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EFFECT OF METALLOPROTEINASES AND CATHEPSINES ENZYMES ON DENTAL ADHESION

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Summary

Introduction

The long-term durability of Dentin-Resin complexes has been a challenge for a long time with consistent bond failure after between 12-18 months from the initial procedure. Restorative procedures are already very technique and skill dependent in which the skill of a clinician can influence greatly the durability of a restoration; however, in addition to this, research has shown that the action of proteases, Matrix Metalloproteinases and Cysteine Cathepsins have a vast internal influence on the durability of dentin-resin complexes due their ability to break down collagen fibrils within the hybrid layer complex, independent of the execution of the restorative procedure and protocols followed.

Materials and Methods

Databases such as PubMed, Google Scholar, NCIM and PMC were used when researching for this article using keywords such as 'MMP', 'Cysteine Cathepsins', 'Dental Adhesion', 'Inhibit MMPs' 'MMP and Dental Adhesion'. Articles than contained these keywords and those that did not mention dental caries were used in an effort to focus this study. 49 articles were used to write this paper.

Discussion and Conclusion

Understanding the mechanisms of action of MMPs and CCs were key in order to identify agents and materials that can be used so as to inhibit their actions within the dentin hybrid layer. Multiple agents were found that had proven to be effective such as the commonly used Chlorhexidine that function via chelation; others such as Glutaraldehyde and Tetracyclines as well as Zinc salts have also been proven to have an inhibitory effect on MMP and CC proteolytic action. However, each agent researched

has its own drawbacks that require further research and testing to be able to be implemented into everyday protocols in restorative procedures.

Resumen

Introducción

La durabilidad a largo plazo de los complejos Dentina-Resina ha sido un desafío durante mucho tiempo con un fallo consistente de la unión después de un periodo entre 12-18 meses desde el procedimiento inicial. Los procedimientos restauradores ya son muy dependientes de la técnica y la habilidad en los que la habilidad de un odontólogo pueden influir en gran medida en la durabilidad de una restauración; sin embargo, además de esto, la investigación ha demostrado que la acción de las proteasas, las metaloproteinasas matriciales y las catepsinas cisteínas tienen una gran influencia interna en la durabilidad de los complejos dentina-resina debido a su capacidad para descomponer las fibrillas de colágeno dentro del complejo de capa híbrida, independientemente de la ejecución del procedimiento restaurativo y los protocolos seguidos.

Materiales y Métodos

Se utilizaron bases de datos como PubMed, Google Scholar, NCIM y PMC para la investigación de este artículo utilizando palabras clave como 'MMP', 'Cysteine cathepsins', 'Dental Adhesion', 'Inhibit MMPs', 'MMP and Dental Adhesion'. Con el intento de enfocar este estudio se utilizaron artículos que contenían estas palabras clave y aquellos que no mencionaban la caries dental. Se utilizaron 49 artículos para escribir este trabajo.

Discusión y Conclusión

La comprensión de los mecanismos de acción de las MMP y de la CC fue importante para identificar los agentes y materiales que se pueden utilizar para inhibir sus acciones dentro de la capa híbrida de la dentina. Se encontraron múltiples agentes que habían demostrado ser eficaces, como la clorhexidina comúnmente empleada que funciona a través de la quelación; otros como glutaraldehído y tetraciclinas, así como sales de zinc, también han demostrado tener un efecto inhibitorio en la acción proteolítica de MMP y CC. Sin embargo, cada agente investigado tiene sus propios inconvenientes que requieren más investigación y pruebas para poder ser implementado en protocolos cotidianos en procedimientos restauradores

Contents

Introduction

Enamel Adhesion

In the matter of adhesion and restorative dentistry, enamel has a distinct advantage over dentin in the fact that 88% (by volume) of enamel is made of inorganic hydroxyapatite crystals, 10% water and 2% organic materials; which allows techniques such as etch-and-rinse (**ER**) with phosphoric acid to be highly effective. The etching creates irregularities in the surface of the enamel increasing its surface free energy; when a fluid resin material is applied to the surface, it is aided by capillary action to penetrate into the surface and creates microtags. This results in a very strong, durable resin-enamel complex (1,2).

Dentin Adhesion

By contrast to enamel, dentin presents a much more difficult task in achieving a good resin-dentin substrate due to its comparatively low mineral content (50% by volume) and high organic and water content (25%/25% respectively)(1,2). With the organic matrix of dentin being comprised of 90% collagen (Mostly Type 1 collagen synthesised by odontoblasts) and 10% non-collagenous proteins; together with the inorganic hydroxyapatite crystals all being embedded into an extracellular matrix (**ECM**).(3,4) Dentin naturally has a higher water content and abundant organic components; one example being dentinal fluid that is found within dentinal tubules.

It actively deters adhesion due to the hydrophilic nature of dentinal fluid; the water content will eventually leach into the resin-dentin interface that will create spaces within the matrix created. These factors affect the durability and strength of bonds when employing the ER system and a newer adhesive system, self-etch adhesives (**SE**)

that contain adhesive monomers that infiltrate the collagen in the dentinal substrate and once polymerised they form an adhesive/co-monomer-collagen complex that is called the Hybrid Layer (**HL**) (4,5).

There are two main factors that hinder dentin adhesion, an extrinsic and an intrinsic factor; extrinsically, when a tooth is prepared using a dental bur the damaged collagen within the debris retained forms a 'smear plug' that infiltrates the openings of the intertubular dentin. The collagen within the smear plugs gelatinise and form a Smear Layer (**SL**) due to the heat generated by the friction of the dental bur. This layer reduces penetration of resins by up to 90% and therefore has to be removed using acid etching techniques; proper removal of the SL is heavily dependent on the etching step in an adhesive system and improper employment of the technique would result in a weaker bond. To achieve the strongest bond possible, removing the SL and exposing the collagen fibrils present in the dentin need to be exposed in order for the adhesive and resin to penetrate and interlock with (6). The ER technique has the ability to remove the SL completely due to having a separate etching step with normally 37% Orthophosphoric Acid (**PA**) for 15-20 seconds; however other acids can be used such as α-hydroxy Glycolic Acid (**GA**) with similar effectiveness in removing the SL and mineralised dentin (7,8). SE adhesive systems contain acidic resin co-monomers that concurrently etch and prime dentin similarly to phosphoric acid in ER systems but not as efficiently and only partially removes the SL. Despite the removal of the SL, there is an increased flow of dentinal fluid from deeper within the tubules that hinder adhesion as the hydrophobic properties of the resin and hydrophilic properties of the dentinal fluid deters adhesion between the two mediums; regardless of the presence of irregularities caused by the acid etching in the dentin's surface that would normally favour adhesion after the etching step.

Intrinsically, the nature of the dentin structure when treated with both types of adhesive systems demonstrate problems. With the ER system, it is shown that there is a decreasing gradient of demineralised dentin that would be infiltrated by the adhesive resin leading to a layer of exposed collagen fibrils towards the bottom of the hybrid layer. SE systems display a similar effect as they can produce water-filled interfibrillar spaces with exposed collagen. The lack of resin in these areas produced by both types of systems contribute to the lack of long-term durability of resin-dentin substrates created in adhesive procedures (2).

Other Factors that Affect Dentin Adhesion

Many other factors can affect the quality of the substrate formed between resin and dentin such as the acid etching agent used (PA or GA), the duration of time that the conditioner is applied can also affect the quality of the substrate; if they were applied for less time (7s) then the remaining dentinal tubules would still retain debris. Any longer than the prescribed 15-20 seconds would result in damaged dentinal tubules and collagen fibrils (8).

The mineralised dentin and SL formed from previous preparation of the tooth are both washed away in a rinsing step using water leaving the collagen fibrils suspended in water. The demineralised dentin can then be air dried which results in the collagen fibrils joining together; this in turn reduces permeability to resins and adhesive due to the lack of space and reduced surface area for the resin to adhere to (6,9,10), which as a result produces lower immediate bond strengths (6,11,12). This process is called ‘Dry Bonding’. Instead, we can maintain a state of hydration of the dentin to avoid the issues we find in dry bonding; this would provide a more porous collagen network and consequently

more efficient penetration of the adhesive (6,9). However, the water present within the collagen must evaporate to provide space for the resin polymers to interlock with the collagen fibrils; any remnants of water within the matrix would make the collagen more prone to degradation by endogenous enzymes over time (6).

Studies have also found that the technique used when applying the conditioner and adhesive greatly affects the quality of the bond to the substrate. Depending on the adhesive system used and acid (PA or GA), rubbing the acid on exposed dentin can produce variable results with some adhesive systems resulting in poorer quality bonds and some higher quality. Rubbing in the adhesive has been seen to overall improve the quality of the bond produced (6,7).

Matrix Metalloproteinase Enzyme

As mentioned earlier, exposed collagen in the hybrid layer (those still surrounded by water especially) are vulnerable to the activity of host-derived matrix metalloproteinase enzymes (**MMPs**) that are usually contained within the dentin. They have an important role in the development of dentin and later become trapped within the dentinal matrix and inactive once the dentin mineralises (13).

MMPs are a group of Ca^{2+}- and Zn^{2+}- dependent endopeptidases that are contained within mineralised dentin during tooth development. There are 23 known types of MMPs that share a common domain arrangement: A signal peptide, pro-peptide domain with cysteine residue, catalytic domain that contains a zinc ion and C-terminal hemopexin-like domain (4,14,15). They have the ability to degrade several extra-cellular matrices (**ECM**) and basement membrane (**BM**) proteins however their primary function

is to hydrolise components of the ECM in physiological processes such as tissue remodelling, angiogenesis and other developmental processes. Normally, several of these MMPs are required in order to convert non-collagenous proteins into signalling molecules that govern other cell functions such as proliferation and differentiation; however, they are also seen to be involved or contribute to the processes of dental caries and periodontal disease as well as adhesive bond failure (1,3,15).

MMPs are secreted primarily as pro-enzymes/zymogens that are released after physical preparation of a cavity in a tooth when removing caries and become active proteinases when activated by other proteases such as MT-MMPs or when exposed to an acidic environment found after treatment with etch-and-rinse or self-etch adhesives from phosphoric acid and acidic monomers respectively, as well as other activation methods that will be discussed later (15,16). When inactive, the zinc ion (catalytic domain) is bound to the cysteine residue (pro-domain) that stabilises the structure of the enzyme by preventing water being able to be bound to the zinc ion (4,16); rendering it unable to bind and cleave appropriate substrates, maintaining it in an inactive state. However, when the above conditions are met then a process called a 'cysteine switch' partially actives the enzyme by breaking the zinc-cysteine bond allowing it to interact with some substrates. It is fully activated by further cleavage of the pro-domain that is either autolytic or governed by other proteases and it is then considered a functional endopeptidase (15).

The most abundant forms of MMPs that are contained within dentin matrices initially in an inactive state (as pro-enzymes) being MMP-2 and MMP-9 that function as gelatinases (17). Although they are found in abundance in dentin that has been subjected to

collagen degradation, MMPs -2 and -9 themselves do not cleave collagen molecules but are key in the process of collagenolysis in dentin. True collagenases MMPs -1, -8, -13 and -18 cannot cleave intact collagen molecules at the cleavage sites due to the orientation of the collagen molecule and position of the C-terminal end, blocking access to the peptide bonds within the cleavage site.

Gelatinases such as MMPs -2 and -9 function as telopeptidases remove the C-terminal domain telopeptides allowing true collagenases access to cleave sites (18). This also removes C-terminal cross-links which leaves collagen more vulnerable to passive or non-specific degradation (19). Once vulnerable, collagenases approach the cleavage site creating fragments into 3/4 N-terminal and 1/4 C-terminal fragments (16).

Cysteine Cathepsins

In addition to MMPs, Cysteine Cathepsins (**CCs**) have been shown to be expressed to a similar degree as MMPs; they are lysosomal cysteine proteases from the C1 family of papain-like enzymes and both these and MMPs can be expressed within similar spaces that is occupied by collagen such as dentin. Additionally, like MMPs, have a wide range of functions including the activation of MMPs themselves, e.g. MMP-1 by Cathepsin B (1,4).

They too are endopeptidases that are capable of breaking down ECM proteins such as collagen I and III and similar to MMPs there are different types of CCs that vary in their ability to cleave collagen into different numbers and types of fragments. However, unlike MMPs that can only cleave collagen I at a single site that will generate N-terminal and C-terminal fragments; CCs can cleave collagen at numerous sites that result in

fragments of various lengths. The most common types that are seen to be expressed within odontoblasts and pulp tissues and that are observed within the process of the breakdown of collagen are Cathepsin B, L and K (15,20).

