Clinical and Experimental Health Sciences

The Effect of Layer Thickness and Light Intensity on the Degree of Conversion, Microhardness and Cytotoxicity of Bulk Fill Composite Resins

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ABSTRACT

Objectives: The aim of this study was to evaluate the effects of polymerizing bulk fill composite resins at different thicknesses and different light intensities on the degree of conversion, microhardness and cytotoxicity of the composites.

Methods: Two different bulk fill composite resins were used in this in vitro study: Sonic Fill 2, Filtek Bulk Fill. Samples prepared from both composites with a thickness of 2, 4 and 6 mm were polymerized in 2 different power modes. The degree of conversion, microhardness and cytotoxicity of these samples were measured.

Results: As the layer thickness of the bulk fill composite resins increased, the degree of conversion of the lower surfaces of the composites decreased significantly (p <.05). When the lower and upper surface microhardness ratios of bulk fill composite resins were examined, 2 mm thick samples of both composites polymerized by both polymerization methods and 4 mm thick samples polymerized in standard power mode exceeded the acceptable threshold value of 0.80. According to the results of the WST-1 experiment; cell viability decreased as the layer thickness of bulk fill composites increased.

Conclusions: The upper surface degree of conversion and microhardness values of the bulk fill composite resins examined were higher than the lower surface values. According to the results of the WST-1 experiment; as the layer thickness of the bulk fill composites increased, the cell viability decreased, the cytotoxic properties increased.

Keywords: Cytotoxicity, filtek bulk fill, hardness, polymerization, sonic fill

1. INTRODUCTION

When developing composite resin materials, which are frequently used in restorative dentistry, the most emphasized issue is the polymerization mechanism, which significantly affects the physical and mechanical properties of the composite. The effectiveness of polymerization affects many properties of the composite such as its mechanical properties, biocompatibility, volumetric shrinkage and the tensile forces formed during this process, degree of conversion (polymerization degree) and polymerization depth (1). When polymerization is not provided sufficiently, the physical and mechanical properties of the composite resin may weaken over time. Manufacturers have produced bulk fill composites that are polymerized in a single layer of up to 4 mm, saving time for the physician and patient, to simplify and speed up the placement of resin-based composites in large layers in the posterior region (2). Bulk fill composites can be applied as a single layer of 4-6 mm thickness, especially with their increased translucency and the presence of polymerization modulators; and have low shrinkage stress and a high degree of conversion (DC) at this depth (3). The percentage of carbon-carbon double bonds (-C = C-) converted into single bonds (-C-C-) to form a polymeric resin is defined as the degree of conversion, and the degree of conversion values reported for conventional composite resins range from 52 to 75% (4). The degree of conversion is an important parameter for evaluating the optimum clinical performance of resin-based composite materials (5). Polymerization depth, degree of conversion, polymerization shrinkage, linear thermal expansion coefficient, elastic modulus, abrasion resistance, the C factor, etc. parameters such as these affect the clinical success of composite restorations.

The clinical success of the composite restoration depends on various parameters such as polymerization depth, polymerization degree, polymerization shrinkage, linear thermal expansion coefficient, elastic modulus, abrasion resistance, C factor, etc. (4) Among these parameters, the degree of conversion is directly related to physical and mechanical properties such as strength, hardness, solubility,

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color changes and biocompatibility (5). Hardness determines the mechanical properties of polymerized restorative materials. Increasing the hardness increases the resistance against scratching and abrasion and increases clinical success by preventing the material from deforming against the incoming forces. In many studies; the polymerization depth of composite resins was defined based on microhardness measurements on the upper and lower surfaces of the composite resin. It has been reported in these studies that 0.80 can be used as a critical acceptable minimum threshold (6). When composite resins are not sufficiently polymerized, their physical and mechanical properties weaken and residual monomer is released into the environment. These residual monomers can cause estrogenic, genotoxic and cytotoxic effects (7). Possible toxicological reactions are evaluated in vitro using cell cultures in cytotoxicity tests. Provides detailed information on cytotoxicity tests, cell membrane and organelles, protein and DNA synthesis, cell division, cell viability and death (8).

