DENTIN DEGRADOMICS IN DENTIN EROSION

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ABSTRACT

Background Dental degradomics are the enzymes found in dentin endogenously and are aimed at attacking organic compounds of the relevant tissue. During dentin demineralization, these enzymes could turn into the reaction phase and may step up the degradation. Thus, their connection with dentin erosion and tissue loss should be explained.

Objective The aim of this review was to describe the mechanisms of dentin degradomics, their relation to dentin erosion, and recent approaches on inhibiting their action.

Data sources A narrative review was performed with a literature search in the PubMed and Google Scholar electronic databases.

Study selection Reference lists included full papers of any study design, published in peer-reviewed journals in English till November 2021.

Data extraction Current literature indicates the term of dentin degradomics, and the mechanism of dental erosion of both enamel and dentin tissues. The inhibition of matrixmetalloproteinase (MMP) enzymes, which constitute the subgroup of dentin degradomics, was gained from the recent papers listed in the reference section.

Data synthesis Biocorrosion covers more of the pathological process of the tissue loss however, most of the dentin degradomics such as MMPs are not covered by the term, biocorrosion. So, the definitions of biocorrosion and dentin degradomics were discussed in detail. Green tea, chlorhexidine and fluorides have the ability to inhibit the reaction of MMPs during dentin demineralization with a different state of mechanisms. Nowadays, other naturally-derived compounds were included in studies such as polyphenols and flavonoids. Still, more studies are necessary to clarify their mechanism of action and rates of efficiency.

KEYWORDS

Dental Erosion; Dentin Degradomics; Biocorrosion; MMP Inhibitors; Polyphenols.

1. INTRODUCTION

With the transformation of lifestyle dynamics and dietary habits, dental erosion has become an increased concern recently. Erosive tooth wear is an important oral health problem when considering the prolongation of human life and the survival of healthy dentition with the overall wellness approach. Regarding the ultraconservative dental concept, updated preventive strategies, and the recent technological improvements in the evaluating methods of enamel surface characteristics at both elemental and physical levels, dental researchers and clinicians have spent significant efforts to clarify the mechanisms of dental erosion. While only a few articles were available during the 1970s, today there are dozens of researches either in vivo or in vitro about dental erosion [1]. Dental erosion was previously defined as a sole substance loss by exogenous or endogenous acids without bacterial involvement. However, it was revealed in 2012 that dental erosion was not only a surface phenomenon but it showed a mineral dissolution beneath the surface [2-4]. It was proved that surface wear in the erosion process was heightened with the friction of acidic solution thus, dental erosion was not only a chemical dissolution but also a pathodynamic surface alteration [5]. Including the whole chemical, biochemical, and electrochemical changes within the dental tissues, ‘bio-corrosion’ was recommended to be used in terms of dental erosion [6]. The term bio-corrosion, which is used in the same sense as the term “microbiological corrosion” in engineering branches, has entered the field of dentistry in its broadest sense under its definition. While corrosion alone describes the chemical, electrochemical, and physicochemical dissolution of inanimate substances, the definition of bio-corrosion includes all the chemical, biochemical, and electrochemical changes seen in both hard and soft tissues and body fluids in living organisms. These changes are seen as either dissolution of the tissue or cell apposition...
by inducing tissue growth. Ulcers, vascular ruptures, or muscle injuries in living organisms as a result of tissue dissolution or induction of tissue growth, even cancer cases may develop [7]. In the field of dentistry, pathologic stages of bio-corrosion reveal mostly on the development of dental caries and erosion. In the following parts of the current review, the term “bio-corrosion”, its relation to dentin degradomics, and recent updates on inhibiting endogenous etiologies of dentin erosion are clarified in detail.

2. METHODOLOGY

The article search for this literature review utilized PubMed and Google Scholar, and the selection included articles published in peer-reviewed journals in English. The terms used for the introduction part were “Dentin Erosion” and “Dentin Degradomics”. Due to explaining the terms in detail and to the terms being highly up-to-date, no time limit was applied and published articles were looked through till November 2021. To reach a clinical point of action, a branch of dentin degradomics, matrix metalloproteinase (MMP) enzymes, which have been appearing in many studies for a while, and recent chemical compounds used to inhibit MMPs were also considered. The search excluded: monographs and case reports.

