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

PART I : A LONG TERM STUDY ACCORDING TO PULSE TYPE

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CONTENTS

I. Introduction

The effect of laser irradiation on the various conditions has been investigated to study the mechanism for the biostimulation of LLLT.[1,2,3,4,5] It is claimed that the low level laser therapy (LLLT) has a biostimulation effect on the proliferation of different cells.

Kim et al. [6] suggest that low level lasers may stimulate the protein and DNA synthesis to accelerate the proliferation of gingival fibroblast. Colin et al. [7] found that LLLT of various wavelengths has a biostimulatory effect on keratinocytes. Rigau et al. [8] reported that the number of fibroblasts following LLLT did not increase, but did reveal significant changes in metabolic rates compared with the unirradiated controls. Lubart et al.[9] reported that they irradiated fibroblasts with various lasers and found that at specific, relatively low energy dose there was acceleration of mitosis, while at higher doses the cells were destroyed.

Kim et al.[10] reported 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. [11] reported Gallium-arsenide(GaAs) laser irradiation at LLLT levels stimulated the increase of *Streptococcus mutans* growth and the rate of increase of bacteria did not coincide with that of laser fluence. They also suggested that LLLT had biostimulation effects for all kinds of cells.

The purpose of this study was to investigate preliminarily the effect of LLLT on the proliferation of *Candida albicans* and to establish the experimental method of the further researches on the fungal effects of LLLT.

II. Materials and Methods

A. Materials

1) Laser Apparatus.

The laser used in this work was the BIOLASER(Dong Yang Medical, Korea) using GaAs semiconductor as the diode. It is a pulsed infrared laser apparatus with a wavelength of 904nm and peak output power is 27 W.

Pulsed lasers used in this study were pulse 1 (5Hz, 1mW of average output power), pulse 7 (500Hz, 1mW of average output power), pulse 9(1500Hz, 3mW of average output power) and

pulse 15(10000Hz, 27mW of average output power). Quasi continuous laser(100000Hz, 15mW of average output power) also was used.

2) Micro-organism

Candida albicans (American Type Culture Collection #28366) used in this study.

3) 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 60nm. Reference liquid was identical brain heart broth media without cells.

B. Methods

1) Culture

Candida albicans was chosen for this study, because this microorganism is common seen as normal flora in oral cavity. This strain had been maintained on Sabouraud dextrose agar plate (Difco, USA) at 4℃.

2) Preparation of cell suspension

Candida albicans was diluted with Sabouraud dextrose broth, and cell concentration was adjusted to 4.0×10^3/ml using hemocytometer. Tissue culture dishes(Cel-Cult, England), of which diameter was 60 mm, were used for this study. All dishes were incubated at 37℃.

3) Laser irradiation

360 samples were used in this study. The samples were randomly divided in 6 groups according to the pulse type : quasi continuous type(CW), pulse 1(P1), pulse 7(P7), pulse 9 (P9), pulse 15(P15) and shame-irradiated control (Co) groups.

The author used the holder to fix the laser probe about 8cm apart from the surface of media(Figure 1). To measure this distance, the author examined the distance from the laser probe to the media that laser beam covered the media completely and accurately, using the laser beam detecter. All samples were irradiated for every 1 minute at 0, 12, 24, 36, 48, and 60 hours. Ten samples of each group were sacrificed at 0 and every 12 hours and then the optical density of all samples was measured with the spectrophotometer. Energy fluences of all experimental groups, P1, P7, P9, P15 and CW were 2.12, 2.12, 6.37, 57.32 and 31.85 mJ/cm^2 respectively.

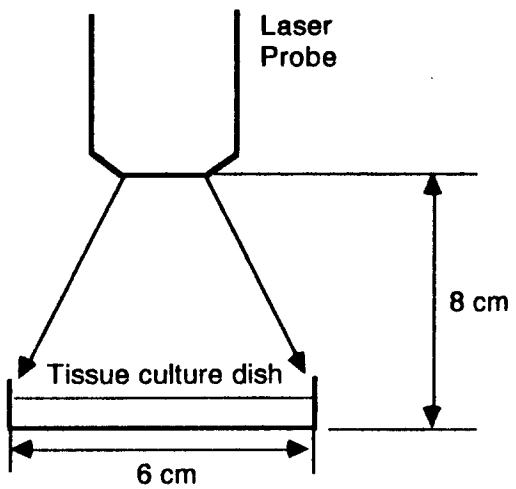

Figure 1. An illustration showing the application of the laser probe.

