UDC: 615.463:616.314-74]:616.311-076

BIOCOMPATIBILITY OF TWO DIFFERENT RESTORATIVE MATERIALS USED IN PAEDIATRIC DENTISTRY

КОМПАРАТИВНА ХИСТОЛОШКА АНАЛИЗА НА БИОКОМПАТИБИЛНОСТА НА ДВА РЕСТАВРАТИВНИ МАТЕРИЈАЛИ

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Abstract

The purpose of our investigation was to make a comparative histopathological analysis of two different restorative materials used in paediatric dentistry. Eighteen male albino rats (Wistar) weighing 200-250 mg were used in this study. Tested material was freshly prepared as advised by the manufacturer and placed in a polyethylene tube. For material implantation, the dorsal skins of the animals were shaved under ketamine (25 mg/kg) anesthesia and disinfected with 5% iodine solution. Three incisions were made on the back of each animal, on the dorsal surface of the front limbs and on the dorsal pelvic area. Each animal received one tube filled by glass ionomer cement and a compomer. For control purposes, empty polyethylene tubes closed from both sides by heat were implanted on the dorsal surface of the left back limb. The histopathological evaluations were performed 1 week, 3 weeks and 45days post implantation. At each period, the rats were sacrificed by anesthetic overdose; the tubes and surrounding tissues were removed by tissue dissection technique and fixed in 10% buffered formalin at pH 7.0. Comparative histopathological analysis were made. One week post implantation at the control and experimental group, microscopic examination revealed the strongest inflammatory reaction despite another three examining periods. All materials in current use are considered acceptable, in terms of their biocompatibility with local tissues, when properly handled and placed.

Апстракт

Цел на нашето истражување беше да ја споредиме хистокомпатибилноста на гласјономерните и компомерните реставративни материјали. Во студијата беа вклучени 18 машки стаорци од видот Wistar со тежина од 200-250g (±20 g) стари 20-24 недели. На грбниот дел од стаорците (субскапуларно) билатерално се обележани 3 оперативни полиња, лево и десно од медијалната линија. Во едното оперативно поле, по соодветната препарација, беше поставен гласјоноер цемент, по соодветната подготовка (според упатството од производителот) и внесен во полиетиленски тубички со должина од 5мм и дијаметар од 3 мм. Во другото оперативно поле се поставува компомерен реставративен материјал и во третото оперативно поле се поставуваат празни полиетиленски тубички со истите димензии, како контролната група. Стаорците се делат во 3 групи, по 6 примероци. Истите се жртвувани по 7, 21 и 45 дена, соодветно по група и е земен примерок од ткивото од местото на имплантацијата. Од земените примероци се изготвуваат хистопатолошки анализи. Резултатите се статистички обработени во статистичката програма STATISTICA 7.1 for Windows. **Резултатите** добиени во нашата студија говорат за појава на ткивна реакција и кај двете групи, поголема кај експерименталната во однос на контролната група. Најбурна ткивна реакција се забележува во првите 7 дена по имплантацијата. Во однос на биокомпатибилноста на гласјономер цементите и компомерите како реставративни средства, применети во нашето истражување, можеме да укажеме на потребата од правилна манипулација од страна на стоматолозите и строго придржување до упатствата за употреба од производителите.

Introduction

Biocompatibility refers to how well the material coexists with the biological equilibrium of the tooth and body systems. Since fillings are in close contact with mucosa, tooth and pulp, biocompatibility is very important, especially in paediatric dentistry. Common problems with some of the current dental materials include chemical leakage from the material, pulpal irritation and less commonly allergy.

To accommodate the bioactive dimension of materials we can use The Williams Dictionary of Biomaterials

which updates the original definition of biocompatibility: "ability of a biomaterial to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most appropriate beneficial cellular or tissue response to that specific situation, and optimizing the clinically relevant performance of that therapy"[1].

Restorative dental biomaterials are designed to recover the shape and the function of the teeth, to protect the pulp tissue and to create adhesion between the tooth surface and the restorative material. Dental materials should not be toxic, irritating or corrosive, and should be easy to use[2].

GJC are "bioactive" materials due to ion exchange with the host, depending on the tissue which reacts, causing a positive response of the host. The term "glass-ionomer" has traditionally been applied to that group of materials which undergo setting through an acid-base reaction between an ion-leachable glass powder and a water-soluble polymeric acid such as the poly-acrylic acid. The traditional (conventional) glass-ionomers are characterized by properties such as brittleness, adhesion and fluoride release[3,4]. Resin-modified glass-ionomers are dental restorative materials of the glass-ionomer family. In addition to the aforementioned components, they contain organic monomers, typically 2-hydroxyethyl methacrylate (HEMA) and an associated initiator system[5,6].

Biocompatibility of GJC is monitored in terms of their cytotoxicity against the cells of the pulp, as assessed by the MTT test. Studies have shown that the concentration of ions and the Sr^{2+}, Al^{3+} and F- are too low to cause any cytotoxic effect. However, it comes from the release of HEMA, which is thought to compromise the biocompatibility of GJC modified resin. Negative biological effects of HEMA include cytotoxicity, induction of apoptosis, persistent inflammation, respiratory problems, allergies and contact dermatitis. It is clear that these kinds of negative effects are possible, although dental literature found very little information about this kind of negative effects in their clinical application[7].

Glass ionomer cements are, in general, cytotoxic shortly after mixing (low pH, between 0.9 to 1.6) with decreasing toxicity as setting occurred. The presence of dentin between filling material and cells (pulp) significantly reduces the toxicity of glass ionomer cements. Conventional glass ionomer cements were not cytotoxic in a dentin barrier test using three-dimensional cultures (Fig. 6.9). Obviously, dentin may act as an acid buffer and as an absorption medium for fluorides[8].

