Effects of curing systems and light units on cytotoxicity of dental composites

Afife Binnaz Hazar Yoruç[1], Kadriye Atıcı Kızılbey[2], Arzu Karaul[3], Özcan Çakmakcıoğlu[4]

[1]Yıldız Technical University, Institute of Science, Division of Metallurgical and Materials Engineering, Davutpasa Campus, Esenler 34210, İstanbul, Turkey
[2]İstanbul Yeni Yüzyıl University, Division of Biomedical Engineering, Cevizlibağ, Zeytinburnu, 34010, İstanbul, Turkey
[3]Yıldız Technical University, Institute of Science, Division of Bioengineering, Davutpaşa Campus, Esenler, 34210, İstanbul, Turkey
[4]Dental Clinic, Nişantaşı, İstanbul, Turkey

Abstract— *The objective of this study was to examine and compare the cytotoxicity behavior of commercial two restorative (light-cured) and three adhesive composites (dual-cured) polymerized by using two different light curing units (LCU). Commercial composites Filtek Z250, Filtek Supreme XT, Rely X Arc, Rely X U100 and Variolink II were polymerized using different light densities of halogen (H) and Light Emitting Diod (LED) curing units. After the polymerization process samples sterilized under UV light for 15 minutes. Dulbecco's Modified Eagle's Minimal Essential Medium (DMEM) containing 200 µL of serum was placed in 96 well cell plates and samples were added in the wells. They were incubated 5% CO_2 incubator for 48 hours at 37°C. Sample surface area/solution volume was adjusted to 2.5 cm^2/ml. Cytotoxicity of samples was examined by the extraction method and the results were evaluated using the MTS test. The extracts of the samples were collected for 24 hours and incubated in L-929 mouse fibroblast cells (MFCs). The data was analyzed with the SPSS statistics program. Samples polymerized by H light source were generally cytotoxic than the samples polymerized by LED light source. Rely X Arc in dual-cure system is the most biocompatible material and Variolink II-LED combination is the most cytotoxic one. Furthermore, there was no statistically significant difference between the cytotoxicity levels of composites using H and LED light sources ($p>0.05$).This study showed that the curing treatment used power density LED affects biocompatibility positively and nano-structures increase the biocompatibility.*

Keywords— Cytotoxicity, dental composites, light curing, dual curing, halogen, LED, light curing units.

I. INTRODUCTION

Light- and dual-curing composite materials have been used with increasing interest as fillers, luting cements and adhesive resin cements in restorative dentistry for many years [1-5]. Increasing usage of these composite materials has recently provided to improvement of new formulations, simplification of bonding procedures and decreasing of aesthetic concern. Therefore physical properties, clinical performance and polymerization degree of resin composites developed. Halogen (H) and light-emitting diode (LED) light-curing units are the most widely used light sources to achieve the sufficient polymerization degree for restorative composites.

Recently, several research teams reported that usage of both composite and light curing unit can influence the cytotoxicity of the material [1-11]. Influence of light curing on the toxic behaviour of composite materials is the interest issue for dental restoration [1-5]. The relation between the type of light curing unit and the degree of polymerization of dental composites is currently being discussed in the literature [1-3]. Recently Siguscha et al. reported on the influence of different light curing units on the cytotoxicity of various dental composites. They proved that the combination of a high power LCU with various composites caused the lowest cell toxicity [4]. Goldberg explained that cytotoxicity mechanisms effected by the short-term release of residue monomers during polymerization and long-term release of soluble substances after polymerization process [12].