3. RESULTS

Dental caries is a pathology caused by bacterial acids that have settled and grown in the biofilm of the dental – mostly enamel – hard surfaces. Dental caries begin with the dissolution of hydroxyapatites of enamel, and a small amount of destruction (proteolysis) occurs in the proteins in the enamel. Simply, the pathology of dental caries is again a bio-corrosion process, as it includes a biochemical beginning (acid production of bacteria) and protein degradation (proteolysis).

The term “erosion” does not include material losses caused by biochemical and electrochemical processes on dental hard surfaces. The biochemical changes induced by “proteolysis” and the electrochemical reactions that occur as a result of the piezoelectric effect on the surface are better defined by the term “bio-corrosion”. To sum up, bio-corrosion is caused by acids coming from both internal and external sources, proteolytic enzymes (pepsin and trypsin), piezoelectric effects - in the dentin because of releasing Ca\(^{2+}\) ions from the tooth surface during dental wear - [5], and factors that cause dissolution in the inorganic and organic matrix of dentin after enamel degradation. Enzymes such as matrix metalloproteinases, which are endogenously found in the structure, are not included in the bio-corrosion mechanism. The biochemical events covered by bio-corrosion are shown in Table 1.

The pathodynamic process begins subsurface by dissolving minerals likewise caries lesions. In the sequel, ionized H\(^{+}\) ions are released from the enamel tissue by acid attacks and non-ionized H\(^{+}\) ions pass through deeper layers of both enamel and dentin tissues [1]. With the non-ionized acidic exposure, the inorganic part of dentin dissolves and collagens of the organic structure are revealed. Thus, the pathodynamic process of the erosion continues with the surface alterations leading to wear and substance losses. Although it was reported that “bio-corrosion”, which reveals all pathologic changes comprehensively, has not yet replaced the term “dental erosion” but is thought to become widespread in the fields of dentistry [6].

Table 1. Processes in bio-corrosion.

<table>
<thead>
<tr>
<th>Processes in Bio-corrosion</th>
<th>Dental plaque (biofilm) &amp; Gingival crevicular fluid</th>
<th>Gastric hydrochloric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Endogenous acid intake</td>
<td>Diet /Nutrition style</td>
<td>Profession/Occupation</td>
</tr>
<tr>
<td>2) Exogenous acid intake</td>
<td>Enzymatic lysis (In dental caries formation)</td>
<td>Proteases (Pepsin and Trypsin)</td>
</tr>
<tr>
<td>3) Proteolysis</td>
<td>Piezoelectric effect on dentin</td>
<td></td>
</tr>
<tr>
<td>4) Electrochemical effect</td>
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</tbody>
</table>

Just as the histology of erosion differs from caries, the morphology of dentin is mainly varied from enamel. Thus, the responses of the two tissues with different contents against acid attacks are highly distinctive. Compared to enamel, the mineral content of dentin diminished, and its organic content is higher. The major component of its organic matrix is Type 1 collagen and other components that are contributed to trace are non-collagenous phosphoprotein, glycoprotein, lipid, and proteoglycan. While the amount of carbonate is approximately 3% in the enamel, this value is 5-6% in dentin, therefore dentin dissolves more easily with acids. On the other hand, the crystals in dentin are smaller than those in enamel; thus, the surface area of dentin exposed to acid attacks is relatively higher [8]. Erosion in enamel tissue, which has 95% inorganic structure, starts with a softening on the surface by the dissolution of the structure and results in permanent loss of demineralized tissue with ongoing acid attacks (Fig. 1) [9]. However, erosion comprises two separate events in dentin, the dissolution of the existing inorganic structure and the realization of proteolytic destruction with the endogenous enzymes (Fig. 2).

Figure 1. Dental erosion limited to enamel tissue of teeth #21 and slight changes in the surface of teeth #11.

The beginning of dentin erosion, inorganic structure, because of their structural differences, acts distinctively as well. At first, peritubular and intertubular dentin begin to dissolve at the same rate. However, after the first minute, the intertubular dentin area remains...
more stable, but the peritubular dentin continues to dissolve rapidly, and the dentinal tubules expand.

