Dental Materials

Biocompatibility of Clearfil Liner Bond 2 and Clearfil AP-X system on nonexposed and exposed primate teeth

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Objective: Recent studies have demonstrated that acid etching of vital dentin and pulpal tissue does not retard pulpal healing, odontoblastoid cell differentiation, or dentinal bridge formation when the pulp is capped with adhesive resins. The purpose of this study was to evaluate the pulpal response in nonexposed and exposed monkey pulps to treatment with the Clearfil Liner Bond 2 and Clearfil AP-X system.

Method and materials: Class V and Class I cavities in nonexposed and exposed pulps were opened at 7 or 27 and 97 days.

Results: There were no differences in pulpal inflammation between the Clearfil Liner Bond 2/Clearfil AP-X specimens and calcium hydroxide controls in either Class V or Class I cavities at the various time periods.

Conclusion: Clearfil Liner Bond 2 and Clearfil AP-X system is not toxic to either nonexposed or exposed pulpal tissues when placed according to manufacturer's directions. (Quintessence Int 1998;29:177-188)

Key words: biocompatibility, Clearfil AP-X, Clearfil Liner Bond 2, pulpal response

Clinical relevance

The results of this study suggest that the correct use of the Clearfil Liner Bond 2 and Clearfil AP-X system will extend the life span of dentinal and pulpal tissues when proper control of hemorrhage is achieved. A 2.5% NaOCl solution is biologically acceptable to enhance wound debridement and hemorrhage control.

A recent survey of the literature associated with cytotoxicity of various dental materials has reported that their general chemical effects will severely diminish, and may even completely inhibit, the healing capacity of the dental pulp. An early in vivo study by Zander and Pejko reported that the low pH of silicate and phosphate cements was primarily responsible for inflammation of the pulp, loss of primary odontoblasts, and eventual necrosis of the deeper pulpal tissues. Schroff reported that pulpal inflammation was greater under silicate cement than under other restorative materials. Lefkowitz et al. later reported that various monomer components of acrylic resins were toxic to primary odontoblasts and the subjacent pulp. Langeland et al. and Stanley et al. reported that pulpal inflammation (ranging from moderate to necrosis) was specifically caused by the low pH (acidity) of the material.

On the other hand, Brännström and Vojinovic demonstrated that microleakage of bacteria at the cavosurface margin is the major cause of pulpal inflammation. Additional studies by Brännström and Nyborg, Hansen and Braun, Dickey et al., Qvist, Mejare et al., Bergenholtz et al., and Cox et al. demonstrated a direct relationship between the presence of bacteria and the onset of pulpal inflammation and necrosis. Direct pulp capping with calcium hydroxide (Ca(OH)₂) has been shown to have not only antimicrobial benefits but also a reported capacity to stimulate deposition of reparative dentin and formation of a dentinal bridge.
Many studies have demonstrated that exposed and inflamed dental pulps will heal to permit formation of a new dentinal bridge directly adjacent to the hard-set Ca(OH)_2 medicament interface with little inflammation. Schroeder\(^1\) suggested that necrosis, created by the chemical effects (high pH) of Ca(OH)_2, causes a slight irritation of the dental pulp, which in turn stimulates pulpal cells to the defense and repair of the exposure. Additionally, Ca(OH)_2 has been reported to stimulate fibroblasts in human cell cultures.\(^1\) However, dissolution of Ca(OH)_2, associated with microleakage in cavities restored with resin composites, reveals that hard-set Ca(OH)_2 medicaments fail to provide an effective long-term (permanent) barrier to bacterial invasion.\(^1\) Additionally, Cox et al.\(^2\) reported that 89% of all dentinal bridges that develop after a hard-set Ca(OH)_2 medicament (Dycal) contain multiple tunnel defects that open to the underlying pulp from the medicament interface. Consequently, they concluded that multiple tunnel defects are morphologic disruptions in the dentinal bridge that fail to provide a permanent barrier or a long-term seal against microleakage.\(^2\)

On the other hand, Rowe\(^3\) and Kozlov and Massler\(^4\) demonstrated that exposed rat pulps are capable of healing and dentinal bridge formation under a wide variety of materials, provided that a biologic seal is maintained to prevent bacterial microleakage. Employing zinc oxide–eugenol to seal pulps under composite restorations, reveals that hard-set Ca(OH)_2 medicaments fail to provide an effective long-term (permanent) barrier to bacterial invasion.\(^4\) Additionally, Cox et al.\(^5\) reported that 89% of all dentinal bridges that develop after a hard-set Ca(OH)_2 medicament (Dycal) contain multiple tunnel defects that open to the underlying pulp from the medicament interface. Consequently, they concluded that multiple tunnel defects are morphologic disruptions in the dentinal bridge that fail to provide a permanent barrier or a long-term seal against microleakage.\(^5\)