C. 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, Two-way ANOVA and Fisher's PLSD test were used.

Ⅲ. Results

The means of optical densities of all groups measured at 0 hour and after 12, 24, 36, 48 and 60 hours are given in Table 1, and the results of 2 way ANOVA tests according to pulse and time are shown in Table 2. From this ANOVA Table in Table 1, it is seen that pulse type has a strong influence on the cell growth, as indicated by the low p-value, ⟨.0001. The interaction of pulse type and time also seems to have a strong influence.

In the interaction line plot of Figure 3 it can be seen that the lines for the different pulse types are spread out over the range of optical density. This confirms that the pulse type has a significant main effect. Concentrating on the order of groups according to the size of mean in Table 1 and on the places in Figure 2 where the lines are not parallel, at 12 hours the optical density(OD) of CW group is higher than those of any other groups, but at 24 hours OD of P7 is and at 36, 48 and 60 hours OD of P7 and P9 are higher than those of any other groups. On the other hand, OD of CW group which bad been the highest at 12 hours decreased gradually with time. It is interesting to see that at 48 hours there were no differences among CW, P1, P15 and Control groups, but at 60 hours there were very significant differences among them and OD of control group was even higher than those of CW, P1 and P15 groups although OD of P7 and P9 were still higher than those of them(Figure 2).

Consequently P7 and P9 which the range of energy density is 2.12 to 6.37 mJ/cm^2 have the biostimulation effects on the proliferation of Candida albicans in this study.

Table. 1 Mean and standard deviations of the optical densities measured according to pulse type and elapsed time.

	12 Hr	24 Hr	36 Hr	48 Hr	60 Hr
Co	1.289 ± 0.135	2.629 ± 0.111	2.771 ± 0.221	2.900 ± 0.300	3.071 ± 0.399
CW	1.633 ± 0.206	2.689 ± 0.247	3.022 ± 0.139	2.767 ± 0.194	2.567 ± 0.206
P1	1.419 ± 0.095	2.820 ± 0.132	2.620 ± 0.169	2.910 ± 0.242	2.790 ± 0.202
P7	1.441 ± 0.068	2.860 ± 0.222	3.180 ± 0.079	3.310 ± 0.208	3.320 ± 0.063
P9	1.282 ± 0.089	2.670 ± 0.142	3.100 ± 0.094	3.220 ± 0.103	3.460 ± 0.097
P15	1.348 ± 0.083	2.820 ± 0.114	2.790 ± 0.110	2.820 ± 0.155	2.550 ± 0.242

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

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Pulse	5	4.305	.861	34.472	<.0001
Time	5	411.682	82.336	3296.141	<.0001
Pulse * Time	25	8.848	.354	14.168	<.0001
Residual	300	7.494	.025		

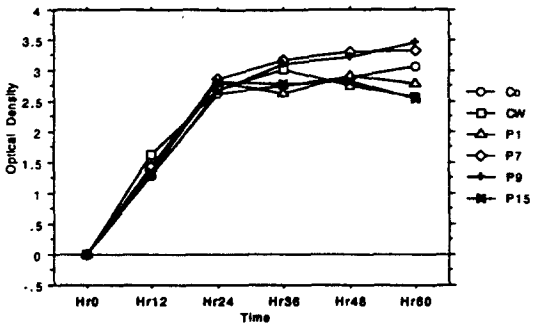

Figure 2. Linear graph showing the changes of growth of Candida albicans(as measured photospectrometrically by optical density of medium) as a factor of pulse type and elapsed time after irradiation, compared with unirradiated control medium

	12 Hr	24 Hr	36 Hr	48 Hr	60 Hr
Co : Cw	S		S		S
Co : P1	S	S	S		S
Co : P7	S	S	S	S	S
Co : P9			S	S	S
Co : P15		S			S
CW : P1	S		S		S
CW : P7	S	S	S	S	S
CW : P9	S			S	S
CW : P15	S		S		
P1 : P7			S	S	S
P1 : P9	S		S	S	S
P1 : P15			S		S
P7 : P9	S	S			
P7 : P15			S	S	S
P9 : P15			S	S	S

s: 99% significant

Tabel 3. Results of multiple comparison test (Fisher's PLSD) for all groups according to the time.

Ⅳ. Discussion

Candida albicans was used in this study because Kim et al.[11] suggested that LLLT has the biostimulation effect for all kinds of cells. This study was performed as preliminary test to detect the general effect of LLLT on the growth of fungus according to pulse type. For this preliminary investigation an irradiation time of 1 minute was chosen because it was within the range of exposure time, which had the stimulatory effect on the bacteria, determined by Kim et al.[11] and irradiating to all samples for 2 minutes respectively as in their study required too much time to perform this study. Consequently one minute of irradiation was enough to obtain the positive results in this study.

