

Research Report

Effect of 405 nm Diode Laser with Varying Irradiation Time on BHK-21 Fibroblast Viability

Kun Ismiyatin¹, Leidy Herlin Rumbiak², Widya Saraswati¹, Sri Kunarti¹, Anuj Bhardwaj³

¹ Staff of Department of Conservative Dentistry, Faculty of Dental Medicine, Universitas Airlangga, Surabaya - Indonesia

² Undergraduate Student of Faculty of Dental Medicine, Universitas Airlangga, Surabaya - Indonesia

³ Staff of Department of Conservative Dentistry and Endodontics, College Of Dental Science and Hospital, Indore, India.

ABSTRACT

Background: Laser is a device that emits light through a process of optical amplification based on the stimulated emission of electromagnetic radiation; it can be used for surgery, detoxification, bio stimulation and antibacterial. However, lasers have Biphasic Dose Response (BDR), which is bio stimulation and bio inhibition. To determine if 405 nm laser diode is biocompatible, viability test is necessary before these lasers can be labeled as viable to use in dental therapy. **Aim:** To prove the variation of radiation time of the 405 nm laser diode radiation can cause bio stimulation and bio inhibition response that affects the viability of BHK-21 fibroblast cells. **Method:** Viability test was carried out using BHK-21 fibroblast cells which were inserted into 96-well microplate, then radiated with 405 nm laser diode with varying irradiation time of 30s, 60s, 120s, 240s and 480s. After radiation, the cells are then incubated for 24h. Cytotoxicity was observed using MTT assay and ELISA reader. Data was analyzed using the Kolmogorov-Smirnov test, Levene Test, Welch ANOVA, and Tukey HSD. **Results:** BHK-21 fibroblast cells radiated with 405 nm laser diode with radiation time of 30s, 60s, 120s, and 240s have the same viability as the control cell, while at 480 seconds the viability exceeds that of the control cell. **Conclusion:** 405 nm laser diode with radiation times of 30s, 60s, 120s, and 240s do not affect the viability of BHK-21 fibroblast cells. Meanwhile, 480s irradiation time of 405 nm laser diode causes bio stimulation response that increases the viability of BHK-21 fibroblast cells.

Kata kunci: Diode Laser; Cell Viability; BHK-21 fibroblast

Correspondence: Anuj Bhardwaj, Staff of Department of Conservative Dentistry and Endodontics, College Of Dental Science and Hospital, Indore, India, dranuj_84@yahoo.co.in, +91 7898540222.

INTRODUCTION

LASER (*Light Amplification by Stimulated Emission of Radiation*) is a device that emits light through a process of optical amplification based on the stimulated emission of electromagnetic radiation; it can be used for many such as surgery, hemostasis, detoxification, biostimulation, and as an antibacterial. Laser has been used widely in medicine and dentistry since 1960⁽¹⁾.

However, laser therapy has Biphasic Dose Response (BDR), which is the response of biostimulation and bioinhibition⁽²⁾. *Biphasic Dose Response* of

laser therapy has been proven by the research of Basso *et al.*, in 2012. This research proves that the duration of laser irradiation 40s increases the viability of gingival fibroblasts while at the time of irradiation 120s cell viability decreases⁽³⁾. Other research by Gkogkos *et al.*, in 2015 proved that the viability of gingival fibroblasts increased at irradiation time 20s, 40s, and 60s while cell viability decreased at 120s irradiation. ⁽⁴⁾.

Biphasic Dose Response of laser affected by power density and time of irradiation. Different wavelength, tissue, redox type,

dan pulse type will produce different BDR⁽⁵⁾⁽⁶⁾.

Therapy used in dentistry must be biocompatible so that it does not have a toxic effect that can endanger the pulp and oral mucosa.⁽⁷⁾ In this research, the viability of BHK-21 fibroblast cells which have irradiated with varying irradiation time (30s, 60s, 120s, 240s, and 480s) of 405 nm diode laser, were tested.