In 1982, Nakabayashi\(^6\) introduced the term hybrid layer to describe the morphologic impregnation of vital dentin with resin. Proper adhesive infiltration of acid-etched dentin leads to formation of a hybrid layer. Nakabayashi\(^6\) demonstrated that the resin-impregnated hybrid layer of dentin does not become altered following either hydrochloric acid or sodium hypochlorite (NaOCl) treatment, suggesting that the hybrid layer serves the sealing function of enamel, providing both a continuous morphologic and biologic seal to the entire resin-dentin interface. It is this hybrid layer that is the basic biologic–cohesive component that provides not only immediately increased bond strength and seal against hypersensitivity, but a long-term biologic seal against bacterial microleakage (recurrent caries). Tay et al.\(^7\) reported the projection of resin tags deep into the dentinal tubule complex with the formation of hollow resin sheaths that appear to envelop odontoblastic processes in the tubule complex. A recent histologic study by White et al.\(^8\) demonstrated that placement of Bisco All-Bond 2 primer system (Bisco Dental) on either damp and/or dry vital dentin does not impair pulpal healing in deep Class V cavities. More recently, Cox and Suzuki\(^9\) reported that etching with either organic or inorganic acid systems failed to cause pulpal inflammation.

In 1996, Clearfil Liner Bond 2 and Clearfil AP-X system (Kuraray) came to the US market. Clearfil Liner Bond 2 is a two-step system of LB primer and LB Bond. LB Primer is a low-pH, self-etching primer, in which both the enamel and dentin are conditioned or etched. Akimoto et al.\(^10\) and Latta et al.\(^11\) reported good results in a 1-year clinical study of the Clearfil Liner Bond 2 system; both studies demonstrated that Clearfil adhesive resin system is clinically acceptable.

The purpose of this study was to evaluate the pulpal response in both nonexposed and exposed monkey pulps restored with the Clearfil Liner Bond 2 and Clearfil APX system. An additional purpose was to observe the pulpal response to an application of 2.5% NaOCl on exposed pulps to provide clinically acceptable hemorrhage control before placement of the adhesive system.

Method and materials

Six adult rhesus macaca monkeys were housed in the UAB Animal Resources facility after the 90-day quarantine period. The monkeys were approximately 4 to 6 years old with a complete dentition of 32 teeth, which provided 90 vital teeth for evaluation of the Clearfil Liner Bond 2 and AP-X System for this study.

Teeth were scaled and polished with a rubber cup and prophylaxis paste prior to each of the definitive operative procedures. Each animal was tranquilized with an intramuscular injection of 10 mg/kg of ketamine hydrochloride (100 mg/mL). Deeper sedation for total muscular immobility was maintained with an intramuscular injection of xylazine hydrochloride as needed.

Quadrants of teeth were isolated with sterile gauge and cotton rolls. Designated teeth received a Class V or Class I cavity, through the enamel and into the dentin, with a sterile No. 330 carbide bur (Midwest Dental), used with copious amounts of water spray and adequate flushing, evacuation, and cooling. A new No. 330 bur was employed on every fourth cavity preparation, to ensure cutting efficiency. No local dental anesthesia was used, thus reducing the potential effects of ischemia on local periodontal and pulpal vasculature.
Clearfil Liner Bond 2 and Clearfil AP-X system (Table 1) were compared against a commercially available Ca(OH)$_2$ direct pulp-capping medicament, Life (Kerr), which has been demonstrated to predictably encourage dentinal bridging when placed on exposed or inflamed monkey pulps$^{9,30,31}$ and nonexposed Ca(OH)$_2$ controls.$^{32}$ All materials were placed according to manufacturer's instructions.

Material placement

Nonexposed pulp cavity with Clearfil Liner Bond 2 and AP-X system. Each Class V cavity was prepared at ultrahigh speed with a No. 330 bur and sterile water spray to within 300 µm or less of the pulp. The thickness of remaining dentin was established with the UAB-modified Endocater Apex Locator (Hygienic). This procedure prevented accidental pulpal exposure.

