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IN VIVO STUDY OF PULP REACTION TO GLASS IONOMER CEMENTS AND DENTIN ADHESIVES

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Abstract: The aim of this study was to evaluate histopathologically the pulp tissue response of the researched materials 7 and 30 days after their application. The reaction of pulp tissue has been examined on first upper molars in 24 Wistar rats, following the previously set parameters. For that purpose, 48 class V cavities were prepared with a high-speed handpiece using a diamond burr under copious water-cooling. The cavities were divided into four groups. In the cavities from the first group we applied Fuji Lining LC, and in the second group cavities we applied Fuji IX as a base. In the third and fourth group cavities we applied Prime&Bond and G Bond as a base. All the cavities were restored with liquid light cured composite. Seven days after the application, 3 rats from each group were killed and the restored teeth were extracted and immersed in a fixative solution, Osteomol. After removing the Osteomol, the specimens were processed according to histological procedures. The histological evaluation was made using a light microscope connected to a video camera. Thirty days after the application of the dental materials we re-did the procedure with the other restored teeth. For Fuji Lining LC and Fuji IX most of the specimens exhibited no pulpal response or slight inflammatory reaction associated with slight tissue disorganization during a seven-day period. A slight to moderate inflammatory pulpal response occurred in the specimens restored with G Bond, while Prime&Bond exhibited the strongest toxic effect on the pulp tissue. After 30 days the pulp tissue in all groups recovered and displayed a normal appearance.

Key words: Biocompatibility, dentin adhesives, glass ionomer cements, pulpal response.

The development of science and technology leads towards the production of a wide range of new dental materials with established physical and mechanical features. Besides the antimicrobial effect and mechanical opportunities it is required of the dental material that it posses biocompatibility towards the pulp and surrounding tissues. According to the research of several authors there are two main causes of inflammation on pulp tissue after the restorative procedures: toxicity of the material and bacterial infection.

The correlation between pulp irritation and the presence of bacteria has been confirmed by several researches. These studies have concluded that the dental materials available on the dental market are biocompatible when applied on dentine or in contact with pulp tissue, and only bacteria and their products might cause pulpal breakdown [8]. On the other hand, inflammation of pulp tissue can be noted also when there is no bacterial microleakage, especially while applying bonding systems with low viscosity [6, 7]. Hypertonic acidic agents applied on dentin remove the smear layer as well as decalcifying the peritubular dentine. It has been demonstrated that uncured residual toxic components released from resin-based materials can diffuse through the dentinal tubules to cause damage to the pulp tissue [4]. The reduction of the remaining dentine leads to increased permeability of dentine as a result of the increasing number and width of exposed dentine tubules. Self-etching adhesive systems which do not completely remove the smear layer and smear plugs and preserve the dentin morphology, might prevent transdentinal diffusion of resin components. Conventional glass ionomer cements present biocompatibility, a nonshrinking setting reaction and chemical adhesion to tooth structure [16]. The newer resin-modified glass ionomer cements, RMGICs, are an alternative to conventional glass ionomer cements, but the light-cured systems of these glass ionomer cements exhibit poor biocompatibility and greater cytotoxicity than conventional cements [10].

Aim of the study

The aim of this study was to evaluate the pulp response to the different dental materials (total-etching and self-etching dentine adhesive, conventional and resin-modified glass ionomer cement) 7 and 30 days after their application.

Materials and method

A total of 48 first upper molars were used for this investigation. Therefore 24 Wistar rats (aged 12 weeks and 250–260 gr weight) received an intraperitoneal injection of Nembutal (pentobarbital). Cavities were prepared on the mesial aspect of the two first upper molars with a high-speed handpiece, under

water-cooling. The burrs were changed after every fourth cavity. No additional retention was made. During the preparation, the depth of the cavity was 1mm, the same in all preparations. The 48 cavities were divided into 4 groups. Cavities in the first group were restored with Fuji Lining LC (GC Corporation), in the second with Fuji IX (GC Corporation), in the third with Prime&Bond (Dentsply, DeTrey) and in the fourth group with G Bond (GC Corporation). After that the cavities of all groups were restored with a liquid light-cured composite. Seven and 30 days after restoration, 24 anaesthetized rats were killed. Block sections including the three molars were dissected from the maxilla, and decalcified with Osteomol (Merk) at room temperature for 48 hours. After dehydration the teeth were embedded in paraffin. Five-micron thick serial sections were prepared through the cavities and pulp using a sliding microtome. These were placed on glass microslides, and stained with hematoxylin and eosin (H/E). Bacteria were evidenced using Brown and Brenn's staining technique. The pulp response was evaluated by light microscope (Nikon-Eclipse E 600, програм Lucia 4,21, Nikon).

