Original Article

A Histopathologic Study on Pulp Response to Glass Ionomer Cements in Human Teeth

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Abstract:

Statement of Problem: Despite the wide range of new dental materials, there is still a need for biomaterials demonstrating high biocompatibility, antimicrobial effects and ideal mechanical properties.

Purpose: The aim of this study was to histologically evaluate the pulpal response to a conventional glass ionomer, a resin modified glass ionomer and a calcium hydroxide in human teeth.

Materials and Methods: Fifty five deep class V cavities were prepared in premolars of 31 patients and were divided into 3 groups based on application of the following liners: resin modified glass ionomer (Vivaglass Liner), conventional glass ionomer (Chembond Superior) and calcium hydroxide (Dycal). After applying varnish, teeth were filled with amalgam. Each group was further divided into three subgroups according to time intervals of 7, 30 and 60 days. Teeth were then extracted and their crowns were fixed in formalin. Each sample was assessed microscopically for odontoblastic changes, inflammatory cell infiltration, reactionary dentin formation, remaining dentinal thickness and presence of microorganisms. Statistical analysis including Kruskal Wallis and Mann Whitney was carried out for comparison of mean ranks. (P=0.05).

Results: In the Vivaglass Liner group, pulpal response was significantly higher on day 7 as compared to days 30 and 60 ($P<0.05$). Reactionary dentin production was significantly lower after 7 days than after 60 days for all materials ($P<0.05$). There was no statistically significant difference in pulpal responses among the three groups during the same time intervals ($P>0.05$). There was no correlation between pulpal responses with micro-organisms and remaining dentin thickness ($P>0.05$).

Conclusion: According to the results of this study, light-cured glass ionomer as well as the other tested lining materials were determined to be biologically compatible with vital pulps in deep cavities of sound human teeth.

Key Words: Pulp response; Inflammatory cell response; Glass ionomer

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INTRODUCTION

Continuous development of new materials provides a wide range of new dental materials with improved mechanical and physical properties and biological compatibility, for various clinical applications. However, despite these advances, there is still a need for biomaterials demonstrating high biocompatibility, antimicrobial effects and ideal 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 [2], non–shrinking setting reaction, chemical adhesion to tooth structure, and fluoride release. New formulations have been successively developed to overcome some clinical drawbacks of the previous ones, especially aiming to improve physical properties [3]. In many clinical situations the resin-modified galss–ionomer cements (RMGICs) are an alternative to the conventional glass ionomer cements.

To evaluate the biocompatibility of dental materials a sequence of tests must be performed including in-vitro assays 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 on cultured cells have shown that the light activated glass ionomer cements exhibit poor biocompatibility and greater cytotoxicity than the conventional ones [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]. Indirect pulp capping employing a RMGIC, has been evaluated in two recent studies; one reported acceptable pulpal response, [7,8] and the other described a less favorable pulpal reaction [9].

This in vivo study histologically evaluated pulp tissue reactions to light-cured resin modified glass ionomer and compared it with a conventional glass ionomer and a calcium hydroxide lining material in deep cavities.

MATERIALS AND METHODS

The study population consisted of 19 females and 12 males, aged between 13 to 32 years old, with a mean age of 18. All patients required the extraction of permanent premolars for orthodontic reasons (Quota sampling was used for this study). The participants and their parents or guardians, received an adequate explanation concerning the experimental rationale, clinical procedure and possible risks. The parents and all volunteers were then asked to read and sign a written consent form explaining the research protocol approved by the ethic committee.

Patients were required to meet the following criteria:

- Permanent first premolars scheduled for orthodontic extraction before applying brackets or orthodontic forces.
- Scores of 2 or less using the community periodontal index for treatment needs (evaluation consisted of examining the premolars with a periodontal probe).
- Completed root formation

Tooth exclusion criteria were as follows:

- Presence of caries
- Presence of restorations
- Presence of abrasions or erosions
- Presence of pulpal symptoms or radiographic periapical lesions

After local anesthesia, the teeth were isolated with a rubber dam. A class V cavity was prepared on the buccal surface of each tooth 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 employed on every fourth cavity preparation. The axial wall was excavated using a carbide round bur at low speed until a pink discoloration was observed due to pulp proximity.

