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The cytotoxicity of resin composites cured with three light curing units at different curing distances

Gulfem Ergun [1], Ferhan Egilmez [1], Isil Cekic-Nagas [2]

[1] Department of Prosthodontics, Faculty of Dentistry, Gazi University, Ankara, Turkiye
[2] School of Dental Technology, Hacettepe University, Ankara, Turkiye

Correspondence:
Suslu Sokak 14/9
Mebusevleri-Tandogan
Ankara / TURKIYE
ergungulfem@yahoo.com

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Abstract

Objective: The purpose of this study was to compare the effect of light curing distance on the cytotoxicity of five resin composites cured with three high-power light curing units.

Study design: Seven cylindrical discs of each material (Grandio ®, Voco; Filtek ™ Z250, 3M ESPE; Clearfil ™ AP-X, Kuraray Co. Ltd.; Aelite ™ LS, Bisco Inc. and Simile ®, Pentron) were cured. For curing, soft-up mode of quartz-tungsten-halogen, exponential mode of light emitting diode for 20 s, and ramp-curing mode of plasma arc light curing units for 6 s were used. The curing tip distances were determined as 2 and 9 mm and controlled via the use of metal rings. After ageing the samples for 24 and 72 hours in Dulbecco's Modified Eagle Medium/Ham's F12 (DMEM/F12), cytotoxicity of the extracts to cultured fibroblasts (L 929) was measured by using MTT (tetrazolium salt 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. The degree of cytotoxicity for each sample was determined according to the reference value represented by the cells in a pure culture medium. Statistical significance was determined using multifactorial analysis of variance.

Results: The type of resin composite ($p<0.05$), light curing unit ($p<0.05$), curing tip distance ($p<0.05$) and evaluation period factor ($p<0.05$) had statistically significant cytotoxic effects on L–929 mouse fibroblast cells. However, when the tested materials polymerized at both distances (2 mm and 9 mm) in both evaluation periods (24h and 72h), there was no significant difference in the mean CSR% values obtained when the quartz-tungsten-halogen, light emitting diode and plasma arc light curing units were used ($p=0.184$, $F=1.448$).

Conclusions: The results of this study suggest that the light curing units and resin composites should be harmonized to one another and the curing distance between the tip of the light curing unit and the restoration surface should be as close as possible in order to achieve maximal biocompatibility.

Key words: *Dental curing lights, cytotoxicity, composite resins.*

Introduction

Effective composite cure is a critical parameter, not only to ensure optimum physical properties of the cured restoration, but also to ensure that clinical problems do not arise as a result of the cytotoxicity of inadequately cured materials (1). If a light-activated resin composite does not receive sufficient energy at the correct wavelengths from the light curing unit (LCU). This could result in wear and greater breakdown of the restoration at the margins, decreased bond strength between the tooth and the restoration, reduced hardness, and greater cytotoxicity (2). Furthermore, this may lead to different releasing rates of unreacted toxic components from resin composites (3).

Nowadays, boosted versions of high intensity quartz-tungsten-halogen (QTH), plasma arc (PAC), light emitting diode (LED) LCUs that possess higher light intensity than conventional LCUs have been developed (4, 5). As it is known, high conversion is important for good mechanical properties and biocompatibility (6). In view of the great variety of LCUs and resin composite materials currently in use, the question is which combinations cause the least toxic effects. In addition, the effective light intensity available for the photoactivation of resin monomers is influenced by the distance between the light curing tip and the resin composite material. In other words, the intensity decreases with the square of the distance (7). Ideally, the LCU tip should be in direct contact with the surface of restoration. However, sometimes cavity design does not allow sufficient polymerization within this distance (8). As a consequence, the distance between the light tip and the restoration becomes a crucial parameter for cytotoxicity.

On these grounds, the aim of this study was to compare the effectiveness of high power QTH, LED, and PAC LCUs on cytotoxicity of five resin composites irradiated at two different curing distances. The null hypothesis was that the LCU type, curing distance and evaluation period affect the cytotoxicity of different resin composites.