Compomers or polyacid-modified resin-based composites are chemically closely related to resin-based composites and GJC, consisting of filler particles and an organic matrix. The filler (a radiopaque, fluoride-containing silicate glass) comprises approximately of 72 wt.% and contains about 13 wt.% fluoride. UDMA (urethandimethacrylate) is used as base monomer together with a special (acidic) monomer with polymerizable acrylate residues and carboxyl groups (trichlorobenzene). Polymerization is initiated by light irradiation. Additions of cetylamine hydrofluoride are intended to increase fluoride release. The material is applied in combination with an adhesive. Compomers are also used for luting inlays, crowns, and bridges. These materials are autopolymerizing[9,10].

Setting of compomers is primarily caused by a polymerization, whereas the acid-base reaction of the carboxyl group, including components of the glass fillers, is of only secondary importance. Thus, the contribution of this acid-base reaction to the entire setting is considered minor (no setting of compomers in the dark!)[11].

The aim of our investigation was to examine histopathologically the biocompatibility of two different restorative materials used in children: Dyract® eXtra and GC Fuji VIII GP.

Material and methods

Materials and manufacture of specimens

For the evaluation of the histological response of the rats'' tissue, two different restorative dental materials were used: compomer Dyract® eXtra and resin-modified GIC, GC Fuji VIII GP (Table 1.).

Experimental animals and implantation procedure

Eighteen male albino rats (Wistar) weighting 200-250 mg were used in this study. Tested material was freshly prepared as advised by the manufacturer and placed in a polyethylene tube (5mm long/3 mm internal diameter). For material implantation, the dorsal skins of the animals were shaved under ketamine (25 mg/kg) anesthesia and disinfected with 5% iodine solution. Three incisions were made on the back of each animal, on the dorsal surface of the front limbs and on the dorsal pelvic area. Each animal received one tube filled by glass ionomer cement and a compomer. For control purposes, empty polyethylene tubes closed from both sides by heat were implanted on the dorsal surface of the left back limb. The histological evaluations were performed 1 week, 3 weeks and 45 days post implantation.

At each period, the rats were sacrificed by anesthetic overdose; the tubes and surrounding tissues were removed by tissue dissection technique and fixed in 10% buffered formalin at pH 7.0. Comparative histological analysis were made.

Table 1. Examined restorative dental materials

Dental material	Name of the material	Manufacturer	Ingredients (compositions)
Compomer	Dyract® eXtra The caries preventive restorative	DENTSPLAY De Trey GmbH Konstanz, Germany	• Urethane dimethacrilate (UDMA) • Carboxylic acid modified dimethacrilate (TCB resin) • Triethylenglicol dimethacrylate (TEGDMA) • Trimethharylate resin • Camphorquinone • Ethyl-4-dimethylaminobenzoate • Butylated hydroxy toluene (BHT) • UV stabiliser • Strontium-Alumino-sodium-fluoro-phosphor-silicate glass • Highly dispersed silicon dioxide • Strontium fluoride • Iron oxide and titanium dioxide pigments
Glass ionomer cement	GC Fuji VIII GP	GC DENTAL PRODUCT CORP. Torimatisu-Cho, Kasugai, Aichi, Japan	Powder • Alumino-silicate glass Liquid • 2-hydroxyethyl methacrylate (HEMA) • Polyacrylic acid • Urethane Dimethacrylate (UDMA) • Distilled water
Control group (empty polyethylene tube)			

Specimen preparation and criteria of histological response evaluation

Following formalin fixation, specimens were routinely processed and embedded in paraffin wax, serially sectioned at a setting of 5 μm, and stained with hematoxylineosin. From each tissue sample, 5 sections presenting the greatest inflammatory reaction were examined with a light microscope. The materials were sign like:

1. Compomer
2. Glass ionomer cement
3. Control

The preparations were analysed by the tissue inflammatory reaction, degree of blood vessels dilatation, fibrosis and presence or absence of giant cells by pathological criteria: 1 (no response), 2 (mild reaction), 3 (moderate reaction), 4 (strong reaction).

The areas of inflammatory reaction, fibrosis and presence or absence of giant cells were evaluated quantitatively and the number of inflammatory cells was scored as:

- 1 (no response),
- 2 (mild reaction),
- 3 (moderate reaction),
- 4 (strong reaction).

The type of inflammatory cells (neutrophils, lymphocytes, macrophages, mast cells and giant cells) was determined.

Fibrous capsule, necrosis and formation of calcification were recorded as present or absent.

Statistical analysis

Statistical evaluation of tissue response to different dental materials, the compomer Dyract® eXtra and the resin-modified GIC, GC Fuji VIII GP, were performed by the statistical software SPSS for Windows. Results were statistically analyzed using ANOVA by ranks analysis (Kruskal-Wallis-test). Differences between groups were statistically analyzed using the Tukey NSD test.

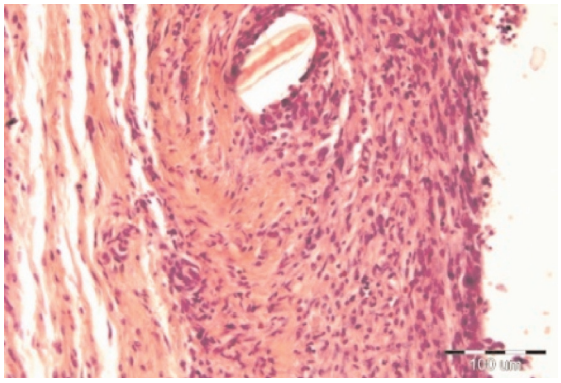
Results

The results obtained in our study show the occurrence of tissue response in all groups, higher in the experimental compared to the control group (Table 2). The strongest tissue reaction was observed in the first seven days after implementation.