The degree of conversion of bulk fill composites has been shown to be comparable to conventional composites (9). However, there are no data on the extent to which a layer thickness of up to 6 mm compared to a layer thickness of 2 and 4 mm affects the degree of conversion, microhardness and cytotoxicity of bulk fill composites. The aim of this study is to examine the degree of conversion, microhardness and cytotoxicity of two different bulk fill composite resins polymerized at different layer thicknesses and different light intensities. The null hypotheses tested are:

(I) The irradiance applied does not affect the degree of conversion, microhardness and cytotoxicity of the composites.

(II) Thickness of composites does not affect their degree of conversion, microhardness and cytotoxicity.

(III) The applied irradiance and composite thickness do not cause an increase in the cytotoxicity of the composite over time.

2. METHODS

Ethical approval is not required for this study. The effect sizes were determined using the results obtained from previously published studies (9-11). G*Power 3.1 (University of Heinrich Heine, Dusseldorf, Germany) indicated that the sample size used in each test exhibited a power of 0.85 (α = .05).

2.1. Preparation of Samples

Sonic Fill 2 (Kerr Corp., Orange, CA, USA) and Filtek Bulk Fill (3M ESPE, Seefeld, Germany) bulk fill composite resins were used in this study. Detailed information on the selected materials is listed in Table 1. For the preparation of the samples, three different teflon molds of 5 mm diameter, 2, 4 and 6 mm thickness were used. Mylar strip was placed on the bottom surface of the teflon mold. Bulk fill composite resins (A2) were placed in the teflon mold. The samples were polymerized from the upper surface of the teflon mold using a light unit (Valo Ultradent, South Jordan, UT). For polymerization, the light device was used in the standard power mode (1000 mW/cm^2-20 sec) in one of the groups and in the extra power mode (3200 mW/cm^2-3 sec) in the other. A total of 60 samples were prepared from two different bulk fill resin composites, with two different irradiances and three different layer thicknesses, 5 samples for each group. While the same samples were used for microhardness and degree of conversion, new samples were prepared for cytotoxicity tests. The samples were kept dry in amber colored bottles at room temperature (25°C) for 24 hours.

Table 1. *Manufacturers and compositions of bulk fill composites used in the study*

Materials	Composition	Filler Ratio (w/v)	Manufacturer
Filtek Bulk Fill (FBF)	Bis-GMA, UDMA, Bis-EMA, ytterbium trifluoride, zircon silica	%64.5 / %42.5	3M Espe, St.Paul, USA Lot number: N899704
Sonic Fill 2 (SF)	Bis-GMA, TEGDMA, EBPADMA, glass oxide, silicon dioxide	%83.5 / %66	Kerr, Orange, CA, USA Lot number: 6599433

Bis-GMA: Bisphenol A-glycidyl methacrylate, UDMA: Urethane Dimethacrylate, Bis-EMA: Bisphenol A Ethoxylate Dimethacrylate, TEGDMA: Triethylene Glycol Dimethacrylate, EBPADMA: Ethoxylated bisphenol a dimethacrylate.

2.2. Measuring the Degree of Conversion

An ATR-FTIR spectrometer (Perkin-Elmer, Waltham, USA) was used to measure the degree of conversion of bulk fill composite resin samples kept in amber bottles. FTIR spectra ranging from 400 to 4000 cm^{-1} were documented by 32 scans at a resolution of 4 cm^{-1}. Measurements were made on the upper and lower surfaces of cured composite resin samples. The degree of conversion (Equation 1) was determined according to the following equation using changes in the absorbance density ratios of aliphatic C = C to aromatic C-C in the cured and uncured states.

$$\text{Degree of conversion}(\%) = \left(1 - \frac{\left(\frac{A_{aliphatic}}{A_{aromatic}}\right)_{cured}}{\left(\frac{A_{aliphatic}}{A_{aromatic}}\right)_{uncured}}\right) x100 \quad (1)$$