Equal amounts of LB primers A and B were mixed and immediately applied on both the enamel and dentin of the cavity for 30 seconds. This layer was gently air dispersed until the surface appeared slightly glossy. No rinsing was done. The cavity walls received a thin, uniform coat of LB Bond resin, gently air dispersed and light cured for 20 seconds with an Optilux 400 (Demetron Research) light-curing unit. Clearfil AP-X resin composite was then placed to the cavosurface margin and light cured for 40 seconds.

Direct pulp capping with Clearfil Liner-Bond 2 and AP-X system. Each cavity was prepared in vital dentin as previously described, and an exposure three times the diameter of the No. 330 bur was placed directly into the dental pulp with the same bur. All hemorrhage was controlled with a 2.5% solution of NaOCl. A damp cotton pellet with NaOCl was held in place from 20 to 50 seconds to control hemorrhage. It was then removed, and the cavity was lightly rinsed and gently air dispersed.

LB primers A and B were mixed with a disposable plastic brush. The entire cavity and axial floor was covered with the LB Primer. The primer was allowed to flow over the exposed pulp for at least 30 seconds. Air was gently directed to the cavity from an angle to prevent pooling; no rinsing was done. All cavity walls received a thin, uniform coat of LB Bond, which was gently air dispersed and light cured for 20 seconds with the Optilux 400 light-curing unit. The remainder of the cavity was filled to the cavosurface margin with AP-X resin and light cured for 40 seconds.

Calcium hydroxide controls. Eighteen nonexposed and 27 exposed Class V cavities were prepared as described above. The Ca(OH)$_2$: material, Life, was placed on the axial floor or over the exposure and Tytin (Kerr) amalgam alloy was placed to the cavosurface margin according to the manufacturer’s instructions. No cavity varnish was used.

Tissue acquisition

All teeth were collected following left ventricular flushing with 0.9% physiologic saline followed by perfusion with a fast-penetrating glutaraldehyde-phosphate buffered formalin (GTA-PBF) fixative at appropriate time periods. After each tooth was cut from its alveolus, the root apex was removed with a No. 5.57 carbide bur. The tooth was immediately placed in GTA-PBF fixative and postfixied for 24 hours at 4°C. Each tooth was demineralized in 0.5M ethylenediaminetetraacetic acid (pH 7.4), which was changed until the specimens were completely demineralized. Demineralization was confirmed radiographically and usually took 3 months.

Tissues were rinsed in distilled water for 4 hours, placed in 30% ethyl and N-butyl alcohol, dehydrated, and embedded in Paraplast Plus (Sherwood Medical), as described by Cox et al.$^{31}$ Serial sections of 7 µm were cut on a rotary microtome and placed on gelatin-coated slides for staining. Glass microslides were stained with hematoxylin and eosin, Masson trichrome, and McKay bacterial stain according to published procedures.$^{32,33}$

Evaluation criteria

Tissue sections were independently evaluated prior to material identification using criteria described by Cox et al.$^{34}$ Histologic evaluation of all the materials was based on the ranking of each tooth, using the specific criteria in the following categories described by Cox et al.$^{35}$

### Table 1  Adhesive materials and their composition

<table>
<thead>
<tr>
<th>Adhesive resin system</th>
<th>Composition</th>
<th>Batch No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearfil Liner Bond 2</td>
<td>A: Phenyl-P 5-NMSA Bis-GMA Photoinitiator Ethanol</td>
<td>B: HEMA water</td>
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<td>Primer</td>
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<td></td>
</tr>
<tr>
<td>Adhesive</td>
<td>MDP, HEMA Bis-GMA Photoinitiator Microfiller (SiO$_2$)</td>
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<tr>
<td>AP-X resin composite</td>
<td>Bis-GMA, TEGDMA Photoinitiator Barium silicate filler</td>
<td>61111</td>
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</table>

Phenyl-P = 2-methylacryloyloxyethyl phenyl hydrogen phosphate; 5-NMSA = N-methylacryloyl-S-aminosalicilic acid; Bis-GMA = Bisphenol A-diglycidylmethacrylate; HEMA = 2-hydroxyethyl methacrylate; MDP = 10-methylacryloyloxydecyl dihydrogen phosphate; TEGDMA = Triethylene glycol dimethacrylate.
### TABLE 2  Histologic and bacterial staining responses in nonexposed monkey pulps

<table>
<thead>
<tr>
<th>Day</th>
<th>Total teeth</th>
<th>Inflammatory cell response</th>
<th>Soft tissue organization</th>
<th>Reparative dentin</th>
<th>Dentinal bridge formation</th>
<th>Bacterial staining</th>
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<tr>
<td></td>
<td></td>
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<td>2a</td>
<td>2c</td>
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</table>

