Original Article

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The effect of LED light on viability and proliferation of periodontal ligament fibroblast cells

Thamonwan Langlongsathit[1], Warunyu Sermkasemsin[2], Orakot Leysak[3], Rudee Surarit[4], Pirasut Rodanant[5]

[1] *Soidao Hospital, Chanthaburi, 22180.*
[2] *Phimai Hospital, Nakhon Ratchasima 30110.*
[3] *Surat Thani Hospital, Surat Thani 84000.*
[4] *Department of Oral Biology, Faculty of Dentistry, Mahidol university, Bangkok, Thailand 10400.*
[5] *Department of Advanced General Dentistry, Faculty of Dentistry, Mahidol university, Bangkok, Thailand 10400.*

Objective: To evaluate the effect of LED blue light on the viability, proliferation and morphology of periodontal ligament fibroblasts (PDLF).

Materials and methods: Human PDLF cells were cultured and irradiated with LED blue light at different intensity and duration. MTT assay was used to determine the cytotoxicity and proliferation activity. Cell morphology was observed under light microscope. Statistical analysis was performed using ANOVA at a significant level of $p<0.05$.

Results: The LED blue light did affect PDLF cell numbers but not its morphology. PDLF cell numbers were significantly reduced in time-dependent manner irrespective to light intensity. The proliferation of PDLF cells was not affected by the duration of light exposure at 20 and 60 seconds.

Conclusion: The healthy periodontal ligament cells are not affected by the use of LED blue light since the exposure time does not exceed 60 seconds.

Key words: LED blue light, morphology, periodontal ligament fibroblast, proliferation, viability

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Introduction

Most success in clinical work inevitably depends on the success of material used. At present, light curing materials are widely used in various fields of dentistry, e.g. composite resin in restorative work; luting cement in prosthodontics; and adhesives in orthodontics. Optimal polymerization of light curing material is the key factor to achieve the required characteristics leading to its effectiveness [1]. Light curing unit (LCU) can be divided by their light producing sources, e.g. Quartz-tungsten-halogen (QTH), plasma arc (PAC) and the light-emitting-diode (LED), which give different wavelength spectra and power outputs. Among all, LED is the most popular according to its broader spectral output and high irradiation and power output which could allow more photons to be absorbed by photoinitiator (eg. Camphorquinone) resulting in producing free radicals to initiate polymerization to a broader range of resins [2]. Nevertheless, the high intensity light resulting from the high power output may cause stress in the material due to too fast polymerization affecting the physical properties of the material [3]. Moreover, the high

Corresponding author: Pirasut Rodanant, Department of Advanced General Dentistry, Faculty of Dentistry, Mahidol university, Bangkok, Thailand 10400. 6 Yothi Street Ratchathewi District Bangkok 10400 Thailand E-mail: pirasut.rod@mahidol.ac.th
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intensity light may harm the surrounding tissues both from the light itself and the heat production from the LCU [4]. The harmful effects of blue light emitting from Quartz-Tungsten-Halogen(QTH)/ Plasma arc/Argon laser on oral cells and tissues have been reported [5]. The effect of rising temperature has also been shown to affect cell viability. With no exception, light energy produced from the LED-LCU, the most popular light source at present, unfortunately produces heat. Nevertheless, previous study reported no harmful effect of LED blue-light on human gingival fibroblast cells [6]. That study demonstrated that there was no change in cell morphology and cell viability following exposure to light produced from light emitting diode. Though an impressive result occurred to the gingival fibroblasts, it could not expect the same result with the different cell type. Periodontal ligament fibroblasts, an essential cell which produces periodontal ligament providing cushion to all teeth and anchoring them to the alveolar socket, may have chance to expose to the light spectrum from LCD-LCU during light curing procedure. Since curing duration and light intensity seem to correlate with heat obtaining from the LCU, this *in vitro* study is designed to observe whether the amount and intensity of the LED blue-light simulating the clinical applications might compromise the cellular activity and characteristic of periodontal ligament fibroblast cells.

Materials and methods

1. Cell and Chemicals

Human periodontal ligament cells were obtained from ScienCell™ Research Laboratories, CA 92011, USA (Catalog#2630). Dulbecco's Modified Eagle Medium (DMEM), 0.25% Trypsin 0.25% and 0.4% Trypan blue solution were purchased from gibco®, USA. Fetal Bovine Serum (FBS) was purchased from Hyclone UK Ltd., Cramlington, UK. 2% $NaHCO_3$ solution, Isopropanol, Thiazolyl Blue Tetrazolium Blue (MTT) were purchased from SIGMA Aldrich Chemical Co., St. Louis, USA. Other chemicals were of analytical grade.

