

## Evaluation of Three Different Posterior Composite Materials in Terms of Microhardness and Cytotoxicity

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**Abstract:** This study aimed to evaluate the polymerization depths of two different bulk-fill posterior composite resins and a conventional posterior composite resin with the microhardness test and there with the in vitro cytotoxicity test. For the microhardness test, 10 samples were obtained for each group using Teflon molds. Microhardness values were measured from the top and bottom surfaces of each sample using the Vicker's microhardness test. For the cytotoxicity test, 12 samples were obtained for each group using Teflon molds. The extraction fluids of samples were obtained by incubating them at 37°C in RPMI 1640 medium for 24, 48, and 72 h. Cell viability was assessed using the MTT assay following incubation. The microhardness values of the top and bottom surfaces of composite resins were aligned as follows: Filtek Z550 Composite Resin > Filtek Bulk-Fill Posterior Composite Resin > Tetric N-Ceram Bulk-Fill Composite Resin. The microhardness ratio of Filtek Z550 Composite Resin was found to be significantly higher than those of other composite resins when the bottom surface–top surface microhardness ratios were compared. The difference between the bottom surface–top surface microhardness ratios of the Filtek Bulk-Fill Posterior Composite Resin and the Tetric N-Ceram Bulk-Fill Composite Resin was not statistically significant. However, all composite resins provided the recommended polymerization depth ( $\geq 80\%$ ). No statistically significant difference was observed between the cytotoxicity values of the composite resins after 24- and 48-h incubation. However, the Filtek Bulk-Fill Posterior Composite Resin showed a significantly higher cytotoxicity compared with the other groups after 72-h incubation. In addition, the cytotoxicity value obtained after 72-h incubation of the Filtek Bulk-Fill Posterior Composite Resin was significantly higher than the cytotoxicity values obtained after 24- and 48-h incubation. Although adequate polymerization depth was obtained in all study groups, the Filtek Bulk-Fill Posterior Composite Resin had significantly reduced cell viability after 72-h incubation.

**Keywords:** Composite resin, cytotoxicity, microhardness.

## INTRODUCTION

Composite resins which are frequently used by the help of the developments in adhesive dentistry should be biocompatible for living tissues and mechanically durable besides the esthetic expectations of the patients [1].

Despite significant developments in composite resins, some limitations still exist in providing ideal polymerization conditions [2]. When the polymerization is not fully achieved, some residual monomers remain in the composite resin because the degree of conversion of monomers into polymers decreases. The monomers are released from the composite resin over time, decreasing the mechanical stability of restoration and affecting the biological compatibility [3].

The traditional approach for ideal polymerization is to apply the material into a cavity with 2-mm layers [4]. However, this approach is associated with some issues, such as gaps or contaminations between layers especially in posterior composite resin restorations, an extended treatment period depending on the application of light in each layer, and a decrease in the depth of polymerization if a composite resin is applied in thicknesses exceeding 2 mm. Due to these disadvantages, the posterior composite resins called "bulk-fill," which can be polymerized even at 4–5 mm thickness, are increasingly used in recent years [5,6].

The depth of polymerization is an important parameter for evaluating the mechanical properties and

clinical success rate of composite resins. The microhardness test is used to determine the depth of polymerization of composite resins, which are usually preferred because of their easy application and reliable results [7,8].

It is necessary to evaluate the compatibility of composite resins with biological tissues to avoid undesired tissue responses that may occur due to the clinical use of these resins. Not only the mechanical properties but also the biocompatibilities of the composite resins need to be questioned due to the deleterious effects of released residual monomers on living tissues. First of all, cytotoxic effects must be understood to be able to protect biological tissues from the effects of released monomers. Therefore, cell culture-based *in vitro* cytotoxicity tests, which were specified by the International Standards Organization, are frequently preferred due to their easy application, low cost, and short-term results [9].

Developments in modern dentistry have made dentists responsible for examining the manufacturers' claims and literature to determine the material and technique that can provide the best service to the patient.