Photopolymerization process uses the light energy to initiate photochemical and chemical reactions in organic molecules and this energy converses the monomer units to macromoleculer polymeric structure with cross linking interactions. Improved photopolymerization process decreases amount of the residue monomer [6], increases the optimization of mechanical properties [7, 8], biocompatibility [9] and color stability [10] of light-activated dental composites. Polymerization of the

organic matrix molecules of light-curing composites is triggered by excitation of a photo initiator system with light [4]. Success of polymerization depends on thickness of the filler material, wavelength of the excitation light, power density and irradiation time [4, 11]. Tuning between excitation wavelength and photoinitiator system has a decisive effect on the degree of polymerization. Sigusch et al. showed that shade of the composite has an influence on its cytotoxicity and cytotoxicity is also influenced by the light curing unit used. It was observed that composites of the darker shade had a higher cytotoxicity which varied with the LCU employed [13]. Beriat et al. compared the cytotoxic effects of various dental composites polymerized with two different curing units and their results exhibited that polymerization of dental composites with a light emitting diode LCU positively influences L-929 mouse fibroblast cell viability [5]. Long curing time and low intensity of light units occur less cytotoxicity than short curing exposure using high intensity of light emitted from the curing light source [14]. Another study investigated p-octyloxy-phenyl-phenyl iodoniumhexafluoroantimonate (OPPI) as a photoinitiator system in combination with camphorquinone/amine (CQ/A) photoinitiation systems for use with di(meth)acrylate-based composite resins. This study suggested that OPPI can be used to replace amine in a given CQ/A photoinitiator system to accelerate cure rate, increase conversion, reduce initial color and increase color stability [15].

Biomaterial cytotoxicity tests require standard protocols to obtain international comparability [16]. Moon et al. evaluated the effect of the various irradiation methods with three light curing units on the leachability of the monomers (Bis-GMA and UDMA) and surface hardness of composite resins as a function of light energy density. Their results presented that a composite resin cured with various curing units and irradiation methods exhibited different amount of leached monomers and hardness values depending on the power density levels of units. These differences disappear when the time or the light energy density increased [17].

Polymerization reaction is never complete according to the results of in vitro studies and side reactions are due to the release of nonpolymerized monomers such as TEGDMA, Bis-GMA and UDMA [18, 19]. In the most studies, comonomer TEGDMA has been identified as the main compound released from polymerized resin composites into aqueous media. However, small quantities of monomers Bis-GMA and UDMA and other comonomers may also be released. Comonomer TEGDMA is cytotoxic and inhibits cell growth [18]. Extracts of various resin composites contain substances of camphoroquinone, benzyl and dimethoxybenzoin as photoinitiators, triphenylphosphan and triphenylstibane as catalysts [20].

Quality of curing light units influences the clinical performance of light-cured composite restorations [21, 22]. Final properties of light-activated composite resins depend on characteristics such as resin chemistry, light source power density and exposure time [23]. Efficiency of H and LED light-curing units in polymerization of resin-based composites has been evaluated in several studies [1-4, 11, 24]. LED unit provides the lowest depth of cure compared to quartz tungsten H units [25]. Material composition and shade, restoration thickness, type of light source and energy level influence the degree of double bond conversion of composite resin. High-power LED light sources have modified to reduce the curing time [21]. Nowadays, four main polymerization type using H units, plasma arc lamps, argon ion lasers and light emitting diodes are preffered in clinical use [26].

H and LED light sources are widely preferred in clinical applications. Consequently, this study tends to examine and compares the cytotoxicity behavior of commercial two restorative (light-cured) and three adhesive composites (dual-cured) polymerized by using H and LED curing units.

II. MATERIALS AND METHODS

2.1 Materials and Sample Preparation

The present study was focused on the examination of potential toxic influences of various dental composites (Table 1) currently used in dental practice as a function of two different curing units (H and LED) (Table 2) and the curing methods (light and dual curing).

TABLE 1
PROPERTIES OF MATERIALS

Composites	Class	Manufacturer	Composition		Curing System
			Organic Matrix	Inorganic Phase	
Filtek Z250	restorative	3M ESPE (USA)	Bis-GMA UDMA Bis-EMA water	78% (w/w) Zirconia/silica	Light
Filtek Supreme XT	restorative	3M ESPE (USA)	Bis-GMA UDMA Bis-EMA TEGDMA water	78.5% (w/w) NanoSilica, NanoZirconia/Silica particles	Light
Rely X Arc	adhesive resin cement	3M ESPE (USA)	Paste A Bis-GMA TEGDMA Paste B Bis-GMA TEGDMA	Paste A 68% (w/w) Zirconia/Silica Paste B 67% (w/w) Zirconia/Silica	Dual
Rely X U100	adhesive resin cement	3M ESPE (USA)	Fluoroaluminosilikat glass powder Phosphoric acid esters with methacrylate groups TEGDMA	72% (w/w) Zirconia/Silica	Dual
Variolink II	adhesive resin cement	IvoclarVivadent (Liechtenstein)	Bis-GMA TEGDMA UDMA	73.4% (w/w) Barium glass Itterbiumtrifluoride Ba-Al-fluorosilicate glass	Dual