Dyract® eXtra

One week post implantation of the compomer (Dyract® eXtra, DENTSPLAY De Trey GmbH Konstanz, Germany), microscopic examination revealed moderate inflammatory reaction (mixed type), the presence of lymphocytes, polymorphs and observed eosinophils, which suggest the possible allergic reaction (Fig. 1 and 2). There is a weak vasodilation, low and moderate fibrosis and occurrence of giant cells.

Exactly 21 days after implantation, a decrease of inflammatory reaction was observed with mild fibrosis (Fig. 3).



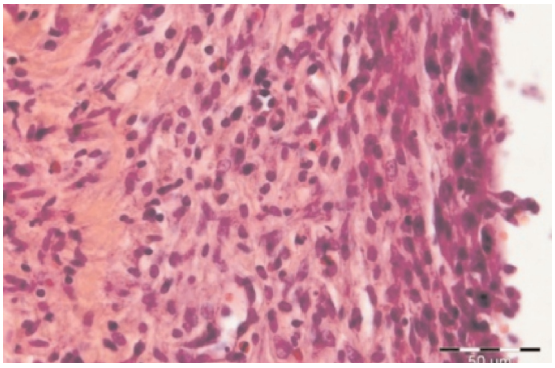


Figure 1./ Figure 2. *Tissue inflammatory reaction one week post implantation of the compomer*

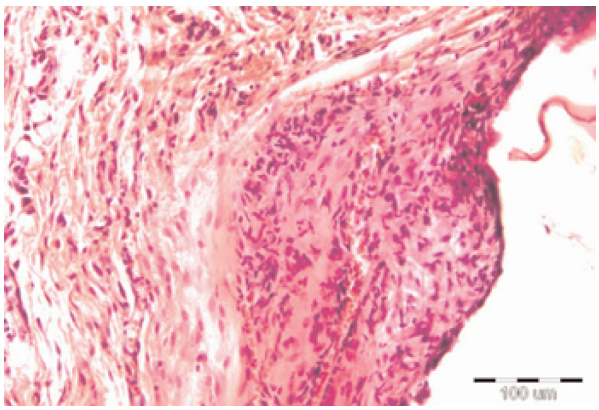


Figure 3. *Tissue inflammatory reaction 21 days after implantation of the compomer*

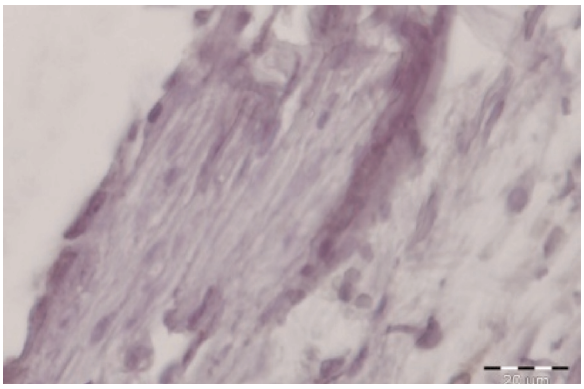
After 45 days of the implantation of the compomer, microscopic examination revealed that there are no serious changes, except moderate to strong fibrosis with calcification and ossification (Fig 4 and 5).

GC Fuji VIII GP

Microscopic examination of the tissue reaction to GC Fuji VIII GP, after one week, revealed a strong neovascularization with occurrence of mild fibrosis, moderate to strong inflammatory cell infiltration, mainly lympho-

Table 2. Tissue inflammatory reaction, degree of blood vessels dilatation, fibrosis and presence or absence of giant cells, necrosis and formation of calcification in the control and experimental groups

Parametar	Blood vesels dilatation			Inflamation			Fibrosis			Giant cells		
Time	7 days	21 days	45 days	7 days	21 days	45 days	7 days	21 days	45 days	7 days	21 days	45 days
1. COMPOMER	2	1	1	3	1	1	2	3	3	1	1	1
2. GJC	3	1	1	4	3	1	2	2	1	1	1	1
3. CONTROL	2	1	1	2	1	2	2	1	1	4	3	1



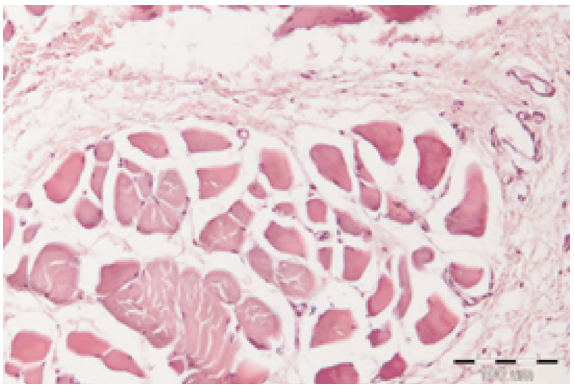


Figure 4./ Figure 5. *Tissue inflammatory reaction 45 days after implantation of the compomer*

cytes (Fig. 6). This is consistent with the results of Souza et al. who think that tissue reaction is caused by the presence of HEMA in the dental material.

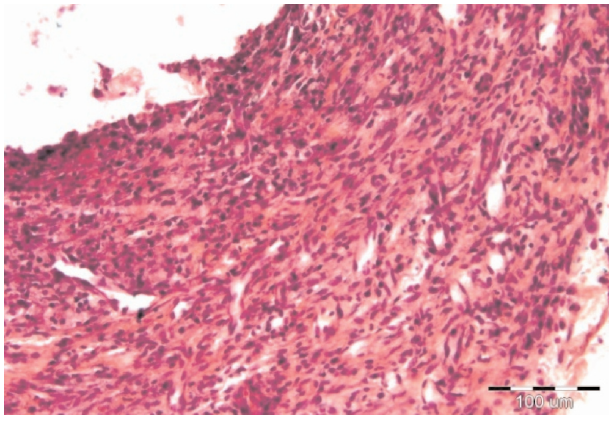


Figure 6. *Tissue inflammatory reaction one week post implantation of glass ionomer cement*

Precisely 21 days after material implantation (GIC), the inflammatory reaction became moderate (decrease), with the presence of calcifications (Fig. 7).