This study aimed to evaluate the polymerization depths of two different bulk-fill posterior composite resins, which were polymerized at 4 and 5 mm thickness and a conventional posterior composite resin, which was polymerized at 2 mm thickness, with the Vicker's microhardness test and their biocompatibility with the 3-(4,5-dimethylthiazol-2)-2,5-diphenyl tetrazolium bromide (MTT) *in vitro* cytotoxicity test.

## EXPERIMENTAL SECTION

This study was carried out by the Dicle University Scientific Research Projects (Project no: DİS.16.014) with the permission of the local ethics committee of the Dicle University Faculty of Dentistry (dated May 12, 2016, and numbered 2016-9). The microhardness of the samples was analyzed at the Department of Mechanical Engineering, Faculty of Engineering and Architecture, İzmir Katip Çelebi University, and the cytotoxicity evaluations were carried out at the Department of Medical Microbiology, Faculty of Medicine, and Mustafa Kemal University. The Filtek Z550 Composite Resin was used as a conventional composite resin material. The Tetric N-Ceram Bulk-Fill Composite Resin and the Filtek Bulk-Fill Posterior Composite Resin were also used as bulk-fill composite resin materials (Table 1). For standardization, A2 color was chosen for the Filtek Z550 Composite Resin and the Filtek Bulk-Fill Posterior Composite Resin materials, and IVA as chosen for the Tetric N-Ceram Bulk-Fill Composite Resin material as the closest color tone to A2.

## Microhardness test

A total of 30 circular Teflon molds having different depths were prepared according to the manufacturer's recommendations. The prepared Teflon molds were sterilized using autoclaving steam at 121°C and 1 atm pressure for 15 min. Glass coverslips and transparent tape were placed at the bottom of the Teflon molds to obtain smooth surfaces. The composite resins were condensed by placing them into molds in one go with the aid of a cement spatula and a plugger, so that the top surface of the Teflon mold was kept upright. Transparent tape and glass coverslips were adapted on the top surface of the mold under a stable hand pressure. The tip of the light device in contact with the mold also needed to be placed perpendicular to the mold to achieve the uniform distance standard and obtain the best polymerization depth. The samples were polymerized only from the top surface using the Coxo DB-685 light device (Coxo, Shishan Town, China) with a light intensity of 1600 mW/cm<sup>2</sup> as recommended by the manufacturer. Only the upper surfaces of the obtained samples were leveled and polished. The samples were separated into groups, placed in light-tight containers, and kept at 37°C for 24 h to complete polymerization. The working scheme in which the samples were divided into groups for the microhardness test is shown in Table 2.

Microhardness measurements were performed with a Shimadzu HMV-II microhardness measurement instrument (Shimadzu Corporation, Kyoto, Japan). A 300 g (2942 N) load was applied using a pyramid diamond tip for 15 s, and a total of six measurements were taken from the top (three measurements) and bottom (three measurements) surfaces of the composite resins. The measurement points were determined to be one in the center and the other two at equal distances from the center. For each sample, a value was obtained for each surface by taking the mean of measurements obtained from the top and the bottom surfaces. The bottom-to-top surface microhardness ratio formula was used to obtain the mean Vicker's microhardness value.

## Cytotoxicity test

A total of 36 circular Teflon molds having different depths were prepared according to the manufacturer's recommendations. Composite resin samples were obtained using the same methods for the microhardness test. The preparation and polymerization stages were carried out in a laminar flow cabinet (Holten, Class II, and Denmark) to provide aseptic conditions. The study scheme in which the samples were divided into groups according to the evaluation periods is shown in Table 3.