The cytotoxicity of the sample extracts was investigated *in vitro*. Commercial Rely X Arc, Rely X U100, Variolink II, Filtek Supreme XT and Filtek Z250 are among the most preferred composite materials in dental restorative practice. Rely X Arc, Rely X U100 and Variolink II are in dual curing composite cement group while Filtek Supreme XT and Filtek Z250 are in light curing restorative composite material group.

For each sample, three tablets [27, 28] with 2 mm of thickness and 4 mm of diameter were prepared in Teflon moulds. Teflon is preferred due to advantages such as homogeneous sample size and flexible structure as mold material. Rely X Arc and Rely X U100 paste form (Paste A + Paste B) materials have been directly mixed and put into molds. However, Filtek Supreme XT, Filtek Z250 and Variolink II were in syringe form tubes and transferred into molds. Samples polymerized by H light source (Blue Luxcer M-835) and LED (Elipar Free Light 2) (Table 2). After the polymerization process, samples were removed from the moulds and sterilized under UV light for 15 minutes. Extraction protocol adapted by Sigusch and coworkers is used to analyze the cytotoxicity of the extracts each sterilized sample. DMEM medium (Dulbecco's Modified Eagle's Minimal Essential Medium) containing 200 µL of serum was placed in 96 well cell plates and then samples were added in the wells. They were incubated in an incubator containing 5% CO_2 for 48 hours at 37°C. Sample surface area/solution volume was adjusted to 2.5 cm^2/mL according to ISO standards [4, 29].

TABLE 2
LIGHT-CURING UNITS

	Halojen	LED
Model	BlueLuxcer M-835	Elipar Free Ligth 2
Manufacturer	Monitex (Taiwan)	3M ESPE (USA)
Light source power (W)	35	5
Fiber-optic light guide (mm)	8-11.5	8
Power density (mW/cm^2)	400-600	1000
Period of application (s)	40	40
Emission spectrum (nm)	400-500	430-480

2.2 Cell Culture

L-929 mouse fibroblast cells (MFCs) were purchased from American Type Culture Collection (ATCC CLL 1, Rockville, MD). They were incubated until they reach the logarithmic phase at 37°C under 5% CO_2 in minimum media including 2 mML-glutamine, 2.2 g/L sodium bicarbonate, 1 mM sodium pyruvate, %10 fetal bovine serum (FBS). Cells were counted using the hemocytometer (Brigth-Line) and each well was fed by 10.000 cells. 96 well plates were left in an incubator for 24 hours at 37°C under 5% CO_2 in order to ensure cell adhesion. Extraction test was chosen to determine the cell-material contact due to the fact that cells will not directly interact with the material in *in vivo* conditions and after contacting with mucosa and saliva, its components will go through the dentinal tubules causing a relative interaction with pulp cells [30].

2.3 Cytotoxicity of Tablets

After the cell adhesion achieved for 24 hours in 96 well culture plates, growth media was removed and 100 µL test extract was added in each well. DMEM and 15% DMSO by volume were used as negative and positive control respectively. After 48 hours long incubations [4], cytotoxicity levels were determined by using Cell Titer 96 Aqueous One Solution Cell Proliferation Assay (MTS) [31].