MATERIALS AND METHODS

Ethical Clearance Certificate: 265/HRECC.FODM/X/2018. BHK-21 fibroblast cell were used as samples in this research because they are the most widely used in the cytotoxicity test of a dental material, because these cells have the morfology and ability of cells similiar to human fibroblasts in producing growth factors.

Fibroblast cells were taken from BHK-21 cell cultures in the form of cell-lines grown in Roux bottles. After full, culture cell is harvested using a solution of Trypsine Versene. The crops were planted in DMEM media containing 20% FBS, and incubated for 24 hours at 37°C. Cells were cultured in each well containing cells and DMEM media with density of 2×10^5 sel/ml as much as 100 μ L.

BHK-21 fibroblast cells that were distributed in wells were divided into 7 treatment groups, group I as a cell control, group II as media control, group III irradiated by laser for 30s, group IV irradiated by laser for 60s, group V irradiated by laser for 120s, group VI irradiated by laser for 240s, adn group VII irradiated by laser for 480s.

Well inserted into the illuminator CNC photodynamic laser diode. The laser is exposed using a CNC photodyamic laser diode illuminator. Distancem irradiation time, and well scheme that will be illuminated by 405 nm laser diode are arranged on the illuminator screen CNC photodynamic laser diode.

The viability test method used in this study was MTT assay with a test basis in the form of the amount of formazan crystal formation which positively correlated with the amount of cell life. The absorbed calorimetric (optical density) values of BHK-21 fibroblast were read using ELISA rader with wavelength 620 nm after 24 hours incubation.

The data obtained were then analyzed and processed using IBM SPSS Statistic Base for normality tests using Kolmogorov-Smirnnov, then homogeneity tests using Levene test, and different tests using welch ANOVA and continued with different tests between groups using Post-Hoc Tukey.

RESULTS

This research aims to prove that varying time of 405 nm laser diode irradiation can cause bio stimulation or bio inhibition response that affects the viability of BHK-21 fibroblast cells using MTT assay method. The sample was divided into 7 treatment groups which were irradiated by 405 nm diode laser with 30s, 60s, 120s, 240s, and 480s of irradiation time, cell control and media control, in which each group replicated 8 times. After ELISA readings, optical BHK-21 Fibroblast Cells were obtained as shown in Table 1.

Table 1. Average optical density and standard deviation

Group	N	\bar{x} <i>Optical Density</i> \pm SD	p value of variance analysis
30s	8	0,388 ^b \pm 0,016	< 0,05
60s	8	0,395 ^b \pm 0,042	
120s	8	0,404 ^b \pm 0,030	
240s	8	0,432 ^b \pm 0,013	
480s	8	0,455 ^c \pm 0,020	
Kontrol sel	8	0,412 ^b \pm 0,004	
Kontrol media	8	0,058 ^a \pm 0,003	

Keterangan:

N = number of samples

\bar{x} = average

SD = Standard Deviation

The data obtained were analyzed using the Kolmogorov-Smirnov test to find out whether the data was normally distributed or not normal. The results of the analysis show $p > 0.05$, which means the data is normally distributed. Then the homogeneity test with Levene Test showed $p < 0.05$ which means the data variant is not homogeneous.

After testing for normality and homogeneity, different tests or significance tests are carried out. Because the data variants obtained were not homogeneous, different tests were carried out using Welch ANOVA, and continued with different tests between groups using *Post-Hoc Tukey* with p value of 0,05.

On the results of the Welch ANOVA test $p < 0.05$. This shows that there are significant or not significant differences in the results of the treatment. The results of the Tukey HSD test (Table 1.) showed that there were significant differences between the cell control group and media control group, cell control group and the treatment group at 480s. However, there

were no significant difference between cell control group and the treatment group for 30, 60, 120 and 240 seconds irradiation.

