Coll. Antropol. **32** (2008) 3: 913–918
Original scientific paper

Dose- and Mode-Dependent Effect of Halogen Dental Curing Blue Light on the V79-Cell Line

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A B S T R A C T

The aim of this study was to evaluate the time and dose dependent effect of halogen light from dental curing unit on the cell viability, colony-forming ability and proliferation of the V79 cell culture. The investigation included the medium mode (M), exponential (E) and standard (S) illumination mode for 20, 40 and 80 seconds. The viability was determined using the trypan blue exclusion test. Colony forming ability was assessed by colony count on post-exposure day 7. Cell proliferation was determined by cell counts during five post-exposure days. The viability of cells was not affected by blue light in view of exposure time and modes. Colony forming ability in treated cells was slightly, but not significantly lower than in control cells. Cell proliferation was lower in cells exposed to the M mode for 80 s on post-exposure day 3 and 4 ($p<0.05$). On the same post-exposure days, the proliferation of cells exposed to modes E and S, showed a significant inhibition after 20, 40 and 80 s of exposure ($p<0.05$). Disrupted cellular functionality and no significant decrease in colony forming ability of V79 cells in addition to time- and dose dependent significant inhibition of cell proliferation might be ascribed to the photocuring blue light activity and/or changes in temperature during the course of experiment in vitro.

Key words: *halogen lamp, photo-polymerization, cell culture, viability, colony forming ability, cell proliferation*

Introduction

The increased use of resin composite fillings, calls for the investigation of their supposed harmful effects on the vital tissue. Most of relevant literatures refer to the cytotoxicity of polymerized materials and its components[1–6]. Wataha[7] studied the contribution of the light source for polymerization to the cytotoxic effect of composite filling. The duration of clinical composite depends on complete and appropriate polymerization, use of quality materials, and suitable dental bonding system. The efficiency of light-initiated polymer curing using halogen lamps is generally discussed in terms of radiation flux density, or light intensity. High light intensities are required for complete composite polymerization, particularly for deep cavities. Thus, the desired polymerization of resin composite materials; that is, their physical properties and clinical performances mostly depend on adequate light curing device[8]. Otherwise, incomplete polymerization may led to the deterioration of mechanical and physical properties and turn to a release of toxic components such as monomer bisglycidyldimethacrylate (Bis-GMA) and trethylene glycol dimethacrylate (TEGDMA) in the pulp[9,10]. There are now a number of commercially available high power dental light curing which provide a greater polymerization quality.

However, it seems that the quality of resin fillings correlates inversely with the light intensity, which, in addition, raises a concern about the damaging effect on the pulp tissue caused by increased temperature. To prevent a tissue this damaging effect, current light curing devices are equipped with a soft-start and low- power mode[11,12].

To avoid heating, a low-power mode is proposed for polymerizing dentin adhesives. Soft start cure is proposed for a lower rate of polymerization and longer flow of resin. This allows compensation of material shrinkage, and the composite is finally optimally polymerized. Soft-start polymerization comes in three varieties: step cure (low intensity level followed by high level), ramp cure (continuous increase from low to high) and pulse cure (successive on and off patterns)[13]. In spite of the efforts

Received for publication January 29, 2007

to minimize blue light radiation flux density or high-intensity dose during resin filling placement, incremental layering technique can multiply the blue light dose, affecting the soft and pulp tissue. The pulp tissue might be affected by blue light in a deep cavities when remains thin layer of dentin. An additional reason for a new photo curing approach certainly is the concern about the rise in temperature in the hard tooth tissue and the pulp. Exothermic polymerization and device heat output both contribute to temperature increase. The quantity of generated heat is related to the wavelength and intensity of emitted light, but this heat generation is not a hall-mark of a device's curing ability. Units of the same light intensity could generate different amounts of heat depending on the wavelengths or spectra used[14,15]. More rapid curing units with a high energy output produces considerably more heat over a shorter period of time.