The Vero (African green monkey kidney epithelial cells) cell line was used to determine cellular cytotoxicity levels of the obtained samples. Samples autoclaved and sterilized at 121°C for 15 min were grown in Roswell Park Memorial Institute (RPMI) 1640

medium containing 10% fetal bovine serum, 10mM 4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES), 4mM glutamine, and 100 IU/mL penicillin/streptomycin. Cell culture incubation was carried out at 37°C in an incubator with 5% CO<sub>2</sub> and 95% air. The cells were seeded and inoculated in 12-well flat-bottomed cell culture plates at a density of 1 × 10<sup>5</sup> cells/mL. A cell culture without any extraction fluid was used as a control group. The control group was also incubated for 24, 48, and 72 h. The effects of the extraction fluids on the cell viability of Vero cells were assessed using trypan blue and MTT assays at the end of incubation. Cytotoxicity studies on cells were performed at least three times for each sample according to the evaluation periods shown in Table 3. The effects of the samples on the cells were transferred to the microplate reader. The absorbance was measured at 570 nm using a spectrophotometer. Cell proliferation was defined as the ratio of the cells in the wells treated with composite resins to the cells in the control group. Concentrations inhibiting at least 50% of the cell proliferation (IC<sub>50</sub>) were then determined using the MTT cytotoxicity assay.

### STATISTICAL ANALYSIS

The Kolmogorov–Smirnov method was used to evaluate the statistical normal distribution of the obtained microhardness values, and the one-way analysis of variance was used to assess differences between groups. The Tukey Honestly Significant Difference (HSD) test was performed to determine the groups from which these differences originated.

The statistical normal distribution of the obtained cell viability values was assessed by the Kruskal–Wallis variance analysis method, and the difference between groups was assessed by the chi-square test. The Mann–Whitney *U* test was used to determine the groups from which these differences originated. Further, 95% confidence interval was applied, and the statistical results were considered significant at  $P < 0.05$ .

### RESULTS

#### Evaluation of the microhardness test results

A statistically significant difference was found between the groups when the top-surface microhardness, bottom-surface microhardness, and bottom-to-top surface microhardness ratios of the

composite resin samples were evaluated ( $P < 0.05$ ) (Table 4). The statistical test results showing the group from which the difference originated are shown in Table 5. The highest top-surface and bottom-surface microhardness values were seen in Group 1, followed by Groups 3 and 2. The order of the top-surface and bottom-surface microhardness values among the groups was found to be statistically significant ( $P = 0.000$ ). The highest bottom surface–top surface microhardness ratio was seen in Group 1, followed by Groups 2 and 3. The differences between Group 1 and Group 2 ( $P = 0.005$ ) and Groups 1 and 3 ( $P = 0.003$ ) were statistically significant when the bottom surface–top surface microhardness ratios between the groups were evaluated, but no significant difference was found between Groups 2 and 3 ( $P = 0.974$ ).

The in-group comparisons showed that the mean top-surface microhardness values for each of the three resin materials were statistically higher compared with the mean bottom-surface microhardness values ( $P = 0.000$ ) (Table 6).

#### Evaluation of the cytotoxicity test results

No statistically significant difference was found between the groups at the end of the 24th and 48th h ( $P > 0.05$ ) when the cell viability values of the groups were evaluated according to the incubation periods. However, a statistically significant difference was found between the groups after 72 h ( $P < 0.05$ ) (Table 7). The statistical test results showing the group from which the difference originated are shown in Table 8. Only the decrease in cell viability observed after 72-h incubation in Group 3 was statistically significant compared with that in the other groups ( $P = 0.024$ ).

The statistical in-group analyses of the number of living cells observed in different incubation periods are shown in Table 9. Within-group comparisons showed no statistically significant difference between the cell viability values in increasing incubation periods of Group 1 ( $P = 0.174$ ) and Group 2 ( $P = 0.067$ ). In Group 3, a statistically significant difference was found between cell viability values in increasing incubation periods ( $P = 0.027$ ). A statistically significant decrease was reported in the cell viability of Group 3 at the end of 72 h compared with 24 and 48 h ( $P = 0.01$ ).