2.4 MTS Test

In this study, MTS method was used to determine cytotoxicity. After incubating for 2 days with 100 µL of test extract, each well was filled by 20 µL of Cell Titer 96 Aqueous One Solution Cell Proliferation Assay solution from Promega [29]and 100 µL of the growth media. Three hours later absorbance values were obtained at 490 nm using BIO-TEK ELX800 ELISA instrument. After obtaining the optical densities, cell viability ratios were calculated using the formula below and the results were shown in figures.

$$\%\ \text{Cell viability} = 100\ \text{x}\ \frac{\text{OD Average of 3 test samples} - \text{OD Average of 3 (+)control}}{\text{OD Average of 3 (−)control} - \text{OD Average of 3 (+)control}}$$

OD: Optical Density

2.5 Statistics

Experimental values were analyzed using the SPSS (Statistical Package of Social Sciences) for Windows 15.0 software. Comparison of the quantitative data were performed using Krukal Wallis and Mann Whitney U tests [28] and the results were studied within 95% confidence interval and at $p<0.05$ levels.

III. RESULTS AND DISCUSSION

3.1 Cytotoxicity Results

The cytotoxicity results of composite samples are provided in Figure 1. According to these results, the biocompatibility of dual curing systems was higher than the biocompatibility of light curing systems.

Comparison of H and LED light sources indicated that polymerized samples with LED light source were more biocompatible. This result attributed to the difference between light intensities (H: 400 mW/cm^2, LED: 1000 mW/cm^2).

Biocompatibility values of composite samples were ranked below from the lowest to highest based on the use of the material-light source:

Rely X Arc-LED > Rely X Arc-H > Filtek Supreme XT-LED > Rely X U100-LED > Filtek Z250-LED > Rely X U100-H > Variolink II-H > Filtek Z250-H > Filtek Supreme XT-H > Variolink II-LED

Polymerized samples using LED light source of Filtek Supreme XT light-cured samples were more biocompatible than Filtek Z250 samples. It was observed that when using H light source Filtek Z250 is more biocompatible than Filtek Supreme XT.

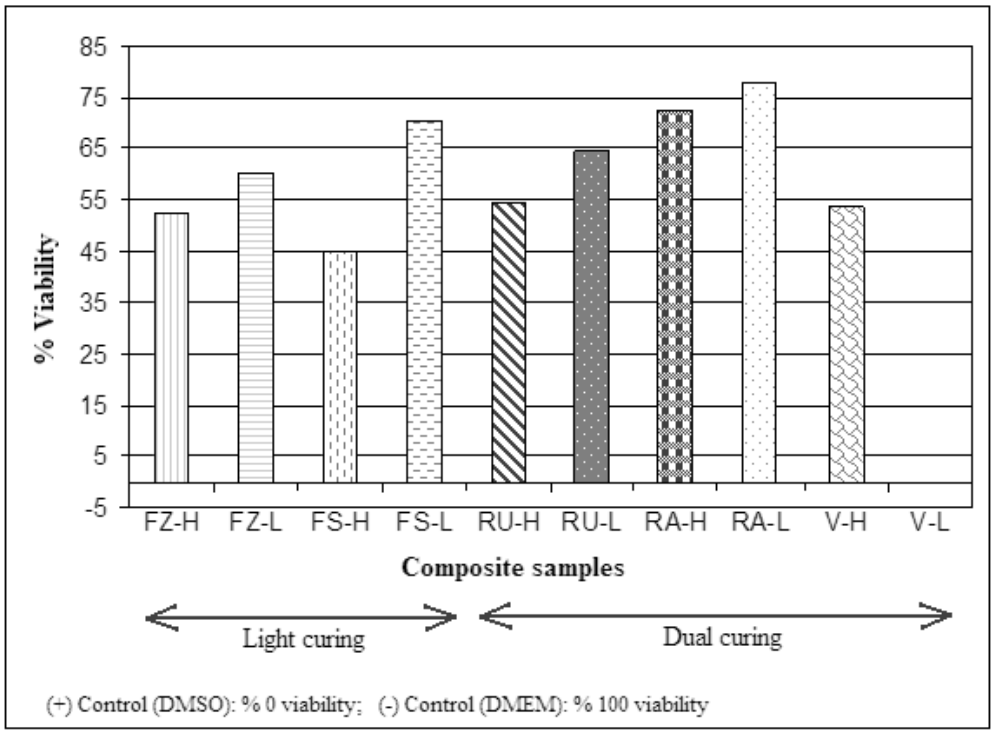

FIGURE 1- CYTOTOXICITY TEST RESULTS OF COMPOSITE SAMPLES (FZ-H: Filtek Z250-H; FZ-L: Filtek Z250-LED; FS-H: Filtek Supreme XT-H; FS-L: Filtek Supreme XT-LED; RU-H: Rely X U100-H; RU-L: Rely X U100-LED; RA-H: Rely X Arc-H; RA-L: Rely X Arc-LED; V-H: Variolink II-H; V-L: Variolink II-LED).