#### Inflammatory cell response
1 = None, or a few, scattered inflammatory cells present in the pulp beneath the cut tubules of the cavity floor
2a = Acute inflammatory cell lesion predominated by polymorphonuclear leukocytes
2c = Chronic inflammatory cell lesion predominated by mononuclear lymphocytes
3 = Severe inflammatory cell lesion appearing as an abscess or dense infiltrate of polymorphonuclear leukocytes involving one third, or more, of the coronal pulp
4 = Necrotic pulp

#### Soft tissue organization
1 = Normal, or almost normal, tissue morphology below the tubules of the remaining dentin or exposure site and throughout the pulp
2 = Lack of complete tissue morphology below the remaining dentin or exposure site, with deeper pulpal tissue appearing normal
3 = Loss of general pulpal morphology and cellular organization below the exposure site
4 = Necrosis in at least the coronal third of the pulp

#### Reparative dentin deposition
1 = No abnormal or reparative dentin below the cut tubules of the cavity preparation
2 = Small, thin rim of reparative dentin below the cut tubules of the cavity preparation
3 = Large bulk of new reparative dentin below the cut tubules of the cavity preparation

#### Dentinal bridge formation
1 = New bridge tissue directly adjacent to some portion of the restorative material
2 = New dentinal bridge some distance from the material interface
3 = No evidence of any dentinal tissue formation in any of the tissue sections

#### Bacterial staining
1 = Absence of bacterial staining in any section
2 = Positive bacterial staining reaction along the cavity walls
3 = Positive bacterial staining reaction within the cut dentinal tubules of the cavity preparation
4 = Positive bacterial staining reaction within the dental pulp

A correlation between presence of inflammation and bacterial staining was tested using regression analysis. A value of $P < .05$ probability was considered statistically significant.

**Results**

Two teeth with artifacts were excluded from this study. The measurements for reparative dentin and the thickness of the dentinal bridge at midpoint were expressed as mean ± standard deviation.

**Nonexposed pulps**

Clearfil Liner Bond 2 and Clearfil AP-X system. The raw histologic data are shown in Table 2. At 8 days, 17 of 20 pulps showed no pulpal inflammation (Figs 1 and 2). Three teeth demonstrated a grade 2 pulpal response, which was associated with bacterial staining, and two teeth showed a loss of primary odontoblasts below the remaining dentin of the cavity preparation. A small, thin rim of reparative dentin was present in four restored teeth at 8 days ($5.8 ± 1.5 \mu m$). At 27 days, one inflamed pulp (grade 2) was visible, again associated with stained bacteria. Sixteen of 17 pulps showed no pulpal inflammation (Fig 3), and 16 of 17 demonstrated a small rim of reparative dentin ($74.1 ± 26.9 \mu m$). At 97 days, no adverse pulpal response was observed, and 9 of 10 pulps showed large amounts of reparative dentin ($265.0 ± 55.1 \mu m$) below the cut dentinal tubules of the cavity preparation (Fig 4).
Fig 1. An 8-day nonexposed pulp (1622-31-6-U6') capped with the Clearfil Liner Bond 2 and AP-X systems. The stain is hematoxylin and eosin to aid differentiation of cellular detail. The cavity (clear area) is seen on the right with a zone of 175 μm of remaining dentin and underlying pulpal tissue in the mid-lower center of the field. The layer of primary blue-stained odontoblastic cells is seen below the remaining dentin of the cavity floor lining the entire pulp chamber. There is no gross break in the normal continuity of these odontoblasts. A thin rim of reparative dentin is present from normal occlusal trauma and not as a result of cavity preparation. The deeper dental pulp is normal, with no inflammatory cells. (Original magnification x10.) *Indicates monkey, tooth, and section number.

Fig 2. An 8-day nonexposed pulp (1622-31-3-L2). Masson trichrome-stained high-powered magnification of a microslide adjacent to Fig 1. The zone of primary odontoblastic cells is seen below the remaining dentin from the cavity with no break in its continuity. The pulpal response is grade 1. The remaining dentin is approximately 175 μm thick, and there is no reparative dentin deposition below the cavity preparation after this short time period; however, some reparative dentin is present from occlusal forces. (Original magnification x25.)

