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Original research

Biocompatibility of different resin composites after polymerization with two light curing units: an immunohistochemical study

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Purpose
The aim of this study is to compare the biocompatibility of two different resin composites after polymerization under two different light sources in three different time periods.

Materials and Methods
72 polyethylene tubes polymerized with 2 different resin composites and 2 different light sources (Elipar S10 and Valo) [Group 1: Kalore Elipar S10 (KE), Group 2: Kalore Valo (KV), Group 3: Essentia Elipar S10 (EE), Group 4: Essentia Valo (EV)] were implanted in the dorsal connective tissue of 18 rats. 24 empty polyethylene tubes [Group 5: (Control group)] were implanted in the dorsal connective tissue of 6 rats. Then, the rats were sacrificed after 7th, 15th and 30th days in each time intervals (n=8). Biopsy samples were stained with H&E and examined for inflammation, necrosis, macrophage infiltrate, giant cell and fibrous capsule criteria. Immunohistochemical staining was performed to evaluate proinflammatory cytokines (IL-1β, IL-6 and IL-8).

Results
When the composite groups and the control groups were compared; there was difference statistically significant for the criteria of inflammation at 7th and 15th days, there was no statistical difference between the time points in terms of fibrous capsule and necrosis. When the composite groups and control groups were evaluated in terms of proinflammatory cytokines; statistically significant differences were found at 7th, 15th and 30th days.

Conclusion
All CRs used in this study showed acceptable biocompatibility in the subcutaneous tissues of rats after polymerization with different light sources.

Keywords: *Composite resins, light sources, biocompatibility, rat, immunohistochemistry*

Introduction

The composite resins (CRs) are the most preferred materials due to their mechanical and optical properties for direct restorations in dentistry (1). CRs contain various organic monomers (BIS-GMA, UDMA, HEMA, TEGDMA) at different concentrations (2, 3). After the polymerization of these monomers, residual monomers pass into the dentinal tubules, resulting in delayed pulpal healing, irreversible inflammatory reaction in the pulp, and insufficient dentin bridge formation (4, 5). It has been reported that the release of residual monomers, oligomers and reduced products can have a adverse effect on the biocompatibility of these materials. These monomers disrupt cell metabolism and can cause cytotoxic effects, allergic reactions and mutagenicity (6). It has been reported that CRs containing BIS-GMA, TEGDMA and UDMA are cytotoxic at the cellular level,

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and in addition the order of cytotoxicity for resin monomers is BIS-GMA>UDMA>TEGDMA>HEMA (7-9). For this reason, free BIS-GMA CRs have been produced in recent years to reduce the cytotoxicity of CRs.

Different types of light-curing units (LCUs), conventional quartz tungsten halogen (QTH) lights or lasers can also be used for the polymerization of light-curing restorative materials while light-emitting diodes (LEDs) are used more often today (10, 11). The polymerization of CRs is a critical parameter that affects both the optimal physical properties and biocompatibility of the material. Inadequate polymerization results in poor physical properties of the restoration, solubility in the oral environment, secondary caries and pulp irritation and increased microleakage. On the other hand, the amount of residual monomers may vary depending on the light source used for curing (12). As it is known, the degree of monomer polymer conversion is very important for good mechanical properties and biocompatibility. The degree of light-induced conversion of monomers to polymers is affected by the wavelength, intensity, irradiation time of light, concentrations, types and mixtures of photoinitiators, stabilizers and inhibitors, as well as types and proportions of monomers and fillers (13).

Biocompatibility can be defined as the non-toxic and physiologically non-reactive of a material or its compatibility with a living tissue or system (14). Since these materials are in direct contact with periapical tissues, alveolar bone, pulp and body fluids, biocompatibility is one of the basic conditions (15). The degree of conversion mainly determines the biocompatibility of composite resins, since this factor can determine the greater or lesser release of unpolymerized/residual monomers during curing processes (16). It has been shown that a decrease in the degree of monomer-polymer conversion can lead to a decrease in the physical-mechanical properties of the material and increase in the release of monomers into the oral environment (17).

The local response from the effect of the materials consists of an accumulation of inflammatory cells, primarily macrophages and giant cells (18). Macrophages are crucial for their capacity to engulf and process foreign body and are involved in the release of chemokines responsible for inflammatory cells (13, 19).