Cytotoxicity results show that nano-structures increase the biocompatibility. Rely X Arc in dual-cure systems is the most biocompatible material in composites. Rely X U100 is more biocompatible than Variolink II and Variolink II-LED combination is the most cytotoxic one.

3.2 SEM Analysis

Wide gaps between cells, cellular remnants and trace cell amount were seen from SEM image of positive (+) control (DMSO) samples. Cellular remnants were indicated with arrows on images (Figure2a-c). Amount of live cell was too much and cell morphologies were regular in negative (-) control (DMEM) samples. Significant differences in cell amount and gaps appeared in SEM images of (+) and (-) control samples. Living cells were indicated with arrows on images (Figure2d-f). Instead of viability, SEM images of Filtek Z250 composite cured with H light source had cell residues as seen in (+) control (DMSO) samples.SEM images were similar but viability was much more than Variolink II composite material polymerized by using LED light source. Also, significant changes in cell morphology were observed.

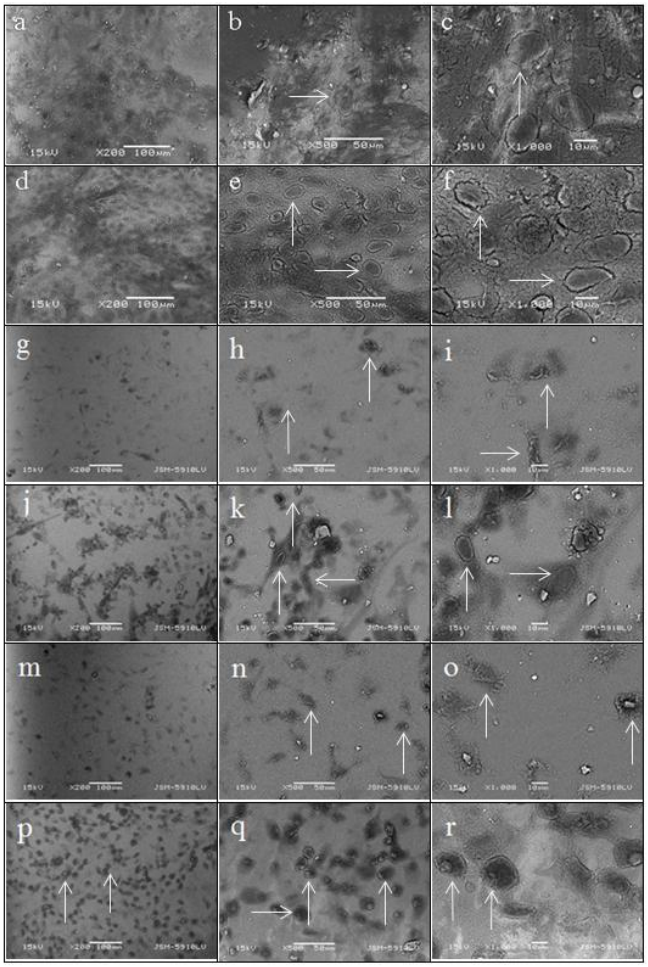

FIGURE 2- SCANNING ELECTRON MICROSCOPE (SEM) IMAGES OF POSITIVE CONTROL (a-c); NEGATIVE CONTROL (d-f); FILTEK Z250 COMPOSITE-H (g-i); FILTEK Z250 COMPOSITE-LED (j-l); FILTEK SUPREME XT COMPOSITE-H (m-o); FILTEK SUPREME XT COMPOSITE-LED (p-r).

