



Histopathology Study on Pulp Response to Glass Ionomers in Human Teeth

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ABSTRACT OBJECTIVE: Evaluation of the pulpal response to a resin-modified glass ionomer, a conventional glass ionomer and calcium hydroxide. METHODS AND MATERIALS: Fifty-five deep Class V cavities were lined with Vivaglass Liner, Chembond Superior and Dycal. After seven, 30, and 60 days the teeth were extracted and a histological assessment was performed. RESULTS: There was no statistically significant difference in pulpal response among the three groups for the same time interval ($P>0.05$). CONCLUSION: Light-cured glass ionomers have similar advantages to conventional glass ionomers.

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Continuous development of new materials provides a wide range of biomaterials appropriate to various clinical conditions in dentistry. Despite all the improvements, there is still a need for a biomaterial that possesses high biocompatibility, antimicrobial effects, and good mechanical properties. Among the recently developed materials, glass ionomer cements, GIC, have gained popularity since their conception in 1972 by Wilson and Kent.¹ Conventional glass ionomer cements present biocompatibility, nonshrinking setting reaction, chemical adhesion to tooth structure and fluoride release.² New formulations have been successively developed to overcome some clinical drawbacks of previous cements, especially aimed at improving physical properties.³ In many clinical situations, the newer resin-modified glass ionomer cements, RMGICs, are an alternative to conventional glass ionomer cements.

To evaluate the biocompatibility

of dental materials, a sequence of tests must be performed including in vitro assay for mutagenesis and cytotoxicity (initial tests), local toxicity reactions by intraosseous or subcutaneous implantation of the material in small laboratory animals (secondary tests) and, finally, the usage tests.⁴

Several studies have shown that the light-cured systems of glass ionomer cements exhibit poor biocompatibility and greater cytotoxicity than conventional cements in cultured cells.⁵ In vitro studies of Vitrebond and Vitremer have shown some cytotoxic and mutagenic effects leading the investigators to conclude that they may cause pulp irritation.^{5,6} Evaluating indirect pulp capping employing a RMGICs, two recent studies reported acceptable pulpal response, and another reported a less favorable pulpal response.⁷⁻⁹

This in vivo study evaluated the histological changes in pulp as a response to light-cured resin-modified glass ionomer and compared it with a conventional

glass ionomer and a calcium hydroxide lining material in deep cavities.

Methods and Materials

The study population consisted of 19 females and 12 males, ranging in age from 13 to 32, with a mean age of 18 years. All of the patients required the extraction of permanent premolars for orthodontic reasons. The participants, and their parents or responsible persons, received an adequate explanation concerning the experimental rationale, clinical procedure, and possible risks. The parents and all the volunteers were asked to read and sign a consent form explaining the research protocol approved by the ethical guidelines.

Patients were required to meet the following criteria.

To be included in the study:

- Permanent first premolars scheduled for orthodontic extraction
 - Scores of two or less using the periodontal screening record (evaluation consisted of examining the premolars with a periodontal probe)
 - Completed root formation
- To be excluded from the study:
- Presence of caries
 - Presence of restorations
 - Presence of abrasion or erosion
 - Presence of pulpal symptoms or radiographic periapical lesions

After local anesthesia the teeth were isolated with a rubber dam. A Class V cavity on the buccal surface of each tooth was prepared with a 440-diamond point (Shofu Inc, Kyoto 605-0983, Japan) in a high-speed handpiece under copious water spray coolant. New diamond points and burs were used after every four teeth. The axial wall was excavated using a carbide round bur at low speed until red feature of the pulp was observed.

The 55 experimental teeth were divided into three groups. In the first

TABLE 1

Evaluation Criteria¹¹

Odontoblastic changes

(Non) Remarkable change was not observed in the pulp

(Slight) Disarrangement of odontoblasts was noted slightly below the cut dentinal tubules

(Moderate) Disarrangement of odontoblasts was seen through most of the cut dentinal tubules

(Severe) Disarrangement of odontoblasts was noted below the remaining dentin.

Inflammatory cell infiltration

(Non) None or a few inflammatory cells were observed through the pulp.

(Slight) A few inflammatory cell infiltrations were noted below the cut dentinal tubules.

(Moderate) Inflammatory cell remarkably observed below the remaining dentin.

(Severe) Severe inflammatory cell infiltration was seen through the pulp.

Reactionary dentin formation

(Non) No abnormal or reparative dentin observed.

(Slight) A small amount of reactionary dentin was noted.

(Moderate) Reactionary dentin was observed below the almost-cut dentin.