The criteria for assessing the physical changes in pulp tissue, adjusted according to ISO standards of evaluation, are shown in Table 1.

Table 1

Histological criteria and grading

Tissue disorganization
0 – Normal tissue
1 – Odontoblastic layer disorganization but central pulp normal
2 – Total disorganization of the pulp tissue morphology
3 – Pulp necrosis
Inflammatory cell response
0 – None or a few scattered inflammatory cells present beneath the cat tubules
1 – Slight inflammatory cell infiltrate with polymorphonuclear or mononuclear leukocytes
2 – Moderate inflammatory cell infiltrate involving coronal pulp
3 – Severe inflammatory cell infiltrate involving the coronar pulp or characterizing abscess.
Hard tissue formation
0 – Absence
1 – Modest hard tissue deposition beneath the axial wall
2 – Moderate hard tissue deposition beneath the axial wall exposed area
3 – Intense hard tissue deposition beneath the axial wall

Results

The results of the histological examinations are shown in Table 2.

Table 2

Number of teeth for each score according to groups and periods

Histopathologic event	Number of teeth							
	Score 0	7 days			30 days			
	Score 0	Score 1	Score 2	Score 3	Score 0	Score 1	Score 2	Score 3
Tissue disorganization								
Fuji Lining LC	0	6	0	0	6	0	0	0
Fuji IX	0	6	0	0	6	0	0	0
G Bond	0	2	4	0	5	1	0	0
P&B	0	0	6	0	5	1	0	0
Inflammatory cell response								
Fuji Lining LC	3	3	0	0	6	0	0	0
Fuji IX	2	4	0	0	6	0	0	0
G Bond	0	2	4	0	6	0	0	0
P&B	0	0	3	3	5	1	0	0
Hard tissue formation								
Fuji Lining LC	6	0	0	0	0	6	0	0
Fuji IX	6	0	0	0	0	6	0	0
G Bond	6	0	0	0	0	6	0	0
P&B	6	0	0	0	0	6	0	0

Changes in the layer of odontoblast

Seven days after the preparation, all experimental groups demonstrated a disruption of the odontoblast layer in areas corresponding to the cut dentinal tubules. Four samples from the first group of teeth treated with G Bond showed moderate changes in the odontoblast layer (OL), while in the other 2 samples changes were slight. All samples treated with Prime&Bond showed moderate changes with significant disruption in OL. In all the examined samples from the third and fourth groups treated with Fuji Lining LC & Fuji IX slight changes in the odontoblast layer were found, with enlarged blood vessels and capillaries in the pulp areas related to the cavity preparation (Fig. 1, Fig. 2). Over a period of 30 days slight changes were noticed in the odontoblast layer in one sample from the G Bond group and in one from the P&B Group. In rest of the specimens the odontoblast layer was unchanged.

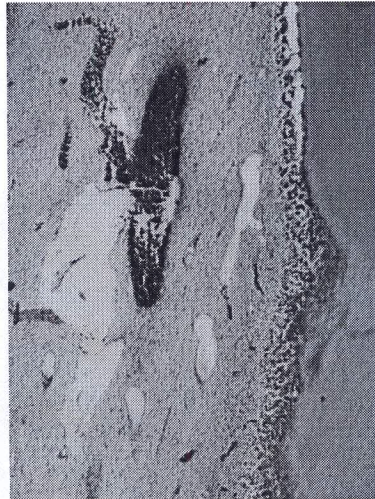


Figure 1 – Fuji Lining LC restored specimens. Pulp tissue is almost normal with disruption of odontoblastic layer (100 x)

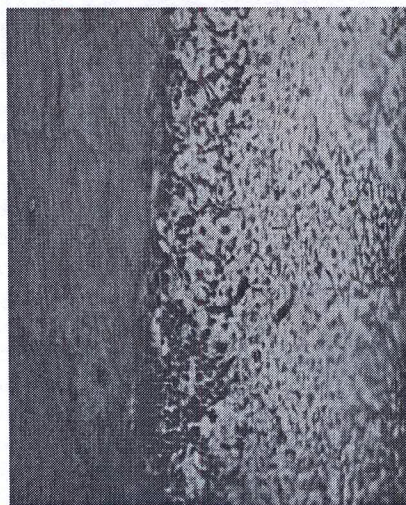


Figure 2 – Fuji IX restored specimens. Pulp tissue is almost normal with slight inflammatory cell infiltration and disruption of odontoblastic layer (100 x)

Inflammatory cell reaction

For a period of 7 days pulp tissue in all the tested specimens exhibited inflammatory cell response. Four specimens from the G Bond group exhibited a moderate inflammatory response (Fig. 3), while the other two cavities showed a slight pulp response.