Materials and Methods

-*Cells*

The cells used for the experiments were L-929 mouse fibroblasts (L-929 An2 HÜKÜK 95030802; Ankara Şap Enstitüsü, Ankara, Turkey). The cells were grown as monolayer cultures in T-25 flasks (Costar, Cambridge, MA, USA), subcultured three times a week at 37°C in an atmosphere of 5% CO_2 in air and 100% relative humidity and maintained at third passage. The culture medium was Dulbecco's modified Eagle medium (DMEM)/Ham's F12 nutrient mixture (1:1; Sigma, St Louis, MO, USA) supplemented with 10% (v/v) foetal bovine serum (FBS; Biochrom, Berlin, Germany) without antibiotics. Adherent cells at a logarithmic growth phase were controlled under an inverted tissue culture microscope (Olympus CK40, Japan) and detached with a mixture of 0.025% trypsin (Sigma) and 0.02% ethylenediaminetetraacetic acid (EDTA; Sigma), incubated for 2-5 min at 37°C and used for cell inoculation.

-*Sample preparation*

Five restorative resin composites (2 mm in thickness and 6 mm in diameter) of shade A1 were used in this study (n = 84/per group) (Table 1). Figure 1 shows the schematic illustration of sample preparation. The composite materials were placed into sterile circular polytetrafluoroethylene moulds. Polyethylene films were added on the top base of the composite materials and a 1 mm glass slide was placed on top of the mould to exclude excessive resin composite material and to eliminate air bubbles. Then the samples were irradiated top through the metal rings used to control irradiation distances (2 mm and 9 mm) by soft-up mode of QTH LCU (Blue Swan Digital, Dentanet, Ankara, Turkey) for 20 s, exponential mode of LED LCU (Elipar Freelight 2, 3M Espe, St. Paul, Minn, USA) for 20 s and ramp-curing mode of PAC LCU (PlasmaStar, SP-2000, Monitex, Taiwan) for 6 s under aseptic conditions at laminar flow (Holten, Class II, Denmark) (Table 2). All samples were prepared by the same operator.

The freshly prepared tested samples were placed immediately at the bottom of six well-plates (Costar, Cambridges, MA, USA). The samples were placed in DMEM/F12 with 10% FBS and incubated at 37 °C in an atmosphere of 5% CO_2 in air without agitation for 24 h and 72 h. After the incubation, the extracts were filtered through 0.22 µm cellulose acetate filters (Millipore, Sigma, St. Louis, MO, USA) and then they were used to evaluate cytotoxicity.

-*Cytotoxicity testing (MTT assay)*

The L-929 cell suspension with DMEM/F12 with 10% FBS and 1% antibiotic was prepared at a concentration of 3 x 10^4 cells mL^{-1} and inoculated onto 96-well cluster cell culture plates (100 µL per well). The multiwell plates were incubated at 37 °C, 5% CO_2 in air for 24 h. After 24 h, the culture medium was removed from the wells and equal volumes (100 µL) of the extracts were added into each well. In control wells, 100 µL DMEM/F12 with 10% FBS and 1% antibiotic was added. After the 24 and 72 h incubation periods, test extracts were removed. Following the removal of the test extracts, 100 µL per well DMEM/F12 with 10% FBS and 1% antibiotic and 12 µL MTT (tetrazolium salt 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) were added to each well and incubated in a dark environment for 4 h at 37 °C. After incubation, 96 wells were checked for formazan crystals with inverted tissue culture microscope. MTT was aspirated and 100 µL per well of isopropanol (Merck, Darmstadt, Germany) was added to each well. Subsequently, the absorbance at 570 nm

Table 1. Resin composites used in this study.

Trade Name	Code	Composition	Type	Filler load* (% by weight)	Lot Number	Manufacturer
Clearfil™ AP-X	**A**	Silanated barium glass, Silanated colloidal silica, silanated silica, [a]Bis-GMA, [b]TEGDMA, dl-Camphorquinone	Micro hybrid	85.5 %	454BA	Kuraray Medical INC. Okayama, Japan
Simile®	**B**	[c]PCBis-GMA, [a]Bis-GMA, [d]UDMA, [e]HDDMA, Silane Treated Barium Boro-alumino Silicate Glass, Silane Treated Nano-particulated Silica, Zirconium Silicate, photoinitiator, accelerator, stabilizer, silane and pigments	Nano-hybrid	75 %	144063	Pentron Clinical Technologies, LLC, Wallingford, U.S.A.
Grandio® Caps	**C**	Inorganic fillers, [a]Bis-GMA, [b]TEGDMA, [f]BHT, [d]UDMA, silicate	Nano-hybrid	87 %	581501	VOCO GmbH, Cuxhaven, Germany
Filtek™ Z250	**D**	[a]BisGMA, [b]TEGDMA. [d]UDMA, [g]BisEMA	Micro hybrid	82 %	20051212	ESPE Dental-Medizin, Seefeld, Germany
Aelite™ Aesthetic Enamel	**E**	[g]Bis-EMA,[a]Bis-GMA, Glass frit, Amorphous Silica	Nano-hybrid	73 %	0500005455	Bisco, Inc. Schaumburg, U.S.A.

