Histopathological response of infected cavities treated with Gluma and Scotchbond dentin bonding agents

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Abstract: The purpose of this study was to observe the pulpal response of bacterially infected cavities restored with either Gluma or Scotchbond. CI V cavities (120) were distributed throughout the dentitions of five adult Macaca mulatta monkeys at intervals of 8 and 90 days. Each cavity was left open to the oral microflora for 48 hours to allow bacterial invasion of the remaining dentin. Six treatment groups consisted of various cavity pretreatments with Gluma or Scotchbond. Each material, treatment procedure and time interval were represented in each animal. Tissues were prepared for light microscopic observation following routine laboratory procedures. Bacteria were present in the remaining dentin of all teeth left open to the oral microflora. Those cavities immediately restored with Gluma showed none to slight pulp responses at 8 and 90 days. Infected cavities (48 hours) which were treated with Gluma showed minor cell irregularities of the odontoblastic zone at 8 days, with healing and repair at 90 days. Most of the teeth treated with Scotchbond presented bacteria at the dentin-resin interface. No bacteria was observed in teeth treated with Gluma, regardless of prior infection for 48 hours.

Key words: Dentin bonding; pulp biocompatibility.

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INTRODUCTION

Studies have demonstrated that pulpal inflammation is a consequence of microleakage of oral bacteria, endotoxins and LPS cell wall factors along the restoration interface.1-7 Thus, the biocompatibility of any dental restorative material is primarily related to its capacity to provide a hermetic seal to exclude oral bacteria at the restoration interface and less from its chemical components.8

Recent studies have evaluated the pulpal response underneath various dentin bonding agents in both human and monkeys.9-12 These data indicate that Gluma8 dentin bonding is non-toxic to the dental pulp when a thin layer of dentin is left at the axial wall of the cavity preparation. Horsted et al15 indicated that treatment of cut dentin with Gluma8 reduces the risk of bacterial microleakage underneath a composite restoration.

Besides providing a mechanism for bonding to the organic (collagen) phase of dentin, does Gluma8 serve as a cavity disinfectant against bacterial infection? Might Gluma8 slow or stop the flow of dentinal serum through the cut dentinal tubules via denaturation of tubule protein depriving bacteria from nutrients? This latter consideration of dentinal fluid hydrodynamics has been shown to be the principal cause of dental pain underneath restorations as well as root scaled dentin.3,13,14

The aims of this study were: 1) to observe the biocompatibility of Gluma8 underneath CI V cavities at short and long time periods. 2) to evaluate the pulpal response of Gluma8 and Scotchbond8 following their placement on intentionally infected dentin of CI V cavities. 3) to assess the long-term disinfecting capacity of Gluma8 and Scotchbond8 in cavities that have been left open to the oral microflora for 48 hours.

MATERIALS AND METHODS

Five Macaca mulatta monkeys approximately 4-5 yrs-old, each with a complete dentition, were housed in the UNC-CH Animal Care Facility. All teeth were scaled and pumice-polished prior to the experimental procedure. Each animal was tranquilized with an IM injection of 0.2 cc/kg Ketamine hydrochloride (100 mg/cc). Deeper sedation was gained with an IM injection of Rompun (20 mg/ml). The teeth were isolated with a buccal shield and cotton rolls and CI V facial cavities were prepared with a carbide bur and copious irrigation providing adequate flushing and cooling. A new #331/2 inverted carbide bur was employed with every fourth cavity to ensure superior cutting efficiency. Each cavity was etched with 6% citric acid for 60 seconds, then rinsed and left open to the oral microflora for 48 hours to allow plaque and bacterial infection of the dentin.15

Following cavity infection, the walls of each cavity were redefined and restored with its respective material.
Table 1. Biocompatibility of bonding agents.

<table>
<thead>
<tr>
<th>Group</th>
<th>8 day</th>
<th>90 day</th>
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</thead>
<tbody>
<tr>
<td>Gluma group 1</td>
<td>n=9</td>
<td>n=9</td>
</tr>
<tr>
<td>Gluma group 2</td>
<td>n=9</td>
<td>n=9</td>
</tr>
<tr>
<td>Gluma group 3</td>
<td>n=9</td>
<td>n=9</td>
</tr>
<tr>
<td>Gluma group 4</td>
<td>n=9</td>
<td>n=9</td>
</tr>
<tr>
<td>Scotchbond group 5</td>
<td>n=9</td>
<td>n=9</td>
</tr>
<tr>
<td>Cl V exposed controls - group 6</td>
<td>n=9</td>
<td>n=9</td>
</tr>
</tbody>
</table>

Total restored teeth = 108
Unoperated control teeth = 12

(Table 1). All materials were placed following the manufacturer’s directions. A dark shade of composite was placed to identify the cavosurface margins for polymerization and later identification. Time intervals of 8 and 90 days provided short and long-term pulp response. Nine teeth per group were restored for each time period per agent for a total of 108 treated teeth. The remaining teeth served as unoperated controls. In order for all teeth to be collected and processed in sequence, each animal received an intentional dentin infection 92 days pre-sacrifice. After 2 days, the cavities were cleaned and materials placed throughout the dentitions as per their grouping. Ten days before sacrifice, the short-term cavities were intentionally infected; at day 8, materials were placed as shown in Table 1.

