

## Original Article

# Histological assessment of pulpal responses to resin modified glass ionomer cements in human teeth

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## ABSTRACT

**Background:** The biocompatibility of resin-modified glass ionomers (RMGIs) as a lining material is still under question. The present study evaluated the response of the pulp-dentin complex following application of resin-modified glass-ionomer cement, calcium hydroxide and conventional glass-ionomer in deep cavities prepared in human teeth.

**Materials and Methods:** In this controlled clinical trial, 30 deep class V buccal cavities (3 mm × 2 mm × 2 mm) were prepared in human premolars treatment planned to be extracted for orthodontic reasons and divided into 3 groups. Groups were lined by a RMGI (Vivaglass), conventional glass ionomer (Ionocid) and calcium hydroxide respectively. The cavities were subsequently filled with amalgam. Each group was then divided into two sub-groups according to time intervals 5 and 30 days. The patients were referred to Kerman Dental School and in accordance with orthodontic treatment plan; premolars were extracted and then prepared for histological assessment. The sections were stained with hematoxylin and eosin and periodic acid Schiff techniques. All of the samples were examined using a number of criteria including odontoblastic changes, inflammatory cells response, reactionary dentin formation and presence of microorganisms. The data were analyzed by Kruskal-Wallis and Mann-Whitney tests.  $P < 0.05$  was considered as significant.

**Results:** There was no significant difference among odontoblastic changes, reactionary dentin, presence of bacteria and inflammatory cells response of the groups ( $P > 0.05$ ).

**Conclusion:** Ionocid and Vivaglass resin-modified glass ionomers can be used as lining materials in human teeth.

**Key Words:** Biocompatibility, calcium hydroxide, glass ionomer, pulp response, tertiary dentin

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## INTRODUCTION

Researches have shown that the placement of restorative materials will induce a response in the tooth dentin-pulpal complex to some degree.<sup>[1]</sup> Thus, the placement of a biocompatible liner on the cavity

floor has been recommended to prevent harmful changes in dentin-pulpal complex.<sup>[2]</sup>

Calcium hydroxide cement (CHC) has long been the material of choice as a liner beneath amalgam restorations.<sup>[3,4]</sup> CHC is an alkaline dental material<sup>[5]</sup> used as a liner under restorations, which acts as a stimuli blocker by filling small gaps by its crystal growth;<sup>[6]</sup> however, concerns exist regarding the solubility of CHC, its lack of chemical or mechanical adhesiveness, its potential accelerated degradation during the adhesion bonding process<sup>[7]</sup> and its inability to provide an effective long-term protection against microleakage. Therefore, scientists proposed glass-ionomer cements (GICs) as alternative lining materials to CHCs.<sup>[1]</sup>

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In early 1970's, Wilson and Kent<sup>[8]</sup> introduced the first GIC, which was based on a single acid. At that time, its biocompatibility was not a big concern; however, with the addition of more acids to enhance certain characteristics and reduce setting time, GICs became more irritating<sup>[9]</sup> and less biocompatible, despite their advantages such as fluoride release, non-shrinking setting reaction, chemical adhesion to tooth structure<sup>[10]</sup> and linear coefficient of thermal expansion similar to tooth.<sup>[11]</sup>

Selection of a new material for clinical use must consider not only the mechanical and physical properties, but also biological compatibility. To evaluate the biocompatibility of dental materials, secondary animal tests and clinical human tests (usage tests) must be performed following initial *in vitro* tests.<sup>[2]</sup> Since late 1980's, when further development in the field of GIC led to the introduction of a hybrid generation of these materials, so called resin-modified glass-ionomer cements (RMGICs),<sup>[12,13]</sup> these materials have passed various *in vitro* and animal tests, nominated for usage tests. Although studies show that RMGICs possess improved mechanical and physical properties and better adhesiveness than conventional glass-ionomers (GIs),<sup>[2,14]</sup> their biological compatibility is still under question. Conducting a systematic review on *in vivo* human researches aiming at assessing the biocompatibility of RMGICs, Mickenautsch *et al.*<sup>[7]</sup> did not come to a definite conclusion about the difference between pulpal reactions in resin-modified glass ionomer (RMGIs) and Dycal (CHC) and researchers have not made a definitive statement about this issue yet. This study was designed to comparatively investigate the biocompatibility of conventional GIC, RMGIC and Dycal to put a short step forward in a better understanding of dental materials.