DISCUSSION

LASER (*Light Amplification by Stimulated Emission of Radiation*) is a device that emits light through a process of optical amplification based on the stimulated emission of electromagnetic radiation; it can be used for many such as surgery, hemostasis, detoxification, biostimulation, and as an antibacterial. However, laser therapy has Biphasic Dose Response (BDR), which are response of biostimulation and bioinhibition. Therapy used in dentistry must be biocompatible so that it does not have a toxic effect that can endanger the pulp and oral mucosa. This research aims to prove the variation of irradiation time of the 405 nm laser diode can cause a bio stimulation and bio inhibition response that affects the viability of BHK-21 fibroblast.

BHK-21 fibroblast cell were used as samples in this research because they are

the most widely used in the cytotoxicity test of a dental material, because these cells have the morphology and ability of cells similar to human fibroblasts in producing growth factors. In addition, BHK-21 fibroblast cells (Baby Hamster Kidney-21) are embryonic cells that can be grown easily, easily cultured, have characters that are quite stable, sensitive and do not experience mutase⁽⁸⁾.

From the results of the study, the viability of BHK-21 fibroblast cells increased with increasing exposure time.

Referring to the Telli standard regarding the toxicity parameters of material, Telli states that a material is said to be non-toxic if the percentage of living cells after exposure to the material is more than 50%⁽⁷⁾. All treatment groups had fairly high average cell viability, which is above 90%, so that it can be said to be non-toxic. The smallest cell viability was obtained in group with 30s irradiation time which was 93.2% and the highest viability was obtained in group with 480s irradiation time which was 112.1% (Figure 1.).

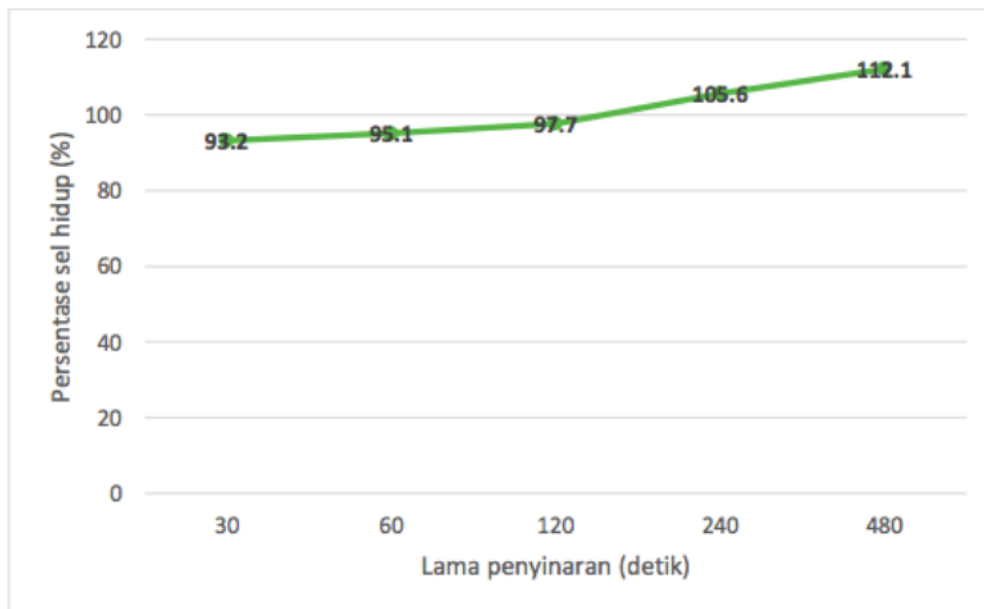


Figure 1. The percentage of live cells after treatment with varying irradiation time of 405 nm diode laser

In the results of data analysis there were no significant differences in the average optical density of BHK-21 fibroblast cells in the treatment group with irradiation times of 30, 60, 120, and 240 seconds with cell control group. This means that 405 nm diode laser with irradiation time 30, 60, 120 and 240 seconds does not affect the life of BHK-21 fibroblast cells.