**Figure 2.** Dental erosion passed through dentin and loss of structure.

As the acid attack continues, the mineral loss is significantly reduced due to the decreasing demineralization rate and the demineralized area reaches a certain thickness [10]. The degree of mineral loss is supplied by the buffering feature of collagens so that further loss of substance is prevented by the dissolved minerals, which brings the ionic level of the environment to the approximate saturation level. While acid attacks continue to a clinically significant concentration and time, the inorganic part dissolves easily as well. Depending on the potential and duration of action of the erosive agent, at first, a completely demineralized layer and then a partially demineralized layer of dentin appears, followed by a completely sound dentin layer. However, the partially demineralized area in the middle is not present in every case [1].

Although the inorganic content dissolves away with the erosive attack, the organic matrix remains intact and forms a barrier against acid attacks, preventing further mineral release from the dental tissue and stopping the progression of the erosive lesion as mentioned above [11-13]. However, it is thought that some of the proteolytic enzymes in the dentin structure are activated by acidic pH and these enzymes increase the rate of erosion by causing the dissolution of the demineralized organic matrix (DOM). For this reason, a new field has emerged to investigate the functions and mechanisms of these enzymes called “Dentin Degradomics” [14]. Subsequently, many studies have been developed to clarify the role of the organic matrix in the stages of erosive demineralization by also considering the histological structure of dentin [15,16]. Ganss et al. (2014) reported that when the organic matrix is chemically removed by either enzymes or mechanical forces (abrasion) [16], the erosive agent directly encounters the mineralized tissue, which dissolves quickly. However, in the presence of an organic matrix, the pH decrease in the environment slows down, and accordingly, the erosion rate reduces as well. Thus, the organic matrix has the feature of limiting the mineral outflow (ionic diffusion) towards the external environment from the tooth surface [17]. For these reasons, it is clear that the organic matrix has a protective role in erosive wear. DOM is resistant to brushing forces up to 4 Newtons (N) so that it can protect the remaining dentin surface against mechanical trauma such as toothbrush abrasion [18]. However, although this layer is resistant to physical factors, it can be dissolved by enzymatic reactions [16]. Considering that erosive demineralization does not occur in the presence of bacteria, it is certain that host-derived enzymes are responsible for the destruction of DOM, which has been proven by clinical studies [14,19].

Recently, a new category of enzymes has been found and named “Dentin Degradomics” which were aimed to degrade the organic matrix, the collagen layer, endogenously [20]. It was shown in the studies that degradomics consist of collagenolytic enzymes and MMPs which are stable in the organic matrix from the formation of dentin tissue. These enzymes are mainly responsible for the catabolic reactions of the organic matrix and their mechanism of action depends on the pH of the environment [21,22]. When the pH decreases at erosive demineralization, these enzymes become activated and when it turns neutral, they start to degrade the collagen of the organic matrix and contribute to the improvement of erosive demineralization [23]. These MMPs are found in various tissues of the body and they have been secreted when tissue remodeling is needed without any pathological circumstances. MMPs are divided into 6 groups according to their structural properties and substrate specificity: Collagenases, Type IV collagenases (gelatinases), stromelysins, matrilysins, membrane-type MMPs (MT-MMP) and others such as enamelysine (MMP-20) [24]. Not all of these enzymes are found in dentin but the ones which are presented in the dentin are shown in Table 2.

**Table 2.** Classification of various degradomic enzymes (Endogenous collagenolytic dental enzymes)* [14,24]

<table>
<thead>
<tr>
<th>Group</th>
<th>Enzyme</th>
<th>Nomenclature</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagenases</td>
<td>Neutrophil collagenase</td>
<td>MMP-8</td>
<td>It is found frequently at dentinal levels and related highly to carious activity in dentin.</td>
</tr>
<tr>
<td>Gelatinases</td>
<td>Gelatinase A</td>
<td>MMP-2</td>
<td>Telopeptidase activity to Type I collagen in organic matrix. Odontoblasts may express these gelatinases.</td>
</tr>
<tr>
<td></td>
<td>Gelatinase B</td>
<td>MMP-9</td>
<td></td>
</tr>
<tr>
<td>Stromelysins</td>
<td>Stromelysin 1</td>
<td>MMP-3</td>
<td>It has the proteoglycanase activity which may affect the activity of some of the cathepsins.</td>
</tr>
<tr>
<td>Other MMPs</td>
<td>Enamelisin</td>
<td>MMP-20</td>
<td>It has shown to process dentinsialophosphoprotein* and found in dentinal tubules of caries-affected dentin.</td>
</tr>
<tr>
<td>Cysteine cathepsins</td>
<td>Cathepsin</td>
<td>Cathepsin B</td>
<td>They show the gelatinolytic activity.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cathepsin K</td>
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</table>