The fifty five experimental teeth were divided into three groups. In the first group, Vivaglass Liner (Ivoclar Vivadent AG, Schaan, Liechtenstein) 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 cavity liner on the axial wall of the cavity, and in the third group (control), Dycal

(Dentsply, Milford, DE, USA) was applied. All materials were used according to the manufacturer's instructions. After application of two layers of a copal varnish, Copalite (Cooley & Cooley LTD,Houston, Texas), the cavities were restored with a high copper amalgam (Oralloy (Coltene Whaledent, USA). After 7, 30 and 60 days, the teeth were extracted under local anesthesia. The mesial and distal 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 in order to facilitate the penetration of the fixative solution. Afterwards, they were fixed with 10% neutral buffered formalin solution for one week. The teeth were demineralized in 10% Ethylene-Diamine Tetracetic Acid (ETDA) with a pH between 7-7.4 at 25^{o}C for sixty days and then were embedded in paraffin. Five-µm-thick serial sections were prepared through the cavities and pulp, obtaining approximately 80-100 sections per cavity, which were placed on glass microslides and stained with hematoxylin and eosin for routine histological evaluation and Taylor's modification of Gram's staining technique for detection of micro-organisms [10]. Pulpal responses and the presence of bacteria in their cavities were evaluated using a light microscope (Zeiss, Germany). The Remaining Dentin Thickness (RDT) between the cavity floor and pulp tissue was measured for each specimen and was divided into three groups as follows: deep (0-0.4 mm), moderate (0.4-0.7mm) and shallow (more than 0.7 mm). Criteria used for the evaluation of odontoblastic changes, inflammatory cell infiltration and reactionary dentin formation are shown in Table I [11].

The results of odontoblastic changes, inflammatory cell infiltration and reactionary dentin formation were statistically analyzed using the Kruskal Wallis and Mann-Whitney tests at a 95% level of confidence. The correlation between pulpal responses with micro organisms and remaining dentin thickness in each group was assessed by Fisher's Exact test ($\alpha = 0.05$).

Table I. Evaluation criteria according to Sonoda [11].

Criterion		Description
Odontoblastic changes	none	Remarkable changes were 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	none	None or a few inflammatory cells were observed through-out the pulp
	slight	A few inflammatory cells were noted below the cut dentinal tubules.
	moderate	Remarkable inflammatory cell infiltration observed below the remaining dentin.
	severe	Severe inflammatory cell infiltration was seen through-out the pulp.
Reactionary dentin formation	none	No abnormal or reparative dentin observed.
	Slight	A small amount of reactionary dentin was noted.
	moderate	Reactionary dentin was observed below the cut dentin.
	severe	A complete and large bulk of reactionary dentin was noted.

RESULTS

Bacterial penetration was observed in 6 cases (5 cases in cavity walls and only 1 case in the pulp) and microorganisms were found in only one specimen in each of the six groups. Pulpal responses did not correlate with dentinal thickness and the presence of micro-organisms ($P>0.05$).

In the Vivaglass Liner group a statistically significant difference was observed in inflammatory cell response among the three intervals ($P<0.05$). Inflammatory cell reaction in the 7-days group was significantly higher than the 30- and 60-days groups (Figs 1 and 2). There was no statistically significant difference in odontoblastic changes among the three intervals. Slight odontoblastic changes were seen in each test period (Fig. 3).

In the Chembond Superior group, there was a significant difference only in reactionary dentin formation among the three intervals ($P<0.05$). The mean rank of reactionary dentin formation in the 7-days group was significantly lower than the 60–days group ($P<0.05$) (Fig. 4).

The results of the Dycal group were similar to that of Chembond Superior.

According to Kruskal Wallis test, statistically significant difference was not observed in odontoblastic changes, reactionary dentin and inflammatory cell response, among the three groups for the same time intervals ($P>0.05$).

Fig.1: Cavity preparation, remaining dentin thickness and pulp tissue (H & E; 40X). The odontoblast layer is disrupted and the cells are displaced into the dentinal tubules. Mild and scattered inflammatory cells are present. (Vivaglass Liner, 7 days)

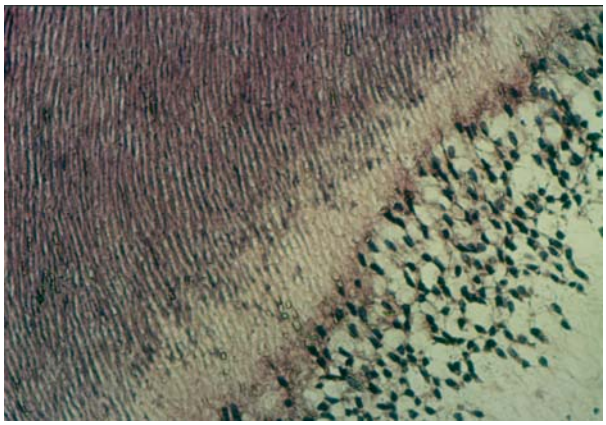

Fig.2: Moderate to severe aggregation of chronic inflammatory cells under the remaining dentin thickness. (Vivaglass Liner, 7 days) (H & E; 200X)

