Degradation of Resin-Dentin Bonded Interface: A Review

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Abstract

Hybrid layer can be described as the resin impregnated demineralized dentin layer. It comprises of the adhesive resin and the collagen fibrils that serve as scaffold for resin infiltration. Degradation of the hybrid layer is one of the major investigated topics in the restorative dentistry. It occurs through the hydrolysis of adhesive resin and the breakdown of collagen fibrils by endogenous proteases especially matrix metalloproteinases (MMPs). Hydrolysis occurs when the water acts as medium for chemical bonds breakdown especially methacrylate based resin containing ester bond that is vulnerable for this process. MMPs are embedded in the dentin during tooth development. These enzymes are activated by acid during dentin demineralization of etch-and-rinse and self-etch adhesive system. Since adhesive resin infiltration is often incomplete, denuded collagen fibrils associated with water can be enzymatically digested, eventually contributing to the hybrid layer deterioration. This review describes the mechanism of hybrid layer degradation and the potential strategies to slow down this process.

Keywords: MMPs, hydrolysis, degradation, hybrid layer.

Introduction

Contemporary dental adhesives can be categorized into etch-and-rinse (ER) and self-etch (SE) adhesives based on their interaction to dentin: removing (ER) and modifying smear layer (SE).1,2 Both systems involve removal inorganic component of enamel/dentin followed by applications of adhesive resins to fill the demineralized space and ultimately create the zone referred as “hybrid layer (HL)”.3 This hybridization consists of adhesive resins and the collagen network that is infiltrated with adhesive monomers. The durability of this layer is a key for determining the longevity of resin composite restorations. Unfortunately, resin composite restorations last approximately 8 years compared to that reported for amalgam restoration (11-12.8 years).4,5 Secondary caries is one of the most reasons for replacement.4,6

Ideally, the adhesive monomer is expected to fully impregnate into the demineralized dentin and create stable bond between dentin and resin composite. Regrettably, whether the different types of adhesive system or technique used for dentin bonding, studies have documented that the HL are not as durable as anticipated due to the degradation of both adhesive resin and collagen matrices in oral environment.1,2,7,8 Over time, this phenomenon weakens the resin-dentin interface and leads to gaps formation between dentin and restoration1 and may ultimately contribute secondary caries formation. Therefore, this review aims to provide an overview of the two unique mechanisms of HL degradation through hydrolysis of adhesive resin and dentinal collagen breakdown by MMPs and the strategies to stabilize the HL.

Incomplete resin infiltration into demineralized dentin

The evidence of imperfect encapsulation of adhesive monomer is observed by nanoleakage studies in which the nanometer-sized molecules (e.g. silver nitrate) diffuse to demineralized dentin, adhesive resin, or the HL. It occurs possibly due to the fluid movement, the

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anastomosis complex of dentin or the continuous etching of the self-etch adhesive system. Nanoleakage is a useful criterion for the evaluation of adhesive performance and often found at the bottom of the HL. Even though these nano-scaled porosities ranged circa 20–200 nm do not permit bacterial penetration, bacterial products, water and enzyme may use the nanoleakage as a pathway for HL degradation of both adhesive resin by hydrolysis and denuded collagen fibrils by endogenous proteinases. In addition, in vitro or in vivo experiments widely reported the reduction of resin-dentin bond strength after the long-term (6-12 months or longer) aging (i.e. water storage, thermocycling) which supports the deterioration of HL.

Hydrolysis of adhesive resins

Dental adhesives are mainly consisted of methacrylate based monomers such as bisphenol Adiglycidyl ether diethacrylate (Bis-GMA), triethylene glycol dimethacrylate (TEGDMA), urethane dimethacrylate (UDMA) and 2-hydroxyethyl methacrylate (HEMA). These monomers contain hydrolytically vulnerable functional groups: ester (R-CO-O-R), carboxy (-COOH), hydroxyl (-OH), phosphate (-PO₃⁻) and urethane (R-NH-CO-O-R). Generally, dental polymers are deteriorated in aqueous environment by two main mechanisms: passive hydrolysis and enzymatic degradation. Hydrolysis is defined as a chemical reaction in which bonds of molecules break down using water. Hydrolysis that is catalyzed by enzymes is called “biodegradation”. It was reported that degradation of dental polymers occurs through oxidation, chain scission and attack of functional groups producing small molecules or degradation products.