Light emitted from a halogen dental curing unit is polychromatic, and it is filtrated to narrow spectrum, λ = 400–500 nm. It is generally accepted that the blue light of these wavelengths is harmless for patients and dental professionals. However, there are a few side effects to be considered, such as reactions to absorbed radiation in the eye, skin tissue or oral mucosa. Furthermore, reports have documented the effects of blue light on the DNA, mitosis of cells, and mitochondria. Blue light could also generate the reactive oxygen species in the cells by absorbing flavins. Other reports have shown effects on monocytes and monocytic antioxidant pathways[7,16,17]. Some of these reports are generally criticized for having used much higher blue light intensity, exposure duration, and light irradiation modes were much higher than are usually used in restorative dentistry. The effects of halogen curing light are generally recognized, but the mechanism of action is still to be determined. There is no doubt however that it involves changes in temperature. Thus, temperature in cell culture medium needs to be measured to assess its role in occurred biological effects for all curing modes at disposal.

The aim of this study was to evaluate the time- and dose-dependent effect of halogen light at the disposed illumination modes on the viability, colony forming ability and proliferation of the cell line V79.

Materials and Methods

Cell culture

Chinese hamster lung fibroblast cell line (V79) was routinely grown in a nutrient medium (RPMI 1640 SIGMA Chemical CO, St. Louis, USA) supplemented with fetal calf serum and antibiotics (110 IU/mL of penicillin and 0.1 mg/mL of streptomycin). The cells were maintained in a humid atmosphere with 5% CO_2 at 37 °C and sub-culture twice a week at 1:5 ratios. Cells samples were pre-incubated for 24 hours before the beginning of the experiment. Control and exposed cells were taken from the same flask for every experimental procedure[18]. Control cell samples were not exposed to the blue light sources.

Exposure conditions

We used a quartz-tungsten-halogen Elipar® TriLight Light Unit (ESPE, Sefeeld, Germany) for intraoral polymerization of dental materials whose wavelengths ranged between 400 nm and 515 nm This light source for intraoral polymerization of dental materials operates in the three illumination modes, medium (M), exponential (E) and standard (S), and five illumination times. In illumination mode M all illumination time options are available; 20 and 60 s time options are available in mode E, and 10, 20, 40 and 60 s in mode S[19]. The total irradiance or the light intensity was measured by the light intensity control area on the control panel of device and mean light intensity reported by the manufacturer 3M ESPE was 800 mW/cm^2.

The prepared cell samples were exposed to the blue light emitted by the Elipar® TriLight Light polymerization unit in the three illumination modes for 20, 40 and 80 s each.

Cell viability

The viability assay is usually used to measure the proportion of viable cells following a potentially traumatic procedure. Cell viability was determined using the trypan blue dye exclusion test[20]. The viability assay is also used to determine the cytotoxicity of agents by comparison the number of viable cells in the exposed and corresponding control samples. Our viability test included cells exposed to blue light in three modes and three duration times. This experiment was repeated three times.

Colony forming ability

Colony forming ability demonstrates the proliferative capacity of several cell generations and newly formed colonies could be considered representative of the entire cell population. To determine the colony-forming ability, cells were plated in the concentration of 40 cells/mL. Initial cell suspension volume was 5 mL per Petri dish. Cell samples were exposed to blue light for 20, 40, or 80 s. They were then cultivated for 7 days and then fixed and stained with Giemsa. The cells were air dried and colonies with more than 50 cells were counted[18].

Cell proliferation assay

In general, the effect of an agent on cell proliferation can be assessed by counting of treated cells after a few days of culture. To avoid any ambiguities that can arise from cell counts at a single point in time in this study, we recorded the complete growth curve of the cells. Cells in the concentration of 1×10^4 /mL of nutrient medium were plated on the culture plates. After adjusting the initial concentration, cell culture plates were exposed to the blue halogen light for 20, 40, and 80 s. Cells were counted every day for five days after exposure. The experiment was repeated three times[20].

Temperature measurements

The temperature of the media was measured during illumination using a thermoprobe device (MD 3150, Beckmann-Eagle GmbH, Kernen, Germany) to see if there were any changes. The thermoprobe, whose sensitivity was 0.1 °C, was placed at bottom of the well filled with nutrient medium and readings were done for each illumination mode and time. The initial temperature was 19 ± 0.5 °C and the peak temperature rise was recorded. There measuring was repeated three times for each experimental condition.

Statistical analysis

Statistical analysis of data was performed using t-test within the program Statistics for Windows, StatSoft 2003 package.