*Not related to carious or erosive demineralization.
Another family of collagenolytic enzymes, cysteine cathepsins (CC), are activated at neutral pH, unlike MMPs. However, they need slightly acidic pH to function [25]. Because of these properties, it is known that MMPs start to function at the point where CCs lose their functions. Since acidic pH is only durable for a while in dentin erosion, MMPs are thought to play a superior role in collagen degradation than cathepsins [26]. Cysteine cathepsins found in the dentin are also shown in Table 3.

<table>
<thead>
<tr>
<th>MMP inhibitor</th>
<th>Type</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyphenols</td>
<td>Epigallocatechin-3-gallate (EGCG)</td>
<td>Found to have inhibitory properties against MMP-2 and -9 and the activation of MMP-8 [1,26,27].</td>
</tr>
<tr>
<td>Phenolic acid</td>
<td>Anacardic acid</td>
<td>Reported to inhibit MMP-2 and -9 [50].</td>
</tr>
<tr>
<td>Natural flavonoids</td>
<td>Quercetin</td>
<td>Showed collagenolytic activity against MMP-2 [52].</td>
</tr>
<tr>
<td>Non-flavonoid polyphenol</td>
<td>Resveratrol</td>
<td>Reported to inhibit MMP-2 and -9 [54].</td>
</tr>
<tr>
<td>Chlorhexidine (CHX)</td>
<td>Bisbiguanide</td>
<td>Has the ability to reduce MMP-9 expression [56].</td>
</tr>
<tr>
<td>Fluorides</td>
<td>Some of the fluoride compounds (eg. NaF)*</td>
<td>Inhibit the activation of MMPs by ion-blocking [32].</td>
</tr>
</tbody>
</table>

*Not all of the fluoride compounds are enlightened to contribute MMP inhibition.

In the acidic environment, dentin demineralization occurs, collagen fibrils are exposed, and the MMPs in dentin and saliva are activated simultaneously. However, when the pH rises to neutral, MMPs degrade the triple helix structure of collagens, start to dissolve organic matrix and increase the rate of dentin loss [26]. In addition, these enzymes cause structural changes in existing collagens. The parts called “telopeptides” at the ends of the collagens are dissolved and removed, thus, spaces are created in the internal structure of the molecule. The relevant structural dissolution prevents interfibrillar remineralization, which is crucial for strengthening the mechanical properties of dentin. It also causes the loss of non-collagen matrix proteins, which act as nuclei for remineralization. Still, the exact contribution of these highly collagenolytic enzymes to the progression of erosion is not known so far. Using specific inhibitors for these distinct classes of enzymes may be better in order to understand their role in the progression of erosive lesions.

4. DISCUSSION

The protection of DOM by MMP inhibitors is the recent approach to the prevention of dentin erosion [26]. Among the different types of MMP inhibitors, chlorhexidine (CHX), and epigallocatechin gallate (EGCG) as a polyphenolic compound, have been the most common compounds evaluated as part of preventive strategies to reduce erosive dentin demineralization. Indeed, their mechanism of action is yet to be estimated. MMP inhibitors that have recently been reported in studies are summarized in Table 3. Polyphenols are used frequently in many research projects and specifically polyphenols isolated from green tea, especially epigallocatechin-3-gallate (EGCG) that was found to have inhibitory properties against MMP-2 and -9 [27] and the activation of MMP-8, which acts for the remineralization in demineralized dentin [1,28]. According to the information obtained, these catechins accumulate on the organic material in dentin [29] and run by masking the catalytic site of MMP-2 or cause structural changes with its hydrogen bonds and hydrophobic linkages to collagenase [28]. The effect of EGCG against degradomics was proven in previous studies [23,29,30,31] and its effectiveness was compared usually to various formulae of fluorides or CHX. These compounds have also shown efficiency against MMPs but with distinctive targeting procedures. To better explain, MMP enzymes are zinc-activated and calcium-dependent enzymes. By chelating these cations, chlorhexidine binds to the sulphydryl groups and/or cysteines in the active parts of MMPs and inhibits the enzyme activity [32]. However, the inhibitory activity of chlorhexidine is directly related to its concentration. CHX can cause protein denaturation at saliva concentrations above 0.2%, reduce the solubility of dentin collagen and prevent the progression of dentin erosion. Besides, chlorhexidine could completely inhibit MMP-2 and -9 at the concentration of 0.03%, and MMP-8 at the concentration of 0.01-0.02% [28]. Furthermore, it was reported that fluorides, thanks to their high electronegativity, prevent Zn\(^{2+}\) and Ca\(^{2+}\) ions, which are necessary for the activation of MMPs, from entering the catalytic activities as similar as the inhibitory activity of CHX [33].