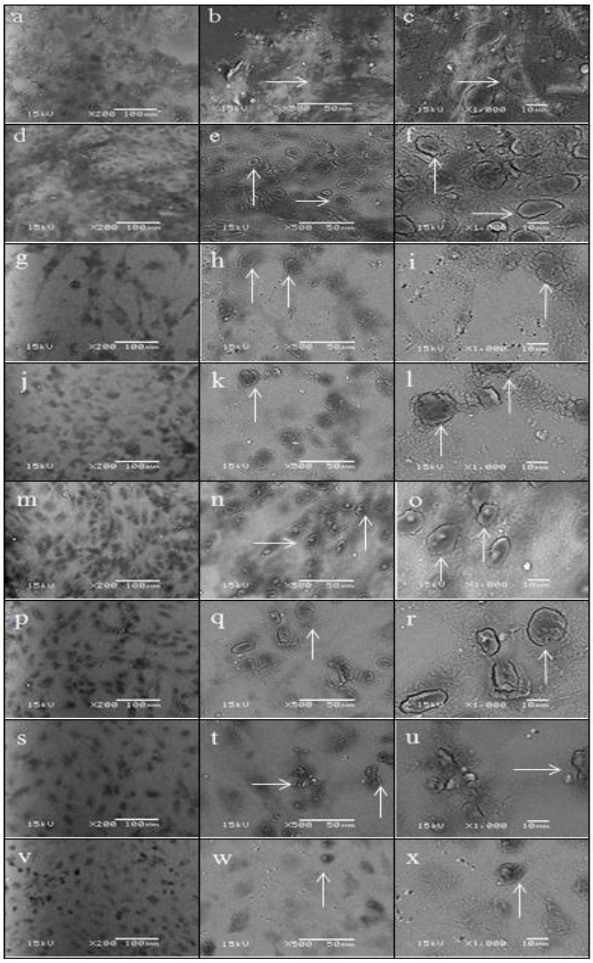

FIGURE 3- SCANNING ELECTRON MICROSCOPE (SEM) IMAGES OF POSITIVE CONTROL (a-c); NEGATIVE CONTROL (d-f); RELY X U100 COMPOSITE-H (g-i); RELY X U100 COMPOSITE-LED (j-l); RELY X ARC COMPOSITE-H (m-o); RELY X ARC COMPOSITE-LED (p-r); VARIOLINK II COMPOSITE-H (s-u); VARIOLINK II COMPOSITE-LED (v-x).

Morphologically changed living cells were marked on images (Figure2g-i). When SEM images of Filtek Z250 composite samples polymerized by using LED and H light sources were compared, viability of cells used LED light source was higher and the morphology of cells was more closely as observed in the negative control (DMEM). It was also clearly seen that compounds left in the cellular environment by disrupted cells. Living cells were indicated with arrows on images (Figure2j-l). Gaps between cells and change in cell morphologies were more in composites polymerized by using LED light source. Number of living cells of composites polymerized by using H light source, was closer to (+) control (DMSO) samples. Morphologically changed living cells were indicated with arrows on photos (Figure2m-o). Amount of living cells in Filtek Supreme XT composites cured by LED light source was close to the living cell density observed in Rely X Arc sample image. Cell morphology was also protected as in (-) control samples that LED light source was used. Living cells were indicated with arrows on photos (Figure2p-r).

SEM image of positive (+) control (DMSO) samples (Figure3a-c) and negative (-) control (DMEM) samples (Figure3d-f) were mentioned before in Figure2a-c and Figure2d-f. SEM images of Rely X U100 composites polymerized by using H light source had less amount of living cell and gaps between cells were wider than observed in LED light source curing polymerization. These results were indicated with arrows on photos (Figure3g-I and Figure3j-l). Morphological change was

not observed in Rely X Arc composite-H treated cells and also living cells were indicated with arrows on images (Figure3m-o). SEM images of Rely X Arc composite cured with LED light source appeared closer to negative control samples, and seem more biocompatible than H cured composites (Figure3p-r). Comparison of Variolink II composite and negative control (DMEM) samples showed that gaps between cells were distinctive and began to increase the use of LED light source (Figure3s-u). Cells left their place to cell debris in SEM images of Variolink II composite material polymerized with LED light source as seen in SEM images of positive control (DMSO). In addition to this, smaller size cells were observed when compared to the negative control. Morphologically changed living cells were indicated with arrows on photos (Figure3v-z).