*According to the manufacturer's instructions.
[a]Bis-GMA: Bis-phenol A diglycidylmethacrylate, [b]TEGDMA: Triethyleneglycol dimethacrylate, [c]PCBis-GMA: Polycarbonate modified-Bis-GMA, [d]UDMA: Urethane dimetacrylate, [e]HDDMA: Hexanediol dimethacrylate, [f]BHT: Butylated hydroxy toluene [g]Bis-EMA: ethoxylated bisphenol A dimethacrylate.
A: Clearfil™ AP-X; B: Simile®; C: Grandio® Caps; D: Filtek™ Z250; E: Aelite™ Aesthetic Enamel

was measured using a UV–visible spectrophotometer (LPB Pharmacia, Bromma, Sweden). Then, the viable cells were counted under a light microscope and calculated as a percentage of the control values at each evaluation period (24 h and 72 h). Each cytotoxicity assay was evaluated three times for each experimental group.

-*Statistical analysis*

Statistical analysis was performed by Statistical Package for Social Sciences (SPSS) 11.5 software (SPSS Inc., Chicago, IL, USA). Whether the data were normally distributed or not were determined by using Shapiro Wilk test. Homogeneity of variances was evaluated by Levene test. Firstly, anscombe transformation was applied to data in order to transform the percentage of cytotoxicity variable in a Gaussian one. Data were expressed as mean ± standard deviation. Multi factorial ANOVA taking such following factors as resin composites (5 levels), light curing units (3 levels), curing tip distances (2 levels) and evaluation periods (2 levels) was performed and post hoc Tukey test was applied for the evaluation of the data. A p value less than 0.05 was considered statistically significant. But, for all possible multiple comparison tests, Bonferroni Adjustment was applied to control Type I error.

Table 2. Light curing units (LCUs) used in the study.

LCUs	Trade Name	Manufacturer	Serial Number	Light Intensity	Polymerization Time*
QTH	Blue Swan Digital	Dentanet, Ankara, Turkey	03-185	1000 mW/cm^2	Soft-up mode: 20 s
LED	Elipar Freelight 2	3M Espe, St. Paul, Minn, USA	939820014022	1200 mW/cm^2	Exponential mode: 20 s
PAC	PlasmaStar, SP-2000	Monitex, Taiwan	P0500206	2250±50 mW/cm^2	Ramp curing mode: 6 s

* According to the manufacturer

Table 3. The mean cell survival rates (CSR%) and standard deviations of the tested materials.

Tested Materials	Light Curing Unit (LCU)	Evaluation Period	Irradiation distance			
			2 mm		9 mm	
			CSR% Mean	Std. Deviation	CSR% Mean	Std. Deviation
A	LED	24 h	18,78	0,22	18,03	0,43
		72 h	17,73	0,45	17,47	0,23
	QTH	24 h	18,77	0,31	18,23	0,14
		72 h	16,98	0,12	17,79	0,34
	PL	24 h	18,79	0,18	17,98	0,34
		72 h	17,63	0,48	17,67	0,41
B	LED	24 h	18,99	0,19	19,05	0,09
		72 h	18,95	0,25	18,74	0,15
	QTH	24 h	19,01	0,36	19,25	0,18
		72 h	18,97	0,21	18,62	0,12
	PL	24 h	19,53	0,17	18,66	0,17
		72 h	19,17	0,13	17,92	0,30
C	LED	24 h	18,75	0,22	19,13	0,09
		72 h	19,64	0,25	19,48	0,08
	QTH	24 h	19,27	0,19	19,82	0,15
		72 h	19,68	0,33	19,71	0,26
	PL	24 h	19,73	0,14	19,36	0,20
		72 h	19,48	0,63	19,53	0,11
D	LED	24 h	17,36	1,28	16,49	0,23
		72 h	17,70	0,32	17,86	0,25
	QTH	24 h	17,17	0,98	16,98	0,75
		72 h	17,50	0,53	17,77	0,54
	PL	24 h	17,62	0,45	17,59	0,45
		72 h	17,11	0,40	17,12	0,44
E	LED	24 h	17,00	0,12	16,15	0,20
		72 h	15,34	0,14	15,16	0,13
	QTH	24 h	17,11	0,22	16,91	0,28
		72 h	16,48	0,20	15,95	0,23
	PL	24 h	15,71	0,20	14,79	0,27
		72 h	14,05	0,21	13,78	0,15