## MATERIALS AND METHODS

### Ethical considerations

This study was approved by Ethical Committee of Dental School of Kerman University of Medical Sciences with registry number 85/17.

### Tooth selection

In this controlled clinical trial, 30 non-carious, intact first premolars of 25 patients - 14 females and 11 males - aged between 14 and 30 referred to Kerman Dental School for tooth extraction for orthodontic treatment were included. The patients were informed

about the procedures and potential risks of the study; written consents were obtained from the participants. The teeth were divided into six groups: V5, V30, I5, I30, D5 and D30.

### Cavity preparation and filling

Prior to tooth extraction, the buccal surfaces of the teeth were cleansed using pumice powder and rubber cap. Each tooth was anaesthetized (2% Lidocaine with 1/100000 epinephrine, Darupakhsh, Tehran, Iran). Isolation was performed, using cotton rolls. Then Diamond fissure bur 245 (DiaTech LLC, Pforzheim, Germany) and high speed rotary instrument with water spray were used to prepare Class V cavities extended 3 mm mesiodistally, 2 mm occlusocervically with 2 mm depth, keeping the gingival floor in enamel, while 3-way syringe was washing the location. Each cavity was then vigorously rinsed and dried with a three-way syringe. After preparing the teeth for filling, the teeth in D5 and D30 groups were first treated with self-cure calcium hydroxide (Dycal, Dentsply, USA); equal amounts of Dycal tubes were mixed and placed on the axial wall of the cavity and left for 2.5-3.5 min to get rigid. Second, the teeth in I5 and I30 groups were treated with conventional GI cement (Ionocid-L30, Salami, Tehran, Iran). A scoop of powder was blended with a droplet of liquid and mixed for 10 s, was applied in the cavity and left for 2-3 min to get rigid. Third, the teeth in V5 and V30 groups were treated with RMGI (Vivaglass, Ivoclar, Germany); 0.25 g of powder was blended with 1 g of liquid and mixed for 20 s. One layer of RMGI was applied in cavity and dried with a stream of air. The RMGI was light cured (light cure unit, Degulux, Germany) with 400 mw/cm<sup>2</sup> intensity for 20 s, according to manufacturer instructions. All of these procedures were performed according to manufacturers' instructions. To prevent dentin sensitivity, two layers of varnish (Copalite, Teledyne Getz, Austria) were applied in the cavities and cavities were filled with lathecut high-copper amalgam (Cina, Faghihi Tehran, Iran), packed, cured and burnished following the conventional method.

### Tooth extraction and histomorphological assay

After 5 days, V5, I5 and D5 teeth were anesthetized and extracted, while V30, I30 and D30 were extracted after 30 days with a minimum trauma. Roots of the teeth were cut-off in the middle by a diamond bur, accompanied by water spray. In order to survey pulpal reactions, histology slides were provided from each specimen. To do this, the specimens were first

