

# How to Increase the Durability of Resin-Dentin Bonds

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## ABSTRACT

Resin-dentin bonds are not as durable as was previously thought. Microtensile bond strengths often fall 30% to 40% in 6 to 12 months. The cause of this poor durability is a combination of the activation of matrix metalloproteinases (MMPs) by weak acids such as lactic acid released by caries-producing bacteria, and acid-etchants used in adhesive bonding systems. These acids uncover and activate matrix-bound MMPs. The other contributing factor is incomplete resin infiltration. If all exposed collagen fibrils were enveloped by resin, the MMPs would not have free access to water, an obligatory requirement of these enzymes. Recently, several inhibitors of MMPs have been added to adhesive primers. Examples include chlorhexidine (CHX), benzalkonium chloride (BAC), and MDPB, an antibacterial monomer used in a two-step self-etching primer adhesive. The advantage of MDPB over CHX and BAC is that it polymerizes with adhesive resins and cannot leach from the hybrid layer. This is an example of what can be termed a "therapeutic adhesive system" that provides anti-MMP activity along with antibacterial qualities.

Although the immediate resin-dentin bond strengths of contemporary adhesives are quite high, these values gradually fall with aging,<sup>1-3</sup> decreasing 30% to 40% in 6 to 12 months. Strategies are, therefore, needed to increase the longevity of resin-dentin bonds. Bond durability is vital for the longevity of esthetic restorations because as adhesive strength falls, gaps form between teeth and restorative materials. The average service length for tooth-colored restorations is only 5.7 years.<sup>4</sup> Replacements of these defective restorations cost about \$5 billion annually in the United States alone.<sup>5</sup> The development of new, more durable bonding systems would potentially save patients and governments a great deal of money. This article examines the problems that cause poor durability and explores possible solutions.

## HISTORICAL

Historically, adhesion of resins to enamel or dentin has been accomplished via hybrid layer formation.<sup>6</sup> That is, the hard tissues are acid-etched to remove smear layers and increase their permeability, and then infiltrated with resin to create hybrid layers. In dentin hybrid layers, collagen fibrils are the only continuous structure between underlying mineralized dentin and the overlying adhesive layer. Early investigations on the durability of resin-dentin bonds demonstrated that their bond strengths decreased over time.<sup>7,8</sup>



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However, the mechanism of the decrease in bond strengths was not known until Armstrong et al<sup>9</sup> published transmission electron micrographs (TEMs) of etch-and-rinse resin-dentin hybrid layers after nearly 4 years of water storage. Those TEMs showed that the cause of decreases in bond strength was degradation of the collagen fibrils in dentin hybrid layers.

#### DEGRADATION DUE TO HOST COLLAGENOUS

That same year, Pashley et al<sup>10</sup> revealed that acid-etched dentin matrices spontaneously degraded over time in vitro when incubated in aqueous buffer, but not if the buffer contained protease inhibitors or 0.2% chlorhexidine (CHX), a known inhibitor of matrix metalloproteinases (MMPs)<sup>11</sup>, or were incubated in oil (ie, in the absence of water). Those authors concluded that the degradation of collagen fibrils in vitro was due to the presence of activated endogenous MMPs that are neutral hydrolases. That is, the enzymes add water across specific peptide bonds to cleave them at neutral pH. The loss of collagen fibrils within the hybrid layer causes a loss of continuity with underlying dentin and a weakening of the coupling of resin composites to dentin.

#### NONPOLYMERIZABLE MMP INHIBITORS

Rapid research progress followed showing that CHX stabilized hybrid layers in vitro<sup>12,13</sup> and in vivo.<sup>14-17</sup> However, although CHX binds to demineralized dentin electrostatically,<sup>18</sup> there is no covalent bonding. It is likely that MMP inhibitors that do not covalently bond with collagen or resins may leach from hybrid layers over the course of 1 to 2 years and will only delay but not stop collagen degradation.<sup>19</sup>

Self-etching adhesives are very hydrophilic because they must contain sufficient water (approximately 35% to 40%) to ionize the acidic monomers used for

self-etching.<sup>20</sup> Self-etching adhesives may absorb less water than two-step etch-and-rinse adhesives,<sup>21,23</sup> but they still absorb significant amounts of water over time. Some of this water is taken up into adhesives where the water weakens the polymers. Water also reaches the MMPs bound to collagen where it can hydrolyze collagen peptides.

The mild self-etching adhesives usually remove the smear layer but leave smear plugs in the tubules.<sup>24-26</sup> This tends to prevent convective and osmotic water movement from dentinal tubules into the bonded interface.<sup>27</sup> However, most self-etching adhesives etch slightly deeper than they infiltrate,<sup>28,29</sup> just like etch-and-rinse adhesives. Because the hybrid

Fig 1.