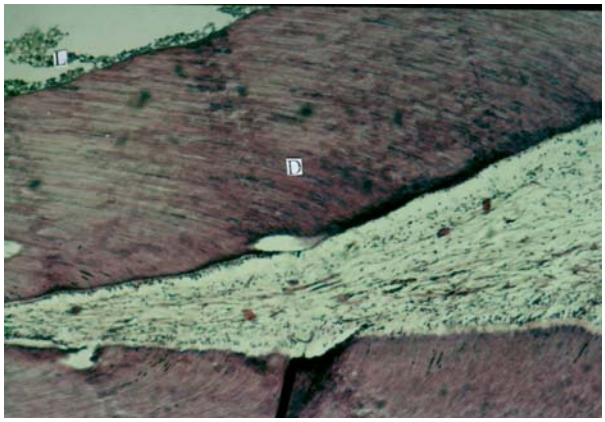

Fig.3: A sample of Chembond Superior, 7days. Remnant of Liner (L) and remaining dentin thickness (D) can be seen. The odontoblast layer is disrupted. (H & E; 40X)

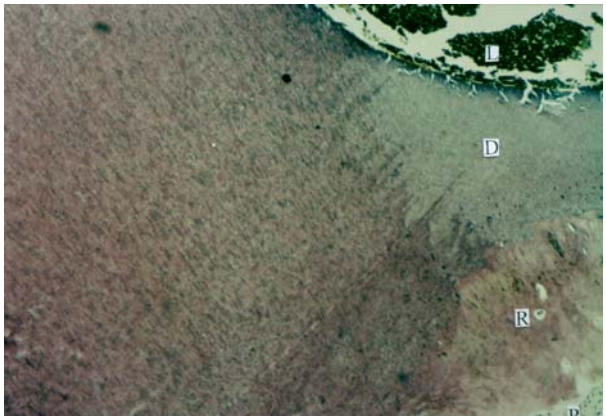

Fig.4: Reactionary dentin formation (R) under the remaining dentin thickness (D). Remnants of the Liner (L) and pulp (P) are also present. (Vivaglass Liner 60 days) (H & E; 40X)

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 [12,13], but in vitro studies often showed some cytotoxicity [5,6].

The purpose of this study was to evaluate and compare the in vivo pulpal response to a resin modified glass ionomer and a conventional glass ionomer and to assess the correlation between the pulpal responses with the presence of bacteria and the remaining dentin thickness. The pulpal responses to these materials were compared with $Ca(OH)_2$ at three time intervals according to the Criag and Powers protocol [4].

According to a number of previous studies, each subgroup consisted of 5 to 8 samples [12,14] and amalgam was used as a filling material [15,16]. Although many 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 dentinal tubules and cause cytotoxicity [5,17,18]. Pervious studies have demonstrated that cellular compatibility of RMGICs, varies significantly [19,20]. Schmalz et al showed that Vitrebond causes a very strong cytotoxic effect when evaluated by dentin barrier tests [21]. Nascimento et al applied Vitrebond as a pulp capping agent in sound human teeth; neither pulp repair nor 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 demonstrated that despite the fact that pulpal response did not differ significantly among the tested materials in the same time intervals, Vivaglass liner showed a significantly higher inflammatory response on day 7 as compared to days 30 and 60.

According to Geurtsen et al, HEMA and TEGDMA may be released from RMGI in the first 24 hours after polymerization [5]. Buillaguet et al also demonstrated the diffusion of HEMA through dentinal tubules even against internal pressure [24]. Cytototxicity of glass ionomer is reduced with time [6] as seen in the present study. RMGIC has a burst release of fluoride and also may have a burst release of monomers that decrease with time. This finding agrees with the results observed by About et al [9].

All tested materials in the present study showed slight to moderate inflammatory reactions, and with the exception of six cases, none of them exhibited bacterial penetration. Bacterial-staining profiles indicated that the studied lining and filling materials provided an almost complete seal against microleakage through all time intervals. There was only a reversible slight to moderate pulpal response, which was due to the excellent biological seal provided by the tested materials. This acceptable pulpal response depended on the prevention of bacterial penetration or the lack of toxicity of glass ionomers.