Fig 3. A 27-day nonexposed pulp (2299-5-2-U3). The stain is hematoxylin and eosin for cellular detail. The darker purple-pink-stained dentin below the cavity on the left is the zone of the remaining dentin (approximately 150 μm thick). A very thin zone of reparative dentin is present. The central view of the entire pulp shows a normal tissue architecture without any disruption of inflammatory pathology throughout its entirety. The pulpal response is grade 1. (Original magnification x25.)

Fig 4. A 97-day nonexposed pulp (8918-26-4-L1). The stain is hematoxylin and eosin (low power). The pulpal response is grade 1. The clear cavity area is seen on the mid-right. The underlying remaining dentin is approximately 300 μm in thickness and with a grade 3 (thick bulk) deposition of reparative dentin below the cavity preparation of approximately 250 μm in thickness. The central pulp is normal without the presence of any inflammation. (Original magnification x10.)
**Ca(OH)₂ control.** Nonexposed Ca(OH)₂ controls showed no pulpal inflammation or necrosis (Table 2). No reparative dentin was present at 8 days. The 22-day teeth showed new odontoblastoid cell profiles adjacent to the new reparative dentin. A thin rim of reparative dentin was present below the remaining dentin at 22 days. None of the Ca(OH)₂ restored pulps showed any stained bacteria at the cavosurface margin interface at these time periods.

**Exposed pulps**

**Clearfil Liner Bond 2 and Clearfil AP-X system.** Table 3 presents the raw histologic data. There were no statistically significant observable differences in inflammatory reactions within the groups at 7, 27, or 97 days. The 7-day histologic responses of 10 of 16 pulps of both Class V and Class I cavities showed no abnormal pulp response (Figs 5 to 8). Three teeth demonstrated a grade 2 response, and two teeth showed a grade 3 pulpal response. Only one necrotic pulp was observed in any of the 41 teeth that received a direct pulp cap with adhesive resin. No reparative dentin was present in any pulps at 7 days. Ten of 16 capped pulps showed clot resolution in the subjacent area directly below the adhesive, with fibroblastic proliferation from the deeper pulp and stratification of pulpoblasts against the resin interface.

At 27 days, 4 of 6 exposed, directly capped pulps had no response (Fig 9), and no necrosis was present. In addition, a new odontoblastoid cell zone had reorganized adjacent to the adhesive interface and had formed a localized, new, thin dentinal bridge in 5 of 6 teeth (180.0 ± 87.2 μm). All specimens showed cellular dentin directly adjacent to the resin interface after 27 days. In 1 of 5 specimens, the dentinal bridge had become organized into a typical pattern of tubular dentin. When the zone of reparative dentin was viewed throughout serial sections, the center of each reparative dentin zone presented the greatest measurable thickness (79.2 ± 40.1 μm), as if the center of a three-dimensional pyramid were being viewed.

At 97 days, only 2 of 19 directly capped exposures showed chronic inflammation, and these also exhibited stained bacteria. Sixteen of 19 pulps showed no adverse pulpal response (Fig 10), and these same pulps showed new dentinal bridge formation (401.4 ± 305.2 μm) directly adjacent to the Clearfil system interface (Fig 11). Sixteen of 17 bridges showed that the initial dentinal structure was that of cellular dentin, directly adjacent to the resin interface. However, the most recently deposited dentin (toward the pulp) presented a tubular pattern in 17 of 19 specimens. No necrosis was present at this time period. None of the adhesively capped pulps showed any pulpal inflammation, and only one pulp showed a grade 2 soft tissue disorganization after 97 days of healing. At 97 days, serial sections of reparative dentin revealed a uniform thickness throughout its entire length (271.1 ± 135.7 μm).

Results showed that 12 of 17 pulps (71%) had tunnel defects in the dentinal bridge at 97 days (Fig 12). One grade 2c and one grade 3 specimen were excluded because they did not exhibit dentinal bridge formation.

**Ca(OH)₂ control.** At 7 days, 5 of 7 pulps showed no inflammation. The original primary odontoblastic layer in two Life-capped pulps showed grade 2 and grade 3 responses, respectively, with slight disorganization of the odontoblastic layer (Table 3). At 21 days, 4 of 5 pulps showed no inflammatory response; however, a new zone of reparative dentin had formed below the cut tubules of remaining dentin. One pulp showed a grade 2 response with slight lack of complete tissue morphology. At 35 days, 10 of 15 pulps showed a grade 1 response, four

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**Table 3: Histologic and bacterial staining responses in exposed monkey pulps**