A: Clearfil™ AP-X; B: Simile®; C: Grandio® Caps; D: Filtek™ Z250; E: Aelite™ Aesthetic Enamel

Results

The cell numbers of all freshly prepared tested materials decreased compared to the control group (culture without sample) (Table 3).

Statistical analysis showed that there was a significant difference amongst the tested materials ($p<0.05$, $F=593.606$). While the test material C exhibited the highest mean cell survival rates (CSR%) (19.465±0.60), E had the lowest mean CSR% (15.702±0.60) values. When the overall mean CSR% values were evaluated, the test materials were ranked as C>B>A>D>E.

Also, there were statistically significant differences for the number of surviving cells in the different LCUs ($p<0.05$, $F=22.102$). While the QTH LCU exhibited the highest mean CSR% (18.098±0.47), the LED LCU 17.891±0.47 and the PAC LCU showed the lowest mean CSR% (17.660±0.47). When the overall mean CSR% values were evaluated, the LCUs were ranked as QTH>LED>PAC.

The differences between the CSR% values were also significant for the curing distances (2mm and 9 mm) ($p<0.05$, $F=18.901$). In addition, the mean CSR% values of the tested materials observed at different evaluation periods (24h and 72h) were statistically different ($p<0.05$, $F=46.963$). In terms of overall mean CSR% of 2 mm results were higher than the 9 mm results (18.00±0.038, 17.66±0.038, respectively) and the 24h results were higher than the 72h results (18.067±0.038, 17.699±0.038, respectively).

When the two factor interaction between the curing tip distances and LCUs was evaluated, it was shown that

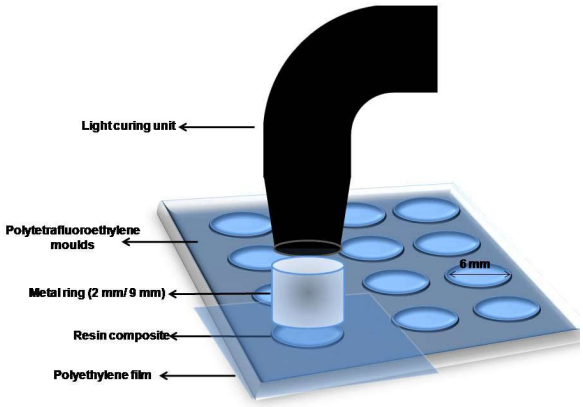

Fig. 1. Schematic illustration of sample preparation.

2 mm and 9 mm curing distances for all LCUs had similar effect on the CSR% values ($p=0.846$, $F=0.168$, $p=0.239$, $F=1.457$, respectively). At the same time, when the QTH, LED and PAC LCUs were used at both curing distances (2mm and 9 mm) overall mean CSR% values were resulted statistically similar ($p=0,976$, $t=-0.03$ for QTH; $p=0.439$, $t=0.78$ for LED and $p=0.35$, $t=0.943$ for PAC LCU).