Cytokines are a broad category of relatively small proteins that are produced and released for the cell signaling purpose. At the beginning of the acute inflammatory process, monocytes reach the damaged tissue following neutrophils. Irritants in the environment cause the production of proinflammatory cytokines such as interleukin 1 beta (IL-1β), interleukin 6 (IL-6) and interleukin 8 (IL-8), and the release of histamines, prostaglandins and metalloproteinases. As a result of the decrease in the proinflammatory activities of macrophages, anti-inflammatory activity and tissue regeneration begin. Evaluation of the inflammatory reaction in biocompatibility tests is performed by histological methods, which give certain results of tissue response, as they are most commonly associated with immunohistochemical methods (19, 20).

In the light of this information, this study aims to evaluate the biocompatibility of BIS-GMA on rats on the 7th, 15th and 30th days after polymerization with different light sources. The null hypothesis of the this study could be stated as the composites free BIS-GMA would not differ in terms of biocompatibility at different time periods after polymerization with different light sources.

Materials and methods

Ethical approval

This study was carried out with the approval of Sivas Cumhuriyet University Animal Experiments Local Ethics Committee (2020/288).

Sample size estimation

Power analysis was performed to determine the sample size before the research. When α=0.05, β=0.20 (1-β)=0.80 was taken, it was decided to take 24 rat for a sample size and the power of the test was found to be p= 0.80837.

Experimental design

CRs and light sources used in this study are shown in the Table 1. In this study, 24 male Wistar albino rats weighing 200-220 g were used. 18 rats for the composite group and 6 rats for the control group were used since a maximum of 4 incisions can be made on the dorsal part of each rat. In this study, a total of 96 polyethylene tubes (PTs) (inner diameter 1.5 mm, 10 mm long) were kept in 96% alcohol for 20 minutes to ensure aseptic conditions. 24 empty PTs were determined as the control group. After filling 36 PTs with ESSENTIA composites using the incremental technique, 18 PTs were polymerized with the Elipar (EE) light source and 18 PTs with the Valo (EV) light source. Within the KALORE composite group, 36 PTs were filled with this expreimental group using the incremental technique, and 18 PTs were polymerized with the Elipar (KE) light source and 18 PTs with the Valo (KV)

Table 1: *Composite resins and light sources used in our study*

Material (Lot number)	Type	Manufacturer	Composition	
Kalore ™ 1906121	Dental Composite	GC, Corporation. Tokyo, Japan	Fluoro-aluminum-silicate glass, Prepolymerized filler, Silicon dioxide, UDMA, BIS-EMA	
Essentia ™ 1906131	Dental Composite	GC, Corporation. Tokyo, Japan	UDMA, BIS-EMA, BIS-GMA, TEGDMA, Barium glass, Prepolymerized filler, Silica	
			Light Intensity	**Wavelength**
Elipar S10	Light Sources	3M Espe	1200 mw/cm^2	430-480 nm
Valo	Light Sources	Ultradent	1600 mw/cm^2	385-515 nm

light source. All composite groups were polymerized from all surfaces for 20 seconds.

Surgical procedure

The mixture of 0.008 mL/100 g ketamine and 0.004 mL/100 g 2% xylazine hydrochloride (Rompun) was administered intramuscularly to the rats to provide anesthesia. The areas where the PT was planned to be placed before the incision were shaved and disinfected using 5% iodine solution. For the composite groups, 4 different incisions were made in the dorsal part of each rat, 2 on the shoulder and 2 on the waist, on both sides of the midline and at a distance of at least 20 mm from each other. After 1 cm incision was made in the upper left side, KE was placed in the upper left pocket, and 1 cm incision was made in the upper right pocket, and the KV was placed. Then, 1 cm incision was made at a distance of at least 20 mm below the left upper pocket, EE was placed in the lower left pocket, and in the lower right pocket, at a distance of at least 20 mm from the upper right pocket, and the EV was placed in the lower right pocket.

For the control group, 6 rats were used to equalize the sample size with the composite groups, and 3 incisions were made on the dorsal part of the rats. 3 incisions were made in the dorsal part of each rat, 2 mm in the shoulder and one waist region. Following implantation of PTs after blunt dissection for both the Composite and Control groups, the incisions were sutured with 3.0 silk sutures. During the 7th, 15th and 30th days of the experiment, 6 rats from the composite group and 2 rats from the control group were sacrificed with overdose anesthesia at each time interval. The dorsal part of the sacrificed rats were shaved and the locations of the PTs were determined by palpation and removed with intact connective tissue.