<table>
<thead>
<tr>
<th>Day</th>
<th>Cavity</th>
<th>Total teeth</th>
<th>Inflammatory cell response</th>
<th>Soft tissue organization</th>
<th>Reparative dentin</th>
<th>Dentinal bridge formation</th>
<th>Bacterial staining</th>
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</table>

Control Ca(OH)₂

|      | Class V | 7           | 5 0 1 1                   | 5 2 0 0                 | 7 0 0            | 0 0 7                   | 4 3 0 0          |
|      | Class V | 5           | 4 1 0 0                   | 4 1 0 0                 | 1 4 0            | 4 0 1                   | 4 1 0 0          |
| 35   | Class V | 15          | 10 0 1 11                 | 4 0 0 0                 | 1 4 1 14         | 0 1 14                  | 0 1 0            |
Fig 5 A 7-day pulpal exposure (8912-9-2-L2). The stain is hematoxylin and eosin at low-powered magnification. The tooth was capped with the Clearfil Liner Bond 2 and AP-X systems. The exposure size is approximately 800 μm in occlusoapical width. The clear area represents that previously filled with adhesive and resin composite. The tissue immediately subjacent to the clear area and deeper pulp is free from signs of dense focal inflammation. The clot has resolved and the pulp has proliterated along the cavity walls. There is no toxic reaction to the adhesive system. (Original magnification x10.)

Fig 6 A 7-day pulpal exposure (8912-9-5-L1). Masson trichrome-stained, high-powered magnification of a section adjacent to Fig 5. The clear area on the left contained resin composite. A few scattered inflammatory cells are present in the tissue directly below the resin-tissue interface; however, there is no deeper pulp inflammation. There is no apparent toxic reaction to the Clearfil Liner Bond 2 and AP-X systems at this time interval. (Original magnification x25.)

Fig 7 A 7-day pulpal exposure (8912-11-10-L1). Hematoxylin and eosin low-powered section. The clear area on the upper mid-right is the cavity and exposure site area. The pink-stained dentin is seen around the periphery. The clot has resolved and the cells of the pulp have migrated to the interface (clear area) of the adhesive system. The exposure size is approximately 1,200 μm in width from an occlusoapical measurement. No pulpal inflammation is present in the tissue subjacent to the exposure or in the deeper pulp. Note that pulpoblasts have migrated along the cut dentinal interface. (Original magnification x10.)

Fig 8 A 7-day pulp exposure (8912-11-7-U3). Masson trichrome-stained section of a higher magnification from a section adjacent to Fig 7. The capped pulp shows complete clot resolution in the area directly subjacent to the adhesive interface, with fibroblastic proliferation from the deeper pulp and stratification of pulpoblasts against the resin interface. The clear areas throughout are vessels that were cleared from the perfusion procedure. The pulp has no inflammation. There is no toxic reaction to the Clearfil Liner Bond 2 and AP-X systems. (Original magnification x25.)
Fig 9. A 27-day pulp exposure (8918-17-6-U4). This is a hematoxylin and eosin section. The exposure site on the mid-left is approximately 260 μm in occlusoapical width, with a new dentinal bridge at the adhesive (clear area) interface. The dentinal bridge is approximately 200 μm in thickness and has reorganized directly adjacent to the (clear) adhesive interface. The light pink area at the periphery to the cut tubules of remaining dentin is newly deposited reparative dentin, continuous with the dentinal bridge. A new odontoblastoid cell zone is present along the dentinal bridge, continuous with the primary odontoblasts. Cellular dentin is visible between the tubular dentin and the cavity. The subjacent and deeper pulp is free from inflammation. There is no toxic reaction to the adhesive system. (Original magnification x25.)

Fig 10. A 97-day pulp exposure (8914-16-9-U4). This is a hematoxylin and eosin section. The exposure size is approximately 500 μm in occlusoapical width, with a new dentinal bridge approximately 250 μm thick directly adjacent to the (clear) adhesive resin interface of the Class I cavity. The new odontoblastoid cells are present at the new bridge, and the subjacent and deeper pulp is free from inflammation. There is no toxic reaction to the Clearfil Liner Bond 2 and AP-X system. (Original magnification x25.)

Fig 11. A 97-day pulp exposure (8914-23-6-L4). Hematoxylin and eosin section. The occlusoapical exposure size is approximately 700 μm wide. A new dentinal bridge, approximately 200 μm thick, is seen directly adjacent to the (clear) adhesive resin interface. The subjacent and deeper pulp is free from inflammation. The new odontoblastoid cells are present, and the deeper pulp is without any inflammation. There is no toxic reaction to the adhesive system at this long time period. (Original magnification x25.)