Cathepsins B and L are known to be able to cleave non-helical telopeptide extensions of collagen molecules whereas Cathepsin K has the ability to cleave collagen in several triple helical regions. In a study by Tezvergil-Mutluay *et al.* measured the collagenolytic activity of Cathepsins B, L, K and S using demineralised dentin beams and measured the change in dry mass after incubating them with 10 µg of each substrate. They found that the beams that were incubated with Cathepsin K had lost 35% of their dry mass compared to only around 4% lost with Cathepsins B, L and S (15).

The fact that CCs are able to cleave collagen at multiple sites that MMPs usually are not able to access, in addition to MMPs and CCs being found in similar spaces suggest that CCs, especially Cathepsin K that has seen to be a powerful collagenase and comprises of 98% of cathepsin activity against collagen (21). Due to the similarity of function and their synergistic nature in regards to location and target substrates; CCs too play a role in the degradation of the HL in dentin-resin complexes (22). As a result, it is assumed that there is a possible enzymatic cascade in the process of the breakdown of collagen fibrils in the HL (1,4).

Activation and Triggers of MMPs and CC

Identifying a cause-and-effect relationship between clinical procedures that are currently used, and MMPs and CC activation is essential in order to formulate new materials and methods that clinicians can employ to prevent hydrolysis of collagen fibres

in the dentin HL and therefore improve the durability of restorations. At this time, physical or chemical treatment via preparation of a tooth for a restoration and subsequent acid etching or the application self-etch adhesives that contain acidic monomers are assumed triggers for the activation of MMPs and CCs due to their demineralising effects (1,23,24). These in turn lead to the activation and release of MMPs and CC, resulting in collagenolytic-gelatinolytic activity and higher probability of bond failure (25). Several strategies to prevent the activation of MMPs and CCs have been proposed in order to produce a more durable dentin-resin interface. One such method being the inactivation of MMPs and CCs via denaturing enzymes using low pH components in adhesive systems.

It was observed in a study with *Hashimoto et al.* that when treating dentin powder with 37% phosphoric acid, the same acid used in the ER technique, the amount of collagenolytic activity was reduced by 65% in mineralised dentin; speculated to be due to the very low pH (-0.7) of phosphoric acid which would partially denature dentin MMPs (26). This suggests that using low pH acids in multiple step techniques or acidic components in convenient one-step techniques have the potential to be used to denature collagenases and inhibit collagen degradation. However, this observation raises further questions about our understanding of adhesive systems and their effects on MMPs and CCs as despite this denaturing effect, we still observe bond failures due to unstable resin matrices as a result of hydrolysis (23,25). This paper will further explore other mechanisms of activation of MMPs and CCs and what materials and methods can be employed in order to deactivate or prevent their activation.

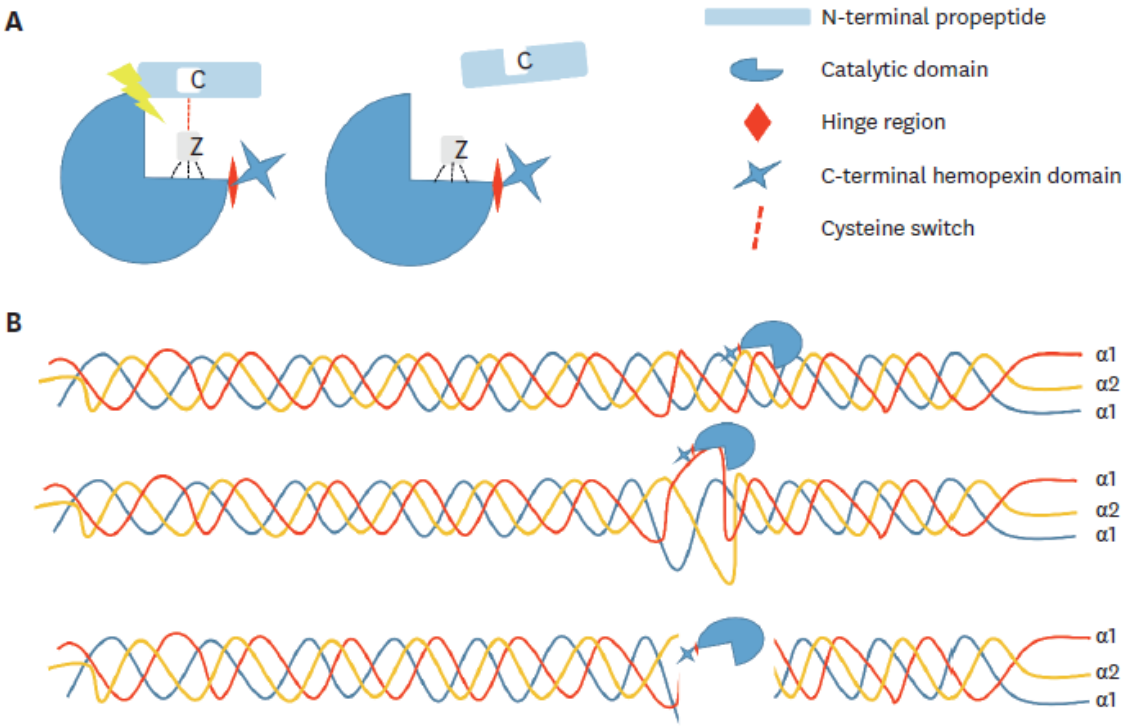

Figure 1. The structure and method of activation and resulting action of MMPs. **A:** Metalloproteinases consist of an N-terminal pro-peptide (Cysteine residue), a catalytic domain (Zinc), hinge region and C-terminal hemopexin domain. Activated when cysteine-switch occurs which is the resulting loss of coordination between cysteine and zinc, due to external factors such as exposure to proenzymes (MT-MMPs), etc. **B:** Once activated, collagenase cleaves the collage causing it to lose its triple helix structure, this results in 1/3 and 3/4 length fragments that are susceptible to action of gelatinases.(27)

Objectives

1. Understand and summarise the role of host-derived Matrix Metalloproteinase enzymes and Cysteine Cathepsines in dental adhesion.
2. Explore the mechanisms and pathways of MMPs and Cysteine Cathepsines in the degradation of collagen fibrils in the hybrid layer.
3. Explore the current options to prevent their adverse effects on collagen within the hybrid layer and ways to increase bond strength.

Methodology

The research for this paper was found using databases such as PMC, PubMed and Google Scholar using keywords such as 'Matrix Metalloproteinase Enzyme', 'MMPs', 'Cysteine Cathepsines', 'MMPs and Dental Adhesion', 'Collagenolytic', 'Collagenolysis'.

Inclusion Criteria

Initially, 40 papers were reviewed that included the keywords listed above and focused on the papers that involved dental adhesion; the mechanisms of action and regulation of MMPs and CC. A further 20 papers were reviewed that focused on the materials used to inhibit MMP and CC action and their mechanisms, such as chelating agents, cross-linkers, competitive inhibitors of MMP and CCs, etc.

Exclusion Criteria

The number of papers used were narrowed down by excluding studies that involved dental caries; as studies including caries would involve external factors such as bacteria and the acidic environments they create and their influence on dentin-composite bond degradation as opposed to only looking at the relationship between metalloproteinases,

cysteine cathepsines and how they affect dental adhesion in restorations. The final number of papers used in writing this review was 49 that adhered to the above criteria.

The papers selected included *In Vitro* studies and review papers that contained the above keywords. The papers were limited to English.

Discussion

Strategies of MMP and CC Inhibition

Chelation (Chlorhexidine)

Chlorhexidine (**CHX**) is a cationic bisguanide agent that has extensive uses in periodontics and endodontics as well as being commercially available at concentrations of 0.2% to be used as a mouthwash to treat gingival inflammation via its bactericidal and bacteriostatic effects that are dependent on its concentration. In addition to being used in other disciplines in dentistry, CHX has been heavily researched and has shown to be effective as an inhibitor of MMPs that are commonly found within dentin (MMPs -2, -8 and -9) as well as exhibit strong inhibition of the activity of CCs within dentin and have a preserving effect in the HL in a dentin-resin complex (15,28,29).

The mechanism behind this MMP inhibiting effect can be attributed to cation chelation, where CHX is able to sequestrate both the zinc and calcium ions from the active sites of MMPs preventing the activation of the catalytic domain; and as a result the ability to modify the three-dimensional structure of the enzyme (30). This effect has been observed in concentrations as low as 0.02% but a concentration that has been more widely researched is 0.2%; both of these concentrations have been observed to preserve the HL and integrity of the dentin-resin bond in restorations (29,31).

CHX has been considered to be used as an inhibitor because it has a strong positive ionic charge that has the ability to bind to phosphate groups in mineralised dentin/enamel or carboxylic acids in demineralised dentin; namely the protonated NH_3^+ in the CHX molecule and the COOH-/OH- in dentin. This binding is further enhanced by acid etching that increases the surface-free energy, resulting in a electrostatic bond (32). In addition, CHX is able to bind to surfaces that are covered in acidic proteins that depending on the concentration used is able to be released at therapeutic levels, enough to inhibit MMPs and CCs in a dentin-resin complex via 'Substantivity'. It allows CHX to remain active after the primary application, maintaining its therapeutic effect; this effect can be prolonged or shortened depending on the concentration of CHX used, with a higher concentration of CHX applied yielding a longer-lasting effect (15).

There have been attempts to integrate the application of CHX into adhesive systems as either a primer to be used before the application of the adhesive as a separate step or into 37% phosphoric acid to reduce the incidence of bond failure in the HL (33). However, being incorporated into an acid etching step presents the problem that a rinsing step follows; in which water is usually used that is able to displace CHX, reducing the amount of CHX to untherapeutic levels or completely removing CHX altogether from the interface. Other protocols advise to use 2.0% CHX for 1 minute after the dentin has been etched and washed as a way to inhibit proteolytic enzymes; alternatively the Peak Universal Bond (Ultradent) has incorporated 0.2% CHX into their adhesive blend that can be applied in the etch-and-rinse two step technique or a self-etch one step technique (15).

Although these techniques and protocols have been researched, developed and suggested; the use of CHX in inhibiting proteolytic activity still presents many issues, as CHX is only able to maintain the stability of dentin-resin complexes in the HL for a maximum of between 12-18 months, severely limiting its capacity to maintain bonds in the HL for long periods of time (34). This may be largely attributed to leaching, making the process reversible; and that the bond between dentin and CHX is electrostatic, leaving it vulnerable to be displaced by competing cations from dentinal fluid or saliva which have been shown to reduce the inhibitory effect of CHX (4,15).

In order to overcome these limitations, several authors have suggested different systems or approaches, however these systems require further research and trials in order to prove its long-term stability. The first suggestion being to chemically graft CHX to resin monomers to create CHX-methacrylates, granting these monomers the inhibitory effects of CHX while not negatively affecting the properties of the resin monomers (15). Alternatively, de Menezes *et al.* suggested a modified release system that uses clay to act as a reinforcing agent and release modulator in adhesives. This system showed improved inhibitory effects on MMPs and CC than CHX on its own and these effects lasted longer than 18 months (35).

Despite the shortcomings of CHX as a primer, it is still the most commonly implemented technique to inhibit the actions of MMPs and CCs in order to preserve the dentin-resin complexes within the HL. Due to its widespread nature that it is already implemented in several other disciplines of dentistry, it is very widely available and can be conveniently implemented into routine restorative procedures as its effects can be seen in applications of as little as 30 seconds (32).

Chelation (Quaternary Ammonium Compounds)

QACs are another positively charged compound that has the ability to inhibit proteolytic activity in dentin using a similar cationic mechanism to CHX; it does this by being able to bind electrostatically to negatively charged hydroxyapatite phosphate and collagen carboxylic groups that are found in collagen, dentin and enamel. The result of which create changes in the three-dimensional structure and block the active site, preventing MMPs to be able to bind and cleave them.; however as these bonds are electrostatic, they are weak (36). This means that QACs are water-soluble and can leach out of the dentin-resin complex, resulting in a similar effect as CHX where the proteolytic inhibitory effects are lost over time (27).

