EFFECTS OF LOW INCIDENT ENERGY LEVELS OF INFRARED LASER IRRADIATION ON THE PROLIFERATION OF *CANDIDA ALBICANS*

PART II : A SHORT TERM STUDY DURING THE CELL CYCLE

Sam-Kun Kim, Phil-Yeon Lee and Ki-Suk Kim

Department of Oral Medicine, School of Dentistry, Dankook University,
Chunan, Choongnam, Korea

CONTENTS

I. Introduction

There has increasing interest in the laser's effect on micro-organisms. Kim *et al.*[1] reported the clinical, microbiological, and histological study on the effect of LLLT in treating early gingivitis. Their study indicated that the LLL irradiation had a favourable influence on the gingival inflammation with the change of rate in the composition of oral flora. Kim *et al.* [2,3] reported that infrared gallium-arsenide(GaAs) laser irradiation at LLLT levels stimulated the increase of *Streptococcus mutans* growth but the growth rate of *Streptococcus mutans* is not always in accordance with the frequency or fluence of LLLT laser. Lubart et al.[4] suggested that LLLT is dependent on wavelength, is not thermal and is limited to a specific energy density. Kim et al. also suggested, in their study for *Candida albicans*, that specific laser pulses are recommanded to have the biostimulation effects on the specific tissue or cells, although the biostimulation effect is dose dependant.[5] Their study however, had some limitations because the effect of LLLT on the growth of *Candida albicans* during the short term of cell cycle was not elucidated in their long term of examination.

This study was performed to investigate the effect of LLLT(GaAs) on the growth of *Candida albicans* during the short term of cell cycle, using various pulsed lasers of infrared GaAs laser.

II. Materials and Methods

Materials

1. Micro-organism

The micro-organism used in this study was *Cadida albicans*, because it is common seen as normal flora in oral cavity. *Candida albicans* ATCC #28366 was obtained from American Type Culture Collection. The strain was maintained by transferring to fresh stock culture media(Sabouraud dextrose agar) each month and storing at 4℃.

2. Culture Media

Media (SDA) was prepared with 20g glucose, 10g Neo-peptone, 15g Agar in liter of distilled water(pH 7.0). A single colony of *Candida albicans* developed on the plate of the stock

culture medium was transferred to 20 ml of the seed culture medium and incubated in a rotary shaking incubator at 30℃. The seed culture was inoculated into 100ml of the medium. Temperature was controlled to 30℃ and culture pH was maintained to 7.0.

3. Laser Apparatus.

The laser used in this work was the BIO-LASER(Dong Yang Medical, Korea) using GaAs semiconductor as a diode. It is a pulsed infrared laser apparatus with a wavelength of 904nm and peak output power is 27W. Pulsed lasers used in this study were pulse 7(500Hz, 1 mW of average output power), pulse 9 (1500 Hz, 3mW of average output power) pulse 11 (P11, 3000Hz, 6mW average output power), pulse 13(P13, 6000Hz, 14mW average output power) and pulse 15(10000Hz, 27mW of average output power).

4. Flask used to measure OD

Special flasks were made to measure OD repeatedly. A pyrex test tube(20ml) is attached in the neck of erlenmeyer flask (300ml) with about 25 degree of slope (Figure 1).

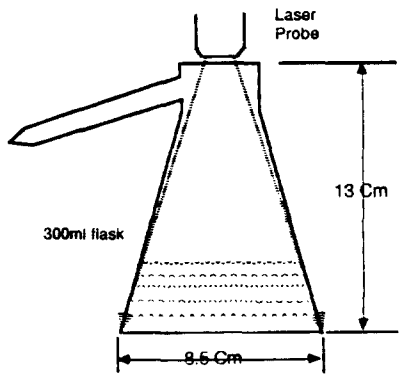

Figure 1. Schematic illustration of the relationship between the laser probe and the tissue culture dish.

5. Spectrophotometer

To evaluate the growth of *Candida albicans*, spectrophotometer(Spectronic 20, Bausch and Lomb, Rochester, NY) was used to measure the optical density of cell culture broth. The spectrophotometer was set at 600nm. Reference liquid was identical brain heart broth media without cells.

Methods

Thirty samples were used in experiment 1. The samples were randomly divided in 6 groups according to the pulse type : pulse 7(P7), pulse 9(P9), pulse(P11), pulse(P13), pulse 15(P15) and shame-irradiated control(Co) groups.

The author used the holder to fix the laser probe vertically about 13cm apart from the middle position between top and bottom of media in the flask(Figure 2). After the inoculation of seed culture, samples were irradiated for 1minute in the begining of experiment and every 2 hours for 27 hours. All samples were cultivated in a shaking incubator(100rpm) at 30℃ in the dark. The optical density of all sample was measured with spectrophotometer every hour for 27 hours of cell cycle. Energy densitiess of all experimental groups for each session, P7, P9, P11, P13 and P15 were 2.12, 6.37, 12.72, 29.68 and 57.32 mj/cm^2 respectively.