The duration of this experiment was 60 hours, the cell cycle of *Candida albicans*, however, was less than about 12 hours in this study using 60 mm diameter petri dishes. The authors should diluted the media of dish to measure OD after 12 hours. It seems that this dilution of media should have an effect on the results of this study. Therefore, further study according cell cycle of *Candida albicans* should be performed to support the results in this study.

Kim et al.[11] reported that the rate of increase of bacteria did not coincide with the increase of laser fluence, although LLL(GaAs) irradiation stimulated the increase of bacterial growth and the biostimulation of LLL irradiation for bacteria cells was most effective when the energy fluence was about 337.0 mJ/cm^2 in their study. They used Streptococcus mutans as subject and only quasi continuous laser in that study. In this study, 6 kinds of pulse were used and all samples were irradiated for 1 miuntes respectively, but the range of their energy fluences were 2.12 to 57.32 mJ/cm^2 because pulsed lasers of the laser apparatus used in this study have different average output powers. Effective pulses for the candida growth in this study are P7 and P9, and the range of their energy fluence is 2.12 to 6.37 mJ/cm^2

Comparing P1(5Hz) and P7(500Hz), both have the same energy fluence, 2.12 mJ/cm^2, OD of P7 after LLLT irradiation, however, was higher than that of Co but OD of P1 was rather lower than that of Co significantly. Young et al.[12] reported that the most effective frequency was 16 Hz to increase the growth rate of macrophage after LLLT(GaAlAs) irradiation. In

this study the effective frequencies to increase the growth rate of candida were 500 Hz and 1, 500 Hz. It is therefore, suggested that specific laser pulses are recommanded to have the biostimulation effects on the specific tissue or cells, although the biostimulation effect is dose dependant as in the previous study[11].

In addition, according to these results, it was assumed that LLLT does not always have biostimulation effects on cells, although the specific pulses with limited does of LLLT have the biostimulation effects, because some pulsed lasers(CW, P1, and P15) in this study rather have the inhibition effect. It will be very interesting for the clinician if some pulses of LLL with the inhibitory effects on the candida were confirmed to have the biostimulation effects on the normal tissue by further researches, because it will be very effective to treat fungal infection in the skin or mucosa using LLLT when this inhibitory effect LLLT on *Candida albicans* is applied clinically in the lesion infected with fungus. Kim et al.[5] suggested, in animal study on the effect of LLLT for the infected wound, that the acceleration of healing in the infected lesion following GaAs LLLT indicates that the cellular activity due to the biostimulation effect of LLLT in the surrounding normal tissue predominates over the destructive irritation due to the bacterial growth in the infected lesion. According to the results of this study, however, it is believed that the experiment to investigate whether the pulse used in their study had stimulatory or inhibitory effects on *Staphylococcus aureus* should be performed in advance to confirm their hypothesis.

Therefore further researches for all types of pulse, different irradiation times and elapsed time should be performed to investigate the most stimulatory or inhibitory pulse and the most effective energy density for LLLT using GaAs infrared laser.

V. Summary and Conclusion

This experiment was performed to confirm the hypothesis that LLLT had biostimulation effect for all kinds of cells and there would be differences in the growth of cells among different types of pulsed laser. *Candida albicans* was used. The samples were randomly divided in 6 groups according to the pulse type. Energy fluences of all experimental groups, P1, P7, P9, P15 and CW were 2.12 2.12, 6.37, 57.32 and 31. 85 mJ/cm^2 respectively.

All samples were irradiated for every 1 minute at 0, 12, 24, 36, 48, and 60 hours. Ten samples of each group were sacrificed at 0 and every 12 hours and then the optical density of all samples was measured with the spectrophotometer. As a result, some types of pulses showed significant differences among groups. The increase of cells were markedly stimulated with laser irradiation in P7 and P9 groups, while inhibited in CW, P1, and P15 groups compared with control group.

It is, therefore, suggested that specific laser pulse should be recommanded to have the biostimulation effects on the specific tissue or cells, although the biostimulation effect is dose dependant.

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Abstract

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All samples were irradiated for every 1 minute at 0, 12, 24, 36, 48 and 60 hours. Ten samples of each group were sacrificed at 0 and every 12 hours and then the optical density of all samples was measured with the spectrophotometer. As a result, some types of pulses showed significant differences among groups. The increase of cells were markedly stimulated with laser irradiation in P7 and P9 groups, while inhibited in CW, P1, and P15 groups compared with control group. It is therefore, suggested that specific laser pulse should be recommanded to have the biostimulation effects on the specific tissue or cells, although the biostimulation effect is dose dependant.