Several studies have observed the effects of different QACs in the effort to avoid this effect and prolong its inhibitory effects against proteolytic activity. Daood *et al.* researched the effects of Quaternary Ammonium Silane (**QAS**) at different concentrations (2%, 5% and 10%) and found that at all concentrations, MMP and CC activity was inhibited. Following this, they used QAS on etched dentin and found that a three-dimensional network was formed after condensation in a restoration when a tetrafunctional organosilane was used as an anchoring unit for trialkoxysilane molecules. This network minimises the leaching effect for the QAS and allows it to act for much longer. Finally, they found that 2% QAS was a good substitute for 2% CHX in order to inhibit proteolytic MMPs and CCs (37,38).

Another compound that was investigated was Benzalkonium Chloride (**BAC**), that is a mixture of alkylbenzyl-dimethylammonium chloride of various chain lengths; it functions similarly to other QACs in that it is cationic and has antimicrobial effects. It is already very widely available commercially in a 1% concentration in combination with

37% phosphoric acid, primarily used for its anti-microbial effects and that it has no adverse effects on bond strength nor dentin bonding when used in this etchant form.(15) BAC too has demonstrated proteolytic inhibitory effects (39). It has a distinct advantage over agents like CHX in that it has the ability to bind strongly to demineralised dentin even after the rinsing step which would prolong the anti-proteolytic effects; however it is still vulnerable to a certain extent in that some BAC is still lost after the rinsing step when used in combination with an etchant (40). Comba *et al.* had attempted to see if there were any improvements if they added BAC into a primer and adhesive formula in effort to avoid the loss of BAC in the rinsing step; although the research did reflect that anti-proteolytic effects were exhibited, the resulting bond strengths after 6 months did not reflect the impression that the effects of BAC were long-lasting (39). This reflects that more research and investigation is required in order to implement BAC in ordinary clinical protocols.

12-methacryloyloxydodecylpyridinium bromide (**MDPB**) is another QAC that has been widely available for a long time; usually used for its anti-microbial effects and as a cavity disinfectant when used in a dentin primer such as Clearfil Protect Bond (Kuraray). Studies have shown that using 5% MDPB, the concentration usually used in commercial adhesion primers, has effective MMP inhibitory effects similar to CHX, leading to more durable dentin-resin complexes. MDPB has the ability to copolymerise with other adhesive monomers that signifies its MMP inhibitory effects can potentially last for many years; however long-term studies have yet to be conducted to confirm this (15,27).

Chelation (Tetracyclines)

Tetracyclines are broad-spectrum antibiotics that have many uses in periodontics; their semi-synthetic analogues and non-antimicrobial analogues have all shown to have some degree of effectiveness when inhibiting the collagenolytic action of MMPs (15,41).

Tetracyclines became an agent of potential inhibitor of proteolysis when the semi-synthetic Minocycline had shown inhibition of collagenase activity in rat gingiva. This led to further research into the semi-synthetic tetracyclines, both doxycycline and minocycline were both shown to have inhibitory effects on collagenases and gelatinases (42). It is thought that their mechanism of action, although not completely understood yet, is zinc chelation in addition to down-regulating MMP mRNA expression; interfering with protein processing leading to irregular protein structures, making it more susceptible to agents that degrade MMPs.

In regard to its clinical use, doxycycline is the only semi-synthetic tetracycline that is available for MMP its ability to inhibit MMPs. 20mg of Doxycycline (Periostat) is used in the treatment for periodontitis in order to reduce the rate of collagen breakdown within periodontal tissues (15). The question remains if it can be effectively integrated into a clinical protocol for restorations however it was observed that doxycycline was not compatible with acetone-based adhesive systems due to it resulting in lower bond strengths in addition to higher silver nitrate penetration in the HL, reducing the overall durability of the dentin-resin complex (41).

The non-antimicrobial chemically modified tetracyclines (**CMT**s) inherently lack anti-bacterial activity but retain their ability to inhibit MMPs; CMT-3 (Metastat) has displayed this effect in carious dental lesions, it too has an inhibitory effect against gelatinases. It’s

mechanism of action is supposed to be calcium chelation in addition to inhibiting enzyme secretion and activity; it does this by binding to the zinc ion active site and changing the three-dimensional structure of the enzyme and blocking any catalytic activity in the ECM.

In spite of these advantageous effects, further research is required in order to integrate these compounds into clinical protocols due to their purple staining effect as a result of photo-oxidation; this makes it not suitable for clinical use. (15,41)

Chelation (Bisphosphonates)

Bisphosphonate derivatives are another class of broad-spectrum proteolytic inhibitor that are thought to be able to inhibit the action of MMPs; their assumed mechanism of action is zinc and calcium ion chelation from MMPs themselves(15), as demonstrated by Tezvergil *et al*. using polyvinylphosphonic acid (**PVPA**) when used in demineralised dentin. They displayed that PVPA was able to inhibit MMP-9 with little hydroxyproline release leading to a more stable collagen structure and dry mass loss (43).

Due its assumed mechanism of chelation, it is thought that PVPA, similar to CHX binds electrostatically to collagen structures; it has an advantage over CHX in that it is able to be trapped within collagen matrices via the application of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (**EDC**). This would allow PVPA to be used for a much longer period of time than the current maximum of CHX of 18 months; when it was applied in combination with EDC before the application of systems such as One-Step and Adpter Single Bond Plus (3M Espe), there were no detrimental effects to the micro tensile bond strengths. This research does show promising results however much more

research is required in order to evaluate its effectiveness in everyday clinical protocols and its long-term effects in inhibiting dentin-resin complex degradation (15).

Collagen Cross-linking Agents

An alternative strategy to inhibiting or reducing proteolytic activity within the HL is to increase the amount of cross-linking between collagen fibrils; it is already a naturally occurring mechanism and if it can be activated or mimicked then this biomodification will be able to enhance the three-dimensional structure of collagen, making it sturdier, improving its biomechanical properties and have a greater resistance to attacks by endogenous proteases including MMPs and CCs (15,44).

It is known that collagenases first unwind the triple helix structure, as the binding sites of collagenase are too narrow to accommodate triple helix collagen; this unwinding allows single peptides to enter catalytic domains resulting in cleavage of the collagen molecule. Cross-linking agents could be applied to collagen that would produce covalent bonds and as a result making it stiffer, enough to prevent unwinding through collagenases; it too can cross-link proteases preventing their mobility and activation. This may be achieved by inactivating C-terminal telopeptidases that have the ability to remove the steric block on the collagenase binding site; if they can be inactivated, then the telopeptidases have a maintained block on collagenase binding sites, preventing collagenase action and collagen attacks (15,18).

Glutaraldehyde (**GA**) is one such cross-linking agent, an aldehyde that is normally used as a tissue fixative is known to produce cross-linking due to its ability to form covalent bonds between the amino groups of proteins and it's two aldehyde groups. It is already

known for its ability to have anti-microbial and anti-MMP benefits; when combined with its ability to cross-link, it improves the resistance of uncross-linked/mildly cross-linked collagen to proteolytic activity (45). A study demonstrated that the use of 5% GA for 1 minute following acid etching improved its modulus of elasticity, inferring that as the mechanical properties of dentin were improved, as were the resistant properties of the collagen against endogenous proteases (46). However, despite these advantages and results, GA still displays cytotoxicity making it insufficient for use in clinical protocols.

Other cross-linking agents such as Carbodiimide (EDC) does not suffer from the level of cytotoxicity that GA does. Cross-linking agents such as EDC in addition to being able to prevent telopeptidase activity, cross-link collagen molecules via covalent amine bonds, increasing their stiffness; are able to cross-link all MMPs, CCs and any other enzymes simultaneously. This ultimately removes the need to apply any other agent in order to inhibit MMP and CC activity in the HL; as if proteases such as MMPs and CCs are covalently bound to the matrix, they do not have the ability to solubilise and diffuse to produce degradation leading to their theoretical complete inactivation. This is attributable to the bonding process that seals dentin tubules with resin tags, preventing any outward diffusion of MMPs that are synthesises by endogenous odontoblasts as they only extend 1/4 of the length of the dentinal tubules (4,15,47).

Again, due to the cytotoxicity of these cross-linking agents, there is also an inability to control cross-linking rates resulting in unstable dentin-resin matrices which could impede their ability to be used in clinical protocols. Another cross-linking agent, Riboflavin (**RF**) is a biocompatible agent that has the ability to cross-link collagen molecules via producing reactive oxygen species such as O_2 and O_2^-; these free radicals

are release when RF is photoactivated and light is absorbed, resulting in covalent cross-linking.

This like other cross-linking agents increases the bond strength of dentin-resin interfaces and inhibits protease activity, increasing the overall durability of these interfaces. It has been seen to be able to be used with UVA or the more commonly used blue light with good success. This fact allows the clinician to have much better control over the degree of cross-linking and achieve more consistent results (15,27,48,49).

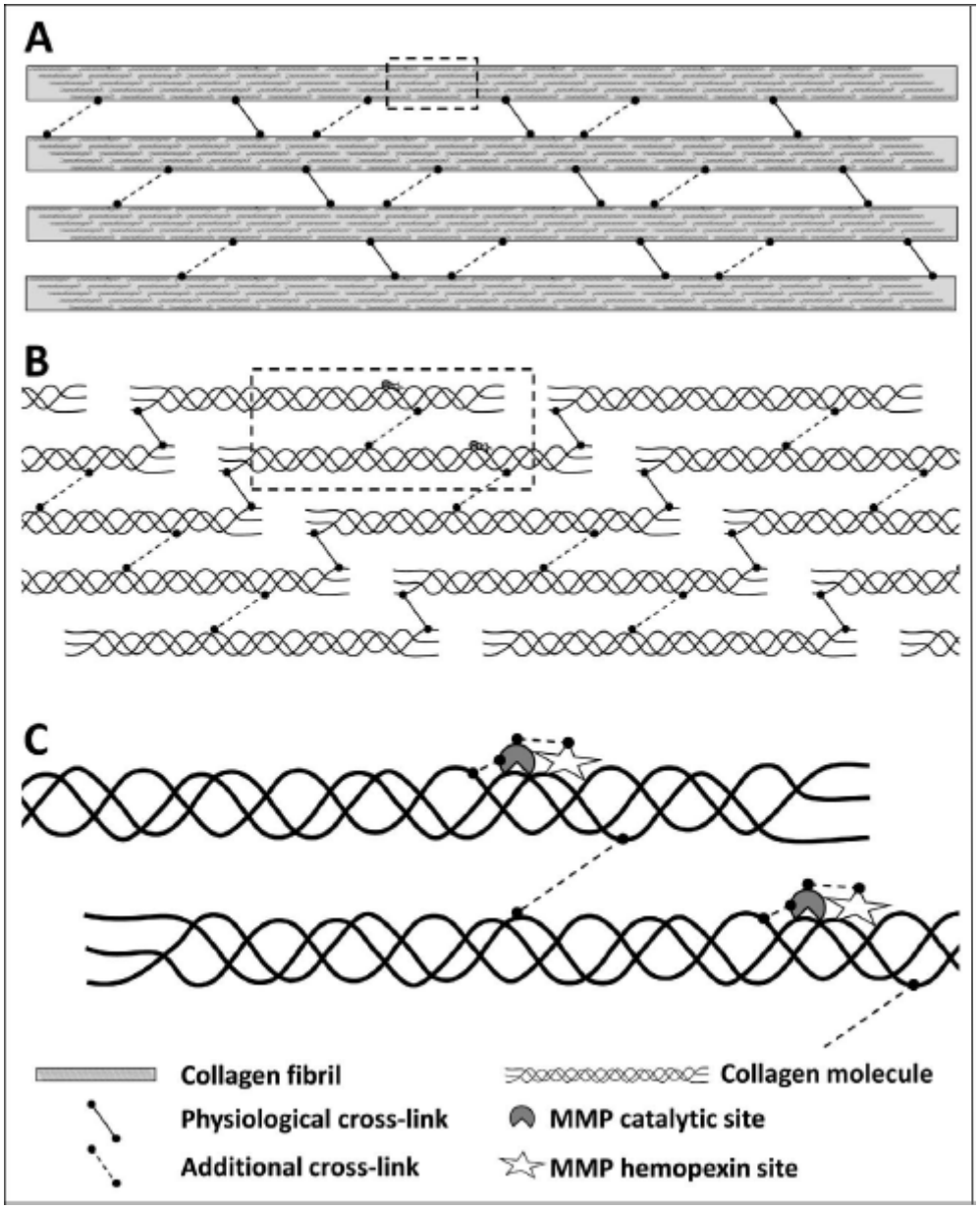

Figure 2: The proposed mechanism of cross-linking agents interacting with collagen molecules. **A:** Presence of additional cross-links between collagen microfibrils increasing the matrix stiffness and possibly improve hydrophobic resin infiltration with fewer possibilities of matrix collapse as a result of drying, due to reinforcement. **B:** A more detailed view off collagen molecules and extra cross-links; reinforcing the collagen matrix structure and decreasing the vulnerability against collagen-degrading enzymes. **C:** Individual collagen molecules highlighted with the dotted line in B; cross-linking may cause structural changes in the active site/catalytic domain and/or binding site of MMPs, possibility eliminating the collagenolytic activity of these enzymes.(5)

Competitive Inhibition (Zinc Salts)

Competitive inhibition is another mechanism proposed and studied in order to inhibit the action of proteolytic MMPs. MMPs require calcium and zinc ions to function properly and maintain their tertiary structure; with zinc being required to bind to the cysteine residue to maintain pro-MMPs in an inactive state by preventing water molecules from binding to the zinc ion in the catalytic site. Therefore trace amounts of zinc are required for proper function of MMPs (4,14).