Material groupings

Group 1. Cl V cavity and 6% citric acid etch for 60 seconds with copious water rinse, intentional 48 hour dentin infection, cavity debridement, and butt-joint cavity redefinition and enamel bevel, 30-second etching with Gluma 1 Etchant (lot #4197B) with copious water rinse, dry with sterile air, cleaning of the dentin with Gluma 2 Cleanser (lot #8191R), rinse and dry, priming of the dentin with Gluma 3 Primer (lot #7952R), dry with sterile air, placement of Gluma 4 Sealer and spread with a stream of sterile air, mylar strip placement and three layer incremental placement, polymerization of Bayer-Lumifor composite for 60 seconds.

Group 2. Cl V cavity and 6% citric acid etch for 60 seconds with copious water rinse, intentional 48 hour dentin infection, redefinition and enamel bevel, exclude 30-second Gluma 1 Etchant and rinse, exclude Gluma 2 Cleanser, rinse and dry, placement of Gluma 4 Sealer and spread with a stream of sterile air, mylar strip placement and three layer incremental placement, polymerization of Bayer-Lumifor composite for 60 seconds.

Group 3. Cl V cavity and 6% citric acid etch for 60 seconds with copious water rinse, intentional 48 hour dentin infection, redefinition and enamel bevel, exclude 30-second Gluma 1 Etchant and rinse, exclude Gluma 2 Cleanser, rinse and dry, placement of Gluma 4 Sealer and spread with a stream of sterile air, mylar strip placement and three layer incremental placement, polymerization of Bayer-Lumifor composite for 60 seconds.

Group 4. (Horsted). Cl V butt-joint facial cavity preparation and enamel bevel, 30-second acid etching with Gluma 1 Etchant, 30-second copious water rinse, dry with sterile air, cleaning of the dentin with Gluma 2 Cleanser (lot #8191R), rinse and dry, priming of the dentin with Gluma 3 Primer (lot #7952R), dry with sterile air, placement of Gluma 4 Sealer (lot #4191B) and spread with a stream of sterile air, mylar strip placement and three layer incremental placement, polymerization of Bayer-Lumifor composite for 60 seconds.

Group 5. Cl V cavity and 6% citric acid etch for 60 seconds with copious water rinse, intentional 48 hour dentin infection, redefinition and enamel bevel, exclude 30-second Scotchbond gel (lot #P860529), 60-second etch with a copious water irrigation, dry with sterile air, Scotchbond dentin bonding (lot #P860529) treatment, mylar strip placement and three layer incremental placement of Silux (lot #P860529) resin with polymerization for 60 seconds.

Group 6. Cl V cavity and 6% citric acid etch for 60 seconds followed with a 60-second copious water rinse, intentional dentin infection for 48 hours. Each animal was sacrificed by left ventricular perfusion 8 days following the last operative procedure.

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Each tooth was immediately cut from its alveolus, placed into individually numbered jars and post-fixed in fresh GTA-PBF at room temperature for 24 hours. After demineralization, each tooth was washed through 20 changes of distilled water over a 48-hour period with agitation, dehydrated in ascending grades of N-butyl alcohol and embedded in Paraplast-Plus. Uninterrupted 7 μm serial sections cut on a rotary microtome were placed on gelatin slides which were alternately stained with hematoxylin and eosin as well as McKay's stain for bacteria. Histopathologic criteria and assessment were the same as defined in previous studies.6,7,17

Analysis of data

Data from all groups were recorded and analyzed using statistical methods to observe any correlation between the degree of severity of lesion development and progression of healing and to compare the efficiency between the agents. Data for each category were analyzed by Chi-square, Kruskal-Wallis and Analysis of Variance (ANOVA) tests (P<0.5). Differences between the groups with the ANOVA tests were compared using the Scheffe test.

RESULTS

The cavities left open to the oral microflora for 48 hours presented pulp tissues with a disrupted odontoblastic zone along the pulpal interface below the remaining uncut dentin (Fig. 1). Most of these infected cavities presented a generalized distribution of various inflammatory cells within the pulp tissue subjacent to the cavity preparation. The tubules of remaining dentin on the axial floor of each cavity presented a large number of stained bacterial profiles (Fig. 1).