Human saliva contains esterase enzymes that can hydrolyze methacrylate dental monomers. These enzymes are produced from bacteria, salivary glands, human cells (i.e. human gingival/pulp fibroblasts, monocytes and macrophages). The esterase enzymes presented in saliva cause hydrolysis of methacrylate based monomers producing carboxylic acid (R-COOH) and alcohol (R-OH). For example, Bis-GMA is hydrolyzed to Bis-hydroxy-propoxy-phenyl-propane (BisHPPP) and methacrylic acid (MA, Figure 1) in aqueous solution. The degree of hydrolytic or enzymatic degradation is related to the degree of conversion, crosslinked networks, formulation of monomer, and specificity and concentration of enzyme to the monomer. Finer and Santerre showed that Bis-GMA is more resistant to enzymatic hydrolysis by pseudo-cholinesterase than TEGDMA in a dose dependent manner.

The enzymatic degradation adversely affects the resin-dentin bond strength, surface hardness, wear resistance and fracture toughness. Furthermore, Kermanshahi et al. found that the exposure of resin bonded dentin specimens to esterase enzymes facilitates bacterial microleakage into dentin and contributes to the progression of secondary caries as the increased bacterial penetration depth was pronounced in the test group compared to the non-exposure group.

Once the dental resins are hydrolyzed and released their breakdown products [(i.e. BisHPPP, MA and triethylene glycol (TEG)], these degradation products also induces the human cells to release more esterase resulting in more leaching of byproducts and creating vicious cycle. The interactions between breakdown products and oral bacteria were investigated. In vitro studies found that the byproducts of dental resins (i.e. BisHPPP, TEG, MA) can upregulate S. mutans specific genes such as gtfB, gtfC, gbpB (bacterial adhesion and biofilm formation related genes), comC and comD (quorum-sensing related genes), yfiV (a putative transcriptional regulator), leading to increased bacterial virulence and cariogenicity. These findings suggest that degradation products can potentially contribute to reduced longevity of adhesively bonded restorations.

![Figure 1. Hydrolysis of Bisphenol A glycidyl methacrylate (Bis-GMA) to Bis-hydroxy-propoxy-phenyl-propane (BisHPPP) and methacrylic acid (MA).](image-url)
Degradation of collagen fibrils by matrix metalloproteinases and other proteases

Matrix metalloproteinases (MMPs) are a group of zinc-dependent endopeptidases that are responsible for regulation of the components of the extracellular matrix (ECM) especially type I collagen which is the main component of dentin matrices.\(^{31}\) MMPs crucially participate in the physiological and pathological remodeling and destruction of living tissue such as arthritis, periodontal disease and tooth development. Based on their internal homologies and substrate specificity, MMPs are classified into six main classes as follows: collagenases, gelatinases, stromelysins, matrilysins, membrane-types, and other MMPs (Table 1).\(^{31,\,32}\)

<table>
<thead>
<tr>
<th>Collagenases</th>
<th>MMP-1 (collagenase-1)*</th>
<th>MMP-8 (collagenase-2)*</th>
<th>MPP-13 (collagenase-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatinases</td>
<td>MPP-2 (gelatinase A)*</td>
<td>MPP-9 (gelatinase B)*</td>
<td></td>
</tr>
<tr>
<td>Stromelysins</td>
<td>MPP-3 (stromelysin-1)*</td>
<td>MPP-10 (stromelysin-2)</td>
<td>MPP-11 (stromelysin-3)</td>
</tr>
<tr>
<td>Matrilysins</td>
<td>MPP-7 (matrilysin, PUMP-1)</td>
<td>MPP-26 (matrilysin-2)</td>
<td></td>
</tr>
<tr>
<td>Membrane type (MT) MMPs</td>
<td>MPP-14 (MT1-MMP)*</td>
<td>MPP-15 (MT2-MMP)</td>
<td>MPP-16 (MT3-MMP)</td>
</tr>
<tr>
<td>Other MMPs</td>
<td>MPP-18</td>
<td>MPP-19</td>
<td>MPP-20 (enamelysin)*</td>
</tr>
</tbody>
</table>

Table 1. Classification of MMPs.

