A scanning electron microscopic study of the effect of Gluma CPS bonding system on dentinal smear layers produced by different bur types and rotational speeds and on the resin-dentin interface

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Objective: The purpose of this study was to investigate the effect of conditioners on smear layers produced by different bur types and rotational speeds and to study the interaction of subsequently applied primer and sealer with these conditioned surfaces.

Method and materials: Smear layers were produced on human teeth immediately after extraction by burs rotating at approximately 6,000 rpm without water spray and 400,000 rpm with water spray. Gluma CPS etchant was applied for 15, 30, and 60 seconds, and a 20% phosphoric acid liquid (control) was applied for 30 seconds to smear layers. The specimens were prepared by critical point drying for scanning electron microscopic imaging within 24 hours. Additional specimens were prepared and treated with Gluma CPS primer and sealer, according to the manufacturer’s instructions.

Results: There were only small variations in the smear layer thicknesses with different bur types or speeds of rotation. Gluma CPS conditioner, applied for the recommended times, did not completely remove the smear layer. An altered smear layer, composed of a reaction product, remained on the dentinal surface. A zone of demineralization did occur; however, beneath the partially removed smear layer in both the 15- and 30-second specimens but was only partly filled by primer and sealer. Smear layers were completely removed by the 20% phosphoric acid liquid to expose a delicate collagen network.

Conclusion: An understanding of the appropriate treatment for smear layers is crucial to the development of improved dentin bonding systems. (Quintessence Int 1998;29:737–747)

Key words: conditioner, Gluma CPS bonding system, resin-dentin interface, smear layer

Clinical relevance

It has been suggested that the less aggressive action of low-concentration acids, reducing the depth of demineralization, may produce a more complete resin infiltration of collagen. With the Gluma CPS system, partially filled demineralization zones, observed in all specimens prepared according to the manufacturer’s instructions, may contribute to microleakage and bond failure in the clinical situation.

Most contemporary dentin bonding systems advocate a total-etch technique and supply an acidic conditioner to be used on cut dentinal surfaces. Pashley1 listed the principal goals of acid conditioning as (1) the removal of the intrinsically weak smear layer; so permitting direct bonding to the dentinal matrix; (2) the demineralization of the superficial dentinal matrix to permit resin infiltration into the prepared surface zone; (3) the uncovering of both the intertubular and peritubular dentin; and (4) the cleaning of any biofilms from the surface.

In spite of the many recent developments in dentin bonding, the mechanisms and optimization of bonding of different systems remain obscure. For instance, it is now generally considered that the primary bonding mechanism is through the entanglement of hydrophilic monomers with the collagen fibers within the demineralized zone, producing a resin-infiltrated dentin layer or hybrid zone.2,3 However, not all conditioners demineralize to the same extent,4 and similar concentrations of phosphoric acid etchants containing thickeners may result in different demineralization depths as well as a dif-

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different morphology of etched dentin. Some conditioners, especially those containing silica thickeners, leave the dentinal surface "coated" and the tubules mostly closed, and therefore do not meet the goals outlined by Pashley. Moreover, it has been suggested that more dilute acids should be used; this would result in a thinner demineralized zone, the collagen network of which would theoretically be more completely infiltrated by adhesive resin.

The controversy surrounding the nature and consequences of the dentin conditioning required prior to the application of resin primers is compounded by the fact that most of the research has been carried out on extracted teeth in the laboratory. Two in vivo studies1-8 tested three proprietary dentin bonding agents, each representing a different method of conditioning and priming. It was concluded that dentin conditioning, as a separate step prior to priming, is an important factor in successful resin-dentin attachment. Preparations were made with a No. 330 bur in a high-speed handpiece, in contrast to most in vitro studies, in which smear layers are usually produced with silicon carbide paper.

An understanding of the appropriate treatment of the smear layer, and the subsequent morphology of the resin-dentin zone, is critical to the development of dentin bonding agents. Gluma CPS (Heraeus Kulzer) uses a 20% phosphoric acid, silica-containing gel applied to the cut dentinal surface for 15 to 30 seconds (Table 1). This is a weaker acid than is used in many other contemporary systems and, as a silica-containing gel, is unlikely to completely remove the smear layer.