The 8-day non-infected cavities which were immediately restored with Gluma* (Group 4) generally presented a normal pulpal response with an absence of inflammatory cells, while the underlying tissues presented a normal architecture. The pulps of 48 hour infected cavities without an acid etch and treated with Gluma* presented a slight disruption of the odontoblastic layer (Fig. 3) and no stained bacterial profiles on the axial wall. Stained bacterial profiles were seen in those teeth restored only with composite resin and no enamel acid etch (Group 3). No stained bacterial profiles were seen in any of the immediately restored Gluma* treated teeth (Group 4). The 48 hour infected cavities restored with Gluma* and/or Scotchbond® and composite...
Fig. 3. A histological section of an 8 day cavity that was infected for 48 hours and then restored with Gluma, however, no acid etch was employed. Note that the odontoblastic zone presents a slight disruption with no inflammatory cells present. (x100)

Fig. 4. A histological section of an 8 day cavity that was infected for 48 hours and then restored with Gluma and composite resin. Note the odontoblastic zone is slightly disrupted. No inflammatory cells are present. (x40)

(Groups 1 and 5) showed a slightly disrupted odontoblastic zone with a few scattered inflammatory cells (Fig. 4). Cavities immediately restored with Gluma* (Group 4) presented a statistically significant improved level of healing to all other groups at this time period.

The 90-day cavities immediately restored with Gluma* presented normal soft tissues and odontoblastic morphology with no inflammation. Other Gluma*-treated teeth showed a none to slight infiltration of inflammatory cells in subjacent pulp tissues. Those intentionally infected cavities treated with Scotchbond® and Silux® resin showed localized nests of inflammatory cell infiltrates (Fig. 5) within the pulp below the cut tubules. In addition, increased numbers of stained bacterial profiles were seen on the cut axial dentin wall of all cavities (Fig. 6) when compared to their Gluma* (Group 1) counterparts. Statistical analysis of the 90 day data showed that Group 3 with 48 hour intentional infection and composite restoration without Gluma* or acid-etching showed a higher degree of inflammation than Groups 1 and 5. Scotchbond® (Group 5) showed more inflammatory cell infiltrates than Gluma* (Groups 1 and 4).

DISCUSSION

The data regarding immediately restored cavities from this study is in agreement with Horsted® who showed that Gluma* is nontoxic to the dental pulp when a thin layer of remaining dentin is left. No stained bacterial profiles were observed in any of the 48 hour infected cavities at either of the time intervals.

Our findings show that the healing capacity of acutely inflamed dental pulps and dentin following 48 hour intentional exposure and treated with Gluma* are similar in their healing response to immediately cut and Gluma* restored cavities, both groups reflecting normal cellular healing events at both the 8- and 90-day time periods. In addition, no bacterial profiles were seen either along the restoration interface or in the subjacent dentinal tubule complex treated with Gluma.* Whereas, those cavities and infected pulps treated with Scotchbond® presented stained bacteria and a mild inflammatory response in the subjacent pulp. In addition, all infected cavities treated with Scotchbond® presented stained bacterial profiles along the restoration interface as well as within the underlying dentinal tubule complex.

A number of reports by Seltzer18, Waerhaug &
Zander\textsuperscript{19} and Zander\textsuperscript{20} speculated that pulp infection and bacterial infection were associated with the restoration gap interface. Well-defined studies have demonstrated that the main reason for pulpal infection is due to bacterial infection at the restoration interface.\textsuperscript{1,2,4,21} These studies now reflect the correlation of bacterial infection and pulpal inflammation.\textsuperscript{8} The absence of stained bacterial profiles beneath Gluma\textsuperscript{8} treated cavities tends to suggest several mechanisms of action. Firstly, the increased bonding capacity of Gluma\textsuperscript{8} with the organic components of the dentin provide a stronger dentin bond than that of Scotchbond\textsuperscript{b} that bonds to the mineral phase of dentin. Secondly, the immediate bacteriocidal and longer term bacteriostatic effect of Gluma\textsuperscript{8} provides a mechanism to exclude bacteria from reentering the contraction gap and infecting the adjacent dentin. However, the long term effect of disinfection is not known beyond 90 days and can only be speculated upon.

CONCLUSIONS
1. Gluma\textsuperscript{8} is a biologically compatible dentin bonding agent when placed in immediately prepared cavities.

2. No stained bacterial profiles were observed in any of the 54 cavities treated with Gluma\textsuperscript{8} dentin bonding agent regardless of preinfection, observation period or etching procedure.

3. Gluma\textsuperscript{8} seems to present a disinfecting capacity by eliminating bacteria resulting from the intentional infection as well as preventing re-colonization at the restoration interface for the observed time.

REFERENCES

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