Light microscopy and immunohistochemical staining protocols

Biopsy samples were kept in 10% formalin solution for 24-48 hours. After fixation, the tissues were embedded in paraffin blocks and 5 µm sections were taken with a microtome (Leica Corp, Germany). Some of these sections were taken from normal slides for hematoxyleneosin (H&E) staining, and some of them were taken as positively charged slides for immunohistochemistry (IHC) staining. After the deparaffinization process, the preparations taken on normal slides were stained with H&E, and the preparations on positively charged slides were stained with DAB detection kit. Entellan was dripped onto the stained preparations and the closure was performed. The groups were evaluated under light microscope (Olympus Bx50) at 100, 200 and 400 magnifications. Histological criteria and scores used in the study are shown in Table 2.

Statistical analysis

The data obtained by the examination were imported to Statistical Package for Social Sciences (SPSS) for Windows software, version 22.0 (IBM SPSS Inc., Armonk, NY, USA). Shapiro - Wilk test was used for the normal distribution test. Accordingly, Kruskall Wallis test was used to compare independent groups, and Mann Whitney U test was used for pairwise comparisons. The Friedman test was used to compare dependent groups. Wilcoxon test was used for pairwise comparisons. The confidence interval was set to 95% and p values less than 0.05 were considered significant.

Table 2: *Histological and immunohistochemical criteria and scores.*

Criteria	Scores			
	0	**1**	**2**	**3**
Inflammation	No detected inflammatory cells	Less than 25 cells (mild)	Between 25 and 125 cells (moderate)	125 or more cells (severe)
Fibrous Capsule	Absent	Thin ≤150 µm	Thick ≥150 µm	
Macrophage Infiltrate	<10 cells	≥10- 20 cells	≥ 20 – 30 cells	>30 cells
Necrosis	Absent	Present		
Giant cell	Absent	Present		
IL-1β	Absent	Mild (<%10)	Moderate (%10-50)	Severe (>%50)
IL-6				
IL-8				

Results

Histological and immunohistochemistry findings

Data for different time periods are presented in Table 3 and Table 4.

Inflammatory cell response

When the comparison between the groups was made on the 7th, 15th and 30th days, the difference was statistically significant on the 7th and 15th days, but the difference was not significant on the 30th day. The highest inflammation value was observed on the 7th day in the EV group, while the lowest inflammation value was observed on the 30th day in the Control group (Figure 1). When the groups were evaluated statistically in three different time periods; the difference was statistically significant in all groups except the control and KE groups ($p<0.05$).

Fibrous capsule, giant cell, macrophage and necrosis

Fibrous capsule thickness was thin in all groups on day 7th, but an increase in capsule thickness was observed on days 15th and 30th. Although giant cell and macrophage infiltration was seen in all groups on the 7th day, it decreased on the 15th and 30th days. Necrosis was observed only on day 7th in all groups (Figure. 1). For fibrous capsule thickness; The difference was not statistically significant in all groups, when the groups were compared on the 7th, 15th and 30th days. When the groups are evaluated statistically in themselves in three different time periods; The difference was statistically significant in all groups except the EE group ($p<0.05$). In terms of giant cell; three was only a statistical difference was found on the 30th day, hen the comparison between the groups

Table 3: *Evaluation of the H&E criteria results for all groups on day 7th, 15th and 30th. In the horizontal column, the same capital letters indicate the difference between the groups, and in the vertical column, the same lowercase letters indicate the difference between the groups (*p<0.05)*