The present investigation had two objectives:

1. To study the effect of the Gluma CPS conditioner, applied for different time periods, to smear layers produced by different burs at various speeds of rotation, with and without water spray, and to study the subsequent morphology of the demineralized zones.

2. To study the morphology of the resin-dentin interface of conditioned dentin, after priming with Gluma CPS primer and the subsequent application of Gluma sealer according to the manufacturer's instructions.

Method and materials

The teeth used in this study were noncarious premolars extracted for orthodontic purposes. Within 30 minutes of extraction, the clinical crowns were cut from the roots and divided along the mesiodistal plane with a high-speed handpiece used with water spray and a medium-grit diamond bur. Enamel was then removed from the occlusal, mesial, and distal aspects of the half crown to produce a block of dentin. A notch was cut on the pulpal aspect of each block to facilitate freeze fracturing.

A smear layer was produced immediately under the dentinoenamel junction on what was originally the buccal or lingual aspect of each tooth. This was done in the following ways:

1. With a dental handpiece used at approximately 400,000 rpm and with water spray, a smear layer was created with each of the following types of bur: (1) tungsten carbide (FG 557; ISO 017007010, Jet, Kerr); (2) diamond (coarse) (B131-018C, Heraeus Kulzer); (3) diamond (extrafine) (pattern 652, HiDi).

2. With a dental handpiece used at approximately 6,000 rpm and without water spray, a smear layer was produced with the following bur: tungsten carbide inverted cone, No. 4 (ISO 018, Ash).

To study the effect of Gluma CPS conditioner applied to smear layers for different durations, 12 specimens were prepared with each bur (total = 48 specimens). Three specimens of each bur type were randomly assigned to groups for the following surface conditioning of the prepared smear layer: (1) conditioning with 20% phosphoric acid gel for 15 seconds (Gluma CPS conditioner); (2) conditioning with 20% phosphoric acid gel for 30 seconds (Gluma CPS conditioner); (3) conditioning with 20% phosphoric acid gel for 60 seconds (Gluma CPS conditioner); or (4) conditioning with 20% phosphoric acid liquid for 30 seconds (control; prepared from AnalaR, analytical reagents, BDH Chemicals).

To study the interaction of conditioned dentinal surfaces with subsequently applied CPS primer and sealer, 10 additional specimens were prepared with a tungsten carbide inverted cone (No. 4) at 6,000 rpm without water spray, and the following treatments were applied to five specimens each: (1) conditioning for 30 seconds (Gluma CPS conditioner) or (2) conditioning for 60 seconds (Gluma CPS conditioner). The conditioned dentin was then treated with Gluma CPS primer and sealer according to the manufacturer's instructions. The sealer was applied to moist, conditioned dentin.

### TABLE 1  Gluma CPS hydrophilic bonding system

<table>
<thead>
<tr>
<th>Component</th>
<th>Composition</th>
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<tbody>
<tr>
<td>Conditioner</td>
<td>Phosphoric acid, 20.0%</td>
</tr>
<tr>
<td>Primer</td>
<td>Hydroxyethyl/methacrylate (36.1%)</td>
</tr>
<tr>
<td></td>
<td>Glutaraldehyde (5.1%)</td>
</tr>
<tr>
<td></td>
<td>Water (58.8%)</td>
</tr>
<tr>
<td>Sealer</td>
<td>Polyfunctional/methacrylic acid esters (100.0%)</td>
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</tbody>
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Batch numbers 036495 and 076741 were used in the present study.
Immediately after treatment, specimens were dropped into liquid nitrogen and fractured by lightly tapping a scalpel placed in the notch, if fracture did not occur spontaneously. The fractured pieces were immersed in absolute alcohol for 2 hours for fixation and dehydration prior to critical point drying. Each critical-point dried specimen was mounted on an aluminum scanning electron microscope (SEM) stub and sputter coated with 15 to 20 nm of gold-palladium in an Emscope SC 500 (Kent) coating unit. Areas of special interest were examined, at appropriate magnification, with a Phillips 505 SEM (operating voltage 30 kv).