It has been demonstrated that zinc can have a protective effect through binding to the collagen-sensitive cleavage sites of MMPs via competitive inhibition; this can be achieved by an excess concentration of zinc ions and results in their reduced effect or inactivation. Studies had shown that an excess of zinc had reduced the amount of MMP-mediated collagenolysis in dentin beams that were incubated in $ZnCl_2$ solution; additionally, MMP-2 and -9 were inhibited with other zinc salts. The inhibitory mechanism can also be attributed to the greater molecular stability caused by the excess ligand resulting in less enzymatic activity.

Toledano *et al.* reported that adding 10 wt% of ZnO particles to Single Bond (3M) had resulted in a dramatic reduction of ICTP collagen peptide fragments that usually are a result from collagenolysis, than compared to just using Single Bond (3M). The same group added $ZnCl_2$ to a final medium concentration of 3.3mg/ml, too inhibited MMP activity in demineralised dentin beams over the period of four weeks. Although $ZnCl_2$ had reduced the microtensile bond strength of other bonding systems such as Clearfil SE Bond (Kuraray), ZnO had no effect on bond strength; however, they observed that the addition of either molecules resulted in a reduction of MMP activity.

It is speculated by several authors that the correct concentration of zinc is important to find in determining the resulting effect on MMPs and collagen molecules while preventing toxicity. As there are many materials used in dentin restorations that incorporate ZnO in their formulas; it is expected that new adhesive systems that use ZnO at correct concentrations with adequate MMP-inhibiting effects will be compatible and viable for us in dental restorations, however more research and experimentation is required (15,27).

Conclusion

1. Identifying the detrimental effect that endogenous MMPs and CCs have on the dentin-resin complex after dentin restorations has allowed us to understand why bond failure after 12-18 months is so common in composite restorations, despite the many advances in adhesive system technology. With MMPs and CCs having strong proteolytic behaviour and being synergistic with one another, they present an extremely detrimental effect on collagen fibrils present in the hybrid layer via cleavage of collagen into fragments; resulting in a more fragile restoration as many of the commonly implemented protocols have little or no means to protect against endogenous MMP and CC activity in the long term.

2. This had created a sense of importance that encouraged more research into exploring the mechanisms of MMPs and CCs, and understanding what activates and triggers these proteases. Such an example was exploring the effect that the commonly used etchant 37% phosphoric acid had on the degree of expression of these enzymes, but this study also identified that low pH acids (~0.2 pH) have

the capacity to produce a denaturing effect on them, discovering a potential vulnerability.

3. Observing the effects of commonly used agents such as Chlorhexidine that is regularly used as an antimicrobial in endodontics and periodontics yielded proof that it had an inhibitory effect on MMPs and CCs via ion chelation. However, CHX's effect is short-lived driving research into exploring different agents and other methods of employing CHX in adhesive systems that could potentially last longer such as grafting CHX into methacrylates or employing a different release system such as clay into the dentin-resin complex, both showing positive results. After attaining a thorough understanding of the mechanisms of MMPs and CCs, other agents that use alternative mechanisms of action such as cross-linking agents like GA or EDC and inhibitory agents like zinc; others and these types of agents have their own respective disadvantages that make it difficult to be able to safely implement them into routine clinical protocols in everyday restorative work. Ultimately meaning that further research is required to create optimal agents that are able to function within the HL and prolong the life and strength of dentin-resin interfaces.

Responsibility

This paper amongst others has emphasised the current limitations of adhesion in composite restorations. Hopefully, this paper raises awareness about which agents and protocols can be followed safely to improve the durability of restorations; as well as provide a platform for others to build upon by researching and investigating the

mentioned materials that still have potential to be implemented in adhesive procedure that can prevent MMP and CC action in resin matrices.

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Annex

- Figure 1: de Moraes IQS, do Nascimento TG, da Silva AT, de Lira LMSS, Parolia A, Porto ICC de M. Inhibition of matrix metalloproteinases: a troubleshooting for dentin adhesion. Restor Dent Endod. 2020;45(3):1–20.
    - Activation and Mechanism of MMPs
- Figure 2: Tjäderhane L. Dentin bonding: Can we make it last? Oper Dent. 2015;40(1):4–18.
    - Mechanism and benefits of cross-linkers.

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Optimizing dentin bond durability: control of collagen degradation by matrix metalloproteinases and cysteine cathepsins

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Abstract

Objectives—Contemporary adhesives lose their bond strength to dentin regardless of the bonding system used. This loss relates to the hydrolysis of collagen matrix of the hybrid layers. The preservation of the collagen matrix integrity is a key issue in the attempts to improve the dentin bonding durability.

Methods—Dentin contains collagenolytic enzymes, matrix metalloproteinases (MMPs) and cysteine cathepsins, which are responsible for the hydrolytic degradation of collagen matrix in the bonded interface.

Results—The identities, roles and function of collagenolytic enzymes in mineralized dentin has been gathered only within last 15 years, but they have already been demonstrated to have an important role in dental hard tissue pathologies, including the degradation of the hybrid layer. Identifying responsible enzymes facilitates the development of new, more efficient methods to improve the stability of dentin-adhesive bond and durability of bond strength.

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4: Fundamental Concepts of Enamel and Dentin Adhesion

CHAPTER 4

Fundamental Concepts of Enamel and Dentin Adhesion

Jorge Perdigão, Edward J. Swift, Jr. and Ricardo Walter

Basic Concepts of Adhesion

The American Society for Testing and Materials (specification D 907) defines adhesion as "the state in which two surfaces are held together by interfacial forces which may consist of valence forces or interlocking forces or both."[1] The word adhesion comes from the Latin *adhaerere* ("to stick to"). An adhesive is a material, frequently a viscous fluid, that joins two substrates together by solidifying and transferring a load from one surface to the other. Adhesion or adhesive strength is the measure of the load-bearing capacity of an adhesive joint.[2] Four different mechanisms of adhesion have been described, as follows:[3]

http://www.ijos.org.cn *Zhang et al*. The Role of MMPs in Dentin Bonding

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REVIEWS

The Role of Host-derived Dentinal Matrix Metalloproteinases in Reducing Dentin Bonding of Resin Adhesives

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Abstract

Shan-chuan Zhang, Matthias Kern. The Role of Dentinal Host-derived Matrix Metalloproteinases in Reducing Dentin Bonding of Resin Adhesives. *International Journal of Oral Science*, 1(4): 163–176, 2009

Dentin matrix metalloproteinases (MMPs) are a family of host-derived proteolytic enzymes trapped within mineralized dentin matrix, which have the ability to hydrolyze the organic matrix of demineralized dentin. After bonding with resins to dentin there are usually some exposed collagen fibrils at the bottom of the hybrid layer owing to imperfect resin impregnation of the demineralized dentin matrix. Exposed collagen fibrils might be affected by MMPs inducing hydrolytic degradation, which might result in reduced bond strength.

Most MMPs are synthesized and released from odontoblasts in the form of proenzymes, requiring activation to degrade extracellular matrix components. Unfortunately, they can be activated by modern self-etch and etch-and-rinse adhesives. The aim of this review is to summarize the current knowledge of the role of dentinal host-derived MMPs in dentin matrix degradation. We also discuss various available MMP inhibitors, especially chlorhexidine, and suggest that they could provide a potential pathway for inhibiting collagen degradation in bonding interfaces thereby increasing dentin bonding durability.

Keywords dentin bonding, matrix metalloproteinases (MMPs), MMP inhibitors, chlorhexidine

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Review

Dentin bonding systems: From dentin collagen structure to bond preservation and clinical applications

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https://doi.org/10.1016/j.dental.2017.11.005

Abstract

Objectives

Efforts towards achieving durable resin–dentin bonds have been made for decades, including the understanding of the mechanisms underlying hybrid layer (HL) degradation, manufacturing of improved adhesive systems, as well as developing strategies for the preservation of the HL.

Operative Dentistry, 2015, **40-1,** 4-18

Buonocore Lecture

Dentin Bonding: Can We Make it Last?

L Tjäderhane

Clinical Relevance

Bond strength to dentin decreases with time because of the hydrolytic degradation of the hybrid layer components dentin collagen and adhesive resin. Inhibition of enzymes responsible for the collagen degradation may improve the bond strength durability.

SUMMARY

In dentin bonding, contemporary dental adhesive systems rely on formation of the hybrid layer, a biocomposite containing dentin collagen and polymerized resin adhesive. They are usually able to create at least reasonable integrity of the hybrid layer with high immediate bond strength. However, loss of dentin-bonded interface integrity and bond strength is commonly seen after aging both *in vitro* and *in vivo*. This is due to endogenous collagenolytic enzymes, matrix metalloproteinases, and cysteine cathepsins, responsible for the time-dependent loss of hybrid layer collagen. In addition, the hydrophilic nature of adhesive systems creates problems that lead to suboptimal hybrid layers. These problems include, for example, insufficient resin impregnation of dentin, phase separation, and a low rate of polymerization, all of which may reduce the longevity of the bonded interface.

Preservation of the collagen matrix integrity by inhibition of endogenous dentin proteases is key to improving dentin bonding durability. Several approaches to retain the integrity of the hybrid layer and to improve the long-term dentin bond strength have been tested. These include the use of enzyme inhibitors, either separately or as incorporated into the adhesive resins; increase of collagen resistance to enzymatic degradation; and elimination of water from the interface to slow down or eliminate hydrolytic loss of the hybrid layer components. This review looks at the principles, current status, and future of the different techniques designed to prevent the loss of hybrid layer and bond strength.

INTRODUCTION

Impact of Adhesive Application to Wet and Dry Dentin on Long-term Resin-dentin Bond Strengths

A Reis • A Pellizzaro • K Dal-Bianco
OM Gomes • R Patzlaff • AD Loguercio

Clinical Relevance

As long as adhesives are vigorously rubbed onto dentin surfaces, high immediate and long-term bond strengths can be obtained to either air-dried or wet demineralized dentin.

SUMMARY

This study compared the effects of moisture and rubbing action on the immediate and one-year microtensile bond strength (BS) of an ethanol/water-based adhesive system (Single Bond [SB]) and an acetone-based system (One Step [OS]) to dentin. A flat superficial dentin surface on 60 human molars was exposed by wet abrasion. Two coats of the adhesives were applied on either a dry (D) or rewetted surface (W) with no (NRA), slight (SRA) or vigorous rubbing action (VRA). After light curing (600mW/cm²/10 seconds), composite buildups were constructed incrementally and the specimens were stored in water (37°C/24 hours). They were longitudinally sectioned in the "x" and "y" directions to obtain bonded sticks (0.8 mm²) to be tested in tension at 0.5 mm/minute. The sticks from each tooth were then divided, stored in water at 37°C and tested immediately and after 12 months (12M) at 0.5 mm/minute. The bond strength values of sticks from the same hemi-tooth were averaged for statistical purposes. The prematurely debonded specimens were included in the hemi-tooth mean. The data from each adhesive was analyzed by three-way ANOVA and Tukey's multiple comparison tests (α=0.05). In the dry groups, high bond strength values were obtained under VRA. When the

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A Novel Enamel and Dentin Etching Protocol Using α-hydroxy Glycolic Acid: Surface Property, Etching Pattern, and Bond Strength Studies

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Summary

Objectives—To determine the use of α-hydroxy glycolic acid (GA) as a surface pretreatment for dental restorative applications. The etching pattern of GA pretreatment of dental hard tissues was assessed by surface microhardness and scanning electron microscopy (SEM). The effectiveness of GA surface etching on the enamel and dentin resin bond strengths was assessed using two etchant application modes (rubbing and no rubbing) and three adhesive systems (Single Bond [SB], One Step Plus [OSP], and Scotchbond Universal [SBU]).

