodontitis, fibrosis [1,3,16,22,23]. Metalloproteinases are multidomain zinc-dependent proteases. Host-derived pro-MMPs are secreted as inactive zymogens and are uncovered and activated by mild acids released by caries-producing bacteria (lactic acid) and acid-etchants [6,9]. Incomplete resin infiltration has also contribution in exposure of collagen fibrils and enables MMPs to have free access to water. Both low pH and water environment are obligatory requirements of these enzymes. [6,15]. It is worth mentioning that self-etch adhesives have pH between 1.6 and 2.9 and this is sufficiently low to demineralize dentin and uncover MMPs and activate them without denaturing them [15]. It is speculated that 37%-phosphoric acid (used in etch-and-rinse technique) which pH is between 0.03 [14] and 0.7 [10], can partially denature these enzymes and it needs to be investigated. Although it is proven that 37%-phosphoric acid removes both extra- and intrafibrillar crystallites, while self-etching (SE) adhesives remove most of the outside-fibrils-crystals and only some of the inside-fibrils-crystals. This phenomenon is reflected in the absorption of water, which in the case of total-etch (TE) technique goes to deeper parts of the collagen fibrils, which facilitates a greater degree of hydrolysis by MMPs. [15].

Compounds that inhibit collagenolytic and gelatinolytic activity of matrix metalloproteinases are inhibitors of metalloproteinases [14]. They can be divided into endogenous and exogenous (synthetic) [26]. The first of them are the tissue inhibitors of metalloproteinases (TIMPs). Among the exogenous ones we can distinguish

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hydroxamic acid derivatives (batimastat, marimastat, SM-25453), SB-3CT, chemically modified tetracyclines (CMTs). Batimastat and merimastat are collagen mimetic compounds and chelating the active site of the enzyme, which have been developed by British Biotech Pharmaceuticals. However, the first one was very poorly soluble in water, while the application of the second one was accompanied by a number of side effects. The created later CT 1166 has proven to be effective in the inhibition of metalloproteinases. The above-mentioned drugs are used to treat cancer and appear to be inappropriate in the inhibition of dental caries of dentin. Although the surface application of those measures may be sufficient in controlling dental caries, however more selective and less toxic inhibitors are sought. Among other inhibitors – SB-3CT is worth mentioning, which blocks the activity of MMP-9. Synthetic forms of tetracyclines (doxycycline, minocycline) inhibit the secretion and activity of metalloproteinases (MMP-1, MMP-2, MMP-12), acting by binding calcium ions, both *in vitro* and *in vivo*. Doxycycline (Periostat®) is a nonspecific inhibitor of MMPs, used in periodontal diseases [19]. It has been shown that small doses of doxycycline did not affect the bacterial flora of the oral cavity; they can inhibit MMPs. For inhibition of metalloproteinases zoledronic acid was also tested (a compound of bisphosphonates), which showed the desired effect [6,8,19,21,26].

Tissue inhibitors of metalloproteinases, including secreted proteins (TIMP-1, TIMP-2, TIMP-3, TIMP-4), consist of two domains. The shape of molecule inhibitors resembles a wedge, which similarly as a substrate penetrates the active site of the enzyme. The N-terminal domain binds to the active site of MMP by non-covalent binding, thus blocking the access of the substrate to the catalytic site. C-terminal domain of TIMP-1 and TIMP-2 joins the hemopexine domain, proMMP-9 and proMMP-2 respectively. TIMPs maintain a balance between destruction and creation of matrix. It has been proven that derived from the saliva TIMP-1s, are active at low pH, and thus may continue to operate on the increased pH and have inhibitory role on MMPs in the course of the dental caries. However, the level of these inhibitors in the active caries may be insufficient to stop the degradation. So the hope lies in increasing the local concentration of inhibitors in carious lesion. It has been shown that α-macroglobulin is a more effective inhibitor of MMP-1 than TIMP-1 [6,19].

Many studies have shown that the chlorhexidine (CHX) has also inhibitory effect on MMPs, by preventing the degradation of the connection between bonding system and a dentin [8]. Other substances that have been investigated in this direction are polyvinylphosphonic acid [24] and quaternary ammonium metacrylates [25]. There is also the view that ethanol used instead of water during the wetting of dentin can also reduce the activity of MMPs. The manufacturers of adhesive systems are considering extending the recommendations of application of the total-etch technique for one minute application of CHX after etching and/or use of CHX as a primer in self-etch technique. CHX similarly as other inhibitors of metalloproteinases can also be added to the self-etch systems [2,4,17].

Chlorhexidine is a broad-spectrum disinfecting agent, isolated form the scorpio's toxin, which is bis-bis-guanide that binds to several proteins by a cation-chelating mechanism. It has excellent antiseptic properties due to acting on ferritine, an iron-storage protein, and destructing the cellular protection from oxyradicals [7,13]. In the similar mechanism, chlorhexidine prevents the binding of metal ions, such as zinc or calcium, to the MMP and so inhibits its catalytic activity. However, the MMPs are probably denaturated at higher concentrations of CHX [7]. Etching of dentin releases a large quantity of calcium salts, which in TE technique are flushed out, while in the SE technique they are incorporated into primer and adhesive layer. Studies have shown that the inhibitory properties of CHX-low- concentration-solutions on MMPs can thus be reduced as a result of displacement of zinc ions by calcium ions [7,8].

CHX has strong antibacterial properties and as an electrically positive-charged molecule has an affinity to negative-charged molecules of bacterial cell membrane. This interaction increases the permeability of bacterial cell membranes, which leads to penetration of destructive agents into the cytoplasm resulting in the ultimate death of the microorganism [5,20]. The studies carried out by Chaharon et al. indicated that the use of 2% CHX has no effect on the shear bond strength of two-step and one-step self-etching adhesive resins [5]. Measurements were performed 24 hours after setting a filling with the use of additional procedure of rubbing into the tooth tissue a preparation Cavity Cleanser™. Further investigations are warranted to evaluate if this additional procedure will be beneficial after 6 or 12 months.

Although CHX binds to demineralized dentin electrostatically, there is no covalent bonding and it is probable that such an inhibitor may reach hybrid layer form over the course of 1 to 2 years and will only delay but not stop collagen degradation. More investigations should be performed in this field [18].

An example of a formulation containing 2% solution of chlorhexidine is Cavity Cleanser™ (BISCO Inc., Schaumburg, IL, USA). As indications - the manufacturer mentions cleaning and moistening. Instructions for use are different for TE technique and SE technique. In etch-and-rinse technique it is recommended to etch (for 15 seconds), rinse and dry the surface of a cavity, leaving it non-desiccated (it should be damp and glossy). Then Cavity Cleanser™ is applied using a brush or foam pellet. The

puddled solution has to be removed with a new foam pellet, leaving the surface slightly moist. The next step is application of adhesive, according to manufacturer's instructions. If self-etch adhesive system is used, application of Cavity Cleanser™ on non-etched, rinsed and dried surface, is followed by application of the adhesive [27].

Despite the introduction of additional procedure in the form of disinfection of the cavity using chlorhexidine and extend by this the valuable chair-time, the fact of preventing microleakage and secondary caries seems to be convincing. Time will tell whether commercialization and financial viability analysis does not move away producers from making every effort to ensure that their materials are the best. This can be achieved for example by the addition of zinc ions to the bonds primers in order to protect the hybrid layer against the effects of metalloproteinases, or by enriching them with MMP inhibitors, such as chlorhexidine. Further detailed studies must be carried out in this area.

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