**Table-1: Information about the composite resin materials used in the study**

Materials	Manufacturer	Shade	Material Type	Matrix Type	Filler Type	Filler Loading
Filtek™ Z550 Composite Resin	3M ESPE, St. Paul, MN, UH.	A2	Nano-hybrid	BisGMA, UDMA, TEGDMA, Bis-EMA, PEGDMA	Zirconia, silica, Zirconia/silica	% 82 (wt), % 68 (vol.)
Tetric N-Ceram Bulk-Fill Composite Resin	Ivoclar/Vivadent, Schaan, LIECHTENSTEIN	IVA	Nano-hybrid	Bis-GMA, UDMA, TEGDMA, Bis-EMA	Barium glass, ytterbium trifluoride, mixed oxide, silicon dioxide ve pre-polymers	%75-77 (wt), %53-55 (vol.)
Filtek™ Bulk-Fill Posterior Composite Resin	3M ESPE, St. Paul, MN, UH.	A2	Nano-hybrid	BisGMA, UDMA, Bis-EMA, AUDMA, DDDMA	Zirconia, silica, Zirconia/silica, ytterbium trifluoride	%76.5 (wt), %58.4 (vol.)

**Table-2: Distribution of the composite resin samples for the microhardness test**

Groups	Composite resin	N	Thickness x Diameter
Group 1	Filtek™ Z550 Composite Resin	10	2mm x 5mm
Group 2	Tetric N-Ceram Bulk-Fill Composite Resin	10	4mm x 5mm
Group 3	Filtek™ Bulk-Fill Posterior Composite Resin	10	5mm x 5mm

**Table-3: Distribution of the composite resin samples according to the evaluation periods for the cytotoxicity test**

Groups	Subgroups	Composite resin	Thickness x Diameter	Incubation Periods	N
Group 1	1A	Filtek™ Z550 Composite Resin	2mm x 5mm	24 h.	4
	1B			48 h.	4
	1C			72 h.	4
Group 2	2A	Tetric N-Ceram Bulk-Fill Composite Resin	4mm x 5mm	24 h.	4
	2B			48 h.	4
	2C			72 h.	4
Group 3	3A	Filtek™ Bulk-Fill Posterior Composite Resin	5mm x 5mm	24 h.	4
	3B			48 h.	4
	3C			72 h.	4

**Table-4: Statistical evaluation of top-surface and bottom-surface microhardness and bottom-to-top surface microhardness ratios of the groups**

	Groups	N	Mean (Micro-hardness)	Standart Deviation	Standart Error	Minimum	Maximum	F	p
Top Surface	Group 1	10	94,2733	1,68931	0,53421	91,50	96,03	492,240	0,000
	Group 2	10	57,8200	2,91904	0,92308	53,10	62,27		
	Group 3	10	70,3967	3,08559	0,97575	66,03	75,10		
Bottom Surface	Group 1	10	88,6967	2,12454	0,67184	85,63	91,53	448,511	0,000
	Group 2	10	50,4667	3,28765	1,03965	46,23	56,70		
	Group 3	10	61,0700	3,27608	1,03599	55,63	66,43		
Bottom Surface / Top Surface	Group 1	10	0,9410	0,02175	0,00688	0,90	0,97	8,357	0,001
	Group 2	10	0,8733	0,04554	0,01440	0,78	0,93		
	Group 3	10	0,8689	0,05756	0,01820	0,77	0,92		

**Table-5: Statistical comparison of microhardness values of the composite resin groups**

		Group 2			Group 3		
		Top Surface	Bottom Surface	Bottom Surface / Top Surface	Top Surface	Bottom Surface	Bottom Surface / Top Surface
Group 1	Top Surface	p=0,000*			p=0,000*		
	Bottom Surface		p=0,000*		p=0,000*		
	Bottom Surface / Top Surface			p=0,005*			p=0,003*
Group 2	Top Surface				p=0,000*		
	Bottom Surface					p=0,000*	
	Bottom Surface / Top Surface						p=0,974 (ns)

**Table-6: Statistical in-group evaluation of top and bottom surface microhardness values**

Groups	N	Surface	Mean	Standart Deviation	Standart Error	p
Group 1	10	Top	94,2733	1,68931	0,53421	0,000
		Bottom	886967	2,12454	0,67184	
Group 2	10	Top	57,8200	2,91904	0,92308	0,000
		Bottom	50,4667	3,28756	1,03965	
Group 3	10	Top	70,3957	3,08559	0,97575	0,000
		Bottom	61,0700	3,27608	1,03599	