Fig 12. A 97-day pulp exposure (8914-23-7-L2). This hematoxylin and eosin-stained section is from a glass microslide adjacent to Fig 11. Note the tunnel defect at middle portion of dentinal bridge—filled with cellular debris. There are new odontoblastoid cells along the pulp-bridge interface, and there is no toxic reaction to the pulp or stained bacterial profiles in the adjacent sections. (Original magnification x25.)
pulps presented a grade 2 response, and one pulp showed a grade 3 response, with a slight disorganization of the new odontoblastic layer. Four of 5 pulps at 21 days, and 14 of 15 pulps at 35 days, showed new dentinal bridge formation directly adjacent to the interface of the Ca(OH)₂ agent. No abscesses or stained bacteria were present in any of the control teeth.

Discussion

Pulpal inflammation, ranging from moderate to necrosis, has been reported to be the result of low pH (acidity) of the applied material. Other studies have indicated that acid etching of vital dentin increases pulpal irritation by facilitating the penetration of acids into the tubules. Clearfil Liner Bond 2 system does not use phosphoric acid to etch the tooth surface but uses the acidity (pH 1.4) of one of its adhesive monomers, Phenyl-P, to dissolve the smear layer and allow hybridization of the tooth substrate. Fujitani et al. reported disarrangement and reduction of the odontoblasts at 3 days, indicating that total-etched dentin showed a greater reduction of primary odontoblasts than unetched cavities. That irritation originated from the initial chemical and mechanical irritation of phosphoric acid etching, rinsing, and air drying.

Stanley and Pameijer recently reported that total etching of exposed primate pulps results in sequential death following adhesive capping, with necrosis in 41% of pulps at 26 and 75 days. In addition, Pameijer reported disastrous histologic effects when exposed primate pulps were etched and direct capped with an experimental adhesive. These data are in direct contrast with those of Akimoto et al., Otsuki et al., and Cox, who all reported that exposed primate pulps heal and resolve with new dentinal bridge formation following direct capping with three different adhesive systems. Histologic data from these studies demonstrated that certain of the adhesive materials are biologically compatible when direct capped on exposed vital pulps, being comparable to Ca(OH)₂ controls. This is evident in the present study of the Clearfil Liner Bond 2 system, because 17 of 19 new dentinal bridges had formed directly adjacent to the adhesive interface at 97 days.

Several reasons may account for these diametrically opposed data. First, in considering the data of Stanley and Pameijer, it is quite probable that their experimental adhesive materials simply contain components that cause pulpal necrosis. Second, following contamination of the exposed pulps with bacterial debris, they treated each exposed pulp with an antimicrobial agent, Consepsis, an agent only suggested for use as a disinfectant on nonexposed cavities. It is probable that one or more components of Consepsis were an irritant to the exposed pulps and that this irritation compromised the healing and bridging mechanisms.

In the present study, 2.5% solution of NaOCl, which has been histologically proven not to cause pulpal inflammation, was used for hemorrhage and infection control. To critically analyze reasons for success and failure of hydrophilic adhesive systems, and to provide an environment for dentinal bridge formation, we suspected that initial control of pulpal hemorrhage was the most critical biologic and clinical issue. Sudo, Hirota, and Katoh all reported that NaOCl produces chemical surgery (surface amputation) of the subjacent pulpal interface, in addition to our original intent of hemorrhage control and disinfection. Katoh et al demonstrated that a 6% solution of NaOCl placed on the vital pulp for amputation provided pulpal healing patterns similar to nontreated controls. They reported no histologic evidence of associated necrosis, especially when NaOCl was used for hemorrhage control. They recommended the use of NaOCl as a routine treatment for pulpal tissues following mechanical exposure. Our study demonstrated that the use of 2.5% NaOCl for hemorrhage control is not toxic to pulp cells or inhibitory to pulpal healing, odontoblastoid cell formation, or dentinal bridge formation.