The top and bottom DC ratio (Equation 2), which shows the change in the degree of polymerization with depth, was calculated according to the formula:

$$\text{Degree of conversion ratio}\ (\%) = \frac{\text{Degree of conversion}_{(\text{bottom})}}{\text{Degree of conversion}_{(\text{top})}} \text{x}100 \quad (2)$$

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2.3. Microhardness Measurement

The hardness measurement of the samples was made after 24 hours. The notching tip of the Vickers (Micromet, Buehler, USA) device was positioned perpendicular to the surface to be measured, a 200 g load was applied for 15 s and microhardness was measured from the lower and upper surfaces of the samples. The average of three measurements made on each surface was determined as the hardness value of that surface.

2.4. Cytotoxicity Test

In the cytotoxicity test, a total of 180 samples were prepared, 5 samples in each group and different samples for the 1st, 7th and 21st days. WST-1 (water-soluble tetrazolium) analysis was applied to determine the cytotoxicity of bulk fill composite resins. The L929 mouse fibroblast cell line to be used in the study (Şap Institute, Turkey) was first stained with blue fluorescent DAPI (4′,6-diamidino-2-phenylindole) dye in terms of Mycoplasma transmission, and the result was negative. Cells were inoculated into 24-well culture dishes at $1x10^4$ cells /cm^2 and kept at 37°C and in an incubator containing 5% CO_2 to adhere to the surface overnight. The discs prepared from bulk fill composite resin materials in a sterile environment were sterilized under ultraviolet light (laminar flow sterile cabinet, Class II, Heraus, Hanau, Germany) for 20 minutes. Bulk fill composite discs were placed in the prepared experimental environment and incubated in low glucose DMEM (Dulbecco's Modified Eagle's Medium) medium containing 10% fetal bovine serum (FBS), 1% penicillin / streptomycin. L929 cells that were not treated with bulk fill composite resin materials were used as the control group. WST-1 test was performed to determine cell viability and cytotoxicity at the end of the 1st, 7th and 21st days after placing the bulk fill composite discs in the experimental environment. After the bulk fill composite resins were removed from the experimental environment, WST-1 Cell Proliferation Assay Reagent (Roche) was added at a ratio of 1:10 (30 µl WST-1 reagent to 270 µl medium). After 2 hours of incubation under appropriate conditions (37°C, in an incubator containing 5% CO_2), absorbances were read using a 450 nm wavelength monochromatic microplate reader (Microplate Reader, VersaMax, Molecular Devices, USA).

2.5. Statistical Analysis

The obtained data were statistically analyzed using the SPSS 22 (IBM Corp., Armonk, NY, USA) package program. Whether the samples were normally distributed or not was examined with the Kolmogorov-Smirnov test. According to the results obtained, Kruskal-Wallis and Mann-Whitney U tests were used for the analysis of microhardness and degree of conversion. According to the results of cell viability measurements, one-way analysis of variance and Tukey multiple comparison test were used to determine the statistical differences between groups. In addition, the Anova test was used for repeated measurements in order to examine the changes in cell viability on the 1st, 7th and 21st days. The significance level for all results was set at $p = .05$.

3. RESULTS

3.1. Results of Degree of Conversion Measurements of Bulk Fill Composites

When the layer thicknesses of Sonic Fill 2 (SF) and Filtek Bulk Fill (FBF) groups polymerized in the standard power mode were compared separately, there was no statistically significant difference among the average degree of conversion ($p>.05$), while there was a statistically significant difference among the groups polymerized in the extra power mode ($p <.05$) (Table 2).

When the effect of irradiance on each composite group and each layer thickness was examined separately, there was no statistically significant difference between the groups of FBF composite prepared only in 2 and 6 mm thickness (Table 2).

As the layer thickness increases in SF and FBF groups polymerized in standard and extra power mode, the average degree of conversion ratio decreased statistically significantly *(p<.05)* (Table 2).