		KE		KV		EE		EV		CONTROL		
Parameters	**Time**	**Mean**	**Min-max.**	**Mean**	**Min-max.**	**Mean**	**Min-max.**	**Mean**	**Min-max.**	**Mean**	**Min-max.**	**p**
Inflammation	**7**	$1.16^{A,D,E,F}$	1-2	$1.83^{B,D,G,a}$	1-2	$2.16^{C,E,a,b}$	2-3	$3.00^{F,G,H,a,b}$	3-3	$0.83^{A,B,C,H}$	0-1	**0.001***
	15	1.00^{B}	0-2	1.33	1-2	1.33^{a}	1-2	$2.00^{A,B,a}$	1-3	0.66^{A}	0-1	**0.017***
	30	0.54	0-1	0.83^{a}	0-1	0.83^{b}	0-1	1.16^{b}	1-2	0.50	0-1	**0.129**
	p	**0.057**		**0.034***		**0.023***		**0.020***		**0.157**		
Fibrous Capsule	**7**	1.16^{a}	1-2	$1.00^{a,b}$	1-1	1.50	1-2	$1.16^{a,b}$	1-2	1.16^{a}	1-2	**0.305**
	15	1.66	1-2	2.00^{a}	2-2	1.66	1-2	2.00^{a}	2-2	1.5	1-2	**0.166**
	30	2.00^{a}	2-2	2.00^{b}	2-2	2.00	2-2	2.00^{b}	2-2	2.00^{a}	2-2	**1.00**
	p	**0.025***		**0.014***		**0.83**		**0.025***		**0.025***		
Necrosis	**7**	0.16	0-1	0.33	0-1	0.33	0-1	0.5	0-1	0.16	0-1	**0.709**
	15	0	0	0	0	0	0	0.16	0	0	0	**1.00**
	30	0	0	0	0	0	0	0	0	0	0	**1.00**
	p	**0.317**		**0.157**		**0.157**		**0.083**		**0.317**		
Giant Cell	**7**	1.00^{a}	1-1	1.00^{a}	1-1	1.00	1-1	1.00	1-1	1.00^{a}	1-1	**1.00**
	15	0.66	0-1	0.66	0-1	0.83	0-1	1.00	0-1	0.66	0-1	**0.578**
	30	$0.16^{B,a}$	0-1	$0.16^{A,a}$	0-1	0.50	0-1	$1.00^{A,B,C}$	0-1	$0.33^{C,a}$	0-1	**0.024***
	p	**0.025***		**0.025***		**0.157**				**0.046***		
Macrophage Infiltrate	**7**	$1.83^{D,a}$	1-2	$2.1^{A,a}$	2-3	$2.33^{B,a}$	2-3	$2.66^{C,D,a,b}$	2-3	$1.16^{A,B,C}$	1-2	**0.02***
	15	$1.16^{D,E}$	1-2	1.5^{A}	1-2	$2.00^{B,D,b}$	2-2	$2.00^{C,E,a}$	2-2	$1.00^{A,B,C}$	1-1	**0.001***
	30	$1.00^{A,D,a}$	1-1	1.16^{a}	1-2	$1.00^{B,a,b}$	1-1	$1.5^{A,B,C,D,b}$	1-2	1.00^{C}	1-1	**0.048***
	p	**0.034***		**0.014***		**0.023***		**0.046***				

Table 4: *Evaluation of the IHC criteria results for all groups on the 7th, 15th and 30th days. In the horizontal column, the same capital letters indicate the difference between the groups, and in the vertical column, the same lowercase letters indicate the difference between the groups (*p<0.05)*

		KE		KV		EE		EV		CONTROL		
Parameters	**Time**	**Mean**	**Min-max.**	**Mean**	**Min-max.**	**Mean**	**Min-max.**	**Mean**	**Min-max.**	**Mean**	**Min-max.**	**p**
IL-1β	**7**	1.00^{A}	1-1	$2.14^{A,B,a}$	1-3	1.28	1-2	1.28	1-2	1.14^{B}	1-2	**0.005***
	15	0.85^{A}	0-1	$1.71^{A,a,b}$	1-2	1.00	1-1	1.00	1-1	1.00	1-1	**0.001***
	30	0.71	0-1	0.57^{b}	0-1	0.85	0-1	0.71	0-1	0.71	0-1	**0.851**
	p	**0.157**		**0.015***		**0.180**		**0.157**		**0.083**		
IL 6	**7**	2.00^{a}	2-2	$1.57^{A,a,b}$	2-3	2.00^{a}	2-2	$2.71^{a,b}$	2-3	$1.28^{A,B,a}$	1-2	**0.001***
	15	1.71	1-2	$2.00^{A,a,c}$	2-2	1.57	1-2	1.85^{a}	1-2	$1.00^{A,B}$	1-1	**0.002***
	30	1.14^{a}	1-2	$1.42^{b,c}$	1-2	0.85^{a}	0-1	1.57^{b}	0-2	$0.57^{A,a}$	0-1	**0.017***
	p	**0.014***		**0.038***		**0.011***		**0.011***		**0.025***		
IL-8	**7**	1.71^{a}	1-2	$1.57^{A,a}$	1-2	2.14^{a}	2-3	$2.14^{B,a,b}$	2-3	$1.28^{A,B,a}$	1-2	**0.010***
	15	1.28	1-2	$1.28^{A,b}$	1-2	2.14	2-3	1.28^{a}	1-2	1.14^{A}	1-2	**0.007***
	30	0.85^{a}	0-1	$0.71^{a,b}$	0-1	1.28^{a}	1-2	0.57^{b}	0-1	0.71^{a}	0-1	**0.113**
	p	**0.014***		**0.014***		**0.034***		**0.014***		**0.046***		