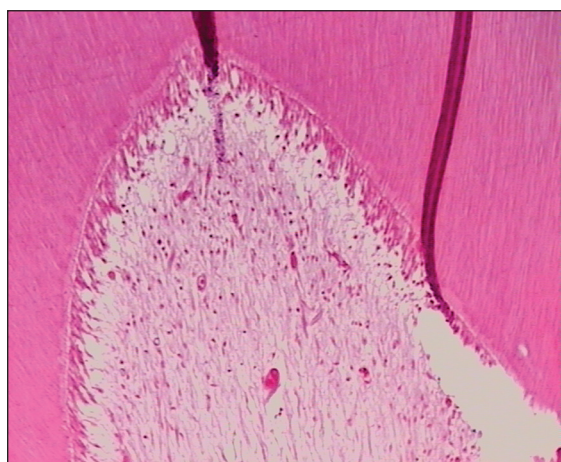
immersed in 10% formalin for 10 days, followed by a 3-day immersion in 5% phosphoric acid. Phosphoric acid was replaced every day.<sup>[7]</sup> Then, the teeth were dehydrated, embedded in series of graded alcohol, finally ending up in Xylene. Then, the specimens were placed into melted paraffin. The melted paraffin was cooled, leaving a salad media for the sectioning of the tissue. After preparing 4  $\mu$ m sections, the specimens were stained, using hematoxylin and eosin technique for routine histological evaluation and periodic acid Schiff staining for detecting microorganisms. At the final step, the slides were observed by means of light microscope (Zeiss, Germany) with  $\times 40$ ,  $\times 100$  and  $\times 400$  magnification [Figures 1-4]. Pulpal reactions were investigated and scored using the criteria illustrated in Table 1. Data were analyzed by Kruskal-Wallis and Mann-Whitney U statistical tests.  $P < 0.05$  was considered as significant.

## RESULTS

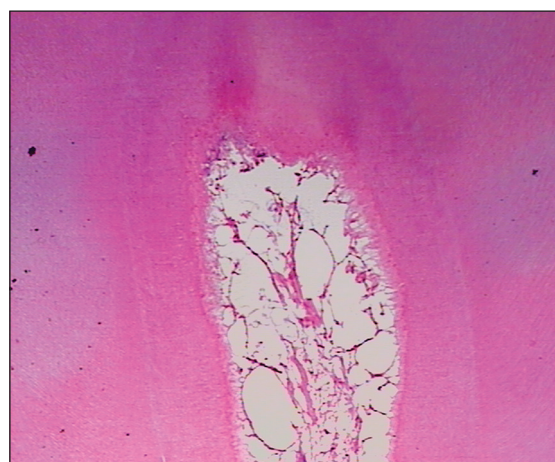
The results of this study are illustrated in Table 2. There was no significant difference among pulpal responses of the applied materials after 5 days ( $P = 0.32$ ) and after 30 days ( $P = 0.81$ ). Odontoblastic changes also showed no significant difference after 5 and 30 days ( $P = 0.07$ ,  $P = 0.35$  respectively). In terms of bacterial presence, no significant difference was seen among the groups both in 5 days and 30 days. No tertiary dentin (TD) formation occurred after 5 days and there was no difference in TD formation among the groups ( $P = 0.34$ ).

## DISCUSSION

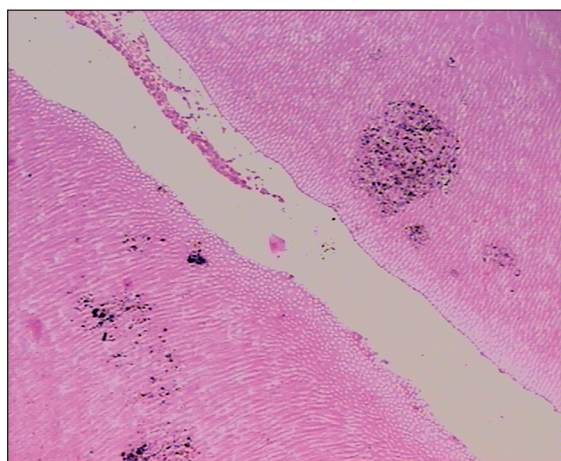
While some studies demonstrate that the RMGIs are less biocompatible than conventional GIs and calcium hydroxide<sup>[15-17]</sup> the biocompatibility of these materials as cavity liners in human teeth is still under question.