Table 2. *The ratio of degree of conversion (%) of SF and FBF groups from the lower surface to the upper surface after polymerization in different light irradiance and different increment thicknesses.*

	Layer thickness	1000 mW/cm² – 20 s (Standart power)	3200 mW/cm² – 3 s (Extra power)
SF	2 mm	78.70 ± 9.31 ^{a,C}	94.40 ± 5.30 ^{a,C}
	4 mm	57.03 ± 7.84 ^{x,B}	24.49 ± 4.54 ^{x,AB}
	6 mm	27.41 ± 6.01 $^{+,A}$	16.28 ± 6.46 $^{+,A}$
FBF	2 mm	83,24 ± 7.65 ^{a,C}	88.36 ± 7.41 ^{a,C}
	4 mm	49.23 ± 8.25 ^{x,B}	33.42 ± 3.12 ^{y,B}
	6 mm	31.13 ± 9.88 $^{+,A}$	34.38 ± 6.37 $^{-,B}$

SF: Sonic Fill, FBF: Filtek Bulk Fill. Different lower case letters and symbols show the statistical difference between SF and FBF samples polymerized at the same thickness and same light irradiance according to the Paired Sample T test. Different capital letters indicate the difference between the groups in the column according to the One Way ANOVA test (p< .05).

3.2. Results of Microhardness Measurements of Bulk Fill Composites

3.2.1. Lower-Upper Surface Microhardness Ratio

When the lower-upper surface microhardness ratios were compared, the clinically acceptable threshold value of 0.80 was exceeded by the 2 and 4 mm thick samples polymerized in the standard power mode of both composites and the 2 mm thick samples polymerized in the extra power mode (Figure 1) (Table 3).

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When the lower-upper surface microhardness ratios of the 2 and 4 mm thick samples of both composites were compared, 2 mm thickness samples prepared in the extra power mode showed statistically higher microhardness ratios than the samples prepared in 4 mm thickness. There was no statistically significant difference between the samples prepared in the standard power mode (p>.05) (Table 3).

Table 3. *Microhardness ratio (%) of SF and FBF groups to the upper-lower surface after polymerization in different light irradiance and different increment thicknesses.*

	Layer thickness	1000 mW/cm^2 – 20 s (Standart power)	3200 mW/cm^2 – 3 s (Extra power)
SF	2 mm	0.90 (0.05) ^{BC}	0.84 (0.05) ^{D}
	4 mm	0.85 (0.05) ^{B}	0.60 (0.05) ^{C}
	6 mm	0.64 (0.18) ^{A}	0.14 (0.05) ^{A}
FBF	2 mm	0.90 (0.06) ^{BC}	0.85 (0.04) ^{D}
	4 mm	0.91 (0.05) ^{B}	0.53 (0.07) ^{C}
	6 mm	0.73 (0.05) ^{A}	0.49 (0.07) ^{B}

SF:Sonic Fill, FBF: Filtek Bulk Fill. Different capital letters indicate the difference between the groups in the column according to the One Way ANOVA test, (p< .05).

When the light intensities were compared, samples polymerized at 4 and 6 mm thicknesses and standard power mode from SF and FBF composite resins showed a higher microhardness ratio than samples polymerized in the extra power mode (p <.05) (Figure 1).

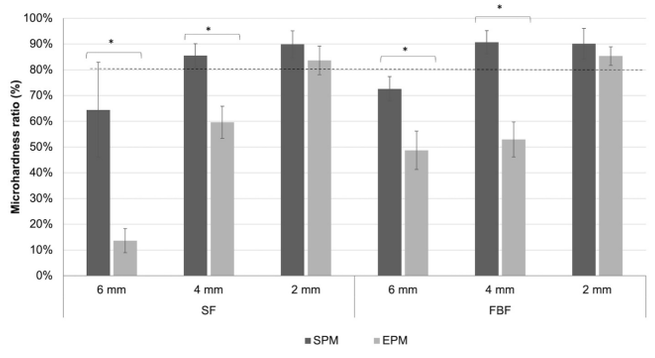

Figure 1. *Upper-lower surface average microhardness ratios (%) of SF and FBF groups. The * sign indicates that there is a statistical difference between columns belonging to the same thickness within the composite groups. The green line indicates the 80% hardness rate, which is considered to be the clinical threshold for microhardness in the literature.*