**Results**

The freeze-fracture technique and critical point drying allowed detailed SEM examination of the smear layers produced by different cutting procedures, the depth of demineralization following different conditioning times, and the character of the resin-dentin interface following priming and sealing. The plane of fracture was unpredictable.

The SEM examination showed that the appearance of the smear layer morphology varied distinctly with the type of bur used and the speed of rotation (Figs 1a to 1d). There was also considerable variation in the plane of fracture (Figs 2a to 2d). This prevented a statistical analysis of the thickness of the smear layer, although the range was approximately 1 to 3 µm. Smear layers produced by rough diamonds were consistently thicker than those produced by tungsten carbide fissure burs, which in turn were thicker than those produced by fine diamonds. The inverted cone, used at speeds of 6,000 rpm without water spray, consistently produced the thinnest smear layers.

The overall effect of conditioning of the smear layers is illustrated in Figs 3 and 4, which show the results of application of CPS conditioner to thick smear layers, produced by a rough diamond, and thinner smear layers, produced by the inverted cone bur. The application of the 20% Gluma CPS conditioner for 15 seconds did not remove the smear layer produced by any of the burs at either rotational speed. A surface coating was always retained; it had a distinct granular appearance that may be due in part to the silica particles contained by the gel. No truly patent dentinal tubules were observed after 15-second conditioning.

After conditioning for 30 seconds with the Gluma CPS gel, this granular reaction product still covered the entire surface of all specimens. A few patent dentinal tubules were observed in all cases.

It was only after conditioning for 60 seconds, twice the manufacturer’s recommended time, that complete removal of the smear layer was achieved with Gluma CPS gel. The degree of removal of the smear layer varied considerably among specimens and also at different locations within one specimen. However, in all cases, the thinner smear layer produced by the inverted cone bur was always more completely removed than the thicker layer produced by the rough diamond.

In contrast to this, the control etchant completely removed all types of smear layers when applied for 30 seconds. A delicate network of collagenlike fibers, intertubular and intratubular, was exposed.

Figures 5a to 5c illustrate freeze-fractured planes that allowed the depth of demineralization to be assessed. Again, the overall pattern was similar for all types of burs at both rotational speeds, although there was considerable variation in depths of demineralization both within and between specimens. The depth of demineralization was generally greatest in specimens prepared with the inverted cone bur, and for this reason these are used to illustrate the morphology at 30-second and 60-second etch times.

Figure 5a is representative of specimens conditioned for 30 seconds with CPS conditioner and shows the following general features: The orifices of the dentinal tubules were funnel shaped and widened from 3.0 to 5.0 µm by the acidic treatment. There was loss of mineral from the hypermineralized peritubular dentin in the 30-second specimens. Intertubular demineralization also occurred to a depth of 1.0 to 1.5 µm, and collagenlike fibers were exposed.

When Gluma CPS was applied for 60 seconds, this general pattern was repeated, although the depth of demineralization was often in excess of 10.0 µm (Fig 5b). This feature was also observed when the 20% phosphoric acid liquid was applied for 30 seconds (Fig 5c). Aggressive demineralization occurred, with total removal of the smear layer and the stripping of the peritubular dentin because of demineralization of the less mineralized intertubular tissue.

Figure 6 illustrates the surface appearance of dentin conditioned with 20% phosphoric acid liquid for 30 seconds. The collagen network of intratubular and intertubular dentin, from which apatite has been removed, is evident and has not collapsed.

Figures 7a and 7b show representative specimens of smear layers produced by an inverted cone bur used at 6,000 rpm without water and conditioned for 30 seconds and 60 seconds, respectively. The conditioned dentin was then primed and sealed according to the manufacturer’s recommended procedures. Priming was done on moist dentin. Nearly-identical micrographs were obtained when smear layers produced by other burs were conditioned and primed.
Figs 1a to 1d  Distinct surface morphology produced by different bur types. (Original magnification x2300. Bar = 10 μm.)

Fig 1a  Coarse diamond, with water spray, at 400,000 rpm.

Fig 1b  Tungsten carbide fissure, with water spray, at 400,000 rpm.