**Table-7: Statistical evaluation of the cell viability of the groups according to incubation periods**

	Groups	Mean (Cell Viability)	Standart Deviation	Standart Error	Minimum	Maximum	Chi-Squared	p
24. h	Group 1	2403333,33	327159,49	188885,62	2100000	2750000	1,248	0,742
	Group 2	2573333,33	352325,61	203415,28	2200000	2900000		
	Group 3	2520000	252388,59	145716,62	2250000	2750000		
	Group 4	2728333,333	326968,4	188775,29	2485000	3100000		
48. h	Group 1	2853333,33	260832	150591,43	2660000	3150000	4,333	0,228
	Group 2	3136666,67	192959,41	111405,17	2920000	3290000		
	Group 3	2961666,67	256238,82	147939,55	2760000	3250000		
	Group 4	3169500	275164,95	158866,56	2900000	3450000		
72. h	Group 1	2501666,67	88928,81	51343,07	2400000	2565000	9,462	0,024
	Group 2	2923333,33	75055,53	43333,33	2850000	3000000		
	Group 3	1520000	252388,59	145716,62	1250000	1750000		
	Group 4	2985000	253820,8029	146543,509	2705000	3200000		

**Table-8: Statistical comparison of cytotoxicity of composite resin groups**

GROUPS		Group 2			Group 3			Group 4		
		24. h.	48. h.	72. h.	24. h.	48. h.	72. h.	24. h.	48. h.	72. h.
Group 1	24. h.	p=0,700 (ns)			p=0,700 (ns)			p=0,400 (ns)		
	48. h.		p=0,200 (ns)			p=0,400 (ns)			p=0,200 (ns)	
	72. h.			p=0,100 (ns)			<b>p=0.01</b> *			p=0,100 (ns)
Group 2	24. h.				p=1,00 (ns)			p=1,00 (ns)		
	48. h.					p=0,400 (ns)			p=1,00 (ns)	
	72. h.						<b>p=0.01</b> *			p=0,700 (ns)
Group 3	24. h.							p=0,200 (ns)		
	48. h.								p=0,100 (ns)	
	72. h.									<b>p=0.001</b> *

**Table-9: Statistical in-group evaluation according to incubation periods**

Groups	Hours	Mean (Cell Viability)	Standart Deviation	Standart Error	Chi-Squared	P
Group 1	24.	2403333,33	327159,49	188885,62	3,832	0,147
	48.	2853333,33	260832	150591,43		
	72.	2501666,67	88928,81	51343,07		
Group 2	24.	2573333,33	352325,61	203415,28	5,401	0,067
	48.	3136666,67	192959,41	111405,17		
	72.	2923333,33	75055,53	43333,33		
Group 3	24.	2520000	252388,59	145716,62	7,2	0,027
	48.	2961666,67	256238,82	147939,55		
	72.	1520000	252388,59	145716,62		
Group 4	24.	2728333,333	326968,4	188775,29	2,756	0,252
	48.	3169500	275164,95	158866,56		
	72.	2985000	253820,8029	146543,5088		

**DISCUSSION**

Ideal polymerization of composite resins provides the increased mechanical strength of restoration and reduces cytotoxic effects [10].

The inorganic filler type, filler size, and filler ratio have an important effect on the polymerization depths and microhardness values of composite resins [11]. Yap *et al.* investigated the polymerization depths of conventional and bulk-fill composite resins and reported that higher microhardness values were obtained in composite resins containing zirconium/silica (Zr/SiO<sub>2</sub>) [12]. Tekçe *et al.* stated that the Filtek Z550 Composite Resin had a high microhardness value due to a high inorganic filler ratio and the presence of Zr/SiO<sub>2</sub> particles in the composition [13]. In this study, the significant superiority of the

Filtek Z550 Composite Resin in terms of surface microhardness values could be attributed to the high Zr/SiO<sub>2</sub> ratio in the inorganic filler content. However, it could be concluded that the microhardness values of the Filtek Bulk-Fill Posterior Composite Resin were significantly lower than those of the Filtek Z550 Composite Resin due to its lower Zr/SiO<sub>2</sub> ratio. The microhardness values of the Tetric N-Ceram Bulk-Fill Composite Resin were significantly lower than those of the other groups due to the absence of Zr/SiO<sub>2</sub> particles in the inorganic filler structure.