was made on the 7th, 15th and 30th days. When the groups are evaluated statistically in themselves in three different time periods; The difference was statistically significant in all groups except EE and EV groups (p<0.05). For macrophage infiltration; the difference was statistically significant in all time periods (p>0.05), when the comparison was made between the groups on the 7th, 15th and 30th days. The highest macrophage infiltration was seen in the EV group on the day 7th. The lowest macrophage infiltration was observed on the day 30th in KE and Control groups (Figure 1). The difference

in the necrosis variable was not statistically significant in all time periods, When the comparison between the groups was made on the 7th, 15th and 30th days. When the groups are evaluated statistically in themselves in three different time periods; the difference was not statistically significant in all groups.

Interleukins

The difference in IL-1β, was statistically significant on the 7th and 15th days, but the difference was not significant on the 30th day, when the comparison was made between the groups on the 7th, 15th and 30th days. The highest IL-1β was seen on day 7th in the KV group, while the lowest IL-1β was on day 30th in the Control group. When the groups were evaluated statistically within themselves in three different time periods; only the difference in the KV group was statistically significant ($p<0.05$) (Figure 2). For IL-6, the difference was statistically significant in all time periods when the comparison between the groups was made on the 7th, 15th and 30th days. The highest IL-1β was seen on the 7th day in the EV group. The lowest IL-6 was observed on the 30th day in the Control group. When the groups were evaluated statistically within themselves in three different time periods, the difference was statistically significant in all groups ($p<0.05$) (Figure 2). For IL-8, when the comparison was made between the groups on the 7th, 15th and 30th days, the difference was statistically significant on the 7th and 15th days, but there was no difference on the 30th day. The highest IL-8 was seen on the 7th day in the KV and EV groups. When the groups were evaluated statistically within themselves in three different time periods; the difference was statistically significant only in the Control group ($p<0.05$) (Figure 2).

