Effects of Different Irradiance Levels of the Light Curing Unit on The Dental Resin Composite's Microhardness and Cytotoxicity

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Abstract

Polymerization of composite resins could be affected by iradiance levels of light curing units. Furthermore, imperfect polymerization of resin composite polymerization could cause low surface hardness and high cytotoxicity.

This study examined the effect of the irradiance levels of the light curing units on the microhardness of cured resin composites and their cytotoxicity A pulse-lighting curing unit with irradiance levels of 800, 900 and 1,000 mW/cm2 in either 10 or 20 sec was employed for this study and a commercially available continuous-lighting curing unit of 900 mW/cm2 in 20 sec was used as comparison. Resin composite specimens were irradiated using the curing units, and the microhardness with Knoop system and cytotoxicity by the MTT were subsequently determined.

The irradiance of 900±10 mW/m2 cm2 emitted from the pulse lighting is the closest value to that emitted by the continuous-lighting curing unit. The resin composites irradiated at higher irradiance levels exhibited significantly higher ($p<0.05$) microhardness than those irradiated at lower irradiance levels. Regarding their cytotoxicity values, higher significant differences ($p<0.05$) were observed with increasing irradiance levels. Increased irradiance of the pulse-lighting curing unit raised the level of microhardness while minimizing the cytotoxicity of the tested resin composites. For clinical work, enough microhardness and cytotoxicity values obtained from the resin composites irradiated using the pulse lighting curing unit might provide a safe tooth condition.

Experimental article (J Int Dent Med Res 2019; 12(1): 138-142)

Keywords: Pulse-lighting curing unit, Resin composite, Microhardness, Cytotoxicity.

Received date: 15 August 2018 **Accept date:** 20 September 2018

Introduction

Owing to advances in technology, tooth-colored resin composites have been developed and have become the predominant materials for dental restorations so as to fulfill patients' increasing desire for an aesthetically pleasing appearance.[1-4] Along with such developments, there have been major advancements in light curing units. Blue light, with a wavelength in the range of 400−500 nm, can trigger a light-sensitive initiator, usually camphorquinone, to polymerize light cured resin-based composites.[5]

To polymerize resin composites, the 3rd Generation of curing units uses LED,[6-7] and it is characterized by its high irradiance, which allows reducing light exposure (irradiating) time.[8] Namely, decreasing irradiation time must be accompanied by the increase in irradiance level to maintain adequate energy density (J/cm2).[9,10] High irradiance levels achieve rapid polymerization, but are associated with higher heat dissipation; thus, when used to irradiate resin composites in deep cavities, it may cause an increase in temperature and thereby damage the pulp tissues.[11,12] To reduce the elevated temperature associated with a high-powered LED, pulse lighting mode has been studied for the development of curing units. The use of a high-powered LED with pulse lighting mode for 40 sec resulted in a dental pulp chamber temperature of 37°C.[13] To achieve more rapid light irradiation and lower pulp chamber temperature than those used in the previous study,[13] our study used a pulse-lighting in 20 sec and has produced lower output temperature from the curing unit.[14] When used for irradiating resin composites, the light emitted from the curing unit should be able to polymerize the material.

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Ideally, polymerized resin composites should show the mechanical and biological according to the ISO standard for resin conposite. Microhardness which is the resistance of a material to indentation under stress also indicates how well the material is polymerized. Insufficiently polymerized resin composites contain unpolymerized (unreacted C=C) resins that are unbound to the material structure; therefore, insufficiently polymerized resin composites may possess inadequate microhardness.[15-17] Unreacted may also cause release from the resin structure.[18-20] Studies have demonstrated the presence of leached resins in the oral cavity, such as in saliva[21] or on gingival.[22] However, the microhardness and cytotoxicity of the irradiated resin composites were not explored. Therefore, the current study aimed to examine the effect of irradiance levels on the microhardness and cytotoxicity of the irradiated resin composites.

Materials and methods

Preparation of the experimental pulse-lighting curing unit

The experimental pulse-lighting curing unit was the same as that used in our previous study.[14] The irradiance levels were checked using an analogue radiometer (Demetron, Kerr, Orange, CA, USA) which showed 800, 900, and 1000 mW/cm^2 in 20 sec. A commercially available continuous-lighting curing unit that only has an irradiance of 900 mW/cm^2 and can only operated at 20 sec (Elipar, 3M ESPE, St Paul, MN, USA). was also used as comparison to the pulse lighting.