A significant superiority is observed in microhardness values of the nano hybrid composite resins having a high inorganic filler ratio due to including particles at nano-size [14]. The increasing the filler ratio by reducing the particle size of the filler has



a positive effect on the polymerization depth and microhardness of composite resins [15]. The microhardness value of Filtek Z550 Composite Resin, which had a higher inorganic filler ratio, was significantly higher than those of the other groups in the present study. Although the inorganic filler ratios of the Filtek Bulk-Fill Posterior Composite Resin and the Tetric N-Ceram Bulk-Fill Composite Resin were similar, the Filtek Bulk-Fill Posterior Composite Resin had a significantly higher microhardness value. The inorganic filler structure of the Tetric N-Ceram Bulk-Fill Composite Resin contained pre-polymerized organic particles (50 µm) called pre-polymer. Recent studies showed that pre-polymer particles added to the Tetric N-Ceram Bulk-Fill Composite Resin structure increased the inorganic filler ratio to increase the polymerization depth, but this structure had an organic content [16]. The statistically significant difference between the microhardness values of the Tetric N-Ceram Bulk-Fill Composite Resin and the Filtek Bulk-Fill Posterior Composite Resin was thought to be related to the pre-polymer particle content.

The light is absorbed and hence loses its energy toward the deep layers of the composite resin [17]. The microhardness measurement results of this study also showed that the upper-surface microhardness of the groups was significantly higher than the lower-surface microhardness.

The percentage of the bottom-surface microhardness value to the top-surface microhardness value ( $\geq 80\%$ ) was taken as reference in the Vicker's microhardness test method used to determine the polymerization depth of composite resins [18]. Abuelenain *et al.* evaluated the bottom surface-top surface microhardness ratios of the conventional and bulk-fill composite resins with different contents and found the bottom-to-top surface microhardness ratios ( $\geq 80\%$ ) to be acceptable, although the hardness values of the bulk-fill composite resins were lower than those of the conventional composite resins [19]. Bucuta *et al.* and Price *et al.* compared the mechanical properties of conventional composite resins and bulk-fill composite resins and found that the microhardness values of the conventional composite resins were significantly higher than those of the bulk-fill composite resins [20,21]. In addition, Garoushi *et al.* and Alkhdhairy evaluated the polymerization degrees of the bulk-fill composites and obtained the Vicker's microhardness ratios of the Tetric N-Ceram Bulk-Fill Composite Resin and the Filtek Bulk-Fill Posterior Composite Resin above "80%," but no statistically significant difference between the two was observed [22,23]. Similarly, although the microhardness ratio of the conventional Filtek Z550 Composite Resin was statistically significantly higher than the microhardness ratio of the bulk-fill Tetric N-Ceram Bulk-Fill Composite Resin and the Filtek Bulk-Fill Posterior Composite Resin, all

groups satisfied the suggested polymerization depth ratio ( $\geq 80\%$ ) in the present study.

Recent studies in the literature showed that the residual monomers released during insufficient polymerization of the composite resins influenced the biocompatibility. In particular, it was necessary to evaluate whether newly developed composite resins were compatible with biological tissues before clinical application [24].

Although many studies have focused on evaluating early cytotoxicity findings, it has been reported that monomer release from composite resins continues 24 h later, and this situation reduces cell viability over time [25,26]. Therefore, this study aimed to evaluate the cell viability and cytotoxic effects of composite resins after 24-, 48-, and 72-h incubation.