According to the ANOVA, there was no significant three-factor interaction amongst the tested materials, LCUs and curing distances ($p=0,099$, $F=1.727$). Additionally, data of all the tested materials for both cur-

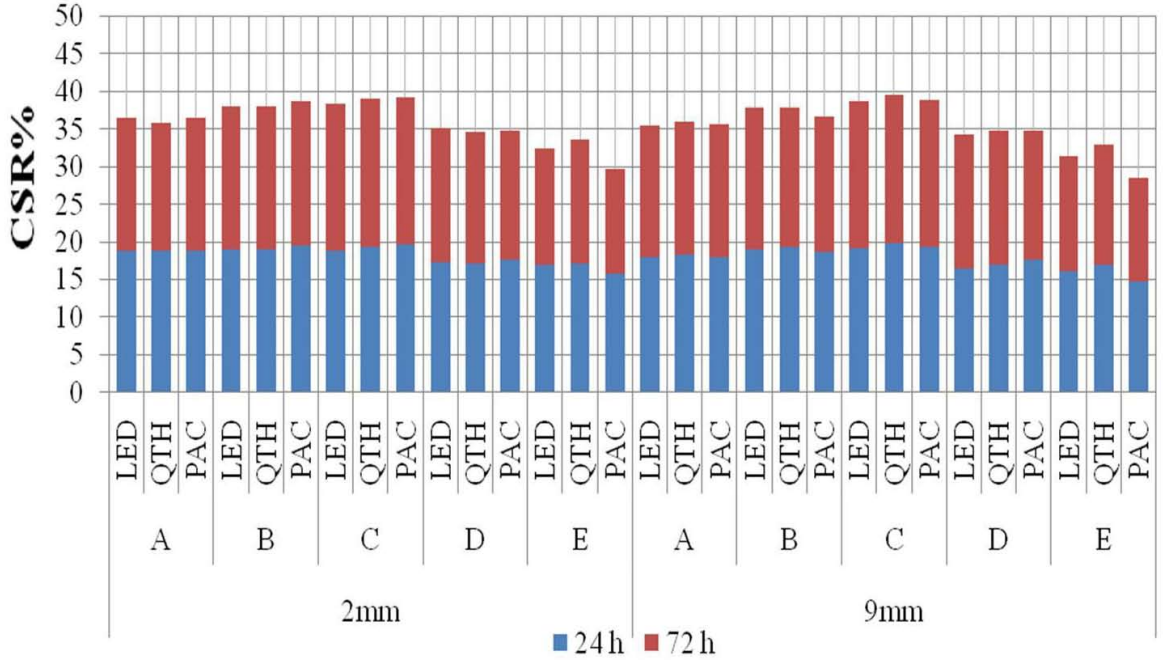

Fig. 2. The distribution of CSR% values at different curing distances in each resin composite. CSR% values were expressed as a percentage of control values (cultures without samples). Bars show the mean of three independent experiments.

ing distances were combined, the overall mean CSR% values were statistically similar between the LCUs at 24h and 72h evaluation periods (p=0.184, F=1.448). However a significant difference was found when the interaction among the tested materials, the LCUs and the evaluation periods for both curing distances (2 mm and 9 mm) was considered (p<0.033, F=2.892). At the 24h evaluation period QTH and PAC LCUs were able to polymerize test material C better than the LED LCU (p<0.008, F=8.832). On the contrary, at the both 24h and 72h evaluation periods, QTH and LED LCUs were able to polymerize test material E better than the PAC LCU (p<0.008, F=25.313, p<0.008, F=125.972, respectively). For other tested materials similar results were obtained when the QTH, LED and PAC LCUs were used for both curing distances (2mm and 9 mm) at both 24h and 72h evaluation periods (p>0.008).

When the tested materials polymerized at both distances (2 mm and 9 mm) in both evaluation periods (24h and 72h), there was no significant difference in the mean CSR% values obtained when the QTH, LED and PAC LCUs were used (p=0.184, F=1.448). So there was no significant four factor interaction among the tested materials, the LCUs, curing distances and evaluation periods. The mean CSR% and standard deviations of the tested materials were given in table 2.

Figure 2 demonstrates the distribution of CSR% of resin composites polymerized with LCUs at two irradiation distances in the evaluation periods.

Discussion

In the present study, the cytotoxic effect of five different resin composites cured with different LCUs at two curing distances were investigated with MTT assay at different evaluation periods (24h-72h). The assay used in this study was MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), which is a widely used functional assay for biocompatibility evaluation, because of its reliability and sensitivity (9-11).