Sample preparation

Sample preparation for the microhardness test was performed according to ISO 4049:2000.[23] A stainless-steel split mold with 6-mm diameter mm and 2-mm thickness was placed on a plastic strip. The materials used in the present study were from one manufacturer, contained the same matrix and filler composition, and were the same shade, i.e., a commercially available resin composite comprising nano filler (Filtek, 3M ESPE, USA). The material was filled in a split mold, covered with another transparent plastic strip, and immediately hand-pressed using a flat surface to extrude excess material for obtaining a smooth, flat test surface. The tip of the light guide was placed as close as possible to the plastic strip without touching its surface. The resin composites in the mold were irradiated using the experimental LED curing unit, with irradiance levels of 800, 900, or 1000 mW/cm^2 in 20 sec, and with the continuous lighting mode with 900 mW/cm^2 in 20 sec. Four groups of sixteen resin composite samples were prepared for the microhardness test. Samples for the cytotoxicity test, with a geometry similar to those used for the microhardness test, were prepared in a sterile laminar hood in the same manner. When irradiating the resin composites, the irradiance were monitored after every five irradiations. All samples were maintained at 37°C for 24 h.

Microhardness test

The microhardness test was performed according to ISO 4049:2000.[23] The test was carried out at the undersurface of each sample using a digital microhardness tester using the Knoop system (Zwick, Kennesaw, GA, USA). A predetermined test force of 100-g load is applied with an elongated pyramid-shaped diamond indenter forced into the sample for a specified dwell time period of 15 sec. After the load is removed, the microhardness value (KHN) was calculated using:

$$KHN = F / d^2Cf$$

where the load L is in gf and the long diagonal indentation d is in μm, and the constanta of the indenter relative to the projected area of the indentation and the length square of the long diagonal Cf is 0.7028. Four groups of ten resin composite samples were prepared, for which each value was an average of a set of five indentations. The recording was conducted at room temperature (24±1°C).

Cytotoxicity determination

The cytotoxicity of resin composite was evaluated in vitro using human HaCaT cells. For quantitative assessment of the cytotoxicity, the viability of HaCaT cells was determined by a modified MTT assay. The cell viability was determined while adhering to the guidelines of the Ethics Committee of the Faculty of Dentistry, Universitas Indonesia, for research purposes using HACaT cells.

HaCaTcells were derived from a cell line of adult human skin. The viability of the HACaT cells was evaluated following exposure to an extract solution of the resin composite samples. The cells were cultured to 80% confluence in Dulbecco's modified Eagle medium (DMEM; Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Biochrom AG, Berlin, Germany) and antibiotics-antimycotics (Gibco, Grand Island, NY, USA) at 37°Cin a humidified atmosphere containing 5% CO_2.The extract solutions were prepared by immersing each sterile resin composite sample in a conical tube containing culture medium for 48 h.

Cytotoxicity was determined from the cell viability using a colorimetric assay according to ISO 10993−5:2009.[24] Cells were seeded in 96-well plates and cultured for 24 h, following which, the medium was removed and replaced with extract solution for further 24 h. Cells without extract solution were considered as control. After removing the extract solution, cells were incubated with dimethylthiazolyl diphenyltetrazolium bromide solution (MTT; Bio Basic, Ontario, Canada) for 4 h. The MTT assay is a good indicator of cell viability. This assay is based on the reduction of the MTT by those cells that remain viable after exposure and incubation. Mitochondrial dehydrogenases at the viable cells convert the yellow water soluble form of the salt to an insoluble, intracellular purple formazon metabolite. Subsequently, the solution was aspirated, acidified isopropanol (Merck, Darmstadt, Germany) was addedto the cell in each well to dissolve purple crystals of formazon and then check the formazan crystals using an inverted microscope (Olympus, Germany). Absorbance (optical density; OD) was determined using enzym-liked immunoabsorbent assay (ELISA). The samples in the 96-well was inserted to the reader and the ELISA read the plates. ELISA readers or micro plate readers do spectrophotometry; they emit light at one wave length around 570 nm, and measure the amount of light absorbed and reflected by the object. All tests were performed with ten specimens and in a laminar hood.

Statistical analyses

The Kolmogorov-Smirnov test with a confidence level of 95% was used to analyze the data. Then data were analyzed using the Two-way analysis of variance (ANOVA) to determine whether there were significant differences in the microhardness and cytotoxicity among groups with p value <0.05. Tukey's *post-hoc* test was used to analyze the difference between groups with different irradiance levels with p value <0.05. The difference in cytotoxicity between the test and control groups was detected using *t*-test. Data analysis was performedby SPSS software program (SPSS, Chicago, USA).

Results

The experimental pulse-lighting curing unit used in the current study produced bright light through the light guide of the unit in 20 sec. The microhardness values with the different irradiance levels of the two curing units are summarized in Table 1. Table 1 presents the differences in microhardness values between different irradiance levels of the curing units. The samples obtained by irradiation using the mid-range irradiance level of the pulse-lighting curing unit showed the value closest to that obtained when an identical irradiance level of the continuous-lighting curing unit was applied.