The structure and amount of organic matrix are the main factors affecting the cytotoxic effects of composite resins. Some methyl methacrylate-based monomers such as bisphenol-A-glycidyl methacrylate (Bis-GMA), triethyleneglycol dimethacrylate (TEGMA), and hydroxyethyl methacrylate have been claimed to have estrogenic, genotoxic, cytotoxic, mutagenic, allergic, and teratogenic effects. The release rate of the monomers in the hydrophobic structure is higher [27]. Al-Hiyasat *et al.* reported that the cytotoxicity increased with a with a increased organic content due to the reduced inorganic filler ratio. They also reported that viscosity-controlling monomers were effective in terms of cytotoxic effect [28]. Huang *et al.* suggested that composite resins with the maximum cytotoxic effect also had the highest organic content [29]. Tuna *et al.* evaluated the residual monomers, such as Bis-GMA, TEGDMA, urethane dimethacrylate, and bisphenol A ethoxylate dimethacrylate, and the amounts released over time from different composite resins and reported that monomers with low viscosity were released more compared with those with high viscosity [30].

The cytotoxicity results indicated that the significant decrease in cell viability in the Filtek Bulk-Fill Posterior Composite Resin after 72-h incubation was related to the fact that the organic matrix structure of this resin included different monomers compared with other composite resins. Although no similar study has been reported in the literature, 1,12-dodecane-DMA monomer, which is hydrophobic and has viscosity control and high molecular reactivity, may be effective when added to the organic matrix of the Filtek Bulk-Fill Posterior Composite Resin.

## CONCLUSIONS

Even if sufficient polymerization depth is obtained for the materials, their cytotoxic effects over time need further investigation. It should also be noted that the cytotoxic effects of the materials may differ

under *in vivo* conditions. Therefore, further studies are needed to increase the biocompatibility of composite resins.