2. Irradiation source and exposure regime

Light emitting diode (LED) device (Bluephase G2, Ivoclar/vivadent) is a blue light emitting source with a wavelength of 385-515 nm, a power output of 650-1200 mW/cm^2 and a frequency of 50-60 Hz. The diameter of the probe is 0.8 cm. The distance from light source to fibroblast was 0.8 cm. Three experimental groups according to light intensity (low, soft and high) were randomly assigned and subjected to treat with LED-blue light at three different time interval (20, 60 and 100 seconds).

3. Viability test

Human periodontal ligament cells were seeded in 96 well-black-opaque plates (200 µl/well at a density of 2×10^4 cells/ml) and were incubated at 37°C in 5% CO_2 atmosphere for 24 hours. At 24 hours, cells were washed with sterile phosphate buffer (1xPBS), treated with LED-blue light in the biohazard safety cabinet (Figure 1) and further incubated at 37°C in 5% CO_2 atmosphere for another 24 hours. The supernatant was discarded and the plate was washed with sterile PBS 1X. After that, 100 µl of MTT solution (0.5 mg/ml) was

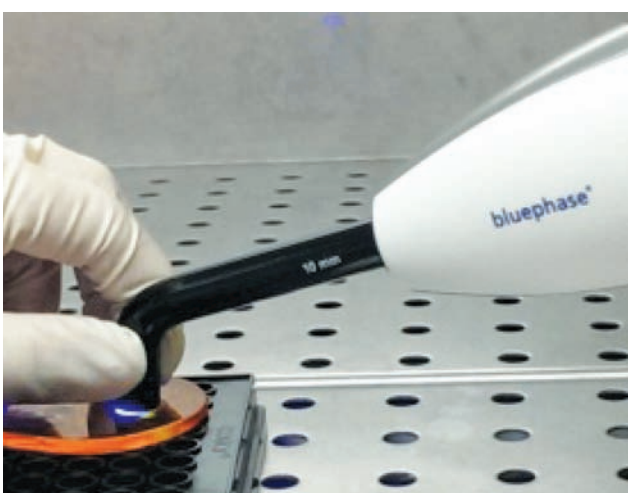

Figure 1 Light Emitting Diode device

added to each well and incubated at 37°C for another 2 hours. Then the medium was aspirated. In each well, the formed formazan crystals were solubilized with 100 µl of DMSO and left for 30 min at room temperature. An absorbance of formazan was reported as optical density which was read at 540 nm by a microplate reader (Model series UV 900 Hdi, USA). Three separate experiments were carried out.

4. Cell proliferation test and morphological study

In brief, 96-well black-opaque plates containing human periodontal ligament cells ($1x10^3$ cell/well) were treated with LED blue-light (the intensity in each duration which reduced the viability of fibroblast to less than 80%) then incubated at 37°c, 5% CO_2 for 24, 72 and 120 hours before estimated cell proliferation was measured utilizing the MTT method. The procedure was done in duplicate. Before commencing MTT staining on day 1, 3 and 5, incubated cells were photographed under inverted microscope (40X).

5. Statistical analysis

Two-way ANOVA was used to evaluate difference in cell viability between experimental groups. Comparison of cell proliferation and cell morphology was analyzed descriptively.

Results

This experiment observed two factors which might affect the human periodontal ligament fibroblast cell viability: light intensity and duration of light exposure. The result demonstrated that there was no statistically significant difference in the percentage of cell viability between cells treated with blue-light for 20 seconds and no light treatment (Table 1). There was a significant decrease in cell viability responding to the increase of light intensity when cells were treated with blue-light for 60 and 100 seconds ($P<0.05$) (Table 1). The percentage of cell viability decreased to below eighty percent when cells were treated with

Table 1 Effect of LED-blue light on viability of periodontal ligament cells

Time (s)	Light (Mode)	O.D. 570 nm (Mean ± S.D.)	%Viability
20	Control	0.495±0.060	100
	Low	0.482±0.035	97.37
	Soft	0.519±0.040	104.85
	High	0.523±0.028	105.66
60	Control	0.520±0.081	100
	Low	0.437±0.083	84.04
	Soft	0.420±0.053	80.77
	High	0.396±0.025	76.15
100	Control	0.490±0.066	100
	Low	0.386±0.047	78.78
	Soft	0.378±0.046	77.14
	High	0.361±0.057	73.67