Methods—Knoop microhardness measurements were carried out on polished enamel and dentin surfaces before and after treatment with 35% GA, 35% phosphoric acid (PA), or distilled water (control group) for 30 seconds. The microtensile bond strength test was carried out on enamel and dentin. Ultrastructural analysis of the surface and interfacial interaction was qualitatively accomplished using SEM.

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Regulatory Statement: This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the University of Illinois at Chicago. The approval code for this study is 2011-0312.

Conflict of Interest: The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

The authors declare no potential conflicts of interest with respect to either the authorship or publication of this manuscript.

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EFFECT OF ETCHING TIME AND ACID CONCENTRATION ON MICROMORPHOLOGICAL CHANGES IN DENTIN OF BOTH DENTITIONS

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ABSTRACT:

Aim: to compare micromorphological changes in primary and permanent dentin after etching with phosphoric acid (20% and 37,5%) for 7 and 15 sec. by SEM.

Material and methods: The study included 42 primary and permanent teeth, divided into 8 groups by etching time and acid concentration. Enamel and dentin were removed from the vestibular area and after the expiration of etching time samples were washed with water-air stream and dried with light airflow. From each sample 10 magnified images were made from central vestibular area. The cleaning effect was measured in percentage, as a ratio between the number of uncleaned tubules to the total tubules. Results were analyzed with One-way and MANOVA. Post hoc Multiple Comparisons test – SPSS 19 was applied.

Results: The proportion of uncleaned tubules in primary teeth was higher than that of permanent teeth at acid concentration of 20%. At a concentration of 37.5% this relationship is reversed. At 7 sec there was a bigger difference between the share of uncleaned tubules for primary and permanent teeth, while at 15 sec this difference virtually disappears. The difference in the proportion of uncleaned tubules between the two acid concentrations at 7 seconds etching is significantly greater compared to the same difference between the two acid concentrations by etching for 15 sec.

Conclusion: Effectively removed smear layer and no precipitate was observed in primary teeth even at 7 seconds etching with 37.5% acid.

Key words: etching time, primary dentin, permanent dentin, smear layer, SEM, peritubular dentin

The etching is a key moment in the preparation of the tooth for application of adhesive systems which are applied with the Total-etch approach [1]. Considering that, the etching of the dentin is of fundamental importance and at the same time is a problematic area for achieveing sufficient bond strength [2, 3].

In dental practice enamel and dentin are both etched. The goal is to create a chemically clean surface and microretentions [3, 4]. Therefore, a micromechanical bond is created - via the formation of "resin tags" of the adhesive into the dentin tubules as well as a nano mechanical bond - via the penetration of the adhesive in the demineralized space between the collagen fibers of the intertubular dentin [3, 5].

At this stage, the data for a better and a long lasting bond with the dentin after the removal of the smear layer, which is achieved by total etching, prevails [1, 6, 7].

The adhesive dentine bond strength is a function of its morphology and the etching agents [5, 8]. The morphology of the dentin substrate can be changed as a consequence of age-related changes, the presence of carious and non-carious lesions, as well as the type of dentition – primary or permanent [4, 6, 7, 9-13]. The effect of the etching agent depends on the type and concentration of the acid, time and the manner of its application [14-18].

The comparison of the composition and the morphology of the dentin of the primary and permanent teeth shows some differences [8, 10, 16, 19-22]. In a study of the hardness of the coronal dentin's central zone it was found that the dentin in permanent teeth is significantly harder than the one of the corresponding areas in the primary teeth [20, 22-26]. The dentin of permanent teeth is with higher mineralization [27], based on the fact that the hardness is directly related to the degree of mineralization [16, 20, 23]. The primary teeth dentin is characterized by a lower hardness, and hence it is with a lower degree of mineralization in comparison with the one in permanent teeth. A lower calcium and phosphorus concentration in the peritubular and intertubular dentin is found as well as lower micromechanical features [21, 23, 25, 26, 28, 29].

Furthermore, there is a difference in the tubular density and the size of the dentin tubules- characteristics which define the dentin permeability. These differences lead to a different amount of intertubular dentin, which is the largest and most significant component of the dentin in terms of bonding procedures [10, 21, 26, 30].

Studies on the hybride layer, performed on primary and permanent teeth, also indicate for differences in the thickness of this layer. The formed hybrid layer in primary teeth is much thicker than the one in permanent teeth when the same protocol of adhesive application is performed [21, 23, 31].

All of this gives grounds to suggest that the probable reason for these results are the different dentin reactions of deciduous teeth to acid used for etching before the application of the adhesive system [8, 22, 25, 28, 32, 33].

All established parameters to achieve adequate dentin

ELSEVIER

Journal of Dentistry

Volume 25, Issue 5, September 1997, Pages 355-372

Review

Dentine permeability and dentine adhesion

D.H. Pashley *, R.M. Carvalho †

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https://doi.org/10.1016/S0300-5712(96)00057-7

Abstract

Objectives: The objectives of this paper are to review the structure of dentine as it pertains to adhesive bonding and to describe the importance of resin permeation into dentinal tubules and into spaces created between collagen fibrils by acid-etching during resin bonding. The advantages and disadvantages of separate acid-etching, priming and adhesive applications are discussed.

The Effects of Acetone, Ethanol, HEMA, and Air on the Stiffness of Human Decalcified Dentin Matrix

K.T. Maciel, R.M. Carvalho, R.D. Ringle, , , , , more... Show all authors

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Article information Altmetric 3

Abstract

During resin-bonding procedures, dentin surfaces are treated with acidic conditioners to remove the smear layer and decalcify the surface to expose the collagen fibrils of the underlying matrix. These decalcified surfaces are then either air-dried or treated with dehydrating solvents, procedures which may modify the physical properties of the dentin matrix. The purpose of this study was to evaluate the effects of dehydration on the stiffness of the decalcified dentin matrix. Small (8 x 1.7 x 0.9 mm) beams of dentin were prepared from mid-coronal dentin of extracted human molars. The ends were covered with varnish for protection, and the specimens were placed in 0.5 M EDTA for 5 days to decalcify. The stiffness was measured by both the cantilever technique and by conventional stress-strain testing. Specimens tested by the cantilever technique were sequentially exposed to water, acetone, alcohol, HEMA, and glutaraldehyde. Specimens tested by

Comparative Study > Am J Dent. 2000 Dec;13(6):324-8.

Comparative microtensile bond strength and SEM analysis of bonding to wet and dry dentin

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Affiliations + expand

PMID: 11764127

Abstract

Purpose: To compare bond strengths of resins to acid-etched wet vs. dry dentin.

Materials and methods: Human third molars were bonded with One-Step (OS), Single Bond (SB) or Clearfil PhotoBond (PB) under control moist or air-dried (5 s air blast) conditions. Tensile bond strengths were tested using the microtensile bond testing method. Scanning electron microscopy was done to evaluate the quality and thickness of the hybrid layers following polishing and acid plus NaOCl-challenge.

Results: The tensile bond strengths of OS, SB and PB were significantly ($P < 0.01$) lower (8-19 MPa) to air-dried dentin than to moist dentin (39-50 MPa). No hybrid layers were seen in the air-dried specimens bonded with OS or SB, while relatively thin hybrid layers were produced by PB. In contrast, moist dentin produced high bond strengths with all bonding systems and created thicker,

The Journal of the American Dental Association
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Improving Bond Strength Through Acid Etching of Dentin and Bonding to Wet Dentin Surfaces

John Kanca III D.M.D.

https://doi.org/10.14219/jada.archive.1992.0248

Dr. John Kanca III describes a dentin-enamel bonding system that bonds to wet surfaces in **"Improving Bond Strength Through Acid Etching of Dentin and Bonding to Wet Dentin Surfaces."**

A bonding system using moisture on the tooth surface can be an enormous benefit as obtaining dentin dryness in the mouth is nearly impossible. The author describes a system that bonds to both wet and dry surfaces.

Minireview

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 274, No. 31, Issue of July 30, pp. 21491–21494, 1999

Matrix Metalloproteinases*

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The timely breakdown of extracellular matrix (ECM)[1] is essential for embryonic development, morphogenesis, reproduction, and tissue resorption and remodeling. The matrix metalloproteinases (MMPs), also called matrixins, are thought to play a central role in these processes. The expression of most matrixins is transcriptionally regulated by growth factors, hormones, cytokines, and cellular transformation (1, 2). The proteolytic activities of MMPs are precisely controlled during activation from their precursors and inhibition by endogenous inhibitors, α-macroglobulins, and tissue inhibitors of metalloproteinases (TIMPs). Table I lists currently known vertebrate matrixins. In addition, non-vertebrate members have been identified in sea urchins (3), *Caenorhabditis elegans* (4), soybean (5), and *Arabidopsis thaliana* (6). Most of these MMPs are the subject of individual chapters in the *Handbook of Proteolytic Enzymes* (7). This minireview focuses on recent progress in regulation of matrixin activities and their biological and pathological implications.

Domain Structure and Function

All matrixins are synthesized as prepro-enzymes and secreted as inactive pro-MMPs in most cases. The primary structures of 20 vertebrate matrixins comprise several domain motifs, as illustrated in Fig. 1; the domain composition for each MMP is listed in Table I.

The propeptide domain (about 80 amino acids) has a conserved unique PRCG(V/N)PD sequence. The Cys within this sequence (the "cysteine switch") ligates the catalytic zinc to maintain the latency of pro-MMPs (8, 9). This sequence is missing in MMP-23 (10). Stromelysin 3 (MMP-11), MT-MMPs, *Xenopus* MMP, and MMP-23 have a proprotein processing sequence RX(K/R)R at the C-terminal end of the propeptide, and MMP-11 (11) and MMP-14 (12) were shown to be activated intracellularly by furin.

The catalytic domain (about 170 amino acids) contains a zinc binding motif HEXXHXXGXXH and a conserved methionine, bronectin-type II domain inserted in the catalytic domain. These repeats interact with collagens and gelatins (15, 16).

The C-terminal hemopexin-like domain (about 210 amino acids) has an ellipsoidal disk shape with a four bladed β-propeller structure; each blade consists of four antiparallel β-strands and an α-helix (17). The hemopexin domain is an absolute requirement for collagenases to cleave triple helical interstitial collagens (18), although the catalytic domains alone retain proteolytic activity toward other substrates (19). The hemopexin domain of MMP-2 is also required for the cell surface activation of pro-MMP-2 by MT1-MMP (20, 21). The function of the proline-rich linker peptide that connects the catalytic and the hemopexin domains is not known, although its interaction with triple helical collagen is hypothesized based on molecular modeling (22). MMP-23 has cysteine-rich, proline-rich, and IL-1 receptor-like regions instead of the hemopexin domain (10). A transmembrane domain is found in the MT-MMPs, which anchors those enzymes to the cell surface.

Regulation of Matrixin Gene Expression

One of the striking features of the matrixins is that many of those genes are "inducible." The effectors include growth factors, cytokines, chemical agents (*e.g.* phorbol esters, actin stress fiber-disrupting drugs), physical stress, and oncogenic cellular transformation, etc., and the enhanced MMP gene expression may be down-regulated by suppressive factors (*e.g.* transforming growth factor β, retinoic acids, glucocorticoids) (1). Induction and suppression through the promoter regions of matrixin genes have recently been reviewed by Fini *et al.* (2).

Recent studies emphasize not only soluble factors but also cell-matrix and cell-cell interactions as keys in gene expression of matrixins. Examples are: induction of MMP-1, -2, and -3 in fibroblasts by EMMPRIN (M6 antigen or basigin), a member of the immunoglobulin family expressed on tumor cell surface (23); induction of MMP-9 in T lymphoma cells through leukocyte function-associated antigen-1 (LFA-1)-intercellular adhesion molecule-1 (ICAM-1)-mediated cell adhesion (24); induction of MMP-2 in T cells through very late antigen 4 (VLA-4)-vascular cell adhesion molecule-1 (VCAM-1)-mediated adhesion to endothelial cells (25); MMP-9 expression in monocytes by the activated T cells through gp39-CD40 interaction (26); and $\alpha_5\beta_1$ integrin-fibronectin interaction for MMP-9 expression during macrophage differentiation (27). Endothelial cells (28, 29), fibroblasts (30), and neoplastic cells (31) cultured in type I collagen gel express MT1-MMP, which appears to be mediated by $\alpha_v\beta_3$ integrin (30).