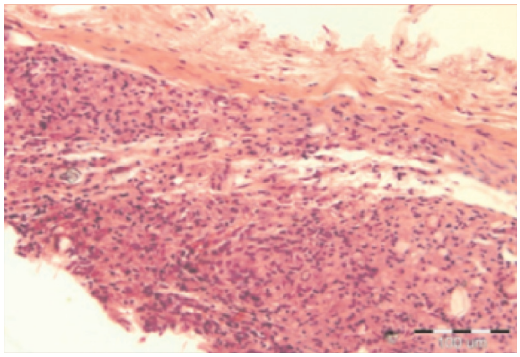


Figure 7. *Tissue inflammatory reaction 21 days post implantation of glass ionomer cement*

At the 45th day of the observation period, inflammatory cells were almost absent with presence of stromal edema (Fig. 8).

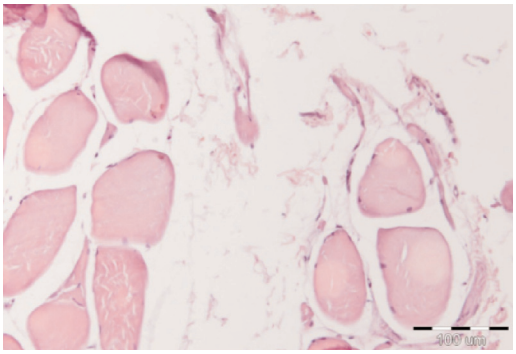


Figure 8. *Tissue inflammatory reaction 45 days post implantation of glass ionomer cement*

Control group (empty polyethylene tube)

One week post implantation of an empty polyethylene tube, microscopic examination revealed a small initial concentration of inflammatory cells in the subcutaneous tissue adjacent to the control, mild vasodilation, quite discreet fibrosis and plenty of giant cells (reaction type foreign body)(Fig. 9).

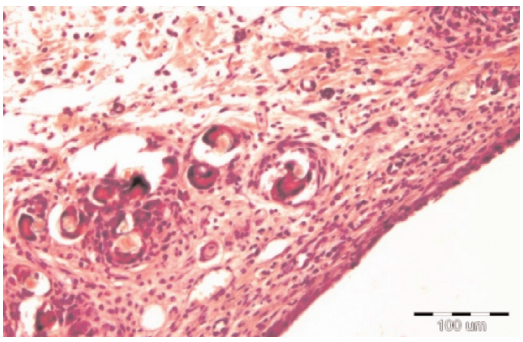


Figure 9. *Tissue inflammatory reaction in the control group one week post implantation*

This reaction quickly calmed down at the 21st day of material implantation, with presence of a thin fibrous capsule that surrounds the tube with a moderate colony of giant cells (Fig. 10).

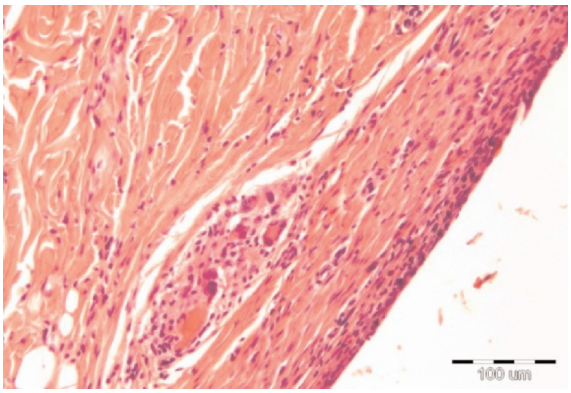
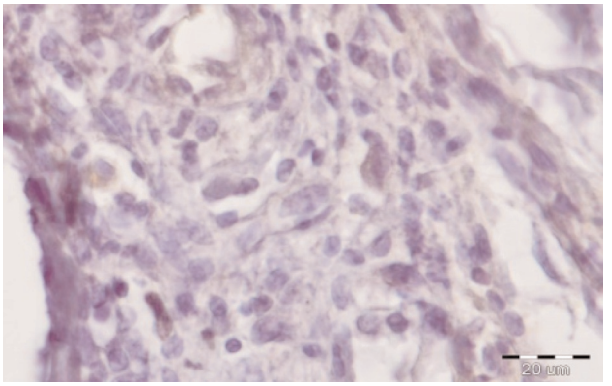


Figure 10. *Tissue inflammatory reaction in control group at the 21st day post implantation*

On the 45th day of the observation period there were no serious changes; all parameters were with response 1 (no response). (Fig. 11 and 12)



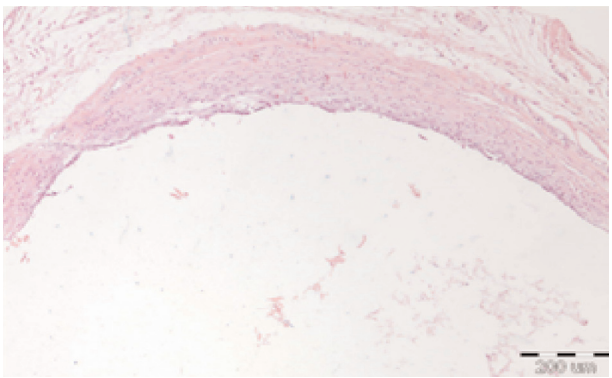


Figure 11. / ***Figure 12.*** *Tissue inflammatory reaction in the control group on the 45st day post implantation*

ANOVA by ranks (Kruskal-Wallis-test) indicate a statistically significant ($p<0.05$) relationship between the degree of inflammation, after 7 days of implementation, and type of the material, which means that there is a statically significant difference between the type of material and thedegree of inflammation in the first 7 days (Table 3.).