3.3. Findings of Cytotoxicity Tests of Bulk Fill Composites

The average cell numbers of the groups (composite resins tested and the control group) are shown in Figure 2. The control group in the WST-1 test referred to the group in which only cells were used instead of bulk fill composite samples. There was a statistically significant difference between the WST-1 test results on the 1st, 7th and 21st days among the samples belonging to the control group (p <.05) (Figure 2).

In the 6 mm thick samples of the SF composite polymerized in the standard power mode, the number of cells decreased statistically significantly from day 1 to day 21 (p <.05) (Figure 2). Although there were changes in cell numbers between the 1st, 7th and 21st days of the FBF composite groups, there was no statistically significant difference (p >.05) (Figure 2).

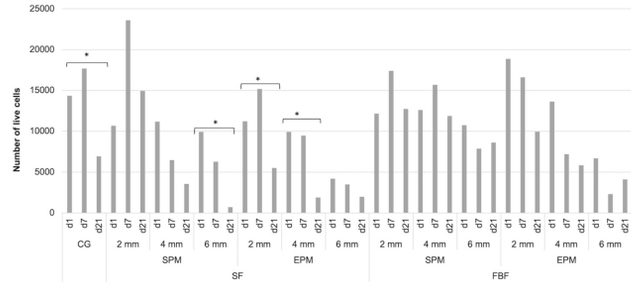

Figure 2. *Comparison of WST-1 test results on days 1, 7 and 21 of the same samples of Sonic Fill 2 and Filtek Bulk Fill composites. (* sign indicates that there is a statistical difference between the columns belonging to the same sample within the composite groups. d1: 1st day, d7: 7th day, d21: 21st day, SPM: standart power mode, EPM: extra power mode, CG: control group)*

There was no statistically significant difference in terms of cell viability at the end of the 1st day between FBF and SF samples prepared in standard power mode at all layer thicknesses (p >.05) (Figure 3). Cell viability from 2 mm to 4 mm did not decrease in samples polymerized in the extra power mode, while cell viability was reduced in samples with 6 mm thickness (Figure 3).

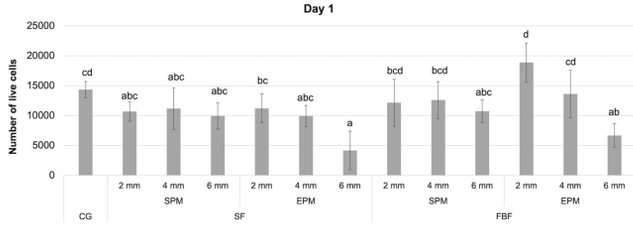

Figure 3. *Comparison of the results of the WST-1 test on day 1 of Sonic Fill 2 and Filtek Bulk Fill composite samples prepared at different light irradiances and 2,4,6 mm thickness. (Different lower case letters indicate statistical difference between columns.)*

At the end of the seventh day, cell viability decreased as the layer thickness increased in the SF and FBF groups polymerized in the extra power mode and in the FBF groups polymerized in the standard power mode (Figure 4).

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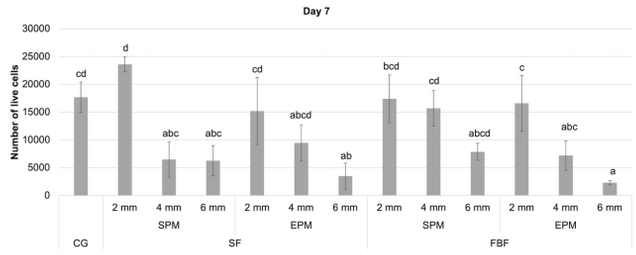

Figure 4. *Comparison of the results of the WST-1 test on day 7 of Sonic Fill 2 and Filtek Bulk Fill composite samples prepared at different light irradiance and 2,4,6 mm thickness. (Different lower case letters indicate statistical difference between columns).*

When compared with the control group at the end of the 21st day, the cell numbers in all groups were found to be similar to the control group (p>.05) (Figure 5).