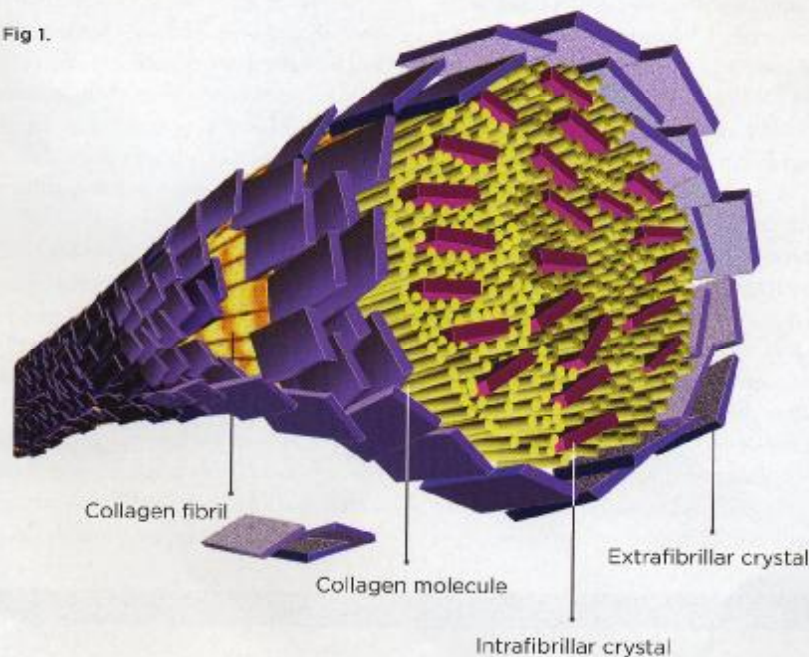


Fig 2.

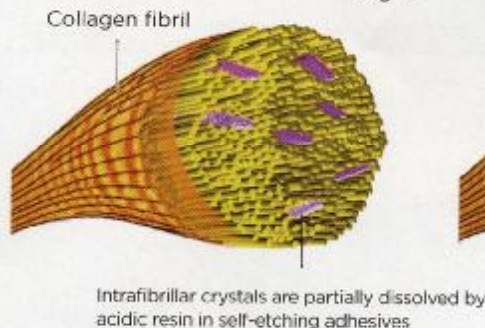
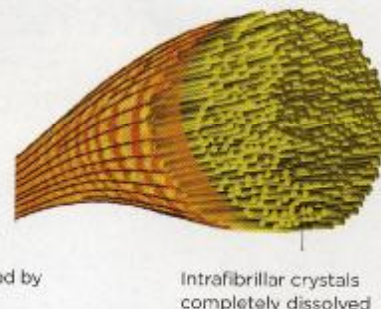


Fig 3.



**Fig 1 through Fig 3.** Schematic of mineralized dentin collagen fibrils. (Fig 1) Before acid-etching. Note two different types of apatite crystals: extrafibrillar and intrafibrillar. (Fig 2) After etching dentin with self-etching adhesives, all of the extrafibrillar apatite fibrils have been removed to provide channels for infiltration of adhesive monomers and space for micromechanical retention. Note the residual presence of intrafibrillar crystals. (Fig 3) After acid-etching with 37% phosphoric acid, all of the apatite crystallites have been solubilized and extracted from dentin, leaving the collagen matrix floating in water.

layers created by mild to moderately aggressive self-etching adhesives are only about 1- $\mu$ m thick, the resin infiltration is more homogeneous than in hybrid layers created by etch-and-rinse adhesives.

The hybrid layers of self-etching adhesives degrade just like those of etch-and-rinse adhesives,<sup>29,30</sup> but it is difficult to demonstrate this even using transmission electron microscopy because the layers are so thin. This is because self-etching adhesives generally have a pH between 1.6 to 2.9 and cannot etch very deeply into dentin. This pH is sufficiently low to demineralize dentin and uncover MMPs and activate them without denaturing them.<sup>31,32</sup>

Schematic drawings of mineralized dentin collagen fibrils are shown in Figure 1 through Figure 3. Prior to acid-etching, the MMPs bound to the collagen matrix are covered with apatite crystallites; about half of the crystals are outside the collagen fibrils and are called "extrafibrillar" crystallites, and the other half of the crystallites are located inside the collagen fibrils and are termed "intrafibrillar" (Figure 1). In this mineralized state, the MMPs are fossilized and inactive. When mineralized

dentin is etched with mild self-etching adhesives, most of the extrafibrillar crystals are removed to provide space for resin infiltration (Figure 2). These acidic self-etching adhesives also remove some of the intrafibrillar crystallites, thereby uncovering MMP enzymes and activating them, allowing them to slowly attack the very collagen fibrils that are used to anchor resin composites to teeth.