Table 3. Results of the Kruskal-Wallis-test for inflammation reaction after 7 days post implantation of the materials

Depend: Inflammation	Kruskal-Wallis ANOVA by Ranks; Inflammation (7 days) Independent (grouping) variable: Material Kruskal-Wallis-test: H(2,N=18)=10,60354 p=0,0050			
	Code	Valid N	Sum of Ranks	Mean Rank
1	1	6	59,50000	9,91667
2	2	6	84,00000	14,00000
3	3	6	27,50000	4,58333

The Tukey HSD test accurately defined the differences between the groups and showed a statistically significant difference only in the 3rd group (control group) compared to the other two groups of materials, compomer and GIC. There was no statistically significant difference between the compomer and GIC (Fig. 13).

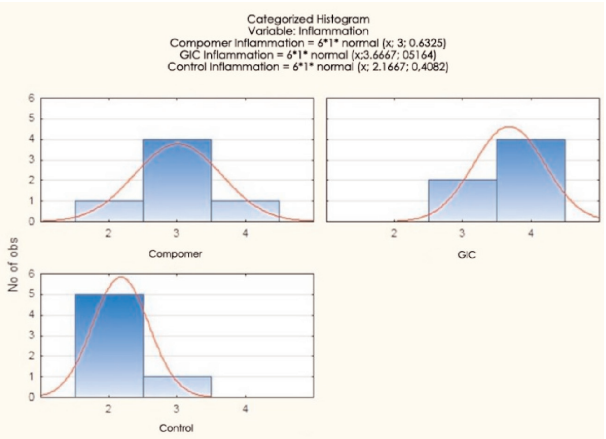


Figure 13. *Categorized histogram for inflammation 7 days post implantation period of the materials*

The inflammatory reaction, at the control group, in the first seven days, was around 2 (mild inflammation)

Table 4. Results of the Tukey HSD test for inflammation 7 days post implantation period of the materials

Material		Unequal N HSD; Variable: inflammation (7 days) Marked differences are significant at p<0,05000		
		{1} M=3,0000	{2} M=3,6667	{3} M=2,1667
1	{1}		0,105398	*0,038318*
2	{2}	0,105398		*0,000637*
3	{3}	*0,038318*	*0,000637*	

while at the other two groups of materials it iwas between 3 and 4 (moderate to severe inflammation) (Tab.4, Fig. 14).

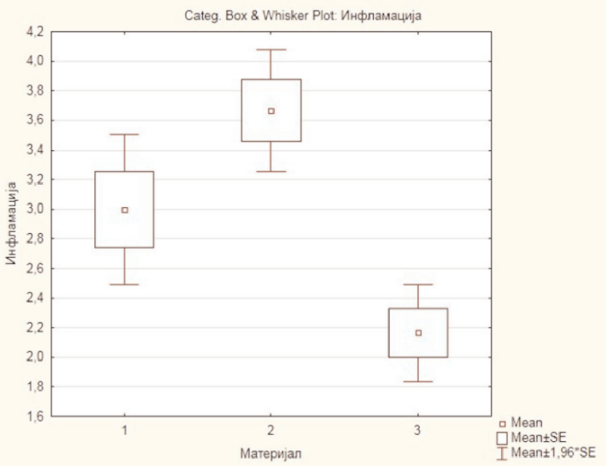


Figure 14. *Results of the Tukey HSD test for inflammation 7 days post implantation period of the materials*

Inflammation 21 days

ANOVA by ranks (Kruskal-Wallis-test) was used to exam the relationship between tissue inflammation and the type of material after 21 days of the implementation. The results indicate that there is a statistically significant ($p<0.05$) difference between the degree of inflammation in different types of materials after 21 days of implantation (Tab.5).

Table 5. Results of the Kruskal-Wallis-test for inflammation reaction after 21 days post implantation of the materials

Depend: Inflammation	Kruskal-Wallis ANOVA by Ranks; Inflammation (21 days) Independent (grouping) variable: Material Kruskal-Wallis-test: H(2,N=18)=10,64074 p=0,0049			
	Code	Valid N	Sum of Ranks	Mean Rank
1	1	6	44,00000	7,33333
2	2	6	89,50000	14,91667
3	3	6	37,50000	6,25000

Tukey HSD test shows a statistically significant difference only in the 2nd group (GIC) in relation to the other two groups of materials. Between the control material and the compomer there is no statistically significant difference (Table 6).

On the 21st day post implantation period, inflammation reaction around the GIC is moderate (3) while in the other two groups it varies between no response (1) and mild reaction (2) (Fig. 15).

Table 6. Results of the Tukey HSD test for inflammation 21 days post implantation period of the materials

Material		Tukry HSD test; Variable: inflammation (21 days) Marked differences are significant at p<0,05000		
		{1} M=1,6667	{2} M=3,0000	{3} M=1,5000
1	{1}		0,002846	0,868421
2	{2}	0,002846		0,001120
3	{3}	0,868421	0,001120	

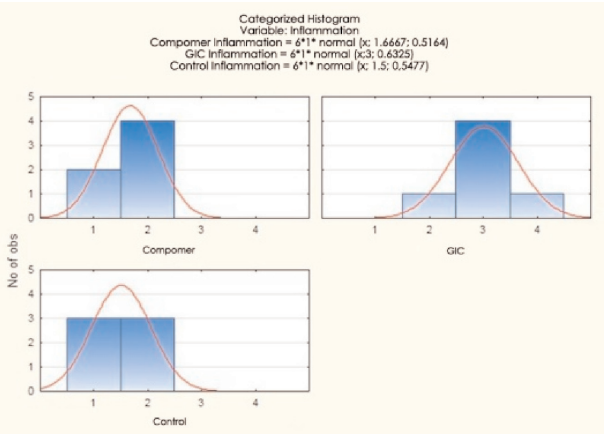


Figure 15. *Categorized histogram for inflammation 21 days post implantation period of the materials*

According to the Kruskal-Wallis-test there is a statistically significant relationship between the degree of vascular dilatation, depending on the type of material in the first 7 days after implantation (Table 7).