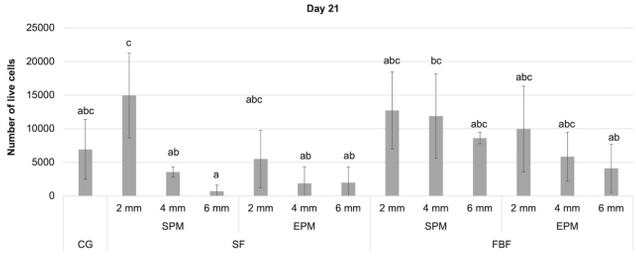

Figure 5. *Comparison of the results of the WST-1 test on the 21st day of the Sonic Fill 2 and Filtek Bulk Fill composite samples prepared at different light irradiance and 2,4,6 mm thickness. Different lowercase letters show that there is a statistical difference between the columns. (CG:Control grup, SPM: Standart power mode, EPM:Extra power mode)*

4. DISCUSSION

The improvements in the mechanical and aesthetic properties of the materials used in the posterior area have brought the expectation of ease of use. The bulk fill technique developed for this purpose and the materials applied with this technique have become very popular today (12). Although bulk fill composites have many advantages, they also have disadvantages. An increase in water absorption and water solubility and a decrease in microhardness may cause weakening of physical and mechanical properties. This may affect the clinical behavior of the restoration due to reasons such as color stability, surface roughness, restoration gloss and hydrolytic degradation (13, 14).The chemical stability, and physical and mechanical properties of composite resins that are not polymerized enough are reduced, and they also have a potentially toxic effect on pulpal tissues (15).

Few studies in the literature have evaluated the cytotoxicity of bulk fill composites. However, different parameters such as cytotoxicity test method, cell type, cell-material contact method and exposure time used in these studies make it difficult to compare the results of these studies (10, 15, 16).

There are studies in the literature showing that monomer release from composites continues in the long term (9, 17-19). The decrease in the number of cells in some groups compared to the control group on the 7th and 21st days in our study suggests that the release of cytotoxic substances from the composite resins may continue after the first 24 hours. These findings support the results in the literature.

Similar to the results of this study, the study of Şişman et al. found a decrease in the number of cells on the 7th, 14th and 21st days compared to the control group. As in our current study, they used FBF and SF bulk fill composites with a diameter of 5 mm and a thickness of 4 mm, differently, they used dental pulp stem cells (10). In the study of Şişman et al., the number of viable cells in the FBF group increased from day 1 to day 7, and decreased on day 21, but this decrease was not statistically significant. This result supports the result of our study since it yields similar results with the 4 mm thick FBF group polymerized in the standard power mode with the same diameter and thickness used in our study. In the same study, while the number of live cells increased from day 1 to day 21 in the SF group, in our study, the number of live cells decreased from day 1 to day 21 in the SF group polymerized in standard power mode, but this decrease was not statistically significant. This difference may be related to the fact that the light intensities (1200 mW / cm^2-1000 mW / cm^2) of the light devices used in both studies is different and the cells used in the WST-1 test are different.

The amount of monomer may increase if the applied light time is insufficient and the wavelength of the light used is not sufficient for the polymerization of the material or the composite is prepared in excessive thicknesses. In our study, it was determined that the number of viable cells in samples prepared with a thickness greater than the manufacturer's instructions and polymerized at high irradiance in a short time compared to the other groups. Based on these results, the parts related to cytotoxicity of the first, second and third null hypotheses were rejected.