ARTICLE PDF AVAILABLE

Mechanisms regulating the degradation of dentin matrices by endogenous dentin proteases and their role in dental adhesion. A review

Sabatini C, Pashley D

American Journal of Dentistry

ISSN: 08948275

Abstract

Purpose: This systematic review provides an overview of the different mechanisms proposed to regulate the degradation of dentin matrices by host-derived dentin proteases, particularly as it relates to their role in dental adhesion. Significant developments have taken place over the last few years that have contributed to a better understanding of all the factors affecting the durability of adhesive resin restorations. The complexity of dentin-resin interfaces mandates a thorough understanding of all the mechanical, physical and biochemical aspects

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REVIEWS

The Role of Host-derived Dentinal Matrix Metalloproteinases in Reducing Dentin Bonding of Resin Adhesives

Shan-chuan Zhang, Matthias Kern*

Department of Prosthodontics, Propaedeutics and Dental Materials, Christians-Albrechts University, Kiel, Germany

Abstract

Shan-chuan Zhang, Matthias Kern. The Role of Dentinal Host-derived Matrix Metalloproteinases in Reducing Dentin Bonding of Resin Adhesives. *International Journal of Oral Science*, 1(4): 163–176, 2009

Dentin matrix metalloproteinases (MMPs) are a family of host-derived proteolytic enzymes trapped within mineralized dentin matrix, which have the ability to hydrolyze the organic matrix of demineralized dentin. After bonding with resins to dentin there are usually some exposed collagen fibrils at the bottom of the hybrid layer owing to imperfect resin impregnation of the demineralized dentin matrix. Exposed collagen fibrils might be affected by MMPs inducing hydrolytic degradation, which might result in reduced bond strength.

Most MMPs are synthesized and released from odontoblasts in the form of proenzymes, requiring activation to degrade extracellular matrix components. Unfortunately, they can be activated by modern self-etch and etch-and-rinse adhesives. The aim of this review is to summarize the current knowledge of the role of dentinal host-derived MMPs in dentin matrix degradation. We also discuss various available MMP inhibitors, especially chlorhexidine, and suggest that they could provide a potential pathway for inhibiting collagen degradation in bonding interfaces thereby increasing dentin bonding durability.

Keywords dentin bonding, matrix metalloproteinases (MMPs), MMP inhibitors, chlorhexidine

Received Aug. 25, 2009; Revision accepted Oct. 15, 2009

Review

This Review is part of a thematic series on **Matrix Metalloproteinases**, which includes the following articles:
Matrix Metalloproteinase Inhibition After Myocardial Infarction: A New Approach to Prevent Heart Failure?
Matrix Metalloproteinases in Vascular Remodeling and Atherogenesis: The Good, the Bad, and the Ugly
Matrix Metalloproteinases: Regulation and Dysregulation in the Failing Heart
Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases: Structure, Function, and Biochemistry

David Kass, Marlene Rabinovitch, Editors

Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases

Structure, Function, and Biochemistry

Robert Visse, Hideaki Nagase

Abstract—Matrix metalloproteinases (MMPs), also designated matrixins, hydrolyze components of the extracellular matrix. These proteinases play a central role in many biological processes, such as embryogenesis, normal tissue remodeling, wound healing, and angiogenesis, and in diseases such as atheroma, arthritis, cancer, and tissue ulceration. Currently 23 MMP genes have been identified in humans, and most are multidomain proteins. This review describes the members of the matrixin family and discusses substrate specificity, domain structure and function, the activation of proMMPs, the regulation of matrixin activity by tissue inhibitors of metalloproteinases, and their pathophysiological implication. **(*Circ Res*. 2003;92:827-839.)**

Key Words: extracellular matrix ■ protease ■ protease inhibitors

Extracellular matrix (ECM) macromolecules are important for creating the cellular environments required during development and morphogenesis. Matrix metalloproteinases (MMPs), collectively called matrixins, are proteinases that participate in ECM degradation.[1,2] Under normal physiological conditions, the activities of MMPs are precisely regulated at the level of transcription, activation of the precursor zymogens, interaction with specific ECM components, and inhibition by endogenous inhibitors.[1,2] A loss of activity control may result in diseases such as arthritis, cancer, atherosclerosis, aneurysms, nephritis, tissue ulcers, and fibrosis.[3] Tissue inhibitors of metalloproteinases (TIMPs) are specific inhibitors of matrixins that participate in controlling the local activities of MMPs in tissues.[4,5] The pathological effects of MMPs and TIMPs in cardiovascular disease processes that involve vascular remodeling, atherosclerotic plaque instability, and left ventricular remodeling after myocardial infarction are of considerable interest and are covered by other reviews in this series.[6–8] In the present review, we give an overview of structure, function, and biochemistry of MMPs and TIMPs.

Members of the Matrixin Family

The first MMP activity discovered was a collagenase in the tail of a tadpole undergoing metamorphosis. To date, 24 different vertebrate MMPs have been identified, of which 23 are found in humans. Matrixins are also found in *Hydra*,[9] sea urchin,[10] and *Arabidopsis*.[11] The sequence homology with collagenase 1 (MMP-1), the cysteine switch motif PRCGXPD in the propeptide that maintains MMPs in their zymogen form (proMMP), and the zinc-binding motif HEXGHXXGXXH in the catalytic domain are the signatures used to assign proteinases to this family. The exception is MMP-23, which lacks the cysteine switch motif, but its

Zymographic Analysis and Characterization of MMP-2 and -9 Forms in Human Sound Dentin

A. Mazzoni[1], F. Mannello[2], F.R. Tay[3], more...

Show all authors

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Article information

Altmetric 0

Abstract

The role and function of dentin matrix metalloproteinases (MMPs) are not well-understood, but they may play a key role in dentinal caries and the degradation of resin-bonded dentin matrices. To test the null hypothesis that MMP-9 is not found in dentin matrix, we used gelatin zymography to extract and isolate all molecular forms of gelatinolytic MMPs in demineralized mature sound dentin powder obtained from extracted human molars, characterizing and identifying the enzymes by Western blotting. Gelatinolytic MMPs were detected in extracts of demineralized dentin matrix and identified as MMP-2 and MMP-9. Acidic extracts (pH 2.3) yielded 3–8 times more MMP activity than did EDTA (pH 7.4). Their activation may contribute to dentin matrix degradation, which occurs during caries progression and following resin bonding. Inhibition of MMP-2 and -9 proteolytic activity may slow caries progression and increase the durability of resin-dentin bonds.

Keywords

dentin, MMP-2, MMP-9, Western blotting, zymography, matrix metalloproteinases, gelatinases

RESEARCH ARTICLE

Collagen fibril architecture, domain organization, and triple-helical conformation govern its proteolysis

Shiamalee Perumal, Olga Antipova, and Joseph P. R. O. Orgel

+ See all authors and affiliations

PNAS February 26, 2008 105 (8) 2824-2829; https://doi.org/10.1073/pnas.0710588105

Edited by Carl Frieden, Washington University School of Medicine, St. Louis, MO, and approved December 21, 2007 (received for review November 7, 2007)

Article | Figures & SI | Info & Metrics | PDF

Abstract

We describe the molecular structure of the collagen fibril and how it affects collagen proteolysis or “collagenolysis.” The fibril-forming collagens are major components of all mammalian connective tissues, providing the structural and organizational framework for skin, blood vessels, bone, tendon, and other tissues. The triple helix of the collagen molecule is resistant to most proteinases, and the matrix metalloproteinases that do proteolyze collagen are affected by the architecture of collagen fibrils, which are notably more resistant to collagenolysis than lone collagen monomers. Until now, there has been

ELSEVIER

Archives of Biochemistry and Biophysics
Volume 535, Issue 2, 15 July 2013, Pages 111-114

Collagen degradation by tumor-associated trypsins

Lynn S. Mirigian [a], Elena Makareeva [a], Hannu Koistinen [b, c], Outi Itkonen [b, c], Timo Sorsa [d, e], Ulf-Håkan Stenman [b, c], Tuula Salo [f, g, e], Sergey Leikin [a]

https://doi.org/10.1016/j.abb.2013.03.008

Highlights

- We examined collagen degradation by non-sulfated trypsins commonly found in tumors.
- Non-sulfated trypsins-1, -2, and -3 cleave only unfolded regions of

JOURNAL ARTICLE

Cysteine Cathepsins in Human Dentin-Pul Complex

Tersariol I, Geraldeli S, Minciotti C et al. See more

Journal of Endodontics (2010) 36(3) 475-481

DOI: 10.1016/j.joen.2009.12.034

Abstract

Introduction: Collagen-degrading matrix metalloproteinases (MMPs) are expressed by odontoblasts and present in dentin. We hypothesized that odontoblasts express other collagen-degrading enzymes such as cysteine cathepsins, and their activity would be present in dentin, because odontoblasts are known to express at least cathepsin D. Effect of transforming growth factor beta (TGF-β) on cathepsin expression was also analyzed. Methods: Human odontoblasts and pulp tissue were cultured with and without TGF-β, and cathepsin gene expression was analyzed with DNA microarrays. Dentin cathepsin and MMP activities were analyzed by degradation of respective specific fluorogenic substrates. Results: Both odontoblasts and pulp tissue demonstrated a wide range of cysteine cathepsin expression that gave minor responses to TGF-β. Cathepsin and MMP activities were observed in all dentin

JOURNAL ARTICLE OPEN ACCESS

The collagenolytic activity of cathepsin K is unique among mammalian proteinases

Garnero P, Borel O, Byrjalsen I et al. See more

Journal of Biological Chemistry (1999) 273(48) 32347-32352

DOI: 10.1074/jbc.273.48.32347

Abstract

Type I collagen fibers account for 90% of the organic matrix of bone. The degradation of this collagen is a major event during bone resorption, but its mechanism is unknown. A series of data obtained in biological models strongly suggests that the recently discovered cysteine proteinase cathepsin K plays a key role in bone resorption. Little is known, however, about the actual action of cathepsin K on type I collagen. Here, we show that the activity of cathepsin K alone is sufficient to dissolve completely insoluble collagen of adult human cortical bone. We found that the collagenolytic activity of cathepsin K is directed both outside the helical region of the molecule, i.e. the typical activity of cysteine proteinases, and at various sites inside the helical region, hitherto believed to resist all mammalian proteinases but the collagenases of the matrix metalloproteinase family and the neutrophil elastase. This property of cathepsin K

ARTICLE OPEN ACCESS

Limitations in bonding to dentin and experimental strategies to prevent bond degradation

Liu Y, Tjäderhane L, Breschi L et al. See more

Journal of Dental Research

DOI: 10.1177/0022034510391799

Abstract

The limited durability of resin-dentin bonds severely compromises the lifetime of tooth-colored restorations. Bond degradation occurs via hydrolysis of suboptimally polymerized hydrophilic resin components and degradation of water-rich, resin-sparse collagen matrices by matrix metalloproteinases (MMPs) and cysteine cathepsins. This review examined data generated over the past three years on five experimental strategies developed by different research groups for extending the longevity of resin-dentin bonds. They include: (1) increasing the degree of conversion and esterase resistance of hydrophilic adhesives; (2) the use of broad-spectrum inhibitors of collagenolytic enzymes, including novel inhibitor

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Reactivation of inactivated endogenous proteolytic activities in phosphoric acid-etched dentine by etch-and-rinse adhesives

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Received 16 November 2005; accepted 31 January 2006

Abstract

Auto-degradation of collagen matrices occurs in resin-infiltrated dentine by the slow action of host-derived matrix metalloproteinases. As phosphoric acid-etching inactivates these endogenous enzymes, it is puzzling how hybrid layers created by simplified etch-and-rinse adhesives can degrade in vivo. This study tested the null hypothesis that there are no differences in the relative proteolytic activities of mineralised dentine, acid-etched dentine, and etch-and-rinse adhesivetreated acid-etched dentine. Powdered dentine prepared from extracted human teeth was treated with 17% EDTA, 10% phosphoric acid, or with five simplified etch-and-rinse adhesives that were applied to 10% phosphoric acid-etched dentine. The gelatinolytic activity of the dentine powder was assayed using fluorescein-labelled gelatine. TEM examination of the air-dried, treated dentine powder was performed to confirm the presence of remnant mineralised dentine after acid-etching. 17% EDTA significantly reduced the relative proteolytic activity (73.2%) of the untreated mineralised dentine powder (control), while 10% phosphoric acid-etched dentine exhibited the highest reduction (98.1%). Treating the acid-etched dentine powder with any of the five simplified etch-and-rinse adhesives resulted in the reactivation of the proteolytic activity, with a significant negative linear correlation ($P<0.05$) between the increases in fluorescence and the corresponding pH values of the adhesives. It is concluded that simplified etch-and-rinse adhesives can reactivate endogenous enzymatic activities in dentine that are previously inactivated by phosphoric acid-etching. The amount of enzyme reactivated may even exceed the original quantity present in untreated mineralised dentine. This provides an explanation for the degradation of hybrid layers after acid-etched dentine matrices are infiltrated with these adhesives.