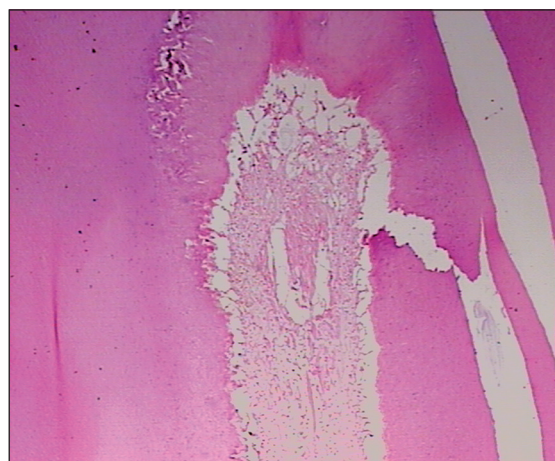
**Figure 1:** Inflammatory response in a specimen of Vivaglass group after 5 days ( $\times 10$ )



**Figure 2:** Tertiary dentin formation in a specimen of Dycal group after 30 days ( $\times 10$ )



**Figure 3:** Presence of microorganisms; periodic acid Schiff staining ( $\times 10$ )



**Figure 4:** Odontoblastic changes in a specimen of Ionocid group after 5 days ( $\times 10$ )

**Table 1: Criteria for investigating pulpal reactions**

Criteria	Intensity	Range	Score
Odontoblastic changes	No changes	No significant change in pulp	0
	Mild	Odontoblastic irregularity in a small area around damaged dentin	1
	Moderate to severe	Odontoblastic irregularity in larger areas around damaged dentin	2
Inflammatory response	No inflammation	0-25 IC×400	1
	Mild	26-50 IC×400	2
	Moderate	51-75 IC×400	3
	Severe	76-100 IC×400	4
TD formation	Without TD formation	—	0
	Mild TD formation	—	1
Presence of microorganisms	No	—	0
	Yes	—	1

IC: Inflammatory cells; TD: Tertiary dentin

**Table 2: Frequency of pulpal reactions to the materials after 5 and 30 days**

Material criteria	Vivaglass		Dycal		Ionocid	
	5 days	30 days	5 days	30 days	5 days	30 days
Inflammatory response						
No inflammation	2	3	4	4	2	3
Mild	2	2	1	0	2	1
Moderate	1	0	0	1	0	0
Severe	0	0	0	0	1	1
Odontoblastic changes						
No changes	0	2	3	4	1	3
Mild	3	1	2	1	3	1
Moderate to severe	2	2	0	0	1	0
Presence of bacteria						
No	3	5	5	5	5	5
Yes	2	0	0	0	0	0
TD formation						
No	5	2	5	2	5	4
Yes	0	3	0	3	0	1

TD: Tertiary dentin

The results of this study showed that there is no significant difference between pulpal responses and TD formation of teeth lined with RMGIs and those lined with conventional GIs or calcium hydroxide.

Studies show that evaluating pulpal responses-inflammatory response, odontoblastic changes, presence of bacteria and TD formation-provides an appropriate index for choosing liner materials, as these criteria estimate the pulpal activity after restoration of teeth.<sup>[1]</sup> Mickenautsch *et al.* in their systematic review study,<sup>[7]</sup> stated that the remaining dentin thickness does not significantly influence the histological pulpal responses. Thus, this criterion was not included in the present study.