There were no significant differences but the difference in average optical density in the treatment group with irradiation time 30, 60, 120, 240 seconds and cell control

occurred because cell cultures in microplates were in the form of stacked cell layers, resulting in differences in MTT salt absorption ability which will color living cells that are on the outer surface and cells that are in the inner cell layer. Cells that are on the inside will be blocked by cells that are on the outer surface of the cell layer, so that living cells inside the cell layer cannot absorb MTT salt properly, which results in different ELISA readings.⁽⁷⁾

The cause of irradiation time 30, 60, 120, and 240 seconds have no effect on the

life of BHK-21 fibroblast cells likely to occur because the energy obtained from photons absorbed by cell chromophore is not enough to stimulate the biological activity of cells. This is consistent with the Ohshiro and Calderhead Low Level Laser Therapy curves adapted from the Arndt-Schultz law (Figure 2.) which explains that at the point A to B there has been no

response due to the small density of energy produced. Arndt-Schultz law states that weak stimuli will excite biological activity, moderate stimuli favor it, strong stimuli will hal increased activity, and very strong stimuli will retard or completely stop biological activity⁽⁹⁾.

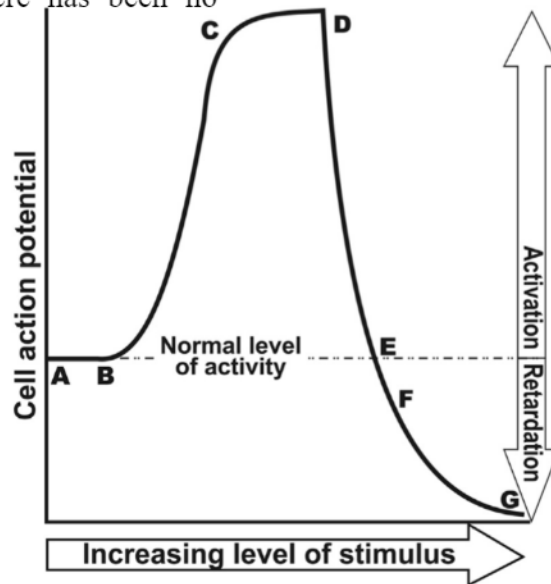


Figure 2. Ohshiro and Calderhead's LLLT-adapted version of the Arndt-Schultz curve.⁽⁹⁾

In the treatment group with 480 seconds irradiation time, the viability of BHK-21 fibroblast cells was increased to 12.1%. Increasing the life of BHK-21 fibroblast cells at 480 seconds is possible because a biostimulation response from 405 nm diode laser has occurred, on the curve (Figure 2.) seen from point B to C⁽⁹⁾. This response occurs because the energy absorbed by the cell is sufficient to stimulate the biological activity of the cell. When the laser beam is exposed to fibroblast cells, the photons emitted by the laser will be absorbed by the cell's chromophore. Cytochrome c oxidase (Cox) is the main photo acceptor in mammalian cells because the absorption spectrum obtained by Cox in various oxidation numbers are found to be very similar to the action spectrum for biological responses to light. Bio stimulation response occurs due to acceleration of the electron transport reaction. The increase in electron

transport reactions causes an increase in ATP production. Increasing ATP synthesis in mitochondria accelerates the speed of cell mitosis. The effects of laser bio stimulation can increase growth factor secretion, such as TGF- β , which is responsible for inducing collagen synthesis from fibroblasts. TGF- β is involved in apoptosis, proliferation of cell differentiation and migration, so that it can cause an increase in the life of BHK-21 fibroblast cells⁽¹⁾.

The results of this research are different from the research conducted by Basso *et al.*, and Gkogkos *et al.*, where cell viability decrease at 120s. This can be caused by different wavelengths, tissue, redox types and pulse types that will produce different Biphasic Dose Response⁽⁶⁾.

response that increases the viability of BHK-21 fibroblast cells.

CONCLUSION

405 nm laser diode with radiation times of 30s, 60s, 120s, and 240s do not affect the viability of BHK-21 fibroblast cells. Meanwhile, 480s irradiation time of 405 nm laser diode causes a bio stimulation

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