The amount of light available to stimulate the photoinitiator is significantly reduced from the upper surface to the lower surface as a result of the absorption and scattering of light by the composite itself or the surrounding tissue (20, 21). The data we obtained in our study support this information. As the thickness of both composites increased, the degree of conversion decreased as the thickness increased, since sufficient light could not reach the lower regions to excite the photoinitiators. For this reason, the part of our second null hypotheses that the composite thickness does not affect the degree of conversion was rejected.

Jain et al. investigated the effects of polymerizing four different bulk fill composite resins at two different layer thicknesses and two different light intensities (1000 mW/cm^2-1400 mW/cm^2) on the degree of conversion immediately after polymerization and 24 hours after polymerization. When the study findings were examined, it was observed that when the irradiance increased, the degree of conversion was higher on the lower and upper surfaces in both thicknesses of

the two bulk fill composite resins (5). When compared with the data we obtained in our study, when the irradiance was increased, the rate of degree of conversion of SF composite samples prepared with 2 mm thickness increased statistically significantly. Except for the samples of FBF composite prepared in 2 and 6 mm thickness, the rate of the degree of conversion decreased significantly in all groups. For this reason, the part of the first null hypothesis related to the degree of conversion was rejected.

In addition, samples of SF composite prepared at 4 and 6 mm thickness and polymerized in extra power mode showed a lower degree of conversion rate compared to FBF samples of the same thickness. This can be explained by the lower translucency confirmed by previous studies compared to Sonic Fill's other bulk fill composites (22, 23). Low translucency affects light transmission and adversely affects the degree of conversion (24).

Comparing the microhardness values of the top and bottom surfaces of composite specimens is another way of assessing the degree of conversion and depth of cure of the material (25, 26).

Previous studies have reported that an increase in the degree of conversion increases the surface microhardness (27, 28).

In examining the polymerization values of composite resins, it is not sufficient to evaluate only the surface microhardness where light is applied. In many studies, it has been reported that the microhardness values of the upper surface closest to the light device and the lower layers should be proportioned, and when this ratio falls below 80%, the polymerization should be considered insufficient (29, 30). According to different researchers, acceptable curing depth is obtained when the bottom-top microhardness ratio of the composite resin is at least 80% (11, 29, 31). According to the data obtained, when the microhardness ratios are examined, it is seen that the bulk fill composite resins used outside the manufacturer's instructions (6 mm layer thickness and polymerization in extra power mode) are below 0.80. The degree of conversion ratios are in parallel with microhardness ratios and groups with microhardness ratio of 0.80 and above have degree of conversion ratios. Clinicians may be advised to pay attention to cavity depth when using these composites. When these results were evaluated, the microhardness parts of the first and second null hypothesis were rejected.

In some cases, clinicians may lose the concept of depth in deep cavities and fall outside the manufacturer's instructions. In this study, a layer thickness of 6 mm was investigated as the worst case for clinicians. Limitations of this study; traditional composite resin was not used as a control group and cytotoxicity was not investigated on dental pulp cells. In this sense, the results obtained in our study give preliminary information about the cytotoxic effects of the tested bulk fill composite resins. Further in vitro tests related to the results obtained from this study should be carried out and the results should be supported by animal experiments.

5. CONCLUSIONS

In this study, the effects of polymerization of Sonic Fill 2 and Filtek Bulk Fill composites at 2, 4 and 6 mm thickness at two different light intensities (standard power mode-20s and extra power mode-3s) on the degree of conversion, microhardness and cytotoxicity of the composite was investigated.

The degree of conversion and microhardness of both bulk fill composites were below the clinically accepted threshold when used at irradiance and thickness not conforming to the manufacturer's instructions. WST-1 test results show parallelism with these results. In addition, it can be said that monomer release continues after the first 24 hours in both bulk fill composites and causes a cytotoxic effect. In line with these results, although bulk fill composite resins provide many advantages to clinicians, they may have insufficient physical and mechanical properties and show cytotoxic effects when the manufacturer's instructions are exceeded.

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Research idea: AD, CB
Design of the study: AD, CB
Acquisition of data for the study: SGB
Analysis of data for the study: CB
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