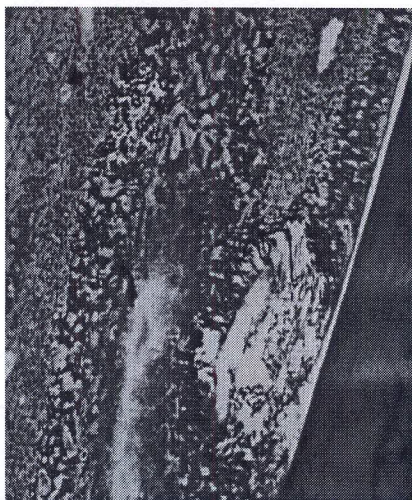


Figure 3 – Moderate reaction in G Bond specimens. Slight tissue disorganization. Infiltration of inflammatory cells in the pulp and disruption of odontoblastic layer (100 x)

The pulp response was greater in specimens restored with Prime&Bond. Three of them exhibited moderated inflammatory reaction at the pulp zone related to the cavity floor. The other three samples showed severe inflammatory cell infiltration involving the coronal pulp (Fig. 4). As for the samples restored with glass ionomer cements, in 3 of the Fuji Lining LC group and in four of the Fuji IX group a slight inflammatory pulp response was observed. The other specimens presented normal histological characteristics. Over a period of 30 days all of the tested specimens resulted in reparation of pulp tissue, with lack of inflammatory response, except in the Prime&Bond group where a persistent slight inflammatory reaction was observed in one of the specimens. Resolution of blood vessel dilatation and odontoblast reorganization were observed next to the thick reparative dentine. No bacteria were observed in any specimens after a 7 and 30 days period (Fig. 5).



Figure 4 – Prime&Bond specimens. Remarkable inflammatory pulpal response (100 x)

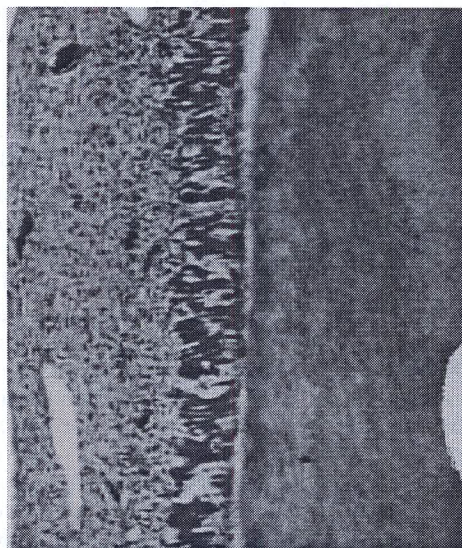


Figure 5–30 days after restavration. Pulp tissue exhibited normal histological characteristics (100 x)

The formation of reactive dentin

For a period of 7 days none of the tested specimens had visible formation of reactive dentin. The occurrence of reparative dentinogenesis was seen in all specimens after a 30-day period. The ANOVA test showed that there was no statistically significant difference in reactive dentine formation between the experimental and control groups.

Discussion

There are two main reasons for the pulp response, the toxic effect of the material and bacterial microleakage. The age of the patient, method of applying the material and remaining dentine thickness also play an important role in the intensity of pulp response and deposition of reparative dentine. Theoretically, a 100% conversion of monomer to polymer is possible, but as much as 25 to 50% of the methacrylate monomer double-bond actually remains unreacted in the polymer [1, 7]. Any unpolymerized monomer in the composite is a potential biological liability if it leaches from the composite toward the pulp of the tooth. Many *in vitro* investigations demonstrate the cytotoxic effects of dental materials and their components [9]. Hanks et al. demonstrate that Bis GMA & UDMA have an extremely toxic effect applied on cultured cells. [6] The result shows an additional cytotoxic effect of HEMA when used as a solvent for Bis GMA [18].