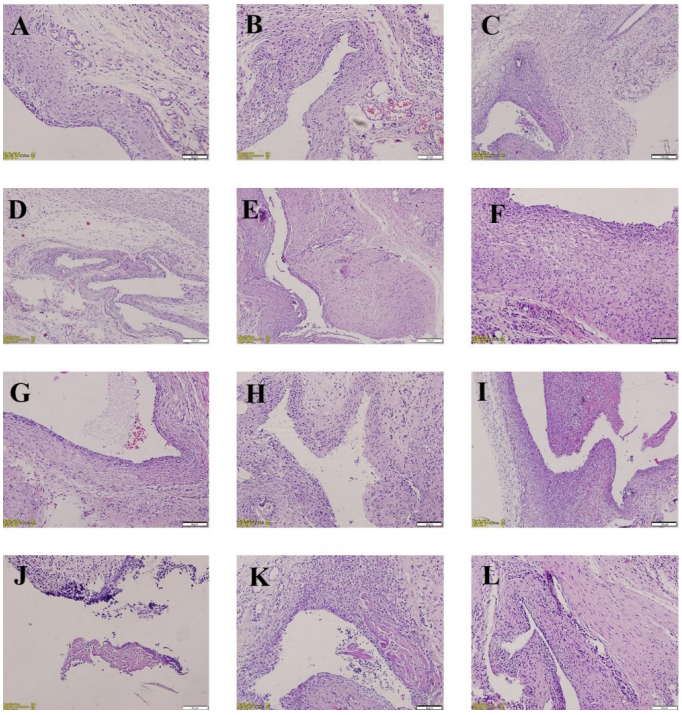

Figure 1. *Histopathological section samples belonging to all groups. A. Mild inflammation, on day 30th Control Group, B. Moderate inflammation, on day 15th KV Group, C. Severe inflammation, on day 7th EV Group, D. Thin capsule, on day 7th KV Group, E and F. Thick capsule, on day 15th and 30th EV Group, G. Mild macrophage infiltration, on day 30th Control Group, H. Moderate macrophage infiltration, on day 15th EE Group, I. Severe macrophage infiltration, on day 7th EV Group, J and K. Necrosis, on day 7th KV Group , L. Giant cells, on day 7th EE Group*

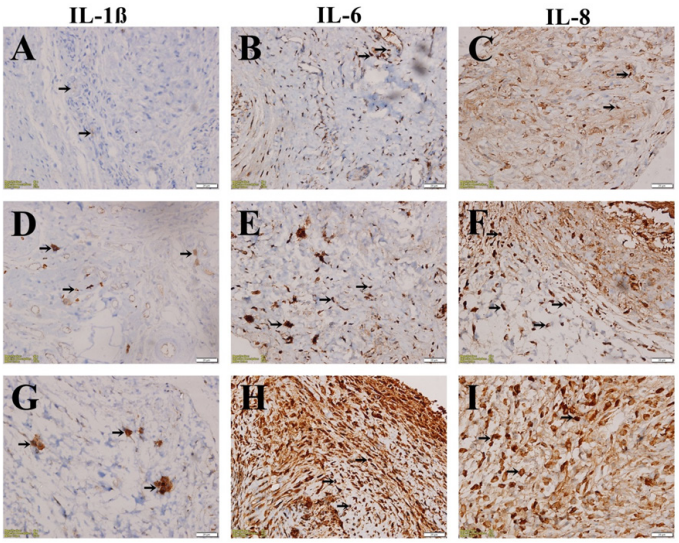

Figure 2. *Immunohistochemical staining images of IL-1β, IL-6, IL-8. A. Mild IL-1β on day 30th Control group, B. Mild IL-6 on day 30th KE group, C. Mild IL-8 on day 30th EE group, D. Moderate IL-1β on day 15th KV group, E. Moderate IL-6 on day 15th EV group, F. Moderate IL-8 on day 15th KE group, G. Severe IL-1β on day 7th KV group, H. Severe IL-6 on day 7th EV group, I. Severe IL-8 on day 7th EE group*

Discussion

Cytotoxicity tests, genotoxicity tests, bone implants and subcutaneous implantation tests are used to determine the effects of dental materials on living tissues. Although cytotoxicity tests are faster and easier, their results can be insufficient for clinical applications (21, 22). It has been stated that local inflammation and toxicities can be determined by implantation of dental materials into subcutaneous connective tissue in experimental animals. Since the inflammatory tissue response after subcutaneous implantation tests is similar to pulp and connective tissue, it is considered a reliable method to evaluate the biocompatibility of dental materials (23). Since PTs are inert in subcutaneous implantation tests, materials are placed in these tubes and their biocompatibility is evaluated (24). For these reasons, different CRs used in this study were filled in PTs and implanted in the subcutaneous connective tissue of the rats.

The standard staining technique with H&E staining is used for histological examination of tissues. Although this simple and cheap staining technique is capable of revealing important cellular details, it identifies only limited protein, enzyme and tissue structure (25, 26). Since IHC involves a specific antigen-antibody reaction, it has a significant advantage over these conventionally used staining techniques, which identifies only limited protein, enzyme and tissue structure. In basic research, this technique is also used to determine the location and distribution of biomarkers within tissues (27). Since more specific results are obtained, IHC staining method was used together with H&E staining in our study and

proinflammatory cytokines (IL-1β, IL-6 and IL-8) were evaluated. CRs are restorative materials used quite frequently in dentistry (28, 29). CRs contain various organic monomers (BIS-GMA, UDMA, HEMA, TEGDMA) at different concentrations (2, 3). It has been reported that these monomers may cause toxic effects due to incomplete polymerization and residual monomer formation (29, 30). BIS-GMA is the monomer with the lowest monomer polymer conversion degree (DC) according to the literature. DC is BIS-GMA < BIS-EMA < UDMA < TEGDMA respectively (31). In this study, Essentia CR containing BIS-GMA and TEDGMA and Kalore CR free BIS-GMA were used.