Fig 1c  Fine diamond, with water spray, at 400,000 rpm.

Fig 1d  Tungsten carbide inverted cone, without water spray, at 6,000 rpm.
Figs 2a to 2d. Freeze-fractured specimens showing variations in the orientation of dentinal tubules resulting from the unpredictability of the plane of fracture, and the smear layer thickness at the top of the picture. (Original magnification, x573. Bar = 0.1 mm.)

Fig 2a. Coarse diamond, with water spray, at 400,000 rpm.

Fig 2b. Tungsten carbide fissure, with water spray, at 400,000 rpm.

Fig 2c. Fine diamond, with water spray, at 400,000 rpm.

Fig 2d. Tungsten carbide inverted cone, without water spray, at 6,000 rpm.
Figs 3a to 3d  Smear layers produced by coarse diamond burs, with water spray, at 400,000 rpm, and conditioned. (Original magnification, x2500. Bar = 10 μm.)

Fig 3a  Gluma CPS conditioner for 15 seconds and washed for 60 seconds.

Fig 3b  Gluma CPS conditioner for 30 seconds and washed for 60 seconds.

Fig 3c  Gluma CPS conditioner for 60 seconds and washed for 60 seconds.

Fig 3d  Twenty percent phosphoric acid liquid for 30 seconds and washed for 60 seconds.
Figs 4a to 4d Smear layers produced by a tungsten carbide inverted cone, without water spray, at 6,000 rpm, and conditioned. (Original magnification, x2500. Bar = 10 μm.)

**Fig 4a** Gluma CPS conditioner for 15 seconds and washed for 60 seconds.

**Fig 4b** Gluma CPS conditioner for 30 seconds and washed for 60 seconds.

**Fig 4c** Gluma CPS conditioner for 60 seconds and washed for 60 seconds.

**Fig 4d** Twenty percent phosphoric acid liquid for 30 seconds and washed for 60 seconds.
Figs 5a to 5c  Freeze-fractured planes showing a lateral view of the fractured dentin with smear layers evident on the cut (top) surface. The smear layers were produced by a tungsten carbide inverted cone, without water spray, at 6,000 rpm, and conditioned. (Original magnification, x2980. Bar = 10 \( \mu \)m.)

Fig 5a  Gluma CPS conditioner for 30 seconds and washed for 60 seconds.

Fig 5b  Gluma CPS conditioner for 60 seconds and washed for 60 seconds.

Fig 5c  Twenty percent phosphoric acid liquid for 30 seconds and washed for 60 seconds.

Fig 6  Dentin smear layer produced by a rough diamond bar, with water spray, at 400,000 rpm, and conditioned for 30 seconds with 20% phosphoric acid liquid and washed for 60 seconds. (Original magnification, x5200. Bar = 10 \( \mu \)m.)

The surface was covered with a dense layer, approximately 2.0 \( \mu \)m thick. There was a gap-free relationship between the resin and the demineralized dentin, but the important feature was the demineralized zone, 1.5 to 2.5 \( \mu \)m deep, which was not completely filled by the sealer. Tags were frequently observed but did not adhere to the walls of the dentinal tubules. The partially unfilled demineralized zone tended to be shallowest beneath smear layers produced by rough diamonds, but this feature was evident in all specimens.

Figure 8a shows dentin etched for 60 seconds with Gluma CPS conditioner, illustrating the structures emerging from within the dentinal tubules. Figure 8b shows the conditioned, primed, and sealed surface with the resin flowing around these structures and into the tubules.

Discussion

The specimens used in this study were prepared by the method described by Titley et al. Critical point drying preserves the morphology of the collagen in the demineralized zone because the method produces a state of equilibrium between gases in the critical point drying chamber and liquids in the specimen. Gross distortion, produced when surface tension forces are created, is thus avoided. Figure 6 shows that the delicate collagen fibers in the demineralized zone have been preserved in an uncollapsed state, attesting to the efficacy of the method.