An adequate degree of conversion is necessary to improve the biocompatibility of composite materials used in restorative dentistry (9). A previous study by Caughman et al. (12) evaluated the correlation between cytotoxicity, filler loading and curing time of dental composites and concluded that when the percentage of monomer conversion increased, the cellular toxicity decreased. It has been reported that the filler content, filler size, and the distribution of the filler particles affect the properties of the resin composites. Thus, increasing the filler content and reducing the average filler size has been one approach in producing resin composites with excellent material properties and a good clinical performance (13). In addition, the nanofilled resin was launched in the market with the intention of offering enhanced curing depth (14). A study by Söderholm et al. (15) indicated that highly filled materials used for indirect resin restorations should exhibit less cytotoxicity because of their lower leachable resin content. In the light of all these information, in the present study, the material C, with the highest filler content by weight (87%), showed the highest CSR% values of all the tested materials. The high filler load might have reduced the amount of resin available for dissolution. Moreover, this flowable universal nano-hybrid composite has lower viscosity facilitating molecular mobility and higher degree of monomer conversion. Lower viscosity results in greater DC% increasing the mobility of molecules (16). Additionally, its silicon-dioxide nano-particles are designed to covalently bond to polymeric resin, increasing the conversion rate of the material C, though reducing the possible releasing of toxic compounds. As for the comparison of CSR% values of the resin composites in the present study, the materials B and C, nano-hybrid composites, exhibited higher CSR% values than A and D microhybrid composites (Table 3). Despite being a nanofilled composite, the material E did not achieve the CSR% values that other nanofiller composites did. This could be related with its lower filler content by weight (73 %, Table 1).

Both resin content and percentage of monomer conversion of dental materials were considered as potential causes of cytotoxicity (12). Methacrylate-based resin monomers released from resin composites, such as bisphenol A diglycidylmethacrylate (Bis-GMA), triethyleneglycol dimethacrylate (TEGDMA), urethane dimethacrylate (UDMA), have been shown to be cytotoxic in sufficient concentrations (17-19). A previous study by Hanks et al. (20) demonstrated cytotoxic effects of some resin components on DNA and protein synthesis on 3T3 fibroblasts and found ethoxylated bisphenol-A-dimethacrylate (Bis-EMA) as the most toxic, then UDMA and Bis-GMA; TEGDMA as slightly less toxic. This was in agreement with the current study in that, materials D and E, which consist of Bis-EMA agent, showed the lowest CSR% values, as this agent might be the most toxic one among the other dimethacrylates, as was demonstrated in the previously mentioned study (20).

In the present study, a high-intensity LED LCU has exposure time options as follows: 5, 10, 15 and 20s according to the manufacturer´s instructions. Because of the high light intensity (1200 mW/cm^2) of this LCU, these time periods corresponds to the time periods (10, 20, 30 and 40 s) of conventional light curing unit that has light intensity of 600-800 mW/cm^2 for halogen technology or 300-400 mW/cm^2 light intensity for LED. Thus, the normal exposure times for conventional units can be cut in half without compromising curing performance. Similarly, according to the manufacturer´s instructions of PAC LCU that has been used in this study, this LCU can produce four times as much of light intensity as the

conventional LCU. Thereby, the curing time could be shortened to as much as ¼ of the curing time of the conventional halogen lamp. Moreover, due to high power capacity of LCUs used in the present study, resin materials were cured with high intensity QTH (soft-up mode) for 20 s, with LED (exponential mode) for 20 s, with ramp-curing mode of PAC for 6 s.

In the current study, when the CSR% values of the tested materials were considered, it was observed that the composite resins cured with QTH, LED and PAC LCUs showed different cytotoxicity values. It was demonstrated that, the QTH LCU resulted in the highest value in the number of surviving cells, while the PAC LCU had the lowest value. Moreover, based on the results of our study, when the QTH, LED and PAC LCUs were used for the polymerization of composites, the CSR% values of materials A, B, C and D were found similar. However, the QTH and LED LCUs polymerize material E better than the PAC LCU ($p<0.01$, $F=25,671$). Therefore, according to the results, the LCU that gave good results for one composite material did not exhibit the same performance for another composite material. This could indicate the presence of cytotoxic monomers, which are inactivated by their conversion during the light curing process with different LCUs. Furthermore, the results revealed that there might be several possible reasons for different effects of composite resins or LCUs on their cytotoxicity such as the light transmission characteristics, the released energy during the curing of the resin composites and the amount as well as the type of released toxic substances from uncured resin composites. For this reason, in this study, it is not possible to grade the performances of the LCUs clinically. Although higher energy density leads to higher degree of conversion (1), a study by Knezevic et al. (21) reported that higher energy density also causes temperature rise. In literature, PAC LCUs, which have high energy densities, are often discussed as an alternative to high-power QTH and LED LCUs (4, 22). However, in the present study, resin composites cured with PAC LCU showed similar to or lower mean CSR% values than QTH and LED LCUs except material E. The special spectrum of this lamp, which has very high light intensities (2250±50 mW/cm^2) at certain wavelengths, might have caused this outcome.

