

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

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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 activation by several non-collagen proteins also released from dentin by the etching. MMPs are released in saliva by salivary glands, by cells in the gingival crevices to crevicular fluid and by pulpal odontoblasts cells to the dentinal fluids. These sources may affect the hybrid layer also. Evidence of the bond strength deterioration over time and the ability of Chlorhexidine to prevent bond deterioration by inhibiting MMP action are discussed. Dentin Bonding procedure utilizing Chlorhexidine for different application times and concentrations are being developed. The application of 2% Chlorhexidine to the phosphoric acid etch surface after rinsing off the acid is the only procedure that has been clinically tested for a longer period of time and shown to prevent bond strength degradation so far. The adoption of this procedure is recommended as means of improving bond stability at this time.

Keywords: Matrix Metalloproteinases, Collagen, hybrid bond layer, bond strength, Chlorhexidine.

INTRODUCTION

Early dentin bonding agents were very prone to failure. As evident by many generations of dentin adhesives that have been developed over the years, perfection has yet to be achieved. Bonds still fail. Recent research has suggested that matrix metalloproteinases released from the dentin by acids during bonding may contribute to eventual bond failure. Reviews have explored the important roles matrix metalloproteinases (MMPs) play in general and dental health [1-3]. We will discuss these roles but focus on recent research utilizing chlorhexidine (CHX) which is a clinically relevant proteinase inhibitor. We will discuss the evidence that it can stabilize the dentin hybrid bond strength of bonded composite resin restorations to dentin and recommend procedures for use in dentin bonding.

BACKGROUND ON MMPS

Matrix Metallo Proteinases (MMPs) are a cell-derived proteolytic enzyme family with 26 identified members [1]. Specific enzymes of this family can function beneficially during tissue remodeling and during formation of the extra cellular matrix or the mineralization of dentin [4].

However, MMPs can act during inflammation to increase the adverse effects of cardiovascular disease [5], cancer metastasis [6], periodontal disease [7] and the carious process

[8] by destruction of the collagen and other proteins of the extra cellular matrix. Normally, the protein cleavage activity of MMPs is balanced in time and spatially by cell secreted inhibitors called Tissue Inhibitors of Metalloproteinases (TIMPs). If the balance is disturbed inflammation, arthritis, cancer and heart problems become manifest [2].

The relationship of active MMPs to the carious process provides a background to recent research on the effects of MMPs on the dentin hybrid layer bond stability [6, 8]. A variety of MMPs have been identified in the carious lesions including MMP-2 (gelatinase), MMP-8 (collagenase), MMP-9 (gelatinase) and MMP-20 (enamelysin) [1, 2]. The dentin protein matrix is composed of 90 % collagen (primarily type I) and 10% non-collagen proteins. The collagen proteins can be clipped in to pieces by MMP-8 and further degraded by MMP-2 and MMP-9 after acid demineralization of dentin in the carious lesion. These MMPs may be present in the saliva, pulp or sequestered in dentin to be released in the local environment during the caries destruction of dentin [1, 2, 9]. The MMPs are located throughout the dentin but appear to be located intensively along the enamel-dentin junction and in the predentin [10]. Increased MMPs presence along the dentin-enamel junction may contribute to the widening of caries along this junction as it progresses into the dentin.

These MMPs are secreted from connective tissue cells (fibroblasts, osteoblasts, and odontoblasts) as zymogens, a pro or inactive enzyme form, which does not cleave extra cellular proteins. The pro-MMPs are trapped or bound in the dentin during its formation [11]. They can be catalyzed to active enzymes after being released from the dentin by low-

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ering the pH to 4.5 or below as in carious lesions [2]. The pro-MMPs after release by the acid can be activated by interactions with specific extracellular matrix proteins also released from the dentin or present in saliva. They will remain active even if the pH is neutralized. The release and increased activation of MMPs can also occur during acid treatment of dentin powders with lactic and citric acids but phosphoric acid greatly decreased activation [12, 13]. For example, a 37% phosphoric acid gel decreased collagenolytic activity by 65% compared to the effect of no gel on mineralized dentin powder [13]. However, application of five etch and rinse dentin bonding acidic resins (Prime and Bond, Optibond Solo Plus, Excite, Single Bond and One Step) raised collagenolytic activities 45% to 260% over the activity of the control non treated dentin powder. In addition, self etching adhesive resin (acidic resins) created a 14 fold increase in MMP enzyme activity when applied to dentin powders [14]. These results suggest that organic acidic molecules play a particularly important role in activating pro MMPs to active proteinases. The lactic acid released by bacteria in a carious lesion has been implicated as an activator of pro MMPs that break down collagen matrix during the caries process [15]. This evidence suggests that organic acids released from plaque or present in the diet could contribute to weakening of the hybrid bond in time. Another possible source of MMP attack on the hybrid layer was found recently when self etching resins placed on prepared dentin produced secretion of MMPs from odontoblasts at the pulp dentin interface. These MMPs may gain access to the hybrid layer via the dentinal tubules [16].