Table 7. Results of the Kruskal-Wallis-test for vascular dilatation reaction after 7 days post implantation of the materials

Depend: Dilatation KC	Kruskal-Wallis ANOVA by Ranks; Dilatation KC (7 days) Independent (grouping) variable: Material Kruskal-Wallis-test: H(2,N=18)=7,990408 p=0,0184			
	Code	Valid N	Sum of Ranks	Mean Rank
1	1	6	43,00000	7,16667
2	2	6	85,00000	14,16667
3	3	6	43,00000	7,16667

The Tukey HSD test accurately defines between which groups the difference in dilatation exist. It is evident, from Table 8 that no significant difference in dilatation exists between the first and the third group of materials, compomer and control group, in the first 7

days. Statistically significant dilation differs in GJC versus the other two materials.

Table 8. Results of the Tukey HSD test for vascular dilatation reaction 7 days post implantation period of the materials

Material		Tukry HSD test; Variable: Dilatation KC (7 days) Marked differences are significant at p<0,05000		
		{1} M=2,0000	{2} M=3,3333	{3} M=2,0000
1	{1}		*0,012739*	1,000000
2	{2}	*0,012739*		*0,012739*
3	{3}	1,000000	*0,012739*	

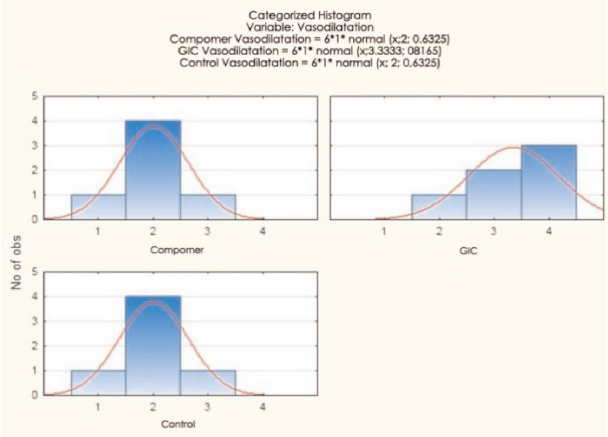


Figure 16. *Categorized histogram for vascular dilatation reaction 7 days post implantation period of the materials*

Results of the Kruskal-Wallis-test for the dilatation of blood vessels and the type of material, after 21 days of implantation of the materials, shows that there is no statistically significant relationship (Table 9).

Table 9. Results of the Kruskal-Wallis-test for vascular dilatation reaction after 21 days post implantation of the materials

Depend: Inflammation	Kruskal-Wallis ANOVA by Ranks; Dilatation KC (21 days) Independent (grouping) variable: Material Kruskal-Wallis test: H(2,N=18)=1,416667 p=0,4925			
	Code	Valid N	Sum of Ranks	Mean Rank
1	1	6	57,00000	9,50000
2	2	6	66,00000	11,00000
3	3	6	48,00000	8,00000

On the 21st day post implantation period of the materials the inflammatory response of the tissue calms down which indicates the decrease of the vascular response of the tissue (Fig. 17).

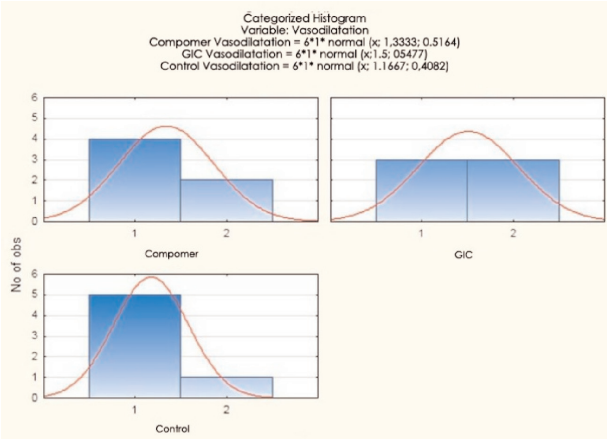


Figure 17. *Categorized histogram for vascular dilatation reaction 21 days post implantation period of the materials*

After seven days observation period, results of the Kruskal-Wallis-test indicated that there was no relationship between fibrosis and the implanted materials in the first 7 days (Table 10).

Table 10. Results of the Kruskal-Wallis-test of tissue fibrosis after 7 days post implantation period of the materials

Depend: Fibrosis	Kruskal-Wallis ANOVA by Ranks; Fibrosis (7 days) Independent (grouping) variable: Material Kruskal-Wallis test: H(2,N=18)=5,059524 p=0,0797			
	Code	Valid N	Sum of Ranks	Mean Rank
1	1	6	67,00000	11,16667
2	2	6	67,00000	11,16667
3	3	6	37,00000	6,16667

Wavy collagen fiber deposits were noted and everywhere there was mild fibrosis present (2) (Fig. 18).

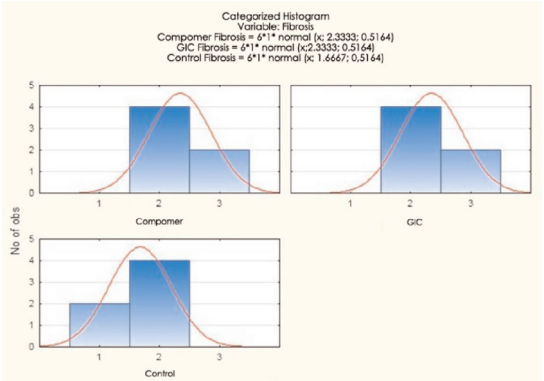


Figure 18. *Categorized histogram of tissue fibrosis after 7 days post implantation period of the materials*

There is a statistical relationship between the degree of fibrosis and the type of implemented material. This signifies that there is a statistically significant difference ($p<0.05$) in the extent of tissue fibrosis in different types of materials after 21 days of implantation (Table 11).