3.3 Statistic Results

Significant difference was not observed between the statistical senses of the cytotoxic effects of dental composites cured with different light sources.

Cytotoxicity levels of Rely X Arc, Filtek Supreme XT and Filtek Z250 composites polymerized by using LED light source were higher than the cytotoxicity levels of Rely X Arc, Filtek Supreme XT and Filtek Z250 composites polymerized by using H light source. This difference was not statistically significant, but very close to significance ($p > 0.05$).

There was no statistically significant result between the cytotoxicity levels of Variolink II and Rely X U100 composites polymerized by using LED light source and H light source ($p>0.05$).

There was no statistically significant difference between the cytotoxicity levels of composite samples using H light source ($p>0.05$). The cytotoxicity levels of composite samples using LED light source had statistically significant difference, but it was not close to significance result ($p>0.05$).

The present study investigated the cytotoxicity of currently used composite-LCU combinations. The in vitro studies showed that the extracts of five commercial dental restorative composites had different toxic effects on the viability of L-929 mouse fibroblast cells (MFCs), which varied with the LCUs used.

According to cell viability results, the biocompatibility of dual curing systems was higher than light curing systems (Fig.1). This result was attributed to the depth of polimerization in dual curing system which was more than 2 mm as in light curing polimerization systems [32]. Inadequate curing of composite was not effective only on the mechanical properties of composite and also effective on biological features by means of residual monomers. Extracted components from composites in *in vitro* testing media increase the cytotoxicity and may have harmful effects on dental pulp and oral mucosa [32, 33]. Both chemical and light curing in dual-cure systems decreases amount of residual monomer and reduces the cytotoxicity.

The power density of the light curing units changes the polymerization rate and the amount of residual monomer [34]. Low polymer/monomer conversion rate results with more residual monomer release [4, 35]. The polymerized samples with LED light source were more biocompatible when Hand LED light sources compared. This difference was originated from the difference between light intensities (H: 400 mW/cm^2, LED: 1000 mW/cm^2). These findings were compatible with recent studies [32] about the effect of light source on polimerization rate in light curing systems. Despite the widespread use of H light sources, they have several disadvantages. The life of H bulbs changes between 40 and 100 hours. The effectiveness of light source on polymerization is reduced in time depending on the operating performance of light bulb [36]. Furthermore, these findings cause high rate cross-linking through the fast polymerization [4] and also polymer structure has a strong release of soluble monomer and increases the cytotoxicity of material. These findings and results of the study are agree with each other [1, 2, 4, 37, 38]. The samples polymerized by H light unit were generally cytotoxic than the samples polymerized by LED light source.

Filtek Supreme XT-LED (FS-L) samples were more biocompatible than Filtek Z250 (FZ-L) samples. Oppositely Filtek Z250-H light source (FZ-H) was more biocompatible than Filtek Supreme XT -H light source (FS-S). They had different biocompatibility due to the polymer matrix components and the chemical structure of filler particles. While Filtek Supreme XT contains nano-silica, zirconia/silica particles as filler, Filtek Z250 contains micro-sized (0.6 to 1.4 m) zirconia/silica filler particles. As a result, the cytotoxicity results presented nanostructures increase the biocompatibility.

Rely X Arc in dual-cure systems was the most biocompatible material in composites. Rely X U100 was more biocompatible than Variolink II and also Variolink II-LED combination was the most cytotoxic one. A common component of dual-cure and light-polymerized systems is usually the Bis-GMA, UDMA, TEDGMA and HEMA are the most toxic monomers [39]. Sigusch et al. reported that the amount of released UDMA and Bis-GMA monomers from composites changes depending on the intensity of the light source [4].

This study showed that dental composites containing Bis-GMA have low cytotoxicity due to the high ratio of inorganic filler (60-78% w/w). This result is consistent with another study in literature [40]. In addition to this result, Bis-GMA, Bis-EMA, UDMA and TEDGMA monomers diffused from partially polymerized resins and they may cause cytotoxic effect [32].