In the present study, frequency of more intensive pulpal responses (odontoblastic changes and inflammatory

response) of teeth lined with Vivaglass within 5 days were higher than 30 days. This is probably due to presence of residual monomers — such as hydroxyethyl methacrylate and triethylene glycol dimethacrylate - in the material within the first few days after cavity lining.<sup>[17]</sup> This finding is in agreement with Mousavinasab *et al.* study stating that pulpal inflammatory reactions after 7 days are significantly higher than those of 30 and 60 days.<sup>[18]</sup>

The results of the present study showed that moderate to severe inflammatory responses occur after 30 days in the absence of bacteria. These findings and the findings of similar studies<sup>[2,18]</sup> emphasize the fact that although microorganisms and their products are considered to be the main etiological factor for dental pulpal inflammation, the restorative materials and liners can also trigger inflammatory responses.<sup>[19]</sup>

There is another variable that influences the intensity of inflammatory responses. Murray *et al.* stated that time elapsed since material placement – as well as bacterial presence and type of material, affects inflammatory response,<sup>[19]</sup> and that level of pulpal inflammation decreases over time. In the present study, the intensity of these responses has decreased over time, when Vivaglass was used.

Studies show that the components of RMGI may be released in wet environment.<sup>[17]</sup> In the present study, Vivaglass did not cause severe pulpal inflammation. Probably an acid-base reaction in the cavity floor has prevented its components from being released. Furthermore, since no acid-etching was used in this study, the remaining smear layer did not allow the formation of a wet environment.<sup>[2]</sup>

For both 5-day and 30-day time periods, mild and moderate-to-severe odontoblastic changes were higher

when Vivaglass was used; nevertheless, the difference was not significant among the groups. This could be attributed to structural differences of deep and superficial dentin; first, number of dentinal tubules per square millimeter in the inner diameter of deep dentin is higher than superficial dentin; second, the inner diameter of deep dentin is larger than superficial dentin. As a result, the continuous out-ward dentin fluid movement interferes with monomer-to-polymer conversion and remaining monomers cause the rupture of odontoblasts.<sup>[2]</sup> Other studies also reported more intensive odontoblastic changes for RMGI compared to Dycal; however, these studies also did not find a significant difference between pulpal reactions to these two materials.<sup>[2,18]</sup>

In a study by Costa *et al.* presence of bacteria in walls of two teeth lined with RMGI was observed, giving no explanations for this observation.<sup>[2]</sup> Furthermore, in the present study, two specimens showed bacterial presence. Since RMGIs provide proper seal,<sup>[20]</sup> inadequate isolation may be the main cause of bacterial presence and bacteria may have penetrated the cavity during restorative procedures rather than having leaked the cavities.

In the present study, no TD formation was detected after 5 days. Since all of the groups revealed TD formation after 30 days, it could be concluded that the elapsed time has had more significant effect than the type of material in TD formation. The results of the present study are in contrary with Murray *et al.*'s study. They reported that in terms of TD formation, the type of restorative material is more important than the time elapsed. This is because the time elapsed between treatment and histometric analysis of teeth in Murray *et al.*'s study was between 28 and 381 days. In fact, the specimens in that study had at least 28 days, which is the average time needed for TD formation,<sup>[21,22]</sup> while in the present study 5 days was not enough time for TD formation. Also, Mousavinasab *et al.* did not find any TD formation after 7 days; however, they observed TD after 30 and 60 days in the specimens, regardless of the material used to line the cavities.<sup>[18]</sup>

Murray *et al.* in their study demonstrated that TD formation in teeth lined with CHC occurs more than teeth lined with RMGI;<sup>[1]</sup> however, the present study and Mousavinasab *et al.*'s study showed that different materials do not cause significant difference in the TD formation. Formation of TD is followed by creating a dead tract in dentin and

complete cessation in the formation of secondary dentin.<sup>[23]</sup> The contrast between these results may be due to the fact that RMGI in Mousavinasab *et al.* and our study was also able to create the dead tract in dentin. Yet we suggest that these materials be evaluated in carious teeth, too. We also suggest conducting similar studies with larger number of samples and longer time periods and with remaining dentin thickness measurement.

## CONCLUSION

The results of this study showed that all of these materials were biocompatible when applied in deep cavities. Due to their improved properties and acceptable biocompatibility, RMGIs can be recommended to be used as lining materials.

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