(Severe) Complete and large bulk of reactionary dentin was noted.

group, Vivaglass Liner (Ivoclar Vivadent AG, Schaan, Lichtenstein) was applied to the axial wall of the cavity and then was light-cured for 20 seconds. In the second group, Chembond Superior (Dentsply, Detry, UK) was applied as a liner in the axial wall of the cavity; and in the third (control) group, Dycal (Dentsply, Milford, Del., USA) was applied. All of the materials were used according to manufacturer's directions. After application, two layers of a copal varnish, Copalite (Cooley & Cooley LTD, Houston, Texas) were added. The cavities were restored with a high copper amalgam, Oralloy (Coltene Whaledent, USA). After seven, 30, and 60 days, the teeth were extracted under local anesthesia.

The mesial and distal approximal surfaces of the teeth were reduced with a high-speed diamond bur under spray coolant until the pulp became almost visible through the remaining dentin to facilitate the penetration of the fixative solution. The surfaces were then fixed with a 10 percent neutral buffered formalin solution for one week. The teeth were demineralized with 10 percent ethylene-

diamine tetracetic acid (ETDA) with PH (7-7.4) as a demineralizing solution at 25 degrees (Celsius) for 60 days, and each tooth was then embedded in paraffin. 5µm-thick serial sections were prepared through the cavities and pulp, obtaining approximately 80 to 100 sections per cavity. They were placed on glass microslides and stained with either hematoxylin eosin for routine histological evaluation or Taylor's modification of Gram's staining technique for detecting microorganisms.¹⁰

The pulpal responses and the presence of bacteria in their cavities were evaluated using a light microscope (Zeiss, Germany). The RDT, remaining dentin thickness, was ranged as deep (0-0.4 mm), moderate (0.4-0.7 mm), and shallow (more than 0.7 mm). Evaluation criteria for odontoblastic changes, inflammatory cell infiltration and reactionary dentin formation are shown in **TABLE 1**.¹¹

The results of odontoblastic changes, inflammatory cell infiltration, and reactionary dentin formation were statistically analyzed using the Kruskal Wallis and Mann-Whitney test at 95 percent level of confidence. Fisher's Exact test ($\alpha = 0.05$) was also used

TABLE 2

Results of Histological Findings		7 days	30 days	60 days
Experimental groups		VCD	VCD	VCD
Number of specimens		875	566	666
Odontoblastic changes	Non	322	224	221
	Slight	121	121	122
	Moderate	432	221	323
	Severe	000	000	000
Inflammatory cell infiltration	Non	022	325	223
	Slight	212	221	313
	Moderate	640	020	130
	Severe	001	000	000
Reactionary dentin formation	Non	875	253	221
	Slight	000	313	445
	Moderate	000	000	000
	Severe	000	000	000

V: Vivaglass Liner; C: Chembond Superior; D: Dycal

for understanding the correlation between pulpal responses with microorganisms and remaining dentin thickness in each group.

Results

Results of histological findings are shown in **TABLE 2**.

Bacterial penetration was observed in only six cases (five cases in cavity walls and only one case in pulp). There was no significant correlation between pulpal responses with dentinal thicknesses and microorganisms ($P > 0.05$).

In the Vivaglass Liner, there was a statistically significant difference in inflammatory cell response among three intervals ($P < 0.05$). Inflammatory cell reaction in the seven-day group was significantly more than in the 30- and 60-day groups (**FIGURES 1 AND 2**). There was no statistically significant difference in odontoblastic changes among three intervals; slight odontoblastic change was seen in each interval (**FIGURE 3**).

In Chembond Superior there was significant difference only in reactionary dentin formation among three intervals ($P < 0.05$). The mean rank of reactionary dentin formation after

seven days was significantly less than in the 60-day group ($P < 0.05$) (**FIGURE 4**).

The results of Dycal were similar to those of Chembond Superior.

■ The Kruskal Wallis test showed that there was no statistically significant difference in odontoblastic changes, reactionary dentin, and inflammatory cell response among the three groups for the same time interval ($P > 0.05$).