The effect of different types of ion-containing fluoride compounds (such as stannous fluoride, titanium tetrafluoride, amine fluoride) on dental erosion is attributed to the protective layer formed on the dentin surface, it is not yet clear whether or not they perform MMP inhibition. Since sodium (Na\(^+\)) ion does not form a layer similar to otherionic fluoride components on dentin surface, the most widely used fluoride compound in studies is NaF. In a study, it was found that by using the gelatin zymography, may inhibit the activity of MMP-2 and -9 in a dose-dependent manner [34]. 200 ppm fluoride can inhibit pro and active forms of MMPs.
of MMP-2 and active forms of MMP-9 by 100%. If these rates are constant at 225 ppm, the pro-form of MMP-9 could be inhibited approximately by 85%; pro and active forms of salivary MMP-9 were inhibited by 55%. While the inhibitory activity of NaF against MMP-2 and -9 is reversible at low concentrations, it has been reported that it is irreversible at high concentrations such as 5000 ppm [34].

There have been studies comparing the effect of fluorides (especially sodium fluoride, NaF), on EGCG, and CHX [35,36]. Regarding the variances differed highly in the methodological section of the studies, most of them could not be compared directly with one-to-another. One of the differences encountered in the studies is the frequent application of the contact profilometer to measure dentin loss [30,36,37]. However, some controversies have arisen regarding its usage at erosive dentin surfaces because of the tip of the profilometer that could cause damage by pressing the DOM [38]. Thus, to overcome this problem, some studies have used non-contact [39,40] or digital microscopy [41]. As another solution, to minimize the shrinkage of DOM, some analysis of the contact profilometer had been done at 100% humidity [38]. Another variation among studies with respect to the method is that the erosive cycles. Most of the cycles were done with Cola [35,42,43] but some studies have used various acidic solutions, such as citric acid [40,44] or hydrochloric acid [45,46]. Moreover, many of the studies have used not only the erosive cycle but also ‘erosive+abrasive’ cycles [38,45] so, within the changes in the methodology, the scores of dentin losses highly vary. Besides, the concentrations of the active ingredients or the ratios of the extractions have varied following the type of formula, such as gels [30], toothpaste [37], and mouthwashes [28,35], as well. Still, the main outcome of these studies is that MMP inhibitors play an active role in reducing dentin loss by protecting DOM.

Within the differences among studies evaluating EGCG, CHX, and NaF, one point is described that EGCG had slightly more action against dentin erosion in another different way. Previous studies [30,47] suggest that the protease inhibitors have the ability to minimize the degradation of DOM against dentin demineralization. Besides, polyphenols are reported to improve the mechanical properties of the organic matrix and resist enzymatic degradation [42]. So recently, plant polyphenols have been investigated against dentin erosion so that potential benefits could be gained. One of them is ‘theaflavin’, which is the most frequent polyphenol in black tea, formed by the oxidation of catechins during manufacturing [48]. Aside from the antifungal [48], antioxidant, and antimutagenic effects of theaflavins, they were also reported to inhibit MMP-2 and MMP-9 [49,50]. In an in vitro study, the aflavins showed similar dentin losses to EGCG and commercial green tea with no significant difference [46]. Anacardic acid is also one of the phenolic acids obtained from the shell of the cashew nut. Accompanied by the antioxidant capacity [50], the collagenolytic activity of anacardic acids against MMP-2 had also been proven by zymographic analysis and in vitro evaluation revealed reduced dentinal wear compared to EGCG and NaF [51,52].