Last, other studies have reported that successful pulp and wound healing is completely predicated on hemorrhage control and adhesive placement, both being technique-sensitive procedures. However, neither Stanley and Pameijer or Pameijer commented on the issue of technique sensitivity. Consequently, it is quite possible that their high failure rate was due to both poor hemorrhage control and material placement. Either of these possibilities, individually or in combination with the other, may ultimately result in bacterial microleakage and eventual necrosis. At 27 and 97 days in the present study, the pulpal responses in Class V and Class I pulps were statistically the same, showing a correlation of pulpal inflammation to the presence of stained bacterial profiles. (P < .05)

Brännström and Nordenvald filled total-etched (37% phosphoric acid) cavities with a resin composite using a bonding agent and reported no pulpal inflammation or stained bacteria. Inokoshi et al. reported that total-etched cavities restored with Clearfil bond system-F showed only a slight pulpal response and no bacterial penetration. Etching of vital dentin for 15 seconds with 40% phosphoric acid and 10% phosphoric acid in deep cavities does not cause pulpal inflammation or necrosis. In the present study, only 1 of 88 pulps showed necrosis. Few inflammatory pulpal responses were observed at each time period in either nonexposed or exposed pulps, and these were generally associated...
TABLE 4 The relationship between inflammation and bacterial existence

<table>
<thead>
<tr>
<th>Finding</th>
<th>7</th>
<th>27</th>
<th>97</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonexposed pulps</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammation (+); bacteria (-)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Inflammation (-); bacteria (+)</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Inflammation (+); bacteria (+)</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Exposed pulps</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammation (+); bacteria (-)</td>
<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Inflammation (-); bacteria (+)</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Inflammation (+); bacteria (+)</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

At 27 days, 5 of 6 direct-capped resin pulps presented tunnel defects. These tunnel defects may simply be the result of incomplete healing and bridge formation at this time period. At 97 days, 12 of 17 specimens showed a more typical type of tunnel defect, one pulp as a grade 2c and one as a grade 3 response. As reported by Cox et al., it appears that these tunnel defects are the result of the presence of vascular channels below the bridging interface. In this study, tunnel defects were always associated with persistence of blood vessels within the defect space. On the other hand, an incomplete bridge appears to be more a consequence of a much larger pulpal interface that has simply not permitted the formation of an initial dentinal bridge interface. In this respect, the actual bridge may be more the consequence of the encroaching deposition of reparative dentin than of new odontoblastic cells at the material interface.

Cox et al. reported that dentinal bridges that form under Ca(OH)₂ medicaments demonstrate a high incidence of multiple tunnel defects (89%) and 45% have inflamed or necrotic pulps. Our study demonstrates that all 27-day pulps with a new dentinal bridge also had tunnel defects, and 71% of the pulps showed tunnel defects in the dentinal bridges at 97 days. However, we found only one grade 2a response at 27 days as well as one grade 2c inflammatory response and one severe inflammatory response at 97 days. Consequently, even though tunnel defects occur in dentinal...
bridges after soft tissue healing, the pulp should present no inflammation when the cavity is completely sealed with the adhesive material.

Finally, to see if there were differences in healing and dentinal bridge formation between the two cavity forms, exposures were placed in both Class I and Class V cavities. The data (see Table 3) showed that the inflammatory cell response and soft tissue organization of Class I cavities were of higher grades than in Class V cavities. However, at 97 days, almost all responses for both cavity forms were the same. These differences at the short-term interval were probably due to the masticatory forces of the animal or the amount of mechanical and vascular trauma to the coronal chamber during the exposure procedure.

Conclusions

1. The Clearfil Liner Bond 2 and Clearfil AP-X System is biologically acceptable to pulpal tissues when placed according to the manufacturer's directions on nonexposed dentin or even directly on mechanical pulpal exposures.

2. Neither Clearfil Liner Bond 2 nor Clearfil AP-X is a biologic irritant to the underlying primary odontoblasts or to the subjacent pulpal tissues when placed in nonexposed cavities, or on mechanical pulpal exposures and restored to the cavosurface margin to effect a biologic seal.

3. Treatment of vital dentin and pulp with the Clearfil Liner Bond 2 and Clearfil AP-X System is not toxic to the primary odontoblasts or the subjacent tissue in the pulps treated in this study.

4. There does not appear to be a correlation between the type of restorative treatment (Clearfil Liner Bond 2 and Clearfil AP-X System) and the thickness of reparative dentin beneath the remaining dentin.

5. When used specifically as a hemostatic agent, 2.5% NaOCl is nontoxic to pulpal cells and does not inhibit pulpal healing, odontoblastoid cell formation, or dentinal bridge formation.

6. The Clearfil LB 2 and AP-X System permits new pulpal cells to stratify, polarize (reorient), and differentiate new odontoblastoid cells to lay down a new dentinal bridge.

This biologic information supports the use of the Clearfil Liner Bond 2 and Clearfil AP-X system for placement on new pulpal exposures.

References