Results

Cell viability

Figure 1 shows the viability of the V79 cells exposed to blue light. The exposed cells did not significantly differ

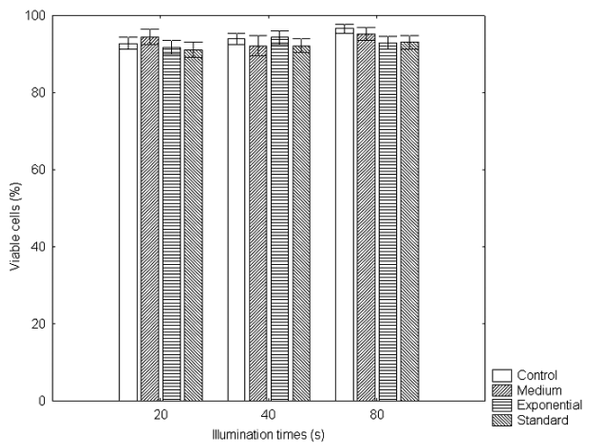

Fig. 1. Viability of the V79 cells after 20, 40 and 80 seconds of exposure to Elipar® TriLight blue dental curing light in three illumination modes (medium M, exponential E, standard, S).

from controls, regardless of the illumination mode (M, E, S) or to the duration of exposure (20, 40, 80 s). In fact their viability was within the range of control values.

Colony-forming ability

Figure 2 shows the colony-forming ability of the V79 cells exposed to blue light for 20, 40, or 80 seconds and matched controls. Regardless of exposure time or illumination mode, the differences were not significant. These results suggest that it is highly unlikely for blue light to procedure a cytocidal effect *in vitro*.

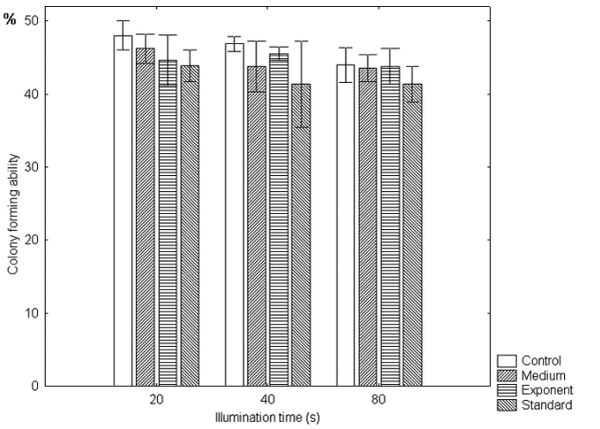

Fig. 2. Colony forming ability of the V79 cells after 20, 40 and 80 seconds of exposure to Elipar® TriLight blue dental curing light in three illumination modes (medium M, exponential E, standard, S).

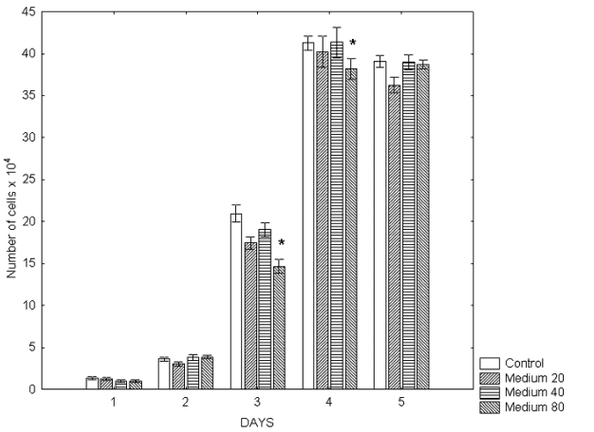

Fig. 3. Growth curve of the V79 cell culture exposed 20, 40 and 80 seconds to Elipar® TriLight blue dental curing light in the medium mode (M).

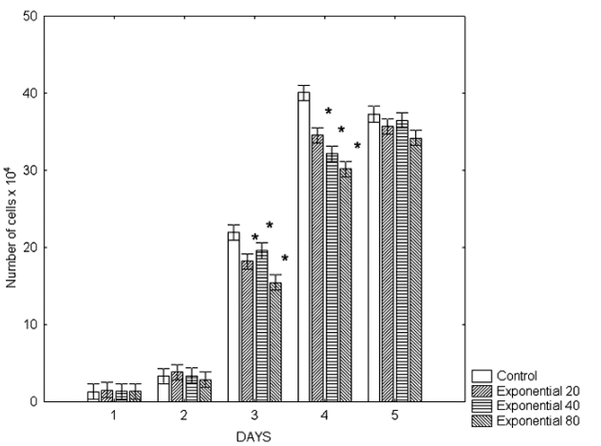

Fig. 4. Growth curve of the V79 cell culture exposed 20, 40 and 80 seconds to Elipar® TriLight blue dental curing light in the exponential mode (E).