LED-light at high intensity for 60 seconds and each mode of light intensity (low, soft, high) for 100 seconds. (Table 1)

According to the viability test, the duration and intensity of light exposure which gave more than 80% of cell viability were used to observe the rate of cell proliferation. Treated cells from both groups showed less proliferation rate compare to the control group in the first three days thereafter it seemed to be at the same rate. (Table 2 and Figure 2).

Photographs showed that there was no particular change in periodontal ligament cell morphology following irradiation with LED light for 5 days. (Figure 3-5)

Discussion

At the same time interval, there was no significant difference in cell viability though cells were treated with different light intensity mode. More interestingly, as time increased, even the use of low intensity mode could significantly decrease the cell number. Accordingly, it could be postulated that human periodontal ligament cells decreased in response to LED blue light in a time dependent manner.

Utilizing the MTT assay, this study revealed that power output significantly reduced the periodontal ligament fibroblast cells viability.

Table 2 Comparing efficacy of periodontal ligament cell proliferation after exposure to different LED-blue light intensity

Light (Time, Mode)	O.D. 570 nm (Mean ± S.D.)		
	Day 1	Day 3	Day 5
None	0.314±0.017	0.531±0.031	0.788±0.127
20 H	0.334±0.034	0.467±0.045	0.682±0.062
60 S	0.322±0.040	0.366±0.060	0.563±0.066

None = control (no light treatment)
H = High intensity mode
S = Soft intensity mode
Time was measured in seconds.

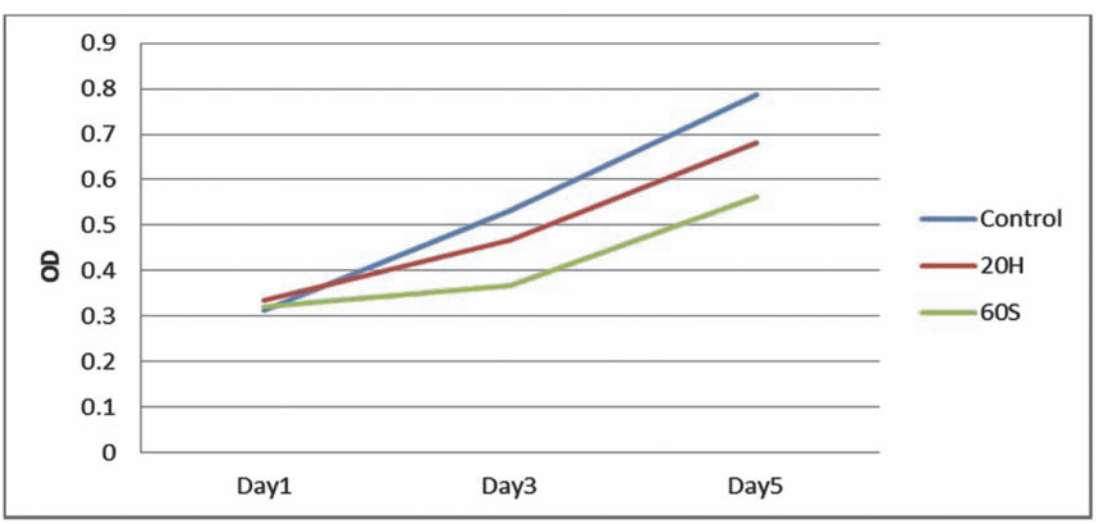

Figure 2 Proliferation activity of periodontal ligament fibroblasts after LED-light exposure

Power output will reflect heat emitting from LCU [7]. Heat produced from LCU might result in the precipitation of protoplasm, the vascular change, or the occurrence of tissue necrosis [8]. Previous studies showed that blue light exerts possible harmful effects on living cells through several means [9]. It could interact with chromophores (photosensitizers), including flavin-containing oxidases, the cytochrome system, heme-containing proteins, and tryptophan-rich proteins, in the mitochondria and peroxisomes resulting in the production of hydrogen peroxide (H_2O_2) leading to cellular dysfunction (DNA damage) [10]. This might explain the decrease in cell proliferation in our study.