Statistical analysis

All measurements in each group were averaged. Statistical comparisons were then made. To determine the significance of differences among groups according to pulse type and elapsed time, Repeated measures ANOVA and Fisher's Protected Least Significant Difference (PLSD) were used.

III. Results

The means of all groups classified according to the pulse type and elapsed time are given in

Table 1, and the changes of OD in all groups are shown in Figure 1. To test whether the pattern of change over time is the same for the different pulse types, Repeated measures ANOVA was used in this study and the result was seen in Table 2. This result means that averaged over the elapsed times there was no difference in the means of the six pulsed groups as measured by the optical densities, although there was weak significant pulse type-by-time interaction indicating that the patterns of changes in means of optical densities over time differ depending

Table 1. Mean and standard deviations of the optical densities measured according to the pulse type and the elapsed time during the cell cycle of Candida albicans.

	14:00	15:00	16:00	17:00	18:00
CON	0.012±4.472E-3	0.022±4.472E-3	0.021±5.477E-3	0.044±0.013	0.088±0.033
P7	0.020±0.012	0.023±9.574E-3	0.024±0.016	0.043±0.022	0.078±0.041
P9	0.020±0.012	0.027±0.020	0.040±0.030	0.079±0.061	0.164±0.124
P11	0.014±5.477E-3	0.012±8.367E-3	0.012±4.472E-3	0.029±0.012	0.050±0.022
P13	0.015±5.774E-3	0.015±5.774E-3	0.013±0.005	0.034±7.500E-3	0.058±0.017
P15	0.012±8.367E-3	0.012±8.367E-3	0.025±0.012	0.036±0.024	0.064±0.042
	19:00	20:00	21:00	22:00	23:00
CON	0.144±0.066	0.286±0.092	0.422±0.104	0.570±0.110	0.708±0.120
P7	0.138±0.070	0.243±0.105	0.417±0.156	0.558±0.191	0.665±0.181
P9	0.240±0.179	0.396±0.260	0.576±0.313	0.648±0.282	0.752±0.279
P11	0.080±0.048	0.156±0.100	0.300±0.142	0.390±0.165	0.492±0.162
P13	0.092±0.046	0.185±0.087	0.337±0.133	0.442±0.134	0.540±0.140
P15	0.128±0.066	0.218±0.102	0.330±0.115	0.470±0.154	0.606±0.163
	24:00	25:00	26:00	27:00	
CON	0.820±0.091	0.850±0.100	0.960±0.096	1.080±0.084	
P7	0.812±0.165	0.900±0.216	0.975±0.171	1.038±0.138	
P9	0.920±0.311	0.960±0.261	1.050±0.212	1.100±0.141	
P11	0.584±0.181	0.698±0.163	0.830±0.160	0.890±0.124	
P13	0.645±0.137	0.752±0.126	0.887±0.111	0.938±0.075	
P15	0.766±0.148	0.868±0.086	0.960±0.152	1.050±0.141	

on the pulse type($p < .0270$). In addition, the significances could be seen only between P9 and P11, and P13($p < .0297$, $p < .0389$), however there was no significant difference between the mean of control group and anyone of the other groups. It is suggested, therefore, that there is no biostimulation effect of LLLT on the *Candida albicans* in the case of irradiating LLLT during only the cell cycle in this study.

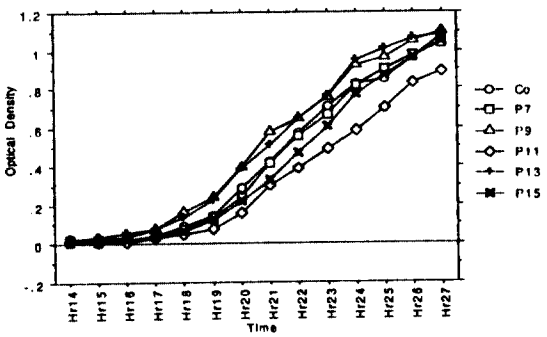

Figure 2. LLLT-activated growth of Candida albicans (as measured photospectrometrically by optical density of medium) as a factor of pulse type and elapsed time after irradiation, compared with unirradiated control medium

Table 2. Results of ANOVA test for optical densities of all groups measured according to the pulse type and elapsed time

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Pulse		1.448	.290	1.478	.2371
Subject(Group)	22	4.310	.196		
Category for Time	13	53.686	4.130	575.762	<.0001
Category for Time * Pulse	65	.678	.010	1.455	.0207
Category for Time * Subject···	286	2.051	7.173E-3		

Table 3. Results of multiple comparison test for the means of optical densities in all groups using Fisher's PLSD