Ten percent of the non-collagen proteins of the dentin matrix proteins consist of three proteins that can bind to specific MMPs. These binding proteins may activate the MMPs when they are released by the acid attack in caries or potentially during acid etching for bonding [2].

These activating proteins are part of the Small Integrin Binding Ligand N-linked Glycoproteins (SIBLING) gene family [17]. The three binding proteins are Bone Sialoprotein (BSP), Osteopontin (OPN) and Dentin Matrix Protein1 (DMP1). The MMPs and binding proteins pair specifically together in the following groups for activation: MMP-2 with BSP, MMP-3 with OPN and MMP-9 with DMP1.

The activation of pro-MMPs to active forms is complex and dynamic for which several mechanisms have been suggested [18]. The N-terminus of these MMP enzymes are thought to be folded over to block the active catalyst site so Zn or Ca ions can not bind to activate MMPs [19]. This N-terminus part of the molecule can be cleaved off the pro-MMP by another enzyme to form an active MMP that can bind Zn and Ca ions from the extra cellular environment [20].

Another mechanism that is similar to the SIBLING binding to activate MMPs is that of the binding of another molecule to an MMP which causes the N-terminus end to be displaced so that Zn or Ca binding site is no longer blocked [21]. A combination of these first two mechanisms may act in concert where another molecule binds to change the molecular configuration to partially activate the MMP or displace the N-terminus allowing the reaction of water with the active site which enhances the removal of the N-terminus by

protease enzymes [22]. On the other hand, CHX is thought to chelate to the zinc or calcium ions on the active site to inhibit the MMPs that had been activated by a mechanisms such as those discussed [23]. If the concentration of zinc ions is high as can occur by release of this ion from zinc containing dental materials, the MMPs can be inhibited [24]. The additional zinc ions may bind to lower affinity sites on the MMPs changing its molecular configuration so it is no longer active or by zinc hydroxide bridge to block the active site [25, 26].

It is of particular interest that application of acid resins of dentin bonding agents after the activation is depleted by the phosphoric acid etchant in the etch and rinse bonding agents more than restores the MMP activity in the dentin. Furthermore, the more acidic the bonding resin, the higher the restored activity of the MMPs [13, 14]. Also, the acidic self etching bonding resins restored activation of the MMPs of dentin powder when used in a manner simulating bonding to dentin. In addition, activated MMPs may find their way to the hybrid bond layer interface via microleakage or nano-leakage at bond gaps, from MMPs in saliva, crevicular fluid and via dental tubule fluid from the pulp over time [2, 27, 28].

THE LINK BETWEEN MMPs AND DENTIN HYBRID BOND STABILITY

With this background above, it is concluded that MMPs can be released by acids (inorganic and organic) and activated by SIBLING proteins and organic acids in the oral environment or in the bonding adhesives. If collagen fibrils are left exposed in the hybrid bond layer unprotected by resin, they can be degraded by the activated MMPs. The reports of nano leakage between the etch and rinse or self etch dentin bonding resins and dentin suggest fibril exposure occurs in clinical placement of resin bonded restorations [29-31]. With time, bond degradation may show up as a loss of retention clinically or a decrease in bond strength with *in vitro* testing. A clinical review of different types of dentin bonding agents showed that for Class V composite restorations that the majority of bonding agents declined in retention by 20% in five years or less [32]. In addition, if the composite shrinkage stress is sufficient, an interfacial gap can form that may lead to tooth sensitivity and marginal staining. These problems may influence a dentist to replace a composite restoration. Replacement is more likely to occur if the dentist did not place the restoration and thus does not know its history [33]. Marginal chipping can also lead to partial or complete restoration replacement [34]. These causes of failure may be influenced by degradation in the bond strength with time.

Microtensile bond strength tests have been performed on clinically placed bonded composites that were orally aged in function and then extracted for orthodontic treatment and tested. Their bond tests demonstrated significant bond strength loss of 36% to 70% at one year and at fourteen months with different bonding agents [35, 36]. Similar significant degradation in bond strength of 40% was measured for dentin bonded composite in extracted teeth, water aged *in vitro*, at a shorter time, 270 days [37]. The degradation of the hybrid bond layer was confirmed by electron transmission microscopy comparison of the hybrid layer for the standard etch and rinse only but not for those treated with 2% chlor-