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Adhesive sealing of dentin surfaces *in vitro*: A review

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Abstract

Purpose—The purpose of this review is to describe the evolution of the use of dental adhesives to form a tight seal of freshly prepared dentin to protect the pulp from bacterial products, during the time between crown preparation and final cementum of full crowns. The evolution of these "immediate dentin sealants" follows the evolution of dental adhesives, in general. That is, they began with multiple-step, etch-and-rinse adhesives, and then switched to the use of simplified adhesives.

Methods—Literature was reviewed for evidence that bacteria or bacterial products diffusing across dentin can irritate pulpal tissues before and after smear layer removal. Smear layers can be solubilized by plaque organisms within 7–10 days if they are directly exposed to oral fluids. It is

JOURNAL ARTICLE OPEN ACCESS

Activation of matrix-bound endogenous proteases by self-etch adhesives

Oguz Ahmet B, Seseogullari-Dirihan R, Tezvergil-Mutluay A

Dental Materials Journal (2020) 39(6) 1044-1049

DOI: 10.4012/dmj.2019-304

Abstract

The study evaluated changes in total enzymatic activity and degradation of demineralized dentin following the application of universal or self-etch adhesives. The universal adhesives — Scotchbond Universal (SU) and All-Bond Universal (ABU) and self-etch adhesives —Adper Easy Bond (EB) and G-aenial Bond (GB) were used for 2 min pretreatment of the dentin beams. Phosphoric acid (PA) treatment as well as no treatment served as controls. Total enzymatic activity was analyzed before and after treatment, collagen degradation was assessed using mass loss, C-terminal telopeptide (CTX) and C-terminal-telopeptide of type I collagen (ICTP) release (24 h, 3-day, 3-week). Over three weeks of incubation, ICTP release of ABU treated beams was significantly higher than other groups ($p<0.05$), except for SU treated beams ($p>0.05$) and CTX release of GB treated beams was the highest among the groups with

NOTE

Inhibition of the Activities of Matrix Metalloproteinases 2, 8, and 9 by Chlorhexidine

Renée Gendron, Daniel Grenier, Timo Sorsa, Denis Mayrand

DOI: 10.1128/CDLI.6.3.437-439.1999

ABSTRACT

Matrix metalloproteinases (MMPs) are a host cell-derived proteolytic enzyme family which plays a major role in tissue-destructive inflammatory diseases such as periodontitis. The aim of the present study was to evaluate the inhibitory effect of chlorhexidine (CHX) on MMP-2 (gelatinase A), MMP-9 (gelatinase B), and MMP-8 (collagenase 2) activity. Heat-denatured type I collagen (gelatin) was incubated with pure human MMP-2 or -9 activated with *p*-aminophenylmercuric acetate (APMA), and the proteolytic degradation of gelatin was monitored by sodium dodecyl

JOURNAL ARTICLE

Chlorhexidine inhibits the activity of dental cysteine cathepsins

Scaffa P, Vidal C, Barros N et al. See more

Journal of Dental Research (2012) 91(4) 420-425

DOI: 10.1177/0022034511435329

Abstract

The co-expression of MMPs and cysteine cathepsins in the human dentin-pulp complex indicates that both classes of enzymes can contribute to the endogenous proteolytic activity of dentin. Chlorhexidine (CHX) is an efficient inhibitor of MMP activity. This study investigated whether CHX could also inhibit cysteine cathepsins present in dentin. The inhibitory profile of CHX on the activity of dentin-extracted and recombinant cysteine cathepsins (B, K, and L) was monitored in fluorogenic substrates. The rate of substrate hydrolysis was spectrofluorimetrically measured, and inhibitory constants were calculated. Molecular docking was performed to predict the binding affinity between CHX and cysteine cathepsins. The results showed that CHX inhibited the proteolytic activity of dentin-extracted cysteine cathepsins in a dose-dependent manner. The proteolytic activity of human recombinant

JOURNAL ARTICLE

Zinc-doped dentin adhesive for collagen protection at the hybrid layer

Osorio R, Yamauti M, Osorio E et al. See more

European Journal of Oral Sciences (2011) 119(5) 401-410

DOI: 10.1111/j.1600-0722.2011.00853.x

Abstract

The aim of the study was to ascertain whether the addition of zinc to adhesives may decrease metalloproteinase-mediated collagen degradation without affecting bonding efficacy. Human dentin beams were treated with phosphoric acid, with Clearfil SE Bond Primer or with Clearfil SE Bond Primer plus ZnCl 2 (2 wt%). Acid-etched dentin was infiltrated with Single Bond, Single Bond plus ZnCl 2 (2 wt%), or Single Bond plus ZnO nanoparticles (10 wt%), and Clearfil SE Bond-primed dentin was infiltrated with Clearfil SE Bonding resin, Clearfil SE-Bonding resin with ZnCl 2 (2 wt%), or Clearfil SE-Bonding resin with ZnO nanoparticles (10 wt%). The C-terminal telopeptide concentrations were determined 24h, and 1 and 4wk after treatment. Microtensile bond strength to dentin was determined for the tested adhesives. Matrix metalloproteinases-mediated collagen degradation occurred in acid-etched and SE-

JOURNAL ARTICLE PDF AVAILABLE

Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo

Hebling J, Pashley D, Tjäderhane L et al. See more

Journal of Dental Research (2005) 84(8) 741-746

DOI: 10.1177/154405910508400811

Abstract

The recent paradigm that endogenous collagenolytic and gelatinolytic activities derived from acid-etched dentin result in degradation of hybrid layers requires in vivo validation. This study tested the null hypothesis that there is no difference between the degradation of dentin bonded with an etch-and-rinse adhesive and that in conjunction with chlorhexidine, an MMP inhibitor, applied after phosphoric-acid-etching. Contralateral pairs of bonded Class I restorations in primary molars of clinical subjects were retrieved after a six-month period of intra-oral functioning and processed for transmission electron microscopy. Hybrid layers from the chlorhexidine-treated teeth exhibited normal structural integrity of the collagen network. Conversely, abnormal hybrid layers were seen in the control teeth, with progressive disintegration of the fibrillar network, to the extent that it was beyond detection by collagen

JOURNAL ARTICLE PDF AVAILABLE

Chlorhexidine-containing acid conditioner preserves the longevity of resin-dentin bon

Stanislawczuk R, Amaral R, Zander-Grande C et al. See more

Operative Dentistry (2009) 34(4) 481-490

DOI: 10.2341/08-016-L

Abstract

The current study evaluated the effect of 2% chlorhexidine digluconate (CHX) on the immediate and six-month resin-dentin bond strength (BS) and nanoleakage pattern (NL) of etch-and-rinse adhesives when applied in aqueous or associated to the phosphoric acid conditioner. The occlusal enamel of 42 caries-free extracted molars was removed in order to expose a flat dentin surface. In groups 1 and 2 (control-C), the surfaces were acid etched with conventional phosphoric acid, and the adhesives Prime&Bond NT (PB) and Adper Single Bond 2 (SB) were applied after rinsing, drying and rewetting with water. In groups 3 and 4 (Ac/CHX), the adhesives were applied in a similar manner, however, a 2% CHX-containing acid was previously applied. In groups 5 and 6 (CHX), the adhesives were applied according to the control group; however, the rewetting procedure was performed with an aqueous solution of

JOURNAL ARTICLE OPEN ACCESS

Review of Matrix Metalloproteinases' Effect or the Hybrid Dentin Bond Layer Stability and Chlorhexidine Clinical Use to Prevent Bond Failure

Moon P, Weaver J, Brooks C

The Open Dentistry Journal (2010) 4(1) 147-152

DOI: 10.2174/1874210601004010147

Abstract

This review describes the relationship between dentin collagen hybrid bond layer degradation and the Matrix Metalloproteinases (MMPs) after their release by acid etch and rinse adhesives and self etching bonding adhesives that can reduce the bond stability over time. MMP-2, MMP-8 and MMP-9 are indicated as the active proteases that breakdown the collagen fibrils in the hybrid bond layer. Phosphoric acid in the acid etch and rinse bonding process and acid primers in the self etch process are implicated in the release of these proteases and their

Delivery Systems | Published: 30 November 2019

The use of clays for chlorhexidine controlled release as a new perspective for longer durability of dentin adhesion

Lívia Rodrigues de Menezes, Emerson Oliveira da Silva, Lizandra Viana Maurat da Rocha, Isabel Ferreira Barbosa & Marina Rodrigues Tavares

Journal of Materials Science: Materials in Medicine **30**, Article number: 132 (2019) | Cite this article

303 Accesses | **2** Citations | Metrics

An Author Correction to this article was published on 05 March 2020

This article has been updated

Abstract

The adhesive systems have the function to establish the connection between the restorative material and dental tissue, therefore it is of fundamental importance, because failures in the adhesive interface can reduce the life of a dental restoration. This study investigated the possibility of using the adhesive layer as a chlorhexidine modified release system evaluating their impact on the properties of these systems as well as evaluating the impact of these systems on immediate and post-aging dentin adhesion. Were used a matrix with BisGMA,

CONFERENCE PROCEEDINGS OPEN ACCESS

The inhibitory effects of quaternary ammon methacrylates on soluble and matrix-bound MMPs

Tezvergil-Mutluay A, Agee K, Uchiyama T et al. See more

Journal of Dental Research (2011) 90(4) 535-540

DOI: 10.1177/0022034510389472

Abstract

Matrix metalloproteinases (MMPs) bound to dentin contribute to the progressive degradation of collagen fibrils in hybrid layers created by dentin adhesives. This study evaluated the MMP-inhibiting potential of quaternary ammonium methacrylates (QAMs), with soluble rhMMP-9 and a matrix-bound endogenous MMP model. Six different QAMs were initially screened by a rhMMP-9 colorimetric assay. For the matrix-bound endogenous MMPs, we aged demineralized dentin beams for 30 days in calcium- and zinc-containing media (CM; control), chlorhexidine, or QAMs in CM to determine the changes in dry mass loss and solubilization of collagen peptides against baseline levels. The inhibitory effects of QAMs on soluble

ELSEVIER

Journal of Dentistry
Volume 60, May 2017, Pages 77-86

Effect of a novel quaternary ammonium silane cavity disinfectant on durability of resin–dentine bond

D. Daood [a], C.K.Y. Yiu [a], M.F. Burrow [b], L.-N. Niu [c], F.R. Tay [d]

Show more

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https://doi.org/10.1016/j.jdent.2017.03.003

Abstract

Objective

The present study examined the effect of a quaternary ammonium silane (QAS)

Effect of a novel quaternary ammonium sila on dentin protease activities

Umer D, Yiu C, Burrow M et al. See more

Journal of Dentistry (2017) 58 19-27

DOI: 10.1016/j.jdent.2017.01.001

Abstract

Objectives Demineralized dentin collagen release C-terminal cross-linked telopeptide (ICTP) and C-terminal peptide (CTX) during degradation. The present study evaluated the effects of dentin pre-treatment with K21, a quaternary ammonium silane (QAS), on matrix metalloproteinase (MMP) and cathepsin K-mediated collagen degradation. Methods Dentin beams were demineralized with 10% H3PO4 for 24 h. After baseline dry mass measurements, the beams were divided into 5 groups (N = 10) according to protease inhibitors. The beams were pre-treated for 2 min with 2% chlorhexidine (CHX), 2%, 5% or 10% QAS; no pre-treatment was performed for the control group. The beams were subsequently incubated in calcium- and zinc-containing medium for 3, 7 or 14 days, after which changes in dry mass were measured and incubation media were examined for ICTP and CTX release. The MMP-2

ELSEVIER

Journal of Dentistry
Volume 85, June 2019, Pages 25-32

Effect of benzalkonium chloride on dentin bond strength and endogenous enzymatic activity

Allegra Comba [a], Tatjana Maravic [a], Lucrezia Valente [a], Margherita Girlando [a], Sandra R Cunha [b], Vittorio Checchi [a], Stefano Salgarello [c], Franklin R Tay [d], Nicola Scotti [e], Lorenzo Breschi [a], Annalisa Mazzoni [a]

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https://doi.org/10.1016/j.jdent.2019.04.008

Abstract

Objective: This *in vitro* study evaluated at baseline (T0) and over time (T12 months), the effect of a multi-mode universal adhesive compared with two experimental formulations blended with different concentrations of benzalkonium chloride (BAC), on bond strength and endogenous enzymatic activity.