The distance between the tip of LCU and resin composite directly affects light intensity on the resin surface (7). Moreover, distances of more than 8 mm between LCU and the cavity have been demonstrated (23). To represent the clinical situations, the curing tip distances of 2 mm and 9 mm were used in the present study and controlled via the use of metal rings. Previous studies investigated the influence of curing tip distances on the microhardness of resin composites and concluded that as the curing tip distance increases, the hardness decreases (7, 24). These results are in line with the results of the present study demonstrating the negative effects of the curing tip distance on cytotoxicity of resin composites. In the current study, in terms of overall mean CSR% of 2 mm results were higher than the 9 mm results (18.00±0.038, 17.66±0.038, respectively). According to the statistical analysis, there was a statistically significant difference among the curing distances, necessitating acceptance of the null hypothesis. The polymerization by LCUs at a distance of 2 mm had always higher CSR% values in all tested materials.

LCUs with higher light intensities have great potential for use in restorative procedures. Decreasing the total cure time may be beneficial for the clinician and the patient. It was reported previously that, higher degree of conversion of the resin containing polymeric materials could be obtained by using the LCUs in high power modes (25). It was considered that, in the present study, high power modes of the LCUs might be able to achieve similar CSR% values in the short curing times even at two different curing distances. What is more, there was a difference between the two evaluation periods on the cytotoxicity of the tested materials. Furthermore, the evaluation periods (24 h and 72 h) had a statistically significant effect on the cytotoxicity of the resin composites cured with different LCUs. According to the results of the present study, the cytotoxicity of resin composites was LCU and time dependent, we are led to accept the null hypothesis. At the 24h evaluation period QTH and PAC LCUs were able to polymerize test material C better than the LED LCU ($p<0.008$, $F=8.832$). On the contrary, at both 24h and 72h evaluation periods, QTH and LED LCUs were able to polymerize test material E better than the PAC LCU ($p<0.008$, $F=25.313$, $p<0.008$, $F=125.972$, respectively). In respect to the CSR% values of material A irradiated from a distance at 2 mm, it was found more cytotoxic at 72 h than at 24 h evaluation period. Also when the material B cured at a distance of 9 mm at 72 h, it was found to be more toxic than at 24 h evaluation period. Furthermore, when the test materials A and E were cured with all the three LCUs, the CSR% results of the 24h evaluation period were statistically higher than the 72 h results. A previous study by Ferracane and Condon (26) reported that the most toxic effects from resin composites occur during the first 24 h of testing. As opposed to these authors (26) the present study showed that the release of the unreacted toxic components from the composite materials continues and that there is no one ideal LCU for all the composite materials.

As the results have indicated, the resin composites cured with the three LCUs at two curing distances may cause harmful effects to the biological tissues. The dentist should always check whether the curing light and irradiation distance used are adequate to polymerize

the particular brand of resin used or not. Nevertheless, from a clinical point of view, LCUs and resin composites should be harmonized to one another for achieving maximal biocompatibility.

Conclusions

Within the limitations of this in-vitro study, the following conclusions could be drawn:

1. The cytotoxic effects in the cell culture showed dependence on the type of resin composite. While significantly highest cytotoxicity was obtained in the tested material E, the lowest cytotoxicity was obtained in the material C. The cytotoxicity of the tested materials can be rank based on the CSR% indicated by C > B > A > D > E.
2. The distance between the tip of the LCU and the restoration surface should be as close as possible. When this approximation is not possible, the more suitable LCU with the restorative material should be selected according to clinical situation.

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Acknowledgments

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