	Mean Diff	Crit. Diff	P-Value	
Co, P7	6.839E-3	.165	.9321	
Co, P9	-.068	.155	.3767	
Co, P11	.106	.155	.1689	
Co, P13	-.068	.165	.4014	
Co, P15	.034	.155	.6499	
P7, P9	-.074	.165	.3590	
P7, P11	.100	.165	.2226	
P7, P13	-.075	.173	.3813	
P7, P15	.028	.165	.7314	
P9, P11	.174	.155	.0297	S
P9, P13	-3.929E-4	.165	.9961	
P9, P15	.102	.155	.1869	
P11, P13	-.174	.165	.0389	S
P11, P15	-.072	.155	.3463	
P13, P15	.102	.165	.2106	

s : 95% significant

Ⅳ. Discussion

This study was performed to investigate the effect of LLLT on the growth of *Cqandida albicans* using the pulse laser, P7, P9, P11, P13 and P15 as in Kim's previous study. Each laser was irradiated for 1 mimute with 2 hours of elapsed time during about 27 hours of cell cycle of *Candida albicans* confirmed by our previous pilot tests. In Kim's previous study[5], laser was irrdiated for 1 minute with 12 hours of elapsed time in the beginning of examination to 60 hours later. The reason that this study was performed for 27 hours different from the duration of previous study was to concentrate on the effect of LLLT during the cell cycle of *Candida albicans* .

Energy densities of P7, P9, P11, P13 and P15 used each session were 2.12, 6.37, 12.72, 29.68 and 57.32 mJ/cm^2 respectively and total energy fluences used in 14 sessions of this study were 29.68, 89.18, 178.08, 415.52, and 802.48 mJ/cm^2. Comparing these doses of LLLT to those of LLLT used in the previous study[5], the dose of each group irradiated for a session in this study was same as the dose used in the previous study, but the total energy fluence of each pulse laser irradiated in this study was larger than that in the previous study respectively because this study has more sessions than previous study.

It is believed that the energy densities of LLLT used for one session and even total energy for all sessions period in this study were included in the range of those of LLLT used in the other study[6 7 8]. This study, however, shows that there was no difference between the control group and one of the other groups. It is suggested, therefore, that frequent irradiation of LLLT on the cell culture has no effects or rather inhibitory effect than biostimulatory effect on the cell growth.

Singlet oxygen at low concentration can modulate biochemical processes taking place in the cell[9]. It was, therefore, suggested that singlet oxygen is a significant biochemical intermediate which may have an important role in biostimulation. At large amounts, on the other hands, singlet oxygen rapidly oxidizes a large variety of biological molecules,[10] damages DNA and is responsible for cell destruction. Lubart et al.[11]. suggested that singlet oxygen can be photoproduced by the natural porphyrins in the cell. Porphyrin has an intense absorption in the violet region around 440nm and four additional absorption bands with decreasing intensity between 500 and 630nm. In this study, however, wavelength of laser used was 904nm, which was not in the violet region. To establish above hypothesis for the effect of frequent irradiation, therefore, the biostimulation effect of LLLT with 904nm of wavelength on the porphyrin should be preceded.

Laser irradiation is also assumed to intensify the formation of a transmembrane electrochemical proton gradient in mitochondria.[12] Thus, at low doses, the additional Ca^{2+} transported into the cytoplasm triggers mitosis and enhances cell proliferation. At higher laser doses too much Ca^{2+} is released, which completely exhausts the cell energy and the intracellular osmotic pressure explodes the cell. Based on these results and previous hypothesis, it is conceivable that frequent irradiation with short interval has rather inhibitory effects on the cell culture, although LLLT is irradiated at low doses.

In Karu's interesting paper, it is suggested that the magnitude of the laser biostimulation effect depends on the physiological state of the cell at the moment of irradiation, therefore if a cell is fully functional, there is nothing for laser irradiation to stimulate, and no therapeutic benefit will be observed. It is believed, in this study, that the cells were in good physiological condition for a short term of cell cycle. Thus, it is also conceivable that this explains why the effect is not detectable in this study, as well as the variability of the results reported in the literature.

To confirm these hypothese, further rese-arches should be performed to investigate the effects of LLLT on the cell culture under different intervals and physiologic conditions of the cell at moment of irradiation.

V. Summary and Conclusion

This study was performed to investigate the biostimulation effects of LLLT on the fungus, *Candida albicans*, for the short term of cell cycle. Samples were divided into 6 groups which were P7, P9, P11, P13, P15 and CO. All samples were irradiated for 1 mimute with 2 hours of elapsed time during about 27 hours of cell cycle of *Candida albicans*, and the optical density was assessed by spectrophotometry every 2 hours. It was found that there were no difference between the control group and any group of others irradiated with 2 hours of short interval.

As a result of this study and previous resear ches, it is assumed that frequent irradiation of LLLT on the cells has no biostimulation effect or rather inhibitory effect on the cell growth and proper interval of irradiation should be investigated for the acceleration of cell growth.

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Abstract

This study was performed to investigate the biostimulation effects of low level laser therapy (LLLT) on the fungus, *Candida albicans*, during the short term of cell cycle. Samples were divided into 6 groups which were P7, P9, P11, P15, CW and CO. All samples were irradiated for 1 minute with 2 hours of elapsed time during about 27 hours of the cell cycle of *Candida albicans*, and the optical density was assessed by spectrophotometry every 2 hours. It was found that there was no difference between the control and any other groups irradiated with 2 hours of short interval.