Acid-etching dentin with 32% to 37% phosphoric acid removes both extra- and intrafibrillar crystallites (Figure 3), thereby uncovering even more matrix-bound MMPs and activating them, allowing them to slowly attack the hybrid layer, especially in regions that are poorly infiltrated with resin.

Nonspecific MMP inhibitors like CHX have been added to two-step self-etching primer adhesives<sup>33,34</sup> and have prevented the usual degradation in bond strengths seen in CHX-free controls. Careful transmission electron microscopy of dentin bonded with mild self-etching primer adhesives showed that a significant amount of residual apatite crystallites remain in the hybrid layer.<sup>35</sup> Indeed, nanoleakage

studies reveal no silver uptake into collagen fibrils (unlike moderate silver uptake in collagen treated with etch-and-rinse adhesive systems), suggesting that self-etching primers may leave intrafibrillar apatite crystallites in place (Figure 2). The authors speculate that this retention of apatite crystallites prevents water permeation into the internal compartments of collagen fibrils where it could accelerate MMP-induced hydrolysis of collagen over time. The bond strengths of most self-etching adhesives decrease over time; this is presumably due to water uptake into extrafibrillar spaces, facilitating MMP hydrolysis of collagen fibrils from the outside only. This is in contrast to the presence of water in both extra- and intrafibrillar collagen compartments, which facilitates even more rapid collagen hydrolysis in etch-and-rinse adhesives (Figure 3).

#### BONDING TO BACTERIALLY CONTAMINATED DENTIN

Most direct esthetic restorations are placed in carious teeth. Whether due to primary or secondary caries, the bacteria-infected dentin must be removed without sacrificing normal dentin. This is not always possible. For instance, as the clinician excavates caries-infected dentin toward a pulp horn, a decision needs to be made; overly aggressive excavation of residual caries-infected dentin may not only cause a pulp exposure but also inadvertently result in forcing chips of bacterially infected dentin into the pulp chamber, requiring expensive endodontic treatment. What is needed is an antibacterial adhesive that would kill bacteria on contact. The pH of self-etching adhesive monomers ranges from 1.6 to 2.9. This is acidic enough to kill many bacteria. However, the pH of these adhesives rapidly rises as soon as it contacts dentin due to the strong buffer capacity of dentin.<sup>36,37</sup> Thus, clinicians cannot rely on low pH to kill all residual bacteria.

In the mid-1990s, Imazato and his colleagues<sup>38-40</sup> synthesized an antibacterial

TABLE 1

#### Effects of Quaternary Ammonium Methacrylates on Soluble and Matrix-Bound MMPs

Inhibitor	rhMMP-9	Matrix-Bound MMPs
5 wt% MDPB	89 $\pm$ 3%	97 $\pm$ 3%
30 wt% METMAC	97 $\pm$ 2%	98 $\pm$ 1%
24 wt% MAPTAC	34 $\pm$ 2%	99 $\pm$ 1%
30 wt% MCMS	100 $\pm$ 2%	96 $\pm$ 3%
30% ATA	101 $\pm$ 6%	94 $\pm$ 2%
30% DDAC	55 $\pm$ 3%	94 $\pm$ 3%

Values are mean  $\pm$  SD inhibition of soluble, recombinant human MMP-9 (rhMMP-9) or the total endogenous MMPs in demineralized dentin beams incubated in the presence of inhibitors for 30 days. Data from Tezvergil-Mutluay et al.<sup>42</sup>

MDPB = 12-methacryloyloxydodecylpyridinium bromide; METMAC = methacryloyloxyethyltrimethyl ammonium chloride; MAPTAC = [3-(methacryloylamino)propyl]trimethylammonium chloride; MCMS = methacryloylcholine methyl sulfate; ATA = 2-acryloyloxyethyl-trimethyl ammonium chloride; DDAC = diallyldimethyl ammonium chloride

analog of a self-etching monomer from Kuraray Medical Inc., 10-methacryloyloxydecylmethacrylate phosphoric acid (MDP), by substituting an antibacterial pyridinium group for the terminal phosphate group. The resulting antibacterial monomer, called 12-methacryloyloxydodecyl-pyridinium bromide, is usually identified by its abbreviation, MDPB. The company incorporated 5 wt% MDPB into its Clearfil™ SE Bond self-etching primer adhesive to create a new product, Clearfil™ Protect Bond. An updated version of that product is currently called Clearfil™ SE Protect. The antibacterial monomer MDPB is incorporated into the self-etching primer, not the adhesive. The product has been shown to have strong bacteriocidal activity while a liquid; as long as it is in liquid form it can diffuse into dentin. After photopolymerization, it copolymerizes with the adhesive, forming an antibacterial polymer that kills any bacteria that contact it.<sup>38-41</sup>

The antimicrobial properties of MDPB reside in its terminal pyridinium group. This group is a member of a broad class of antimicrobial agents termed *quaternary ammonium compounds* such as benzalkonium chloride (BAC).

