

Healing Effect on Infected Wound by Two Different Wavelengths and Output Energies of GaAs Semiconductor Diode Lasers : A Comparative Study

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1. INTRODUCTION

There are two principle ways in which photon energy is used in medicine, i.e. average heating used for tissue destruction (surgical methods such as coagulation, ablation, etc) and photochemical conversion of the energy absorbed by the photoreceptor (methods like photodynamic therapy (PDT), low level laser therapy (LLLT), etc).¹⁾

Over the past two decades, low level laser (LLL) has become widely used for the treatment of a variety conditions, including wound healing^{2,3)}, healing of aphthous ulcers⁴⁾, inhibition of plaque formation on teeth⁵⁾, enhanced healing of dental extraction sockets⁶⁾, recovery from nerve injuries⁷⁾, the reduction of edema, the relief of pain of various etiologies⁸⁾ and its use in painful temporomandibular disorders and other fibromyalgic conditions^{9,10)}.

Many studies demonstrated that LLL irradiation (LLLI) involves biostimulation effects on wound healing in cellular and molecular levels, including enhanced cell proliferation¹⁾, stimulation of mitochondrial activity^{11,12)}, enhanced adenosine triphosphate (ATP) production^{11,13)}, stimulation of

DNA and RNA synthesis¹⁰⁾, increased protein production^{11,14)}, modulation of enzymatic activity^{11,15)}, variation of intracellular and extracellular pH^{11,16)}, stimulated cell growth¹⁶⁾, increased revascularization¹⁶⁾, increased tensile strength^{19,20)} and acceleration of cellular metabolism^{19,20)}.

Of variable LLL systems, HeNe laser and GaAs laser have been utilized commonly. Sima et al.²²⁾ studied the effects of 780 nm GaAlAs diode laser for wound healing and suggested that it promoted wound healing by enhancing proliferation of fibroblasts and keratinocytes.

In a study using 800 nm diode laser, Katsuni and Teshio²³⁾ demonstrated healing effect for a diachronic wound in the rat model, possibly resulting from increasing the levels of IL-6 in the healing tissue and inducing an earlier proliferative phase of wound healing. Lee et al.²⁴⁾ reported the increased wound healing rate of infected skin wounds in rats and the decreased incidence of swelling by using 904 nm GaAs laser irradiation.

Another animal and human study by Simunovic et al.²⁵⁾ demonstrated that LLLI (632.8 nm HeNe combined with 904 nm diode laser) on postoperative

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wounds of soft tissue injuries promoted wound healing and relief of pain with subsequently restored functional ability. In addition, Kim's study²⁰ on extraction wounds in human exhibited that 904 nm LLLI led to relief of pain and decreased use of analgesics compared to non-irradiated controls.

On the basis of many researches on healing effect of LLLI, it is generally accepted that LLLI accelerates wound healing process and that its effects on cells are wavelength- and dose-dependent. The existence of a "window-specificity" at certain wavelengths and energy dosages has been postulated. Therefore, it is still important to find optimal irradiation parameters including wavelengths and output energy for variable clinical situations²⁰.

The aims of this study were to evaluate the effect of LLLI on healing process of infected wounds, and to compare the difference in effect of wound healing between two types of GaAs diode lasers with different wavelength and output energy.

II. MATERIALS AND METHODS

1. Laser Apparatus

800 nm GaAs diode lasers (L-Dr890, Pros International Co., LTD, Korea) was compared to 904 nm GaAs diode lasers (Dens BIO-LASER, Dong Yang Medical, Korea), as a control laser system in this study, which has already been proved to be effective in the treatment of wound(Fig. 1,2) 890 nm lasers used in this study has an average output power of 2 mW and pulse frequency of 2000 Hz, whereas 904 nm lasers has peak output power of 27 W and its irradiation for this study was carried out within a combination of pulse frequency of 6000 Hz and average output energy of 14 mW.

2. Subjects and Methods

26 adult Sprague-Dawley male rats (300 g) used



Fig. 1. 904 nm GaAs diode lasers (Dens BIO-LASER, Dong Yang Medical, Korea)



Fig. 2. 890 nm GaAs diode lasers (L-Dr890, Pros International Co., LTD, Korea)

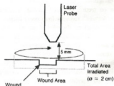


Fig. 3. An illustration showing the application of the laser probe

in this study were anesthetized with Ketamine® (Ketamine HCl, 50 mg/ml). Identical wounds were punched out bilaterally in the gluteal regions of the rats by using a nasal cutting forcep after shaving the skin over the target region and disinfected with 75% ethanol. Each wound consisted of the dermal, epithelial and fascial layers.

Staphylococcus aureus employed in this study were cultured on BHI slant and suspended with 1 ml of BHI broth, and then 10 μ l of bacterial suspension was inoculated on the wounds, respectively.