Table 11. Results of the Kruskal-Wallis-test of tissue fibrosis after 21 days post implantation period of the materials

Depend: Fibrosis	Kruskal-Wallis ANOVA by Ranks; Fibrosis (21 days) Independent (grouping) variable: Material Kruskal-Wallis test: H(2,N=18)=7,870370 p=0,0195			
	Code	Valid N	Sum of Ranks	Mean Rank
1	1	6	82,00000	13,66667
2	2	6	54,50000	9,08333
3	3	6	34,50000	5,75000

According to the Tukey HSD test, which accurately defines between which groups the difference exists, statistically significant was the difference in tissue fibrosis between the compomer and the control group (Table 12).

Table 12. Results of the Tukey HSD test for tissue fibrosis reaction after 21 days post implantation of the materials

Material		Tukey HSD test; Variable: Fibrosis (21 days) Marked differences are significant at p<0,05000		
		{1} M=2,6667	{2} M=2,0000	{3} M=1,5000
1	{1}		0,138272	*0,007666*
2	{2}	0,138272		0,307518
3	{3}	*0,007666*	0,307518	

The tissue fibrosis reaction at the compomer ranges between mild to moderate (2-3), at the control, empty polyethylene tube, it varies between no response and mild response and at the GIC the degree is that of mild reaction (Fig. 19).

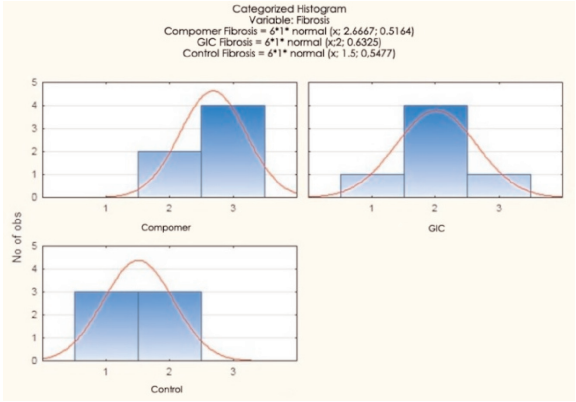


Figure 19. *Categorized histogram of tissue fibrosis after 21 days post implantation period of the materials*

Analysis of variance ranking (ANOVA by ranks), Kruskal-Wallis test, shows the dependence between the amount of giant cells and the type of material, after 7 days observation period. There is a relationship between the amount of giant cells in the tissue around the implemented dental materials and the type of the dental material, statistically significant difference ($p<0.05$).

Table 13. Results of the Kruskal-Wallis for the amount of giant cells in the tissue around the implemented dental materials and the type of the dental material after 7 days post implantation period of the materials

Depend: Giant cells	Kruskal-Wallis ANOVA by Ranks; Giant cells (7 days) Independent (grouping) variable: Material Kruskal-Wallis-test: H(2,N=18)=13,18038 p=0,0014			
	Code	Valid N	Sum of Ranks	Mean Rank
1	1	6	39,50000	6,58333
2	2	6	39,50000	6,58333
3	3	6	92,00000	15,33333

Tukey HSD shows that there is a statistically significant difference between the amount of giant cells only around the 3rd material (control, empty polyethylene tube) compared to the other two materials (Table 14).

Table 14. Results of the Tukey HSD test for the amount of giant cells in the tissue around the implemented dental materials and the type of the dental material after 7 days post implantation period of the materials

Material		Tukey HSD test; Variable: Giant cells (7 days) Marked differences are significant at p<0,05000		
		{1} M=1,1667	{2} M=1,1667	{3} M=3,5000
1	{1}		1,000000	*0,000186*
2	{2}	1,000000		*0,000186*
3	{3}	*0,000186*	*0,000186*	

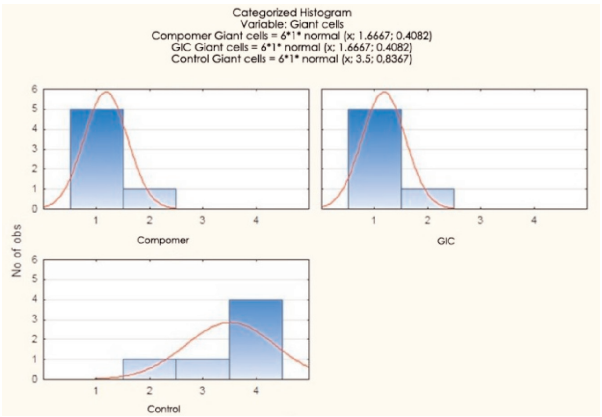


Figure 20. *Categorized histogram of the amount of giant cells in the tissue around the implemented dental materials and the type of the dental material after 7 days post implantation period of the materials*

According to the Kruskal-Wallis test, there is a relationship between the amount of giant cells in the tissue around the implemented dental materials and the type of the dental material after 21 days with statistically significant difference ($p < 0.05$).

Table 15. Results of the Kruskal-Wallis for the amount of giant cells in the tissue around the implemented dental materials and the type of the dental material after 21 days post implantation period of the materials

Depend: Giant cells	Kruskal-Wallis ANOVA by Ranks; Giant cells (21 days) Independent (grouping) variable: Material Kruskal-Wallis-test: H(2,N=18)=11,47171 p=0,0032			
	Code	Valid N	Sum of Ranks	Mean Rank
1	1	6	37,00000	6,16667
2	2	6	44,00000	7,33333
3	3	6	90,00000	15,00000

Tukey HSD indicates that there is a statistically significant difference between the amount of giant cells present in the tissue around the implemented dental materials only in the 3rd material (control) compared to the other two materials (Table 16).