#### USE OF POLYMERIZABLE INHIBITORS OF DENTIN MMPs

The authors screened a number of quaternary ammonium compounds, including BAC, for their ability to inhibit endogenous MMPs in dentin as well as for their antimicrobial activity.<sup>42,43</sup> These matrix-bound MMPs are responsible for the degradation of hybrid layers over time. If they could be inhibited by anti-MMP compounds that contain polymerizable acrylate or methacrylate groups, they might remain in hybrid layers for many years. If their anti-MMP activity compounds remain effective for years, then the durability of the hybrid layers or resin-dentin bonds would likely be extended, thereby making resin-bonded composites more durable over time.

In a recent paper, the authors screened the anti-MMP activity of MDPB along with a number of other quaternary ammonium methacrylates.<sup>43</sup> Among the quaternary ammonium methacrylates tested, 5 wt% MDPB showed the highest inhibition of soluble recombinant human MMP-9 (rhMMP-9) and matrix-bound MMPs compared to all others tested (Table 1). Thus, in addition to its antimicrobial activity, MDPB also has potent anti-MMP activity. This is highly desirable because the caries process involves demineralization of the dentin matrix by bacterially derived organic acids. These acids lower the pH of the matrix to around 4.5 to 5, which can both uncover and activate MMPs.<sup>44</sup> Once activated, the MMPs can destroy the exposed collagen and deepen the lesion. The presence of MDPB in the self-etching primer of Clearfil SE Protect should inhibit the endogenous MMPs of dentin and prevent further progression of the lesion; however, this assumption is speculative, and future experiments must be designed to confirm these potential actions.

#### HOW DENTIN MMPs ARE ACTIVATED

The dentin matrix contains MMPs-2, -8 and -9.<sup>45-52</sup> These host-derived proteases contribute to the breakdown of collagen matrices in dental caries.<sup>44,53,54</sup>

The acid-etching step in bonding is thought to uncover and activate pro-MMPs trapped within mineralized dentin.<sup>10</sup> However, 37% phosphoric acid is highly acidic (pH 0.4 to -1) and may denature exposed MMPs due to their low acidity. The acidic resin components of etch-and-rinse adhesives<sup>55</sup> and self-etch adhesives<sup>38,39</sup> have

been shown to increase the gelatinolytic and collagenolytic activities of completely or partially demineralized dentin. A self-etching adhesive was also shown to increase the synthesis of MMP-2 in human odontoblasts,<sup>56</sup> which may secrete MMP-2 into dentinal fluid and then into hybrid layers. Mildly acidic self-etching monomers seem to activate latent forms of MMPs (pro-MMPs) via the cysteine-switch mechanism that exposes the catalytic site of these enzymes that were blocked by propeptides.<sup>57</sup> These same self-etching monomers may also activate dentin MMPs by displacing tissue inhibitors of metalloproteinases (TIMPs)<sup>58</sup> in MMP-TIMP complexes.<sup>46,59</sup> Solubilization of collagen from the hybrid layer can result in the loss of mechanical properties of the collagen matrix,<sup>42,60</sup> and a loss in resin-dentin bond strength.<sup>15</sup>

Cysteine cathepsins are another class of endopeptidases that participate in intracellular proteolysis with the lysosomal compartments of living cells.<sup>61</sup> However, they are also trapped in the dentin matrix during dentinogenesis but are inactive because they are covered with mineral crystallites.<sup>62,63</sup> During acid-etching, they become uncovered and active and can serve as proteases by cleaving to collagen in hybrid layers. Recent unpublished research has shown that chlorhexidine can inhibit cathepsins as well as it inhibits MMPs. Future studies are planned to determine if MDPB inhibits cathepsins as well as it inhibits MMPs.

#### THERAPEUTIC ADHESIVES

In a recent review of three-step etch-and-rinse adhesives,<sup>37</sup> the authors suggested that such adhesives provide a number of therapeutic opportunities to inhibit bacteria as well as dentin MMPs. Two-step primer adhesives provide similar therapeutic opportunities.

The incorporation of MDPB into the self-etching primer of a self-etching primer/adhesive product as described herein is an example of a so-called “therapeutic



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adhesive." In addition to simply bonding to dentin, this new class of dental adhesives provides specific, value-added therapeutic activity that kills residual bacteria in caries-infected dentin and inhibits any endogenous MMPs that are activated by the caries process or exposed and activated by the self-etching adhesive system. It is the authors' hope that the dental industry will emulate this innovative approach to product development.

## DISCLOSURE

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