Mesquita *et al.* (32), evaluated the biocompatibility of three different resin-based cements and reported that resin cements containing BIS-GMA had the highest cytotoxicity and CD68 levels compared to other cements. It has been reported that resin-based dental materials with a high BIS-GMA concentration cause an increase in phagocytic cells such as CD68, overexpression of proinflammatory cytokines, and cause a long-term inflammatory process in rats (33, 34). In our study, both inflammation and proinflammatory cytokines (IL-1β, IL-6 and IL-8) were found to be high on the 7th day in the EV and EE groups. In addition on day 15th, both the cytokine and the inflammatory process continued, while at day 30th both decreased.

Jun *et al.* (35), evaluated the biological properties of the biomonomer without BIS-GMA, which they developed to avoid the estrogenic and cytotoxic effects of BIS-GMA, reported that the biomonomer they developed had a high viability level of human oral keratinocyte and MCF-7 cells. Shinkai *et al.* (36), evaluated the toxicity of resin-containing adhesives in the pulp reported that dental adhesives containing HEMA and TEDGMA showed more severe inflammation. However, they reported that the permeability of the adhesive monomers to the pulp tissue and the degree of polymerization of the adhesives may also be related to the irritation of the pulp tissue. In present research, low inflammation, proinflammatory cytokines (IL-1β, IL-6 and IL-8) were observed in the KV and KE groups free BIS-GMA and free TEDGMA. This may be caused by the release of lower amounts of residual monomers (37). Castaneda *et al.* (38), evaluated the cytotoxicity of silorane-based resin composite and BIS-GMA-containing resin composite, and reported that BIS-GMA containing composite resin caused higher inflammation. Similarly, in our research, EE and EV groups showed higher inflammation and proinflammatory cytokines (IL-1β, IL-6 and IL-8) compared to KE and KV groups on the 7th day. Kamalak *et al.* (39), evaluated the cytotoxicity and biological properties of bulk-fill composites reported that inflammatory cytokines such as IL-6, IL-8 and TNF-α were high in cell culture. Silva et al. (24), evaluated the biological properties of BIS-GMA containing endodontic canal filling paste and silicate-based root canal filling pastes, reported that BIS-GMA-containing canal filling paste had higher IL-6 than other root canal filling pastes. Similar to the results of the study evaluated the effects of resin-containing composites on inflammatory cytokines such as IL-6, IL-8 and TNF-α in our research, IL-1β, IL-6 and IL-8 were found to be high in all resin composite groups on the 7th day (40). Different light sources and curing modes affect the release of resin monomers that have an impact on the biocompatibility and cytotoxicity of dental composites (41-43). Feiz *et al.* (44), evaluated the biocompatibility of resin composites after polymerization with different light sources, and reported that high-intensity light source caused toxic effects on inflammation and fibroblasts.

Munksgaard *et al.* (45), compared the residual monomer amounts of BIS-GMA and TEDGMA containing resin composites polymerized using different light sources, and reported that the high-intensity light source left less residual monomer. However, it has also been reported that a high-intensity light source may cause damage to the pulp and periodontal tissues during polymerization (46). Ergun *et al.* (47), evaluated the cytotoxicity of resin-based luting cements after polymerization at different times, and stated that strong LED light for a long time caused a toxic effect on fibroblasts. Yap *et al.* (48) evaluated the cytotoxicity of resin composites after polymerization with different light sources, and observed that the high-intensity LED light source caused a more cytotoxic effect than the QTH light source. When they compared the LED light sources within themselves, reported that the resin composite polymerized with the high-intensity LED light source was more cytotoxic.

Tunç *et al.* (49) evaluated the cytotoxicity of compomers on pulp fibroblasts after polymerization with different light sources, reported that the LED light source with high light intensity was more toxic on fibroblasts. They stated that the cytotoxicity of hydrophobic monomers such as BIS-GMA and UDMA is greater than that of hydrophilic monomers such as 2-hydroxyethyl methacrylate and TEGDMA (6). In addition, higher light intensity and higher temperature rise is another factor that causes this situation (50).