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#### REFERENCES

1. Alshali, R. Z., Silikas, N., & Satterthwaite, J. D. (2013). Degree of conversion of bulk-fill compared to conventional resin-composites at two time intervals. *Dental Materials*, 29(9), e213-e217.
2. Fortin, D., & Vargas, M. A. (2000). The spectrum of composites: new techniques and materials. *The Journal of the American Dental Association*, 131, 26S-30S.
3. Moore, B. K., Platt, J. A., Borges, G., Chu, T. G., & Katsilieri, I. (2008). Depth of cure of dental resin composites: ISO 4049 depth and microhardness of types of materials and shades. *Operative dentistry*, 33(4), 408-412.
4. Ilie, N., & Stark, K. (2014). Curing behaviour of high-viscosity bulk-fill composites. *Journal of dentistry*, 42(8), 977-985.
5. Jackson, R. D. (2014). Posterior composites and the new bulk-fill materials. *Inside Dentistry*, 10(8), 68-75.
6. Colak, H., Ercan, E., & Hamidi, M. M. (2016). Shear bond strength of bulk-fill and nano-restorative materials to dentin. *European journal of dentistry*, 10(1), 40.
7. Fronza, B. M., Rueggeberg, F. A., Braga, R. R., Mogilevych, B., Soares, L. E. S., Martin, A. A., ... & Giannini, M. (2015). Monomer conversion, microhardness, internal marginal adaptation, and shrinkage stress of bulk-fill resin composites. *Dental materials*, 31(12), 1542-1551.
8. Shahdad, S. A., McCabe, J. F., Bull, S., Rusby, S., & Wassell, R. W. (2007). Hardness measured with traditional Vickers and Martens hardness methods. *Dental Materials*, 23(9), 1079-1085.
9. Wever, D. J., Veldhuizen, A. G., Sanders, M. M., Schakenraad, J. M., & Van Horn, J. R. (1997). Cytotoxic, allergic and genotoxic activity of a nickel-titanium alloy. *Biomaterials*, 18(16), 1115-1120.
10. Tsai, P. C., Meyers, I. A., & Walsh, L. J. (2004). Depth of cure and surface microhardness of composite resin cured with blue LED curing lights. *Dental Materials*, 20(4), 364-369.
11. Kim, K. H., Ong, J. L., & Okuno, O. (2002). The effect of filler loading and morphology on the mechanical properties of contemporary composites. *Journal of Prosthetic Dentistry*, 87(6), 642-649.
12. Yap, A. U. J., Pandya, M., & Toh, W. S. (2016). Depth of cure of contemporary bulk-fill resin-based composites. *Dental materials journal*, 35(3), 503-510.
13. Nedeljkovic, I., Teughels, W., De Munck, J., Van Meerbeek, B., & Van Landuyt, K. L. (2015). Is secondary caries with composites a material-based problem?. *Dental Materials*, 31(11), e247-e277.
14. Badawy, R., & Aboalazm, E. (2015). Microhardness of two bulk-fill resin composites. *Dental journal*, 61(5573), 5582.
15. Ilie, N., Bucuta, S., & Draenert, M. (2013). Bulk-fill resin-based composites: an *in vitro* assessment of their mechanical performance. *Operative Dentistry*, 38(6), 618-625.
16. Al-Mansour, K., Al-Sada, A., & Al-Sinan, H. (2015). Curing depth of bulk-fill composites-an *in vitro* study. *Pakistan Oral & Dental Journal*, 35(2).
17. Santos, G. B., Medeiros, I. S., Fellows, C. E., Muench, A., & Braga, R. R. (2007). Composite depth of cure obtained with QTH and LED units assessed by microhardness and micro-Raman spectroscopy. *Operative dentistry*, 32(1), 79-83.
18. Santos, M. J. M. C., Passos, S. P., Encarnação, M. O. L. D., Santos Junior, G. C., & Bottino, M. A. (2010). Hardening of dual-cure resin cement using QTH and LED curing units. *Journal of Applied Oral Science*, 18(2), 110-115.
19. Abuelenain, D. A., Neel, E. A. A., & Al-Dharrab, A. (2015). Surface and mechanical properties of different dental composites. *Austin J Dent*, 2(2), 1019.
20. Price, R. B., Felix, C. A., & Andreou, P. (2005). Knoop hardness of ten resin composites irradiated with high-power LED and quartz-tungsten-halogen lights. *Biomaterials*, 26(15), 2631-2641.
21. Bucuta, S., & Ilie, N. (2014). Light transmittance and micro-mechanical properties of bulk fill vs. conventional resin based composites. *Clinical oral investigations*, 18(8), 1991-2000.
22. Garoushi, S., Vallittu, P., Shinya, A., & Lassila, L. (2016). Influence of increment thickness on light transmission, degree of conversion and micro hardness of bulk fill composites. *Odontology*, 104(3), 291-297.
23. Alkhudhairy, F. I. (2017). The effect of curing intensity on mechanical properties of different bulk-fill composite resins. *Clinical, cosmetic and investigational dentistry*, 9, 1.
24. Geurtsen, W. (1998). Substances released from dental resin composites and glass ionomer cements. *European journal of oral sciences*, 106(2p2), 687-695.
25. Abe, K., & Matsuki, N. (2000). Measurement of cellular 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) reduction activity and lactate dehydrogenase release using MTT. *Neuroscience research*, 38(4), 325-329.
26. Issa, Y., Watts, D. C., Brunton, P. A., Waters, C. M., & Duxbury, A. J. (2004). Resin composite monomers alter MTT and LDH activity of human gingival fibroblasts *in vitro*. *Dental Materials*, 20(1), 12-20.



27. Alshali, R. Z., Salim, N. A., Sung, R., Satterthwaite, J. D., & Silikas, N. (2015). Analysis of long-term monomer elution from bulk-fill and conventional resin-composites using high performance liquid chromatography. *Dental Materials*, 31(12), 1587-1598.
28. Al-Hiyasat, A. S., Darmani, H., & Milhem, M. M. (2005). Cytotoxicity evaluation of dental resin composites and their flowable derivatives. *Clinical oral investigations*, 9(1), 21-25.
29. Huang, F. M., & Chang, Y. C. (2002). Cytotoxicity of resin-based restorative materials on human pulp cell cultures. *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology*, 94(3), 361-365.
30. Tuna, E. B., Aktoren, O., Oshida, Y., & Gencay, K. (2010). Elution of residual monomers from dental composite materials. *Eur J Paediatr Dent*, 11(3), 110-4.