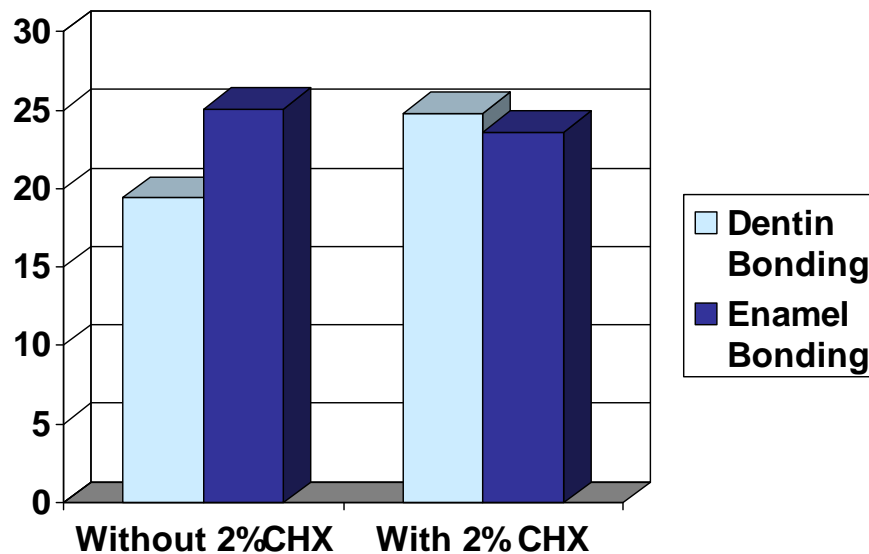


Fig. (1). Chart of shear bond strengths 24 hours after bonding of OptiBond Solo Plus to enamel and dentin with and without the application of 2% chlorhexidine to bond Filtek Z250 composite to extracted teeth.

hexidine (CHX) after acid etching, water rinsing and drying before bond resin application [38].

Chlorhexidine had been used primarily as a disinfectant for cavity preparations and oral irrigation in dentistry. There have been reports which indicate that chlorhexidine application to the acid etched surfaces of enamel and dentin does not decrease bond strength *in vitro* of composite resin in the short term (24 hours) [39-46]. Also, the early bond strength to caries affected dentin *in vitro* is reported to be unchanged by application of 2% chlorhexidine after acid etching with phosphoric acid. The caries affected dentin produced similarly lower bond strengths with or without the application of chlorhexidine [47-49].

We initially measured an increase in shear bond strength of 24 % with 2% CHX applied to etch and rinse dentin after water rinsing [45]. The composite cylinder bond was formed against a recently extracted molar tooth (n=10) whose dentin was prepared flat by a diamond saw, treated with and without 2% Chlorhexidine after acid etching. The bonding procedure to dentin used Scotch Bond (3M ESPE St. Paul, MN, USA) phosphoric acid etchant for 15 sec., rinsing 15 sec. and drying to moist dentin before applying the 2% Chlorhexidine (Consepsis, Ultradent Inc., South Jordan, UT, USA) for one minute with redrying to a moist dentin surface. Next, two coats of Optibond Bond Solo Plus were applied with 5 seconds of air drying after each before being visible light cured (VLC) for twenty seconds to which a 2mm high cylinder of Filtek Z250 (3M ESPE St. Paul, MN, USA) was placed using an Ultradent bonding jig. The Filtek Z250 was visible light cured (VLC) for forty seconds. The VLC were performed at 400 mw/cm² with an Optilux Model VCL 401 curing light (Kerr Inc., Danbury Ct, USA). The average shear-bond strength to dentin increased from 19.4 ± 1.4 MPa for the control without chlorhexidine to 24.1 ± 3.1 MPa with 2% Chlorhexidine after storage in water at 100°f for 24 hours as listed in Fig. (1). Retesting this procedure but with 10 seconds of air pressure drying after application each layer raised the bond strength at 24 hours to 29.6 ± 4.6 MPa

without CHX and to 30.0 ± 4.1 MPa with CHX. We now use clinically 2% Chlorhexidine with OptiBond Solo Plus for bonded direct composite restorations as of the Fall of 2007 in Virginia Commonwealth University Dental School Clinic. We started using Optibond Solo Plus in 2005 as it was available in unidose which we judged from experience would make student bonding procedures more effective and efficient.

A variety of etch and rinse dentin bonding resins have been tested with chlorhexidine without reporting a short term detrimental affect on bond strength [39-46]. Other bonding materials and procedures may work equally well with chlorhexidine. It is advisable to test at least their 24 hour bond strength *in vitro* to determine CHX does no harm immediately and to follow with extended time tests to show their longer term benefit as we are in the process of doing. It is expected that many bonding agents, procedure modifications and MMP inhibitors will be tested in the search for more efficient ways to stabilize the hybrid bond against MMPs.