*MMPs indicated MMPs identified in dentin

MMP activity is controlled at the level of transcription, activation of zymogens (inactive MMPs), interaction with specific extracellular matrix component and inhibition of activated enzymes by tissue inhibitor of metalloproteinases (TIMPs).\(^{33}\) Failure of their control can lead to several pathological conditions such as atheroma, arthritis, cancer and inflammation.\(^{34,\,35}\) MMPs is secreted as zymogen or inactive enzyme, and therefore, they have to be activated by proteinases, heat, low pH or other MMPs for their fully function.\(^{36}\) The activation occurs through the “cysteine switch” mechanism where the bond between Zn\(^{2+}\) in the catalytic domain and the cysteine (-SH) in the prodomain of MMP structure is disrupted leading to the active MMPs (Figure 2). MMPs participate in dentin matrix formation during tooth development and secondary dentin formation. After collagen matrix calcification, the MMPs remain trapped within the mineralized dentin matrix and inactive.\(^{2}\) Studies reported that odontoblasts can secrete MMP-2,-8,-9,-13,-20 and MT1-MMP.\(^{37}\) So far, at least MMP-1,-2,-3,-8,-9,-20 and MT1-MMP have been identified in dentin.\(^{36,\,38-43}\)

MMPs have been suggested to be responsible for HL degradation.\(^{1-\,3,\,44-47}\) Even though published studies demonstrated the presence and activity of endogenous enzymes in human dentin\(^{39,\,44,\,48,\,49}\), the direct evidence of MMP activity within the HL after acid exposure was firstly revealed in the in situ zymography.\(^{40}\) It showed that the MMP activity was intense at the bottom of the HL where the exposed collagen occurred.\(^{40}\) Interestingly, this observation also correspond to the area of interfacial nanoleakage which is the initial area of HL degradation. During the dentin pretreatment for dentin bonding, phosphoric acid or acidic primer etching in ER and SE adhesive systems is not only demineralized dentin for microretention, but low pH would also expose the collagen networks and activate matrix bound MMPs.

![Figure 2. The activation of inactive MMPs.](image-url)
The active MMPs slowly degrade the exposed collagen fibrils (uninfiltrated by resin monomers)\textsuperscript{50,51}, weakening the resin-dentin bonds and reducing longevity of the resin restorations.

Cysteine cathepsins are a group of lysosomal cysteine proteinases. There are at least 11 human cysteine cathepsins (B, C, F, H, K, L, O, S, V, X and W).\textsuperscript{52} Similar to MMPs, cysteine cathepsins take part in extracellular matrix degradation and remodeling. Unlike MMPs that work at neutral pH, most of cysteine cathepsins function at acidic environments except Cathepsin K that possesses gelatinolytic activity at pH 4.0–7.0 and collagenolytic activity at pH 5.0–6.5.\textsuperscript{53} Human odontoblast can express a number of cysteine cathepsin gene.\textsuperscript{54} To date, cysteine cathepsin B and cysteine cathepsin K are found in human dentin.\textsuperscript{52} Cysteine cathepsin K is the only endopeptidase that is able to cleave multiple sites of the triple helix region of the collagen chain generating different fragments of collagen molecules. Similar to bacterial collagenases, this distinct property is not found in human MMPs as they can cleave collagen chain at one unique site.\textsuperscript{52} These observation supports the possible role of cysteine cathepsins in HL degradation.

**Strategies to preserve bonded interface**

A number of approaches are introduced to overcome the deterioration of adhesive-dentin bonded interface including the modification of dentin bonding protocol (i.e. dentin pretreatment after acid etching), use of protease inhibitors, crosslinkers, chelating agents and a combined treatment.