Table 16. Results of the Tukey HSD test for the amount of giant cells in the tissue around the implemented dental materials and the type of the dental material after 21 days post implantation period of the materials

Material		Tukey HSD test; Variable: Giant cells (21 days) Marked differences are significant at p<0,05000		
		{1} M=1,1667	{2} M=1,3333	{3} M=2,6667
1	{1}		0,823575	*0,000361*
2	{2}	0,823575		*0,000799*
3	{3}	*0,000361*	*0,000799*	

Around the compomer and GIC, after 21 days of implementation of the materials, the giants cells are almost gone, while the control still gives moderate response (Fig. 21).

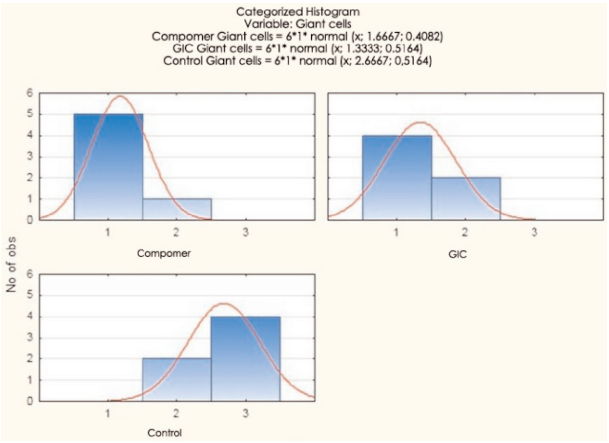


Figure 21. *Categorized histogram of the amount of giant cells in the tissue around the implemented dental materials and the type of the dental material after 21 days post implantation period of the materials*

Discussion

Dental restorative materials can affect oral health in several ways: by components that are soluble in water and reach saliva and oral media in general, and through direct interaction with pulp, gingiva or periodontal bridle[12].

Therefore, selection and evaluation of any material or device intended for use on humans requires structural assessment in four stages: toxicity (cell culture), local tissue irritation (implantation in animals), preclinical (animal tests) and clinical evaluation (testing on patients)[13].

There is no proof that glass ionomer cements cause systemic toxicity. Only Mjor reported an outbreak of generalized urticaria after application of glass ionomer cements. The main reason for this reaction is thought to be HEMA (known allergen)[14].

Microscopic examination of the tissue reaction to GC Fuji VIII GP, after one week, revealed a strong neovascularization with occurrence of mild fibrosis, moderate to strong inflammatory cell infiltration, mainly lymphocytes (Fig. 6). This is consistent with the results of Souza

et al. who think that tissue reaction is caused by the presence of HEMA in the dental material[15].

Nicholson and Czarnecka, 2006 considered that resin-modified glass-ionomers can't be biocompatible as conventional glass ionomer cements because of the presence of 2-hydroxyethyl methacrylate (HEMA) which has harmful biological properties and is known to be part of these restorative materials[9].

Exactly 21 days after material implantation (GIC), the inflammatory reaction, in our study, became moderate (decrease), with the presence of calcifications (Fig. 7) and at the 45th day of the observation period, inflammatory cells were almost absent with presence of stromal edema (Fig. 8).

Several studies have revealed various adverse biological effects caused by the HEMA ingredient in dental materials. The study of Buoillaguet et al. (2000) clearly shows that extremely small amounts of HEMA are capable of causing disorder of cells function, inhibiting growth and reducing mitochondrial activity by 60-80%[16].

Experiments of Becher et al, 2006 showed that HEMA causes apoptotic death in peripheral blood mononuclear cells. Moreover, Schweikl et al, 2006 show that micronuclei develop in cells affected by HEMA and TEGDMA, and that HEMA causes damage to chromosomes and errors in DNA molecules[17].

One week post implantation of an empty polyethylene tube, microscopic examination revealed a small initial concentration of inflammatory cells in the subcutaneous tissue adjacent to the control, mild vasodilation, quite discreet fibrosis and plenty of giant cells (reaction type foreign body) (Fig. 9). This reaction quickly calmed down on the 21st day of material implantation, with presence of a thin fibrous capsule that surrounded the tube with a moderate colony of giant cells (Fig. 10). On the 45th day of the observation period there were no serious changes; all parameters were with 1 response (Fig. 11 and 12).

Yaltirik et al, 200418 and Zmener 200419 reported initial low concentration of inflammatory cells in the subcutaneous tissue adjacent to the control. This reaction quickly appeased and over time, a delicate fibrous capsule surrounded the tissue reaction. This reaction was probably the result of surgical trauma of implantation. However, microscopic analysis made by Batista et al, 200720 showed that the empty polyethylene tubes do not cause an inflammatory reaction.

No evidence of necrosis could be detected in all groups throughout the experiment except at the group of GJC were we found presence of calcifications.

The reactions observed in our study represent a preliminary stage in evaluating the potential of irritating abilities of restorative materials investigated. They cause different inflammatory reactions depending on the time of implantation. However, better tolerated by the tissue itself is GJC despite the stormy reaction in the one week period after inoculation of the material and the presence of calcifications associated with pulp tissue proper defense response.

Conclusions:

- The results obtained in our study show occurrence of tissue reaction in both, control and experimental group, depending on the time of implantation of the restorative material. The strongest tissue reaction was observed in the first week after implantation, which was gradually reduced until the 21st and the 45th day.
- The reactions observed in our study represent a preliminary stage in evaluating the potential of irritating abilities of restorative materials. They cause different inflammatory reactions depending on the time of implantation.
- However, GIC are better tolerated by the tissue despite the strong reaction on the 21st day of the period after implantation and the presence of calcifications.
- After 45 days of observation, every parameter was with value 1- no response, only mild inflammatory reaction at the control, and moderate to strong fibrosis with calcification after compomer implantation.
- We advise dentists to perform proper manipulation and usage of dental restorative materials guided by the strict manufacturers recommendations.

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