Of the several variations in procedures so far reported, one showed that the 24 hour bond strength of a self etch bonding agent was decreased by approximately 20% if the 2% CHX was applied to the dentin before self etch resin. But it was not affected by the addition of up to 1% CHX when added to Clearfil SE Bond (Kuraray America Inc., New York, NY, 10022) resin [50,51]. Further research on the long term effect of adding CHX to Clearfil SE bonding resin at concentrations of 0.1%, 0.5% or 1% proved at one year *in vitro* to protect the bond against degradation but 0.05% did not [52]. It should be recognized that MMPs from saliva, odontoblasts via dentinal tubules and from crevicular fluid are not present during *in vitro* bond conditioning in water baths. This suggests that there may be a need for a higher CHX concentration *in vivo* to prevent bond degradation clinically. However, one product, Hemaseal & Cide with 4% CHX, (Advantage Dental Products Inc., Lake Orion, MI, 48361) that is described as a desensitizer and disinfectant agent suggests it can be used for self-etch as

well as with etch and rinse adhesive systems. Research using chlorhexidine reported by Christensen Research Associates as a disinfectant did not weaken the 24 hour bond for five dentin bonding agents [53]. Of those tested, two were self-etch and three were etch and rinse systems. Molecularly, chlorhexidine can be chemisorbed on the hydroxyapatite of the tooth or react as an ion to form an insoluble compound with phosphate ions in plaque, saliva and hydroxyapatite. It may be released later without apparently interfering with bonding to enamel or dentin at the concentrations tested [54].

The clinical application of 2% chlorhexidine for one minute to the etched dentin after rinsing off the acid and before applying the dentin bonding primer and resin is able to stop significant *in vivo* degradation of bond strength from MMPs for at least 14 months [36-38]. Chlorhexidine was applied after rinsing off the acid with water and air drying the dentin to just a moist surface. This method is the only one proven clinically, is easy to adopt and will likely be first to gain wider acceptance initially. However research is expected to develop simpler and more efficient methods of applying CHX or other MMP inhibitors. For example, recently it has been demonstrated for six months *in vitro* that addition of 2% CHX to a conventional 37% phosphoric acid prevented degradation of the bond strength of an etch and rinse bonding system [55]. The influence of concentration and application time of CHX have been evaluated to show that times less than one minute and concentrations less than 2% may prevent degradation *in vitro* [56]. These results are yet to be verified *in vivo*. For resins that might be used in a self etch bonding resin systems, research has shown that CHX influences the degree of cure and the elastic modulus on curing. These results suggest that the success of adding CHX to self etch systems to prevent hybrid bond degradation will vary with the resin used in the commercial products [57]. *In vitro* application of concentrations of greater than 0.12% CHX to dentine before applying the primer is contraindicated as it can reduce the 24 hour bond strength [58]. The use of 0.12% CHX mouth wash to rub on the preparation might seem to be the most immediate way to treat self etch dentin bonds.

More research is needed to show that treatment does not weaken the 24 hour bond and works for a long time *in vitro* and *in vivo* for the variety of self etching agents available. Moreover, *in vitro* tests simulating pulp pressure and thermo-mechanical action exerted on the self etch adhesive dentin bond to which the CHX was applied prior to the adhesive showed loss of bond strength at 6 months. But the loss was much less with the 2% CHX compared to 0.2% CHX and no CHX [59].

It is hoped that knowledge to improve bonding procedures for stability of self etch systems will evolve from further research so bond stability of these products will improve.

RECOMMENDED CLINICAL APPLICATION PROCEDURE

With the best *in vitro* and limited clinical research evidence verifying no immediate bond weakening and improve long term stability with the use of etch and rinse dentin bonding adhesives, we suggest the clinical use with 2%

chlorhexidine at this point of time. The reported *in vivo* test were performed with Single Bond (3M) using 2% Chlorhexidine digluconate solution (CHX) by Proderma, Peracica SP, Brazil.

Based on convenience and what was available at our clinics, we selected the 2% chlorhexidine solution, Consepsis (Ultradent, Inc., South Jordan, UT 84095), applied it for one minute after etching with 37% phosphoric acid for 15 seconds, water rinsing thoroughly and drying to moist surface before applying CHX. The CHX treated surface is again dried to a moist surface followed by the resin application of adhesive resin like Optibond Solo Plus (Kerr U.S.A., Orange, CA 92867) which we use. In addition, to enhance the bond strength, we recommend at least two separate coats of the bonding agent resins applied without pooling of the resins in the prep [29]. Also, air pressure drying is required to evaporate the solvent out of the bonding resins for 10 seconds between coats and after the last coat to insure thorough light curing. Better solvent evaporation increases the degree of cure and prevents excess water absorption by water displacement of the remaining solvent. This increases bond strength, decreases nano leakage, hydrolysis of the resin and MMPs attack on the collagen of the hybrid layer with time [60]. These procedures are design to minimize sensitivity, marginal staining, or chipping and improve retention of direct bonded composite restorations.

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