Methods and materials: Specimens were assigned to the following groups according to the adhesive protocol: G1) All-Bond Universal (ABU) self-etch (SE); G2) ABU + 0.5% BAC SE; G3) ABU + 1% methacrylate BAC SE; G4) ABU etch-and-rinse (E&R);

Journal of Dentistry
Volume 39, Issue 1, January 2011, Pages 57-64

The anti-MMP activity of benzalkonium chloride

Arzu Tezvergil-Mutluay [a], M. Murat Mutluay [a], Li-sha Gu [b], Kai Zhang [b], Kelli A. Agee [c], Ricardo M. Carvalho [d], Adriana Manso [e], Marcela Carrilho [f, g], Franklin R. Tay [h], Lorenzo Breschi [i, j], Byoung-In Suh [e], David H. Pashley [c]

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https://doi.org/10.1016/j.jdent.2010.10.003

Abstract

Objective

This study evaluated the ability of benzalkonium chloride (BAC) to bind to dentine and to inhibit soluble recombinant MMPs and bound dentine matrix metalloproteinases (MMPs).

original research article

THE EFFECTS OF HOST DERIVED METALLOPROTEINASES ON DENTIN BOND AND THE ROLE OF MMPS INHIBITORS ON DENTIN MATRIX DEGRADATION

M. LONGHI, L. CERRONI, S.G. CONDÒ, V. ARIANO, G. PASQUANTONIO

Department of Clinical Science and Translational Medicine, University of Rome "Tor Vergata", Rome, Italy

SUMMARY

Objectives. The work has the objective to analyze the literature on the degradation of the adhesive interface. In particular the study is focused on the role of the metalloproteinase in the hydrolytic degradation of collagen matrix in the bonded interface. The survey will concern also the latest innovations to improve and increase the link between dentin and the restorative materials through the MMPs inhibitors.

Methods. The research has been carried out in the MEDLINE database by choosing keywords as "metalloproteinases" and "dentin bond" and "degradation". *In vitro* studies were included in the research, excluding studies with no human and deciduous teeth. Language was limited to English.

Results. The collagenolytic enzymes in mineralized dentin have been demonstrated to have an important role in dental hard tissue pathologies, including the degradation of the hybrid layer.

Conclusion. The preservation of the collagen matrix integrity is a key issue in the attempts to improve the dentin bonding durability.

Key words: metalloproteinases, dentin bond, MMPs inhibitors.

Introduction

Most of the studies carried out in adhesive dentistry in recent years have aimed to simplify the clinical procedures (1).

The modern adhesive techniques provide for a reduction in the number of steps during the application of the adhesive: these allow, in addition to simplification, a reduction of working time and make the process less operator dependent (2).

ability and degradation of metalloproteinases (MMPs). All these factors contribute to the degeneration of the hybrid layer and the failure of the restoration (1, 2).

After dentin bonding with resins collagen fibrils are exposed at the bottom of the hybrid layer owing to imperfect resin impregnation of the demineralized dentin matrix. Exposed collagen fibrils might be affected by MMPs inducing hydrolytic degradation, which might result in reduced bond strength (3).

Tetracyclines Inhibit Connective Tissue Breakdown: New Therapeutic Implications for an Old Family of Drugs

Lorne M. Golub, N.S. Ramamurthy, Thomas F. McNamara, , , , more... Show all authors

First Published July 1, 1991 | Other | Find in PubMed

https://doi.org/10.1177/10454411910020030201

Article information

Abstract

Tetracyclines have long been considered useful adjuncts in peridontal therapy based on their antimicrobial efficacy against putative periodontopathogens. However, recently these drugs were found to inhibit mammalian collagenases and several other matrix metalloproteinases (MMPs) by a mechanism independent of their antimicrobial activity. Evidence is presented that this property may be therapeutically useful in retarding pathologic connective tissue breakdown, including bone resorption. The experiments leading to this discovery are described and possible mechanisms are addressed, including the specificity of tetracyclines' anti-collagenase activity, the role of the drugs' metal ion (Zn2+, Ca^{2+})- binding capacity, and the site on the tetracycline molecule responsible for this nonantimicrobial property. Of extreme interest, the tetracycline molecule has been chemically

ELSEVIER

Acta Biomaterialia

Volume 6, Issue 10, October 2010, Pages 4136-4142

The inhibitory effect of polyvinylphosphonic acid on functional matrix metalloproteinase activities in human demineralized dentin

Arzu Tezvergil-Mutluay [a, b], Kelli A. Agee [b], Tomohiro Hoshika [c], Franklin R. Tay [b, d], David H. Pashley [b]

Show more

https://doi.org/10.1016/j.actbio.2010.05.017

Abstract

This study has examined the use of polyvinylphosphonic acid (PVPA) as a potential matrix metalloproteinase (MMP) inhibitor and how brief cross-linking of demineralized dentin matrix that did not affect its mechanical properties enhanced the anti-MMP activity of PVPA. The anti-MMP potential of five PVPA

JOURNAL ARTICLE

Changes in stiffness of demineralized dentin following application of collagen crosslinkers

Bedran-Russo A, Pashley D, Agee K et al. See more

Journal of Biomedical Materials Research - Part B Applied Biomaterials (2008) 86(2) 330-334

DOI: 10.1002/jbm.b.31022

Abstract

It is thought that increasing the strength of the dentin matrix using crosslinking agents may improve both the strength and the durability of resin-dentin bonds. The purpose of this study was to evaluate the effect of two collagen crosslinking agents (glutaraldehyde, GD and grape seed extract, GSE) on the modulus of elasticity of demineralized dentin. Sound molar fragments were fully demineralized and divided into five groups according to the type and concentration of crosslinking agents: 2.5% GD; 5% GD, 25% GD; 0.65% GSE; 6.5% GSE. Specimens were immersed in their respective solution and tested at baseline, 10 min, 30 min, 1 h, 2 h, 4 h. The elastic modulus of dentin was significantly affected by the treatment ($p < 0.01$) and exposure time ($p < 0.01$). There was a statistically significant interaction between the two factors evaluated (treatment vs. time $p < 0.01$). Mean baselines values varied between 4.8

JOURNAL ARTICLE PDF AVAILABLE

Inhibition of endogenous human dentin M by Gluma

Sabatini C, Scheffel D, Scheffel R et al. See more

Dental Materials (2014) 30(7) 752-758

DOI: 10.1016/j.dental.2014.04.006

Abstract

Objective The objective of this study was to determine if Gluma dentin desensitizer (5.0% glutaraldehyde and 35% HEMA in water) can inhibit the endogenous MMPs of dentin matrices in 60 s and to evaluate its effect on dentin matrix stiffness and dry mass weight. Methods Dentin beams of 2 mm × 1 mm × 6 mm were obtained from extracted human third molars coronal dentin. To measure the influence of Gluma treatment time on total MMP activity of dentin, beams were dipped in 37% phosphoric acid (PA) for 15 s and rinsed in water. The acid-etched beams were then dipped in Gluma for 5, 15, 30 or 60 s, rinsed in water and incubated into SensoLyte generic MMP substrate (AnaSpec, Inc.) for 60 min. Controls were dipped in water for 60 s. Additional beams of 1 mm × 1 mm × 6 mm were completely demineralized in 37% PA for 18 h, rinsed and used to evaluate changes on the dry weight and

JOURNAL ARTICLE PDF AVAILABLE

Stabilization of dentin matrix after cross-linking treatments, in vitro

Scheffel D, Hebling J, Scheffel R et al. See more

Dental Materials (2014) 30(2) 227-233

DOI: 10.1016/j.dental.2013.11.007

Abstract

Objectives To evaluate the effect of EDC on elastic modulus (E), MMPs activity, hydroxyproline (HYP) release and thermal denaturation temperature of demineralized dentin collagen. Methods Dentin beams were obtained from human molars and completely demineralized in 10 wt% H3PO4 for 18 h. The initial E and MMP activity were determined with three-point bending and microcolorimetric assay, respectively. Extra demineralized beams were dehydrated and the initial dry mass (DM) was determined. All the beams were distributed into groups (n = 10) and treated for 30 s or 60 s with: water, 0.5 M, 1 M or 2 M EDC or 10% glutaraldehyde (GA). After treatment, the new E and MMP activity were redetermined. The beams submitted to DM measurements were storage for 1 week in artificial saliva, after that the mass loss and HYP release were evaluated. The collagen thermal denaturation

Dental Materials
Volume 32, Issue 3, March 2016, Pages 423-432

Use of crosslinkers to inactivate dentin MMPs

R. Seseogullari-Dirihan [a], F. Apollonio [b], A. Mazzoni [c], L. Tjaderhane [d], D. Pashley [e], L. Breschi [c], A. Tezvergil-Mutluay [f, g]

https://doi.org/10.1016/j.dental.2015.12.012

Abstract

Objectives

This study evaluated the endogenous matrix metalloproteinase (MMP) activity of demineralized dentin matrix following 1 or 5 min pretreatment by various collagen crosslinkers. Generic MMP activity assay, total protein analysis, *in situ* zymography, gelatin zymography and multiplex bead technology were used to evaluate matrix-

JOURNAL ARTICLE

Riboflavin as a dentin crosslinking agent: Ultraviolet A versus blue light

Fawzy A, Nitisusanta L, Iqbal K et al. See more

Dental Materials (2012) 28(12) 1284-1291

DOI: 10.1016/j.dental.2012.09.009

Abstract

Objectives: To investigate the effect of photo-activation of riboflavin either by ultraviolet (UVA) or visible blue light (BL) on the biodegradation resistance, strength of demineralized dentin matrix, bond strength to dentin and resin/dentin interface morphology. Methods: Dentin beams were demineralized, treated with 0.1% or 1% riboflavin solution for 5 min and photo-activated with UVA or BL for 20 s. The ultimate tensile strength (UTS) and hydroxyproline (HYP) release were assessed after 24 h collagenase challenge. For micro-tensile bond strength (μTBS) testing and resin/dentin interface morphology investigation, dentin was acid-etched, crosslinked with riboflavin and bonded with an etch-and-rinse adhesive system. Riboflavin was photo-activated separately with UVA or BL followed by photo-polymerization of the bonding resin with BL (two-step) or both riboflavin photo-activation and bonding resin photo-

JOURNAL ARTICLE

Characterization of riboflavin-modified dentin collagen matrix

Fawzy A, Nitisusanta L, Iqbal K et al. See more

Journal of Dental Research (2012) 91(11) 1049-1054

DOI: 10.1177/0022034512459053

Abstract

Crosslinking is considered a possible approach to increasing the mechanical and structural stability and biodegradation resistance of the dentin collagen matrix. The aim of this study was to investigate the mechanical and chemical variations and collagen degradation resistance associated with crosslinking of the dentin collagen matrix with UVA-activated riboflavin. Dentin collagen matrix specimens were treated with 0.1 and 1% riboflavin for 2 min and photo-activated with 7 mW/cm2 UVA (368 nm) for 2 min. The structural change of the dentin collagen network with collagenase exposure was investigated by AFM and SEM at different time-points. The variations in surface/bulk mechanical properties and biodegradation resistance were characterized by nano-indentation, conventional mechanical testing, and hydroxyproline liberation at different time-points. Chemical changes associated with