Discussion

Certain controversy persists regarding the biocompatibility of various RMGIC systems. Some studies have reported an innocuous histologic pulp response to RMGICs in Class V cavities, but in vitro studies often showed some cytotoxicity.^{5,6,12,13}

The purpose of this study was to compare the in vivo pulpal response to a resin-modified glass ionomer and a conventional glass ionomer and to evaluate the correlation between the pulpal responses with the presence or absence of bacteria and the remaining dentin thickness. The pulpal responses to these materials were compared with a $\text{Ca}(\text{OH})_2$

at three time intervals in accordance with the Craig and Powers protocol.⁴

According to previous studies, each subgroup consisted of five to eight samples and amalgam was used as a filling material.^{12,14-16} Although a number of studies claimed that pulp tissue response is caused only by the presence of bacteria, in vitro studies have demonstrated that resin monomers diffuse through the dentin tubules and cause cytotoxicity.^{5,17,18} Previous studies have demonstrated that cellular compatibility of RMGICs varies significantly.^{19,20} Schmalz and others showed that Vitrebond causes a very strong cytotoxicity effect when evaluated by dentin barrier tests.²¹ Nascimento and others applied Vitrebond as a pulp-capping agent in sound human teeth, and no pulp repair or dentin bridge formation was observed even after 300 days.²² They concluded that Vitrebond is not an appropriate pulp-capping agent to be used in mechanically exposed, sound, human pulps. However, it has been reported that the pulpal response to visible light-activated glass ionomer cements may be quite favorable when applied as a cavity liner.^{7,23}

The present study showed that al-



FIGURE 1. Cavity preparation, remaining dentin thickness and pulp tissue. The odontoblast layer is disrupted and the cells were displaced into the dentinal tubules. Mild and scattered inflammatory cells are present. (Vivaglass Liner, seven days.) (H & E; 40X.)

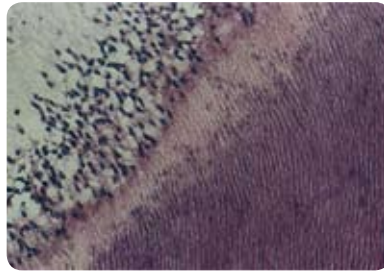


FIGURE 2. Moderate to severe aggregation of chronic inflammatory cells under the remaining dentin thickness. (Vivaglass Liner, seven days.) (H & E; 200X.)

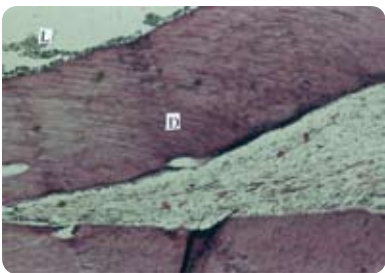


FIGURE 3. A sample of Chembond Superior, seven days. Remnant of liner (L) and remaining dentin thickness (D). Odontoblast layer is disrupted. (H & E; 40X.)



FIGURE 4. Reactionary dentin formation (R) under the remaining dentin thickness (D). Remnant of liner (L) and pulp (P). (Vivaglass Liner, 60 days.) (H & E; 40X.)

though pulp responses in the same time intervals did not differ significantly among materials, inflammatory cell response in Vivaglass Liner after seven days was significantly more than in the 30- and 60-day groups. According to Geurtsen and others, HEMA and TEGDMA may be released from RMGI in the early 24 hours after polymerization.⁵ Buillaguet and others also demonstrated the diffusion of HEMA through dentinal tubules, even against internal pressure.²⁴ The cytotoxicity of glass ionomer is reduced with time, as seen in the present study.⁶ RMGIC has a burst release of fluoride and may also have a burst release of monomers that will be decreased with time. This finding agrees with the results observed by About and others.²⁵

All of the testing materials in this study showed slight-to-moderate inflammatory reactions and no bacterial presence except in six cases. In this study, bacterial-staining data indicated that the lining and filling materials provided an

almost complete seal against microleakage through all time intervals. There was only a reversible slight-to-moderate pulp response, since the testing materials provided an excellent biological seal. This acceptable pulp response was dependent upon the prevention of bacterial penetration or the lack of toxicity of glass ionomers.

The results of this study showed there was no correlation between the presence or absence of microorganisms and remaining dentin thickness with the pulp response. The authors' finding corroborates the results of a study done by Sonoda and others.¹¹ This is probably due to the minimal changes in dentinal thickness prepared in this study and also due to a biologic seal which prevented the bacterial penetration through the pulp tissue.

If in this study the pulp response to resin-modified glass ionomer after elimination of carious lesion had also been evaluated, the results of the study could better imitate clinical conditions. It

is suggested that a study for evaluation of pulp response to glass ionomer in deep carious lesions be done in the future.

Conclusions

The glass ionomer systems that were tested provided an almost complete seal against bacterial microleakage through all time intervals. No serious inflammatory reaction of the pulp was observed. The pulp response to the Vivaglass Liner in seven days was significantly higher than the other intervals.

In all groups, reactionary dentin formation after 60 days was more than other intervals. There was no significant difference in odontoblastic changes, reactionary dentin formation and inflammatory cell response among the groups for the same interval. There was no correlation between pulp response with dentinal thickness and microorganisms. ■■■■

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