The difference in methodology might be another explanation for the result's contradiction. The previous study assessed the vitality of hamster's lung fibroblast cells using the tryphan blue dye exclusion test, which reveals the late stage of apoptosis whereas our study assessed the mitochondria function through the enzymatic activities, which detects the early changes in cell function [11]. The production of reactive oxygen species from the photoreduction of flavin within peroxyzome and mitochondria might also harmful to mitochondria [12]. Power generation from LCU using different apparatus might also affect cellular response.

In conclusion, the healthy periodontal ligament cells is not affected, in term of cell viability and cell proliferation, by the use of LED blue light in curing dental tooth-colored materials since the exposure time does not exceed 60 seconds.

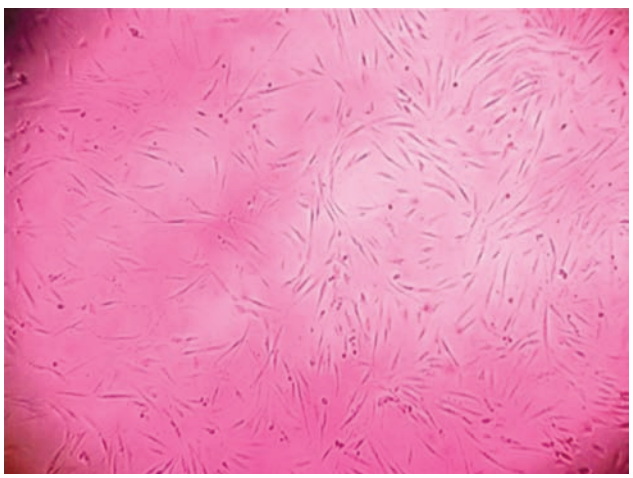

Figure 3 Human Periodontal Ligament Fibroblast cells Morphology observed by inverted microscope (x40): 1 day after light exposure (60 seconds at soft intensity mode)

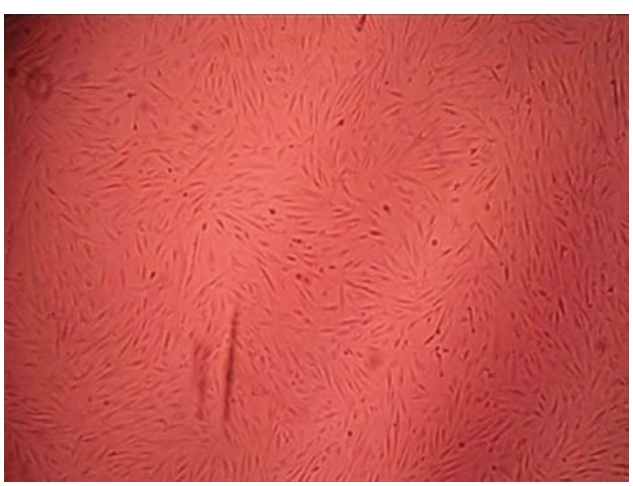

Figure 4 Human Periodontal Ligament Fibroblast cells Morphology observed by inverted microscope (x40): 3 day after light exposure (60 seconds at soft intensity mode)

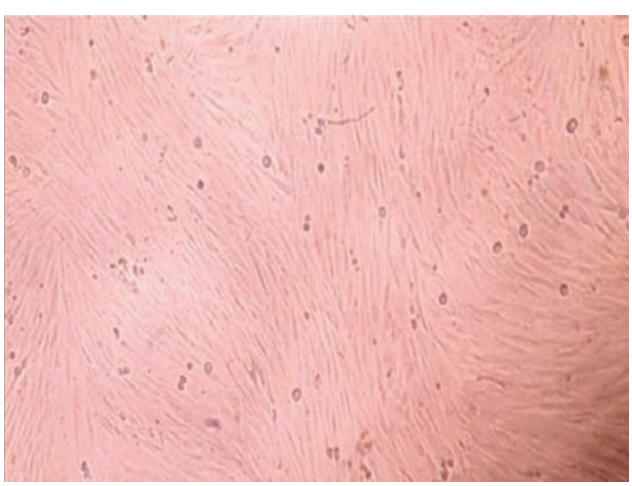

Figure 5 Human Periodontal Ligament Fibroblast cells Morphology observed by inverted microscope (x40): 5 day after light exposure (60 seconds at soft intensity mode)

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