Laboratory studies on dentin bonding do not replicate physiologic reality, and few studies have been conducted in vivo in humans. In the present study, it was
Figs 7a and 7b Specimens produced by a tungsten carbide inverted cone No. 4, without water spray, at 6,000 rpm. The specimens have been freeze fractured to give a lateral view of the primed and sealed dentin (top), the zone of demineralization, and the infiltration of primer and sealer into the conditioned dentin and dentinal tubules.

Fig 7a Etched for 30 seconds with Gluma CPS conditioner and washed for 60 seconds. Primer applied to moist dentin and sealed according to the manufacturer's instructions.

Fig 7b Etched for 60 seconds with Gluma CPS conditioner and washed for 60 seconds. Primer applied to moist dentin and sealed according to the manufacturer's instructions.

Fig 8a Dentin smear layer produced by a tungsten carbide inverted cone No. 4, without water spray, at 6,000 rpm, and conditioned for 60 seconds with CPS conditioner. A constant feature was the emergence from the dentinal tubules of frayed rope-like structures. (Original magnification, x8800. Bar = 10 μm.)

Fig 8b Dentin smear layers produced as described in Fig 8a and primed (moist dentin) and sealed according to the manufacturer's instructions. Resin is seen to flow around the structures and into the tubules, creating craterlike indentations (arrows). (Original magnification, x655. Bar = 0.1 mm.)

considered inappropriate to subject patients to cavity preparation prior to extraction. However, specimens were prepared and conditioned within minutes of extraction and were processed for SEM imaging within 24 hours. In other studies, teeth were stored for up to 12 months prior to use. How much the process of storage in formalin, thymol, or other media affects the hydration of tooth structure, alters the apatite structure, or causes degradation of collagen is largely unknown. Second, in the current study, the smear layers were produced by burs in clinical handpieces and not by saws or grinding wheels, as in the above-cited studies. The present study, therefore, more closely mimicked clinical procedures and conditions, although the presence of dentinal fluid under physiologic pulpal pressure could affect the conditioning, priming, and sealing of dentin.

The methodology of the present study does not lend itself to quantifying the various zone thicknesses or subjecting these to statistical analysis because only one plane of fracture is produced with each specimen and the fracture plane is unpredictable. The fracture plane was not always perpendicular to the smear surface (see Figs 2a to 2d). In accordance with similar studies, approximate values are given for descriptive purposes. The results agree with previous studies, which showed that the depth of smear layers depended on the type of bur, the speed of rotation, and whether the cutting was accompanied by water spray or not and that coarse dia-
monds with water spray produced thicker layers than carbide fissure burs.\textsuperscript{12,13} The depth of the smear layers produced in the present study also agreed with the figures cited by Brännström.\textsuperscript{14}

Previous studies have shown that acid etching of dentin with silica-thickened gel leaves a layer of denatured collagen and residual smear on the dentinal surface, even after vigorous washing, preventing the collagen network from being completely exposed.\textsuperscript{9} This view is supported by the present study, which showed that Gluma CPS conditioner does not remove smear layers when applied according to the specifications of the manufacturer, for 15 or 30 seconds. After it was rinsed with water, the surface remained covered with a granular coating, which contained silica particles left by the gel. No patent dentinal tubules were detected, and no exposed collagen fibers could be seen at 15 seconds. At 30 seconds, a few patent tubules were evidenced, but the majority of the surface remained occluded by the granular coating. Patent dentinal tubules were only observed after conditioning for 60 seconds and washing, although even this did not completely remove the residual coating.

The manufacturer's promotion material\textsuperscript{15} shows SEM of a dentinal surface free of smear layer and with patent dentinal tubules. This is used in support of the claim that Gluma CPS conditioner provides ideal bonding by dissolving the smear layer, by superficial demineralization of dentin, and by exposing dentin collagen to secure adhesion of the resin restoration. The article cited by the manufacturers\textsuperscript{16} concluded that an experimental etching agent containing 20% phosphoric acid gel with 5% silicon dioxide as a thickening compound can be used with a 30-second etch time to produce a frosty appearance of enamel without adversely affecting retention of dental materials. Furthermore, it was claimed that the "acid conditioner is probably a suitable compromise regarding the gel's function as a dentin demineralizing uni-etch conditioning agent," although this was not addressed in the aforementioned study. The extent to which manufacturers rely on other research is unknown, but it is incumbent on manufacturers to investigate every aspect of all materials prior to marketing.