The rats were randomly divided into two groups with 13 rats in each. In one group, one wound in each animal subject was irradiated by 890 nm laser and the other wound was sham-irradiated as a control. In the other group, one wound of each animal was irradiated by 890 nm laser and the other by 904 nm laser (Fig. 3). Each irradiation was done for 2 minutes daily during the experiment period of 9 days and it was photographed on days of 1, 3, 5, 7 and 9, while keeping a constant distance of subject-lens of camera to obtain the images of the wounds under the identical condition.

All images of the wounds taken during the experimental period were projected through a reflector projector and the wound areas were traced and then measured by a planimeter (Kouffel and Esser Co, Germany). All the measurements were performed twice and the mean values were obtained. In each wound, the wound areas on consecutive days were expressed as a percentage of their initial area before irradiation.

3. Statistical analysis

Repeated measures ANOVA was used to determine the significance of differences among the results according to time elapsed and LLLJ groups with different output energy and different wavelength. Paired t-tests were also employed to compare the difference of healing rate between 890 nm LLLJ group and controls and between 890 nm LLLJ and 904 nm LLLJ groups at the given time.

III. RESULTS

Table 1 shows the results of ANOVA test for the wound healing rate in rat models between 890 nm LLLJ group and sham-irradiated controls. Laser irradiation and time affected wound healing rate ($p=0.005$ and $p<0.001$, respectively) but there's no interaction between group and time. When the wound healing rate in 890 nm LLLJ group during experimental period were compared to those in the sham-irradiated group, the means and standard deviations of the wound areas (%) at each time point were given in Table 2 and Fig. 4. The wound areas in 890 nm LLLJ group were decreased more rapidly than control group ($p=0.005$), but paired t-test, at a given time, showed that significant difference between two groups existed only on 9th day.

The differences related to two irradiated groups (890 nm and 904 nm LLLJ) and time elapsed were analyzed by ANOVA and the results were given in Table 3. Types of laser irradiation (with different wavelength and different output energy), as well as

Table 1. The results of repeated measures ANOVA for the wound healing rate between 890 nm low level laser irradiation and sham-irradiated control groups.

	Square sum of type III	df	Average square	F	p-value
Group	2005.984	1	2005.984	8.104	0.005
Time	99470.040	4	16328.340	46.091	<0.001
Group*Time	235.169	4	125.861	0.352	0.968

Table 2. Comparison of the wound healing rate related to time elapsed between 890 nm low level laser irradiation (LLLI) and sham-irradiated control groups. (%)

	TIME (day)					p-value (ANOVA)
	1st	3rd	5th	7th	9th	
890nm LLLI	100.00	51.46±15.82	43.01±17.81	38.30±17.37	17.15±18.93	0.005
control	100.00	66.20±27.06	55.15±23.83	40.98±25.14	34.61±26.68	
p-value (paired t-test)	-	0.399	0.038	0.302	0.030	

Table 3. Results of repeated ANOVA test for the wound healing rate between 904 nm and 890 nm low level laser irradiation groups.

	Square sum of type III	df	Average square	F	p-value
Group	2727.848	1	2727.848	5.242	0.034
Time	130564.6	4	32641.146	62.723	<0.001
Group* Time	4804.869	4	1201.167	2.307	0.082

Table 4. Comparison of the wound healing rate related to time elapsed between 904nm and 890 nm low level laser irradiation(LLLI) groups(%)

	TIME (day)					p-value (ANOVA)
	1	3	5	7	9	
904 nm LLLI	100.00	81.43 ± 21.07	74.99 ± 25.28	21.52 ± 29.94	15.26 ± 30.47	0.034
890 nm LLLI	100.00	57.55 ± 25.76	53.59 ± 20.81	38.22 ± 27.51	12.34 ± 18.42	
p-value (paired t-test)	-	0.048	0.023	0.362	0.674	

time, affected wound healing rate ($p=0.034$ and $p<0.001$, respectively). However, there was no interaction between group and time.

Table 4 and Fig 5 exhibit the comparison of the wound healing rate between 890 nm and 904 nm LLLI groups at a given time. Significant differences between two groups existed on 3rd and 5th days but there were no significant differences on 7th and 9th days.

IV. DISCUSSION

Many researches on medical lasers have concerned wavelengths both in the visible region (340-700 nm) and the infrared region (700 nm-1000 nm) of the electromagnetic spectrum. It is accepted that the biological effect of low level visible light therapy is through photochemistry (probably the photoactivation of enzymes) while the biological

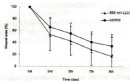


Fig. 4. Comparison of the wound healing rate related to time elapsed between 890 nm low level laser irradiation (LLLI) and sham-irradiated control groups. * stands for $p < 0.05$.