In our experiment, inflammation and IL-1β, IL-6 and IL-8 proinflammatory cytokines were found to be higher in the EV and KV groups polymerized with a high light intensity VALO light source, compared to the control group, on the 7th day. Mild to moderate levels of inflammation and proinflammatory cytokines were observed in the KV and EV groups on the 15th day. This may be due to the continued release of unreacted monomers. In addition, the response of the immune system of rats to foreign bodies may be delayed (44). In addition, from the 7th to the 30th day, the thickness of the fibrous capsule increased, while the macrophage infiltration and giant cell decreased. It is also stated that the cytotoxic effect increases depending on the dose and changes over time. Inflammation and proinflammatory cytokine levels of all composite groups used in our study decreased over time. Necrosis is defined as the uncontrolled death of the cell and is associated with the resulting increase in non-viable cells and increased release of inflammatory cytokines. In our study, necrosis was observed on the 7th day in all composite groups. On day 15th, necrosis was seen only in the EV group.

Conclusion

Within the limits of this animal experiment, it can be stated that all composites used in the present study demonstrated acceptable biocompatibility in the subcutaneous tissues of rats. However, pulp protective materials should still be considered for deep dentin caries which are close to the pulp tissue, due to the high inflammation rate observed on the 7th day.

Türkçe özet: *Rezin kompozitlerin iki farklı ışık cihazıyla polimerizasyonu sonrası biyouyumluluğu: immünohistokimyasal çalışma. Amaç: Bu çalışmanın amacı, iki farklı rezin kompozitin, iki farklı ışık kaynağı ile üç farklı zaman diliminde polimerizasyon sonrası biyouyumluluğunu karşılaştırmaktır. Gereç ve Yöntem: 2 farklı rezin kompozit ve 2 farklı ışık kaynağı (Elipar S10 ve Valo) ile polimerize edilmiş 72 polietilen tüp [Grup 1: Kalore Elipar S10 (KE), Grup 2: Kalore Valo (KV), Grup 3: Essentia Elipar S10 (EE), Grup 4: Essentia Valo (EV)] 18 ratın dorsal bağ dokusuna implante edildi. 24 adet boş polietilen tüp [Grup 5: (Kontrol grubu)] 6 ratın dorsal bağ dokusuna yerleştirildi. Daha sonra ratlar 7., 15. ve 30. günlerden sonra her zaman aralığında (n=8) sakrifiye edildi. Biyopsi örnekleri H&E ile boyandı ve inflamasyon, nekroz, makrofaj infiltratı, dev hücre ve fibröz kapsül kriterleri açısından incelendi. Proinflamatuar sitokinleri (IL-1β, IL-6 ve IL-8) değerlendirmek için immünohistokimyasal boyama yapıldı. Bulgular: Kompozit grupları ile kontrol grupları karşılaştırıldığında; 7. ve 15. günlerde inflamasyon kriterleri açısından istatistiksel olarak anlamlı fark bulunurken, fibröz kapsül ve nekroz açısından günler arasında istatistiksel fark yoktu. Kompozit grupları ve kontrol grupları proinflamatuar sitokinler açısından değerlendirildiğinde; 7., 15. ve 30. günlerde istatistiksel olarak anlamlı fark bulundu. Sonuç: Bu çalışmada kullandığımız tüm kompozit rezinler, farklı ışık kaynakları ile polimerizasyon sonrası ratların deri altı dokularında iyi biyouyumluluk gösterdi ve böylece klinik restoratif tedavilerde bu materyallerin güvenle kullanılabileceği kanısındayız. Anahtar kelimeler: kompozit rezin, ışık cihazları, biyouyumluluk, rat, immünohistokimya*

Ethics Committee Approval: This study was carried out with the approval of Sivas Cumhuriyet University Animal Experiments Local Ethics Committee (2020/288).

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Author contributions: II, MU, TK participated in designing the study. II, MU participated in generating the data for the study. II, MU participated in gathering the data for the study. II, MU participated in the analysis of the data. II, MU, TK wrote the majority of the original draft of the paper. II, MU, TK participated in writing the paper. has had access to all of the raw data of the study. II, MU, TK have reviewed the pertinent raw data on which the results and conclusions of this study are based. II, MU, TK have approved the final version of this paper. II guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

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