Results from *in vitro* studies do not always correspond with the results obtained under *in vivo* conditions. The dentine, to some extent, acts as a barrier which slows and prevents the penetration of toxic substances toward the pulp.

Schmaltz reported that a remaining dentine thickness of 0.5mm should be enough to protect the pulp tissue against the cytotoxic effects of dental materials. Furthermore, pulp circulation *in vivo* reduced the concentration of cytotoxic agents [19].

In our research, over a period of 7 days, the inflammatory pulp response was most extensive in teeth treated with Prime&Bond. In 3 of the samples there were signs of severe inflammatory pulp response while the other 3 samples showed moderate inflammatory response. The results observed in the G Bond group showed slight inflammatory changes of pulp tissue and odontoblast layer. Considering the fact that in our research dental materials were applied on dentine in cavities with approximately the same depth, we can conclude that differences in the pulp response are due to the toxicity of the material and the technique of application. The outward fluid flow through the tubules and the plasma proteins present in the dentinal fluid is the first line of defence against the inward diffusion of noxiousness from the cavity floor to the pulp [17]. The wet bonding technique associated with a high dentine permeability in acid-etched deep dentine can lead to increased penetration of unreacted monomers

towards the pulp. G Bond is a self-etching dentine adhesive which is less aggressive compared to phosphoric acid and during its application a slight decalcification of dentine is formed. Sousa Costa et al. reported that when the smear layer was removed by total etching technique and the enlarged dentinal tubule openings in the cavity floor received the bonding agents as a liner, a remarkable inflammatory response and tissue disorganization was observed. According to these authors a remaining dentine thickness of 300 μm may not be enough to protect the pulp tissue against the cytotoxic effects of dental materials [3–4]. Sonoda demonstrated only a little initial chemical and mechanical irritation from self-etching/priming/bonding system, providing a slight reason for irritation dentine formation at 90 days [21]. The other study showed that when microleakage is prevented, some adhesive resin systems and their accompanying composite system could elicit tissue responses comparable to calcium hydroxide [11]. Contrary to these views, Murray considers that the presence of bacteria increases the severity of pulp inflammation and the smear layer appeared to prevent the further microleakage of bacteria through cut dentinal tubules in a pulpward direction [15]. Consequently, based on the histological findings observed in the present study, glass ionomer cements seem to be more biocompatible with pulp tissue than dentine adhesives, without a significant difference in pulp reaction between conventional and resin-modified glass ionomer cement. Many researchers have confirmed the biocompatibility of GJC. The blandness of the GJC was thought to be due to the fact that they avoided the stronger acids and toxic monomers. Polyacrylic acid and related polyacids are much weaker than phosphoric acid and, as polymers, they possess higher molecular weights that supposedly limit their diffusion to the pulp through the dentinal tubules [22]. The other reasons that explain the biological tolerance of the pulp to the glass ionomer cements is considered to be a good adhesion to cavity walls and capability to prevent microleakage. Major et al. used GJC to restore the class V cavities of dog teeth and found only a slight pulp reaction and disturbance of mineralization of predentin. The presence of a dentine barrier bigger than 0.6 mm, that limits the direct contact of pulp cells with GJC, may partially explain the results of this study [12]. Some researches demonstrated that resin-modified GIC and GC Lining cements were more toxic to dental pulp cells than conventional GIC, so they do not recommend that resin-modified GICs be directly applied onto dental pulp cells [9–10]. Over a period of 30 days, most of the pulps returned to their normal appearance in all examined groups. The results show that changes in the pulp tissue that we observed after a period of 7 days are reversible. A recent *in vivo* study has noted that filling of rat upper molar class V cavities with Fuji IX GIC induced disruption of the odontoblast layer, dilatation of blood vessels and transient inflammatory reactions after 8 days. 30 days after Fuji IX filling, the pulp tissue recovered and displayed a normal appearance [20]. According to some authors some dental materials show a stronger cytotoxic effect on pulp tissue in the early phase after application [2]. This may be caused by a higher concentration of unreacted monomers that