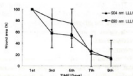


Fig. 5. Comparison of the wound healing rate related to time elapsed between 904 nm and 890 nm low level laser irradiation (LLLI) groups. * stands for $p < 0.05$.

effect of IR radiation is through molecular rotations and vibrations³⁰. However, laser therapy with the two wavelength regions produces similar clinical results, in spite of great difference in their photochemical and photophysiological properties³⁰. For example, Abergel and coworkers^{31,32} found that the irradiation of fibroblasts in culture either at 632 nm or at 904 nm stimulated the synthesis of collagen. To lead to photoreponse, irradiation by using wavelengths in IR region initiated the response at the membrane level (probably through photophysical effects on Ca^{2+} channels) at about halfway through the total cascade of molecular events leading to biostimulation³⁰. In addition, it is proposed that the magnitude of the laser biostimulation effect depends on the physiologic state of the cell at the moment of irradiation, which means that the cells with a lowered internal pH, pHi respond more strongly than the cells with the normal pHi value. Therefore, it is suggested that such pathologic conditions as chronic inflammation and indolent wounds respond to irradiation because of their lowered pHi value and hypoxia³³.

This can explain beneficial effect of LLLI on wound healing in a number of studies³⁴⁻³⁷ including this study that compared the healing effect on open skin wounds infected with *S. aureus* by 890 nm

diode laser at 2000 Hz and 2 mW to sham-irradiated controls. The wound healing rate relative to time elapsed was accelerated by 890 nm LLLI as compared to sham-irradiated controls in this study ($p < 0.05$). However, it is noteworthy that irradiation affect not only wound healing but also growth of *S. aureus*. Several *in vitro* studies demonstrated that 904 nm and 890 nm lasers stimulated bacterial growth of *Streptococcus aureus*³⁸, *Candida albicans*³⁹⁻⁴¹, *S. aureus*⁴² and *Escherichia coli*⁴³ in a dose-dependent fashion.

Since infection of tissue is one of the most common factors that interfere with wound healing, stimulated bacterial growth by LLLI would compromise healing process. Nonetheless, there existed the positive effect on wound healing by irradiation in this study and another study by Lee et al.²⁰, which probably suggests that laser stimulation of the host tissue predominated over stimulation of bacterial growth. In addition, it might be another explanation for the accelerated healing process that irradiation effect on cellular level *in vitro* differs from that in living body. In other words, the dose of LLLI leading to enhanced growth of microorganism *in vitro* situation may offset its growth potential *in vivo* situation, possibly due to the photoinactivation of living body's natural bacteriocidal armamentarium⁴⁴.

There still existed controversy regarding the effect of lasers in wound healing, some of which stem from the different types of lasers used and a wide variety of irradiation measurements. In this study, we employed 890 nm LLL with average output energy of 2 mW to compare with healing effect by 904 nm LLL at 14 mW. Overall ANOVA showed that there was significant difference of wound healing effect between both of them and, in addition, irradiation effect at 890 nm was greater than that at 904 nm LLL in the early stage of the total experiment period. Although it is unclear what mechanism contributes to this result, it unlikely comes from the difference of wavelength between 890 nm and 904 nm and our attention was paid to the difference of power.

Previous studies have shown that higher energy of irradiation was less effective, possibly due to damage to various light receptors, formation of cytotoxic products and stimulated release of fibroblast-inhibitory growth factors.^{41,42} In addition, Osanai et al.⁴⁰ exhibited that the shorter time and the weaker output power was given in irradiation field, the stronger phagocytic and chemotactic action of neutrophils was induced. These findings indicate that lower energy of irradiation would be favorable for biostimulation. However, it is necessary to remind that the researches reporting inhibitory effect of irradiation had mostly done by using high energy density of more than 1 J/cm^2 and *in vitro* trials.^{40,42}

Although this study encompassed 890 nm and 904 nm with low energy density of less than 1 J/cm^2 , there existed difference of output energies between both of them. It is generally accepted that light with a weak power penetrates only a limited depth. Therefore, 890 nm LLL with a weak power might be advantageous to be absorbed into superficial wound artificially made in this study, which can, at least in part, contribute to the enhanced healing effect by irradiation at 890 nm observed at the early stage of healing process.

V. CONCLUSIONS

On the basis of above results, 890 nm LLL with an average output energy of 2 mW exhibited accelerated healing effect on infected wound in rats as compared to sham-irradiation and 904 nm with an average output power of 14 mW. In addition, the wound healing effect by 890 nm LLL was greater than that by 904 nm LLL in the early stage of healing process. Therefore, it is suggested that LLL is effective on the infected wound healing and, in addition, the weak power of 890 nm LLL is more effective in a superficial wound healing than the stronger power of 904 nm irradiation.

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