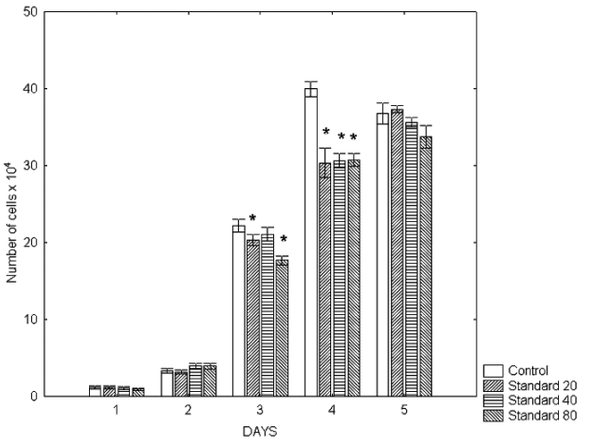

Fig. 5. Growth curve of the V79 cell culture exposed 20, 40 and 80 seconds to Elipar® TriLight blue dental curing light in the standard mode (S).

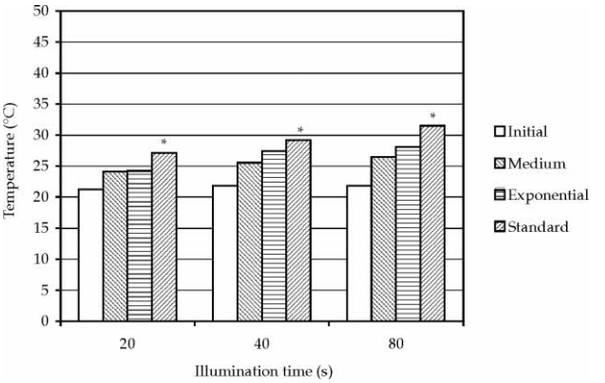

Fig. 6. Temperature changes in the nutrient medium exposed 20, 40 and 80 seconds to Elipar® TriLight blue dental curing light in the medium, exponential and standard illumination mode (– statistically significant).*

Cell proliferation assay

Figure 3–5 show the growth of the V79 cell culture exposed to blue light in the medium, exponential and standard illumination mode for 20, 40, or 80 seconds. The cultures exposed to blue light in M mode for 80 s showed a significantly lower cell count than controls on post-exposure days 3 and 4 ($p<0.05$). The inhibition of cell proliferation was also significant in the cell culture illuminated in the exponential and standard mode in all exposure times on post-exposure days 3and 4 ($p<0.05$).

Temperature rise

Figure 6 shows temperature changes in the media exposed to blue light for 20, 40, or 80 seconds in all illumination modes with respect to the baseline temperature. An increase was recorded for all illumination modes applied and the most was related to blue light exposure in the standard mode for 80 seconds ($p<0.05$).

Discussion

Our results give an insight into the biological potency of halogen blue dental curing light *in vitro*. As the cell viability assay *in vitro* usually reveals traumatic and cytocidal action, whose mechanism is mainly that of breaking the membrane integrity, it has been reasonable and expected to be negative for all modes and energy parameters of light illumination which is widely used in daily dental practice. The cell viability of the samples illuminated by blue light remained in the control range between 92 and 97% (Figure 1). The main findings of this study are that the colony forming ability of the exposed V79 cells has been insignificantly reduced and that their proliferation was significantly inhibited. The effect on the colony-forming ability was not time- or dose-dependent. In contrast, cell proliferation, over the five post-exposure days strongly depended on illumination modes (notably, E and S) and on all applied exposure times. The illumination mode M, which has a reduced light intensity and is recommended for curing of bonding and where gingival irradiation cannot be avoided, showed significant effect on cell growth only when exposure lasted 80 s.