leaches from the composite towards the pulp in the early phase after polymerization. While some researchers find the toxicity of the material to be responsible for the inflammatory pulp response, others consider it to be result of the bacterial microleakage. In our research there was an inflammatory pulp response of different intensity without the presence of bacteria. This leads to the fact that the reason for pulp reaction is the toxicity of the material. One of the parameters for determining biological compatibility was the tertiary dentine formation. Tertiary dentine formation can be classified as reactive dentine or reparative dentine. Reactive dentine is secreted from prime odontoblasts while reparative dentine is from newly separated odontoblast-like cells. Both mechanisms of deposition of dentine depend on the intensity of the initial response and the conditions under which the deposition of the dentine matrix is formed. Seven days after preparation, it was difficult to evaluate the presence or absence of osteodentine. Osteodentine or reparative dentine formation were seen in both control and experimental groups after a period of 30 days. Since no statistical difference in reparative dentine formation was found between the four experimental and control groups, this implies that cell recruitment, synthetic activity and secretion were neither stimulated nor inhibited by glass ionomers and dentine adhesives in this model. This is an acceptable proof that odontoblasts and odontoblast-like cells have kept their potentialities for reparative dentine formation and the examined materials do not alter this function. Mounasavab reported that there is no difference in reactive dentine formation between glass ionomer cements and dycal. The deposition of dentine was larger after a period of 30 days compared with 7 days [13]. According to Six the reparative dentine thickness scored after 30 days supports that about 6.6–7,1 μm were formed daily [20]. Murray et al. demonstrated a quantitative relationship between the remaining dentine thickness and the stimulation of reactionary dentinogenesis that may support the theory that a molecular stimulus for reactionary dentinogenesis is an effector molecule released from endogenous growth factors contained within the dentine. The reduction in RDT would increase the pool of released growth factors, which may stimulate odontoblast dentinogenic activity beneath the cavity floor [14]. In contrast to these views, Sousa Costa suggests that the irritative stimulus during cavity preparation rather than effector molecule solubilized from cut dentine is responsible for the initiation of a reactionary dentinogenesis [3]. In our research all the cavities were shallow, having approximately the same depth, which could be a reason for the slight differences in the deposition of dentine.

Conclusion

At short-term evaluation, 7 days, Prime&Bond caused a greater inflammatory pulpal response than did glass ionomer cements and G bond. However, a slight or no inflammatory response occurred for all dental materials after

a 30-day period. Based on the histological findings observed in the present study, it could be concluded that Prime&Bond, G Bond, Fuji IX and Fuji Lining LC are biocompatible materials to be employed as liners. Glass ionomer cements seem to be more biocompatible to pulp tissue than dentin adhesives.

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Резиме

**IN VIVO СТУДИЈА ЗА РЕАКЦИЈАТА НА ПУЛПНОТО ТКИВО
НА ДЕНТИН АТХЕЗИВИ И ГЛАС ЈОНОМЕР ЦЕМЕНТИ**

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Апстракт: Целта на овој труд ни беше да го испитаме патохистолошкиот одговор на пулпното ткиво на испитуваните материјали 7 и 30 дена по нивната апликација. Испитувањето го правевме на први горни молари кај 24 Wistar стаорци, по претходно утврдени параметри. За таа цел со дијамантски борер под водено ладење испрепариравме 48 кавитети В класа. Кавитетите ги поделивме во

4 групи. Во првата група кавитети врз дентинот аплициравме Фуџи Лининг ЛЦ, кај втората група Фуџи IX, кај третата група Приме&Бонд а кај четвртата група аплициравме Г бонд. Потоа сите кавитети беа затворени со светлосно полимеризирачки течен композит. Седум дена по апликација на материјалот по 3 стаорци од секоја група беа жртвувани, а третираните заби беа извадени и аплицирани во Остеомол. По вадење на забите од фиксативот вршена е стандардна постапка за изработка на препарати за хистолошка анализа. Препаратите беа евалуирани со светлосен микроскоп поврзан со видео камера. Триесет дена по апликацијата на материјалот и со останатите заби беше повторена истата постапка. Резултатите од нашето испитување покажаа присуство на слаба воспалителна реакција на пулпното ткиво кај забите реставрирани со Фуџи IX и Фуџи Лининг ЛЦ. Слаба до умерена инфламаторна реакција на пулпното ткиво беше забележана кај забите реставрирани со Г бонд, додека Приме&Бонд покажа најјак токсичен ефект кон пулпното ткиво. По триесет дена кај ниеден од примероците не забележавме појава на воспалителна реакција на пулпното ткиво.

Клучни зборови: биокомпатибилност, дентин атхезиви, глас јономер цементи, реакција на пулпата.

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