SEM images of positive (+) control (DMSO) samples indicated wide gaps between cells and cellular remnants and negative (-) control (DMEM) samples showed regular behavior between cells and cell morphologies. Cell amount and differences between cell gaps in (+) and (-) control samples was significantly appeared in SEM images (Fig.2a-f and Fig.3a-f). SEM images of Filtek Z250 composite cured with H light source (FZ-H) were similar to (+) control (DMSO) samples. SEM images were similar but viability was much more than Variolink II composite material polymerized by using LED light source. Also, significant changes in cell morphology were observed (Fig.2g-i). When SEM images of Filtek Z250 composite samples polymerized by using LED and H light sources were compared, viability of cells used LED light source was higher and the morphology of cells was more closely as observed in the negative control (DMEM). It was also clearly seen that compounds left in the cellular environment by disrupted cells (Fig.2j-l).

SEM images of Filtek Supreme XT composites polymerized using H or LED light sources (FS-H and FS-L) exhibited significant differences. Composites polymerized by using LED light source had more gaps between cells and changes in cell morphologies when compared with H light source, is closer to (+) control (DMSO) samples (Fig.2m-o). Filtek Supreme XT composites cured by LED light source (FS-L) had similar living cell density when compared with Rely X Arc sample image (R-L). Cell morphology was also protected such as (-) control samples that LED light source was used (Fig.2p-r).

SEM images of Rely X U100 composites polymerized by using H light source (R-H) had less amount of living cell and gaps between cells were wider than observed in LED light source (R-L) curing polymerization (Fig.3g-I and Fig.3j-l). Rely X Arc composite-H (R-H) treated cells showed morphological change (Fig.3m-o). SEM images of Rely X Arc composite cured with LED light source (R-L) appear closer to negative control samples and seem more biocompatible than H cured composites (Fig.3p-r). Comparison of Variolink II composite and negative control (DMEM) samples showed that gaps between cells were distinctive and began to increase with use of LED light source (Figure3s-u). SEM images of Variolink II composite material polymerized with LED light source included cell debris as seen in SEM images of positive control (DMSO). In addition to this, smaller size cells were observed when compared to the negative control (Fig.3v-z).

Significant difference was not observed between the statistical senses of the cytotoxic effects of dental composites cured with different light sources.

Cytotoxicity levels of Rely X Arc, Filtek Supreme XT and Filtek Z250 composites polymerized by using LED light source were higher than the cytotoxicity levels of Rely X Arc, Filtek Supreme XT and Filtek Z250 composites polymerized by using H light source. This difference was not statistically significant, but very close to significance ($p > 0.05$). There was no statistically significant result between the cytotoxicity levels of Variolink II and Rely X U100 composites polymerized by using LED light source and H light source ($p>0.05$). There was no statistically significant difference between the cytotoxicity levels of composite samples using H light source ($p>0.05$). The cytotoxicity levels of composite samples using LED light source had statistically significant difference but it was not close to significance result ($p>0.05$).

IV. CONCLUSION

In general all materials used in experimental studies decreased the biocompatibility in certain amounts. Cytotoxicity test results of composite samples indicated that biocompatibility of dual cure system was higher than light curing system. When H and LED light sources effects on in vitro biocompatibility compared, less cytotoxicity was observed in the samples polymerized with the LED light source. The most biocompatible material-curing method combination was Rely X Arc polymerized by LED curing unit in all composites.

According to composite types of materials using H and LED light sources, no significant statistical difference was found between the levels of cytotoxicity ($p>0.05$).

One of the most important features of restorative materials used in dental practice is biocompatibility. In vitro biocompatibility study results showed chemical structure of material, degree of polymerization, residual monomer amount in the structure, clinical setting conditions (temperature, humidity, etc.) and properties of the curing method (light source type, curing type etc.) stimulate the cytotoxicity formation.

These results revealed the necessity of determination of residual monomer amount and effects on in vitro biocompatibility. In

conclusion, it was well understood that in vivo experiments should be done beside in vitro studies to achieve the closest data as in clinical practice. We were able to prove that the combination of a high power LCU with various composites caused the lowest cell toxicity.

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