Although most investigators have studied combined exposures, such as those of adhesives plus halogen light, our findings are in agreement with Bruzell Roll et al.[21]. They found a higher percentage of apoptosis and necrosis after *in vitro* cell irradiation from a dental curing lamp (Polofil Lux; Voco) with wavelength range between 350 and 550 nm. Increased cytotoxicity toward rat submandibular salivary gland acinar cells was observed as a result of irradiation[21]. Our study differed from Roll's in the cell culture type and experimental protocol. It is possible that hamster lung fibroblasts are not as radiosensitive to the blue light irradiation as submandibular salivary gland acinar cells, as our findings are less pronounced than Bruzell Roll's results. The difference may also have been contributed by different light sources and different efficiency of the filters. The deleterious effects of the shorter wavelengths (ultraviolet light), included in emission spectrum of the lamp used in previous study, is well known. It is obvious that clinically relevant exposures of dental curing lights affect the growth kinetics of our cell line, which implies the use of blue light make the exposed cells more vulnerable. Another consequence of composite resin photopolymerization is a temperature rise in the tooth tissue. It depends not only on the illumination time, but also on light intensity. The highest temperature rise in the composite resin recorded in a study of Knezevic at al.[22] was 13.3±1.31 °C after 40 seconds of exposure to Elipar® Trilight halogen curing unit in standard curing mode[22]. Our results show a temperature rise in the nutrient medium exposed to Elipar® TriLIght blue light for 20, 40 and 80 seconds in all operation modes

(Figure 6) with the most prominent in standard mode for 80 seconds. A rise in temperature during photopolymerization could trigger cell-death mechanisms. There are also two major mechanisms which could lead to the impairment of cell integrity, that is, photo-induced degradation of flavins and oxidative stress. Blue light with the wavelength of 460 nm wavelength, could be absorbed by flavins. Flavins which are essential coenzymes for succinic dehydrogenase activity of mitochondria have been found to be suppressed by blue light[7]. It is well known that the transport of hydrogen ions in redox processes within the cell depends on riboflavins. Other evidence suggests that specific cell responses result from redox changes induced by exposure to blue light. It was shown that blue light exposure arrested monocyte cell growth and increased the levels of peroxiredoxins[23]. Blue light exposure generally induces higher levels of total reactive oxygen species and significantly suppresses succinate dehydrogenase activity in oral tumor cells[23]. Spagnuolo et al.[24] found that light source could contribute to the intracellular production of reactive oxygen species in combination with »one bottle« adhesive system, leading to the reduced cell survival.

Conclusion

In conclusion, our results indicate that the blue curing light and/or changes in temperature may lead to disrupted cell functionality, slight decrease in colony-forming ability, and significant time- and dose-dependent proliferation inhibition of the V79 cells.

Acknowledgement

This study was supported by Grants No. 0022005 and 0065999 of the Ministry of Science, Education and Sports of the Republic of Croatia.

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UČINAK PLAVOG HALOGENOG STOMATOLOŠKOG SVJETLA NA STANIČNU V79 LINIJU OVISNO O NAČINU RADA I DOZI

SAŽETAK

Svrha ovog rada je bila ispitati učinak halogenog svjetla iz stomatološke polimerizacijske lampe na preživljavanje, sposobnost stvaranja kolonija i proliferaciju V79 stanica ovisno o vremenu i dozi. Istraživanje je uključivalo srednji (M), eksponencijski (E) i standardni (S) način rada tijekom 20, 40 i 80 sekundi. Preživljavanje je određeno uporabom testa ekskluzije tripanskog modrila. Sposobnost stvaranja kolonija je procijenjena brojem kolonija 7 dana nakon ekspozicije. Proliferacija stanica je određena brojem stanica tijekom pet dana nakon osvjetljivanja. Plavo svijetlo nije utjecalo na preživljavanje stanica u ispitivanim uvjetima vremena i načina ekspozicije. Sposobnost stvaranja kolonija osvijetljenih stanica je bila slabo, ali ne i značajno niža nego kod kontrolnih stanica. Proliferacija stanica je bila niža kod stanica eksponiranih M načinu rada tijekom 80 s treći i četvrti dan nakon obasjavanja ($p<0,05$). Istog dana nakon obasjavanja, proliferacija stanica izloženih E i S načinu je pokazala značajnu inhibiciju nakon 20, 40 i 80 s obasjavanja ($p<0,05$).

Ometanje celularne funkcionalnosti i beznačajno smanjenje sposobnosti stvaranja kolonija V79 stanica uz inhibiciju proliferacije stanica ovisno o vremenu i dozi, može se pripisati aktivnosti polimerizirajućeg plavog svjetla i/ili promjenama u temperaturi za vrijeme pokusa *in vitro*.