On the other hand, another approach to inhibit erosive wear has exhibited promising results which aimed to enhance protecting properties of acquired pellicle. The adsorption of polyphenolic compounds (EGCG, epicatechin-3-gallate (ECG), and theaflavin) onto the pellicle may lead to stabilize the structure [38] and increase its thickness [53] resulting in an anti-erosive effect. So that, dental materials such as gels or varnishes including polyphenols were demonstrated in studies [33,38] which were tested against both enamel and dentin erosion. However, due to the structural variations of enamel and dentin, such as the higher porosity of dentin, the preventive effect of the acquired pellicle could be reduced. Methodologies involving gels usually engage polyphenolic compounds as active compounds and compare their effect against a fluoride gel [30,38]. However no commercial products have figured yet, except the mouthwashes with green tea aromas. For instance, gels containing EGCG and CHX showed to increase a protein (Statherin) in the acquired pellicle, which increased the saturation of oral fluids by releasing Ca²⁺ and PO₄⁻ ions following acid attacks [38]. Another study reported that resin materials containing EGCG increased basic isoforms of salivary proteins which may perform to improve the acid resistance of demineralized surfaces [54].

More recently, flavonoids, which are from the subgroups of polyphenolic compounds, have been frequently investigated in studies comprising MMP inhibition [44,55]. Quercetin is one of the natural flavonoids which is found highly in fruits and vegetables and has been reported to have the potential to protect against degradation of the collagen matrix by inhibiting MMP-2 and MMP-9 [56]. An in vitro study showed that quercetin showed significantly lower microhardness loss than CHX, EGCG, and NaF groups and revealed a thicker DOM than control dentin [44]. This dose-dependant outcome of quercetin was explained by its improving effect on collagen resistance as a result of inhibiting both free- and collagen-bound degradomics (MMPs) in dentin [57]. On the other hand, previous studies have shown that resveratrol significantly reduces MMP-9 expression [58], which is a non-flavonoid polyphenol found in many of the plants. Since there is no study that has investigated its protective effect against MMPs, there are studies reporting its benefit on dentin bonding durability [55,59] and as anticaries agent [60].

5. CONCLUSION

Since dental erosion is a complex situation, there are debates on terming it as “bio-corrosion” in order to explain the process more comprehensively. Besides, endogenous enzymes called degradomics have
also detrimental effects on the process if they reach exposed dentin surfaces. There have been inhibitory materials such as fluorides, chlorhexidine, and green tea extracts that were proved to protect demineralized collagen matrix. Studies are being carried out on the novel polyphenolic compounds that could be beneficial to collagenolytic processes. The effect should be further investigated and compared evaluated with recently known MMP inhibitors in various concentrations. So that, research may solve the inhibition mechanism and clinicians may benefit from the enhancement of their process.

REFERENCES

Dentin degradomics


CV

Günçe Ozan received her DDS in 2011 from the Faculty of Dentistry at Istanbul University, Istanbul, Turkey. She immediately started her PhD at the Restorative Dentistry Department of the same university. After studying on the fields of dental erosion and preventive dentistry, she had her PhD degree in 2017. She worked at the Young Dentists’ Commission of the Turkish Dental Association for 2 years. She is now continuing her career at the Istanbul University as a Research Assistant.

Questions

1. The difference between the terms, “Dental erosion” and “Bio-corrosion” distinguishes from the certain reactions that were occurred during dental erosion?
   - a. Piezoelectric effect;
   - b. Proteolysis;
   - c. Piezoelectric effect & Proteolysis;
   - d. Electrochemical effect.

2. Demineralization occurs... in dental erosion.
   - a. Subsurface;
   - b. Surface and subsurface;
   - c. Surface;
   - d. Beneath the surface.

3. Which of the enzyme groups of degradomics are not localized in the “dentin”?
   - a. Enamelisin;
   - b. MT-MMP;
   - c. MMP-2;
   - d. MMP-3.

4. MMP enzymes activated at ... ph but, functioned at... ph.
   - a. Acidic / basic;
   - b. Basic / neutral;
   - c. Neutral / acidic;
   - d. Acidic / neutral.