Figure 5a shows a demineralized zone of approximately 1.5 \(\mu m\), created beneath the altered smear layer, and exposed collagen fibers. Viewed longitudinally, dissolution of the highly mineralized peritubular dentin is apparent. The distinctive funnel appearance, which tapers for approximately 3.0 to 6.0 \(\mu m\) to normal peritubular dentin, is evident.

Van Meerheek et al\textsuperscript{17} stated that the tubular orifices are reduced or partially occluded by the collapse of the intertubular collagen network. Although some collagen collapse cannot be ruled out, a distinctive collagen network is clearly visible in Fig 5a, and it is the altered smear layer that occludes the orifices. This layer is a "reaction" product of the original smear layer and the silica-containing phosphoric acid gel, composed of fragments of collagen fibers, broken up by the cutting instruments; inorganic salts derived from the apatite crystals; and silica particles.

The present study does not address how this reaction product is implicated in the bonding process or how bond strengths are affected, if at all, although it has been claimed that the presence of silica particles does not interfere with bonding of resin to tooth structure.\textsuperscript{18} In contrast to the CPS gel, 20% phosphoric acid liquid, applied for 30 seconds, removed the smear layer when rinsed with water and exposed a delicate, collagen network. Present theory is that bonding principally takes place by the formation of a hybrid zone created by the infiltration of unfilled resins into such a network. With the retention of a reaction product, resins must diffuse through and incorporate such a product as well as infiltrate the collagen fibers.

Bond strengths of Gluma CPS, applied with the moist dentin bonding technique, are approximately 18 MPa.\textsuperscript{19} Finger and Uno\textsuperscript{19} stated that the high bond strengths are achieved when the smear layer, as well as apatite, is removed to expose an open collagen network. Yet they stated that, when the wet technique was used, "the bonding resin has obviously completely penetrated the conditioned dentin surface," suggesting that they realize that the smear layer was not completely removed. This suggests that, although satisfactory bond strengths may be obtained, the mechanisms of bonding are far from fully understood. This reinforces Pashley's opinion\textsuperscript{10} that careful SEM examination of dentin treated with different conditioners will provide new insight into the mechanisms of bonding of resins to dentin.

A hybrid zone was created when Gluma CPS primer and sealer were applied to dentin conditioned for 30 seconds. A demineralized zone could always be observed beneath this and was usually less than 0.5 \(\mu m\) but could be as deep at 5.0 \(\mu m\) (see Figs 7a and 7b). This unfilled demineralized zone was best observed in the 60-second specimens and corroborates previous reports.\textsuperscript{15,15} It also substantiates the view that the failure of unfilled resins to fully infiltrate a demineralized zone indicates a possible weakness in currently used dentin bonding systems as a possible route of microleakage and failure of function of the subsequently placed restoration.

Structures emerging from the dentinal tubules were consistently observed at high magnification (see Fig 8a).
The sealer flowed around them and into the tubules, leaving a craterlike structure (see Fig 8b). These may be remnants of odontoblastic processes, although no odontoblastic processes were observed in the unprimed, unsealed sections. Although their precise source remains obscure, they do constitute a possible weakness in the bonding system and a route for ingress of oral fluids to the underlying unfilled, demineralized dentin, which could lead to bond failure.

Gluma CPS conditioner, when applied for times recommended by the manufacturers, did not completely remove smear layers produced by burs used at 6,000 rpm without water spray or at 400,000 rpm with water spray. However, the zone of demineralization produced by the 20% silica-thickened gel when applied for times recommended by the manufacturers was much shallower than that produced by an application for 60 seconds, in which the sealer more completely infiltrated the produced demineralized collagen fibers. The actual clinical significance has not been determined, but hypothetically, the elimination of a partially filled demineralized zone may eliminate microleakage and so reduce the incidence of pulpal inflammation and recurrent caries.

Conclusion

The study shows that an understanding and recognition of appropriate treatment of smear layers is crucial to the development of improved dentin bonding systems.

References