The statistical analysis indicated that there was a significant difference (p<0.05) in microhardness values between different groups, with the exception of the difference in micro-hardness of the specimens irradiated by the mid-range of the pulse-lighting and that of the continuous-lighting curing units (p>0.05) (Table 1).

Concerning cytotoxicity (Table 2), the values were increased when higher irradiance levels were applied. Cytotoxicity of the control group was 100%. There was a significant difference (p<0.05) among the cytotoxicity with different irradiance levels, with the exception of the difference between the cytotoxicity obtained using the mid-range of the pulse-lighting curing unit and that obtained using an identical irradiance level of the continuous-lighting curing unit. The differences between the treatment and control groups were also significant, with the exception of when the highest irradiance level was used.

Curing unit, lighting	Irradiance level (mW/cm^2)	Micro hardness (KHN)
Pulse-lighting	800	61.17(0.72)[d]
Pulse-lighting	900	70.51(0.66)[e]
Pulse-lighting	1000	79.75(0.85)[f]

Continuous-lighting	900	70.71(1.04)[e]

*The mean values (standard deviation) of ten independent specimens per group are presented. Identical letters indicate that mean values were not significantly different (p>0.05).

Table 1. Microhardness of the resin composites cured using different irradiance levels.

Curing unit, lighting	Irradiance level	Cytotoxicity (%) Test group	Control group
Pulse-lighting	800	86.6(0.99)[g]	100(0.0)[i]
Pulse-lighting	900	96.8(0.15)[h]	100(0.0)[i]
Pulse-lighting	1000	99.5(0.21)[i]	100(0.0)[i]
Continuous-lighting	900	96.3(0.82)[h]	100(0.0)[i]

*The mean values (standard deviation) of ten independent specimens per group are presented. Identical letters indicate that mean values were not significantly different (*p*>0.05).

Table 2. Cytotoxivity of resin composites cured using different irradiance levels.

Discussion

The microhardness of resin composites should be between 22 and 80 KHN.[25] Results of the present study demonstrated that the pulse lighting with the irradiance of 800, 900 and 1,000 mW/cm^2, and the continuous lighting curing units were able to polymerize the resin composite samples as were shown by the microhardness and cytotoxicity values (see Tables 1 and 2). Microhardness and cytotoxicity related to the samples irradiated using the mid-range irradiance level of the pulse-lighting curing unit was close to that of the samples irradiated using the continuous-lighting curing unit. Regarding the pulse lighting, the irradiance levels produced high microhardness in the cured resin composite samples, even when the lowest irradiance level was applied.

The energy density from the light is assumed to activate the camphorquinone to produce radicals that convert C=C into C−C groups, thereby accelerating the polymerization reaction in the resin composite samples. The use of higher irradiance levels, i.e., 1,000 mW/cm^2 may produce the highest C−C groups, consequently, highest microhardness values of the cured resin composites.

With respect to the cytotoxity, the pulse-lighting curing unit with the irradiance level of 1,000 mW/cm^2 could have the least number of (C=C) groups that may release from the samples to the extract solution at the time during the 24-hour immersion in the culture solution, compared with those irradiated at the lower irradiance level of 800 and 900 mW/cm^2. During the 48-h immersion in the culture medium to produce the extract solutions, the unreacted C=C groups in the specimens may have leached, which furhter could damage the cells during incubation. The use of the irradiance levels of 800 and 900 mW/cm^2 resulted a difference in cell viability between the treatment and control groups. The non-significant difference in cell viability between the treatment and control groups regarding the use of the highest irradiance level of 1000 mW/cm^2 was most probably due to the increase in the degree of polymerization of the resin composite samples.

When used for mastication, a relatively low microhardness resin composites, that hs high unreacted C=C groups, may have less ability to withstand functional stresses, such as when used for cutting or scratching. Also, when in mouth, unreacted C=C groups could release from the resin structure into saliva, normally within the first few hours following polymerization.[18-20] Although the occurance is rare, leached resins may potentially act as irritants and cause cytotoxicity in the oral environment.[21,22]

Conclusions

Irradiance of the pulse-lighting curing unit influences the microhardness and cytotoxicity of the resin composites tested. Resin composites polymerized using the lowest irradiance level have been shown to have enought microhardness; nevertheless, the lowest cytotoxicity is gained when higher irradiation levels are chosen.

Acknowledgments

The research was supported by the Ministry of Research and Technology and Higher Education, the Republic of Indonesia. The authors thank the Oral Biology Laboratory, Faculty of Dentistry, Universitas of Indonesia for providing the HaCaT cell line and appreciate Prof. Kazuhiko Endo of the Biomaterials and Bioengineering Department, Health Sciences Hokkaido University for the valuable discussion.

The publication of this manuscript is supported by Universitas Indonesia.

Declaration of Interest

None.

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