Chlohexidine (CHX) is an MMP inhibitor that is extensively investigated in vitro and in vivo studies. CHX can be applied directly on demineralized dentin or can be incorporated into adhesive system (i.e. acidic primer, adhesive) either as a polymerizable chlorhexidine-methacrylate\textsuperscript{55} or CHX solution.\textsuperscript{56} Generally, the immediate dentin bond strength showed no significant different between 2% CHX and the control (without CHX) but the use of 0.2% CHX negatively affected the bond strength. After aging, the preservation of dentin bonding seems to be promising with CHX (0.2–2%) since CHX had higher bond strength compared to the control. However, this trend does not remain after long term aging.\textsuperscript{45} Similarly, a clinical study on class V lesion with 2% CHX application using split-mouth design showed no long term advantage of CHX since restorations of the control (no CHX) started debonding after 6 months and the CHX treatment group was not debonded until 12 months.\textsuperscript{57} These results correspond to other clinical studies.\textsuperscript{58, 59}

Tetracyclines derivatives are broad spectrum antibiotics with an MMP inhibitory effect though the Zn\textsuperscript{2+} chelating property. Doxycycline at subantimicrobial level incorporated into adhesive resin using aluminosilicate clay nanotube as a carrier showed potent MMP-1 inhibition without negative effect on physico-chemical properties of adhesive.\textsuperscript{51} Similarly, application of 2% minocycline pretreatment on demineralized dentin significantly improved microtensile bond strength reduction and nanoleakage after 24 months of aging compared to the non-treated group.\textsuperscript{60}

Crosslinkers such as carbodimide, glutaraldehyde, and proanthocyanidin strengthen the HL by forming the covalent bonds between collagen molecules, improving mechanical properties of dentin and making more resistant to degradation.\textsuperscript{61} Proanthocyanidin has a dual function as a crosslinker and MMP inhibitor. Proanthocyanidin does not only improve the mechanical properties of dentin, but it can also stabilize the dental collagens against enzymatic digestion\textsuperscript{62} possibly due to the induction of conformational change of enzyme structure\textsuperscript{63} and the noncovalent nature of its interaction with collagen molecules.\textsuperscript{63}

Gallardin, synthetic MMP inhibitor, exhibits a collagen-like backbone facilitating the binding to MMP active site on the catalytic domain of MMP structure resulting in MMP inhibition.\textsuperscript{65} Treatment of gallardin reduced interfacial nanoleakage after 1 year aging compared to the no treatment group.

Polymerizable quaternary ammonium compounds (QAMs) such as 12-methacryloxyloxydodecylpyridinium bromide (MDPB) significant decreased the loss of dry mass of the dentin beams incubated with QAMs (0.2–6.0% loss of dry mass) compared to the no QAMs group (29% loss of dry mass).\textsuperscript{64} Moreover, the unique benefit of QAMs over other MMP inhibitors is the methacrylate-polymerizable property which makes the QAMs not leaching out of the bonded interface.

Finally, polyphenol compounds (i.e. green tea polyphenol epigallocatechin-3-gallate),
benzalkonium chloride, chelating agents (ethylenediaminetetraacetic acid, EDTA) and other MMP inhibitors have all been employed and demonstrated successful protease inhibition properties. However, although these strategies provide promising results, most of the inhibitor are not methacrylate polymerizable and will leak out to the environment after aging. Moreover, some inhibitors are reversible in nature (i.e. doxycycline, EDTA). Therefore, a long term inhibition may not be achieved unless the carrier is used for their sustained release. Thus, well-designed long term clinical trials (i.e. randomized clinical trial) has not been fully investigated and should be determined in the future.

Conclusions

Degradation of the HL is unavoidable. It reduces the longevity of the resin restoration. Indeed, studies have provided the strategies and clinical procedure to stabilize the resin-dentin interface. Although this review may not be addressed all available information, the key evidences are discussed. Further studies are needed on new knowledge, technique and invention to improve the stability of the adhesive bonded interface.

References

3. Porto IC, Nascimento TG, Oliveira J, Freitas PH, Haimeur A, Porto IC, Nascimento TG, Oliveira J, Freitas PH, Haimeur A, et al. Specificity of protease inhibition may not be achieved unless the carrier is used for their sustained release. Thus, well-designed long term clinical trials (i.e. randomized clinical trial) has not been fully investigated and should be determined in the future.