The results of this study indicate that there was no correlation between the presence of micro-organisms and remaining dentin thickness with pulpal response. This is probably because of the minimal changes in the dentinal thickness prepared in this study and also due to the perfect seal which prevented bacterial penetration through the pulpal tissue. This finding corroborates with the results of a study conducted by Sonoda et al [11].

If the pulpal response to resin modified glass ionomer had been evaluated after the elimination of carious lesions, the results could have better imitated clinical conditions. It is recomended that further studies be performed in the future, to evaluate pulpal response to

glass ionomer in deep carious lesions.

CONCLUSION

Within the limitation of this study, the following conclusions were drawn**:**

1- The tested glass ionomer systems provided an almost complete seal against bacterial microleakage through all time intervals. No serious inflammatory reaction was observed in the pulp. The pulpal response to the Vivaglass Liner on day 7 was significantly higher than the other intervals.

2- In all groups, reactionary dentin formation was higher after 60 days as compared to all other time intervals. There was no significant difference in odontoblastic change, reactionary dentin formation, and inflammatory cell response among the groups for the same intervals. Pulpal responses did not correlate with dentinal thickness and the presence of micro-organisms.

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مطالعه هیستوپاتولوژیک پاسخ پالپ به سیمان گلاس‌آینومر در دندان انسان

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چکیده

بیان مسأله: علی‌رغم تمامی پیشرفتهایی که در زمینه مواد دندانپزشکی انجام شده است، هنوز هم نیاز به موادی با سازگاری زیستی بالا، اثرات ضدمیکروبی و خواص مکانیکی ایده‌آل وجود دارد.

هدف: هدف از این مطالعه ارزیابی هیستولوژیک پاسخ پالپ به یک نوع گلاس‌آینومر معمولی، یک نوع رزین گلاس‌آینومر مدیفای شده و یک نوع کلسیم هیدروکساید در دندانهای انسان است.

روش تحقیق: پنجاه و پنج حفره عمیق cl v بر روی دندانهای پرمولر ۳۱ بیمار تهیه و جهت کف‌بندی با سه نوع لاینر (liner) به سه گروه تقسیم شدند. در گروه اول از رزین گلاس‌آینومر مدیفای شده (Viva glass liner) در گروه دوم از گلاس‌آینومر معمول (Chembond superior) و در گروه سوم از کلسیم هیدروکساید (Dycal) استفاده شد. پس از زدن وارنیش، ترمیم دندانها با آمالگام صورت گرفت. نمونه‌ها براساس دوره‌های هفت، ۳۰ و ۶۰ روز به سه زیرگروه تقسیم شدند سپس دندانها کشیده شده و در فرمالین ثابت گردیدند. تمامی نمونه‌ها از جهت شاخصهای تغییرات ادنتوبلاستیک، ارتشاح سلول‌های آماسی، شکل‌گیری عاج ترمیمی، ضخامت عاج باقیمانده و حضور میکروارگانیسم‌ها مورد بررسی میکروسکوپی قرار گرفتند.

جهت مقایسه Mean ranks از آزمون‌های آماری kruskal-wallis و Mann-whitney با درنظر گرفتن ∝=۰/۰۵ استفاده شد.

یافته‌ها: در گروه Viva glass پاسخ پالپی در روز هفتم به طور معناداری بیش از روزهای سی‌ام و شصتم بود (P<۰/۰۵). شکل‌گیری عاج در تمامی مواد در روز هفتم به طور معناداری کمتر از روز شصتم بدست آمد (P<۰/۰۵). هیچ‌گونه اختلاف آماری معناداری بین میزان پاسخ پالپی در سه نوع ماده در زمانهای یکسان دیده نشد (P>۰/۰۵). ارتباط آماری معنی‌داری بین میزان میکروارگانیسم‌ها و ضخامت عاج باقیمانده وجود نداشت (P>۰/۰۵).

نتیجه‌گیری: با توجه به نتایج مطالعه اخیر، گلاس‌آینومر light cure همچون دیگر مواد liner از نظر بیولوژیک سازگاری مناسبی با بافت پالپ زنده در حفرات عمیق دندانهای سالم انسانی دارد.

واژه‌های کلیدی: زیست سازگاری؛ گلاس آینومر؛ کلسیم هیدروکساید؛ پاسخ پالپی

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