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Research Article

Effects of 980nm Diode Laser Irradiation on Gingival Fibroblasts and Periodontal Ligament Stem Cells

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Abstract

Purpose: Diode lasers with a 980 nm wavelengths have become popular in oral surgeries. Their cellular phototherapy effects are a topic needing further investigations. The aim of this study was to examine the effects of this wavelength on human periodontal ligament stem cells (PDLSCs) and Human gingival fibroblasts (HGF), which play an important role in periodontal tissue regeneration and oral wound healing.

Methods: HGF cell line was purchased from the Pastour National cell bank of Iran (NCBI: C-165), PDLSCs were isolated from adult human third molars and their Mesanchymal nature was confirmed by flow cytometric evaluation of CD90,CD105 and CD45 cell surface markers. Cells were irradiated with Fulences (Energy densities) of 0.5, 1.5, 2.5 J/cm2 with a 200 mw output power of a continuous wave 980 nm diode laser to determine the effect of a single session of laser therapy on the viability and proliferation of these two cell types after 24 and 48 hrs. A non irradiated group of each cell was considered as control.

Results: The PDLsc cells were positive for CD 90 and 105 and negative for CD 45. Laser radiation had a positive stimulatory effect on the viability of both cell types within 24 hrs, although only the 0.5 J/cm2 HGF group shad ignificantly higher MTT activity after 24 h (P=0.017). This trend did not go on 48 h later and a reduction in the proliferation of both cell types compared to control groups was observed.

Conclusion: A single session of 980 nm laser application with the settings used in this study did not have a prolonged positive effect of HGF and PDLsc viability and proliferations and repeated sessions of irradiation seems to be necessary.

Keywords: Laser; Cell; Photobiomodulation Therapy; Periodontal Ligament

Introduction

Phototherapy or use of Lasers with Low intensity irradiation has been shown to effect the activity of cells in the tissue without modifying the living tissue's structure. These effects are not mediated through thermal induction but, rather, through a process named as photobiostimulation [1]. The first medical application of phototherapy can date back to about a century ago by Niels Finsen for the treatment of dermatological diseases [2]. Ever since Low-intensity laser therapy has been performed in many clinical conditions in order to accelerate the process of tissue regeneration, with its stimulatory effects on the proliferation of different types of cells [3]. It is also used to create a range of beneficial therapeutic effects on wound healing and pain relief in various fields of medicine, including applications in dentistry [4,5].

On the other hand, regenerative dentistry has stepped into a new era with the discovery of dental stem cells. These cells have MSC-like characteristics and self-renewal, multi differentiation potential [6]. Periodontal ligament stem cells (PDLSCs) are a type of mesenchymal stem cell with a high potential for differentiation into

periodontal structures and play an essential role in periodontal tissue regeneration [3,7]. Fibroblast cells are also the most important cells in the normal repair process of the skin and mucosal wounds. The effects of laser irradiation on fibroblast proliferation *in vitro* are not very well established yet and further investigations on this process are still needed.

Low-intensity or low level laser therapy, with its ability to accelerate the recovery process less invasively, has attracted a lot of attention in medicine and dentistry in recent years leading to many laboratory and clinical studies. Positive effects of the laser may be due to increase of collagen production, activity of enzymes, mitochondrial activity, DNA synthesis and cell proliferation, anti-inflammatory effect and pain relief [5,8,9]. The tissue repair process depends on factors such as high blood supply, cell proliferation and transition in the affected area following local release of growth factors and cytokines [10]. Proper enhancement and bio-stimulation of cells can be a promising adjunctive treatment in oral tissue regeneration and healing. The effect of laser as a cell stimulating factor depends on parameters such as wavelength, energy and power density, exposure time [11,12].

Most previous studies on wound healing and effect of laser therapy have focused on skin fibroblasts and there are a few studies on the oral mucosa and gingival fibroblasts and PDL stem cells [13,14]. Further investigations still seem to be necessary in order to pave the way for correct clinical applications.

Diode lasers with wavelengths in the red and near infrared part of the electromagnetic spectrum. Because of their price and device size they are becoming popular in routine dental practice. Most previous studies on cell bio-stimulation with lasers has focused on red light sources and there are less studies on near infra red and 980 nm lasers [3]. Since 980 nm lasers are useful tools in oral surgeries due to their thermal effects and good absorption in haemoglobin and melanin, we decided to examine the effect of low power/intensity effect of this wavelength with the same device used for oral surgeries with different energy densities on the proliferation and survival of gingival fibroblast cellsand periodontal ligament stem cells.

Materials and Methods

In this *in vitro* study, Human gingival fibroblasts were purchased from the Pasteur Institute's cell bank of Iran National cell bank of Iran (NCBI: C-165). This cell category was cultured in αMEM medium containing 10% FBS in sterile flasks. Their culture medium was replaced every 2-3 days and after one week they were passaged. The third passage cells were irradiated 12 h after they were cultured into 96 well plates with 5×10^3 cells/well.

Periodontal Ligament stem cells were obtained from the mid root periodontal ligament tissue of healthy third molar teeth of a patient extracted for orthodontic reasons. This study was approved by Zahedan University of Medical Sciences Ethics committee (IR.ZUMS.94.5.11-7348) and the patient was informed about the study and cell isolation and signed a consent form prior to this procedure. After polishing the tooth prior to extraction the roots were immediately sectioned with a sterilized rotating metal disk and dropped into a tube containing. The mid rood periodontal ligament tissue was scraped using a scalpel and the stem cells were isolated by a method previously described [7]. The cells were investigated for Mesenchimal stem cell surface markers by flow cytometry (Becton Dickinson, Franklin Lakes, NJ, USA).

Laser irradiation

A 980 nm diode laser (Velure S9, Lasering s.r.l, Italy) was used with a 200 mw power setting and continuous wave (CW) mode 600 micron fiber. Cells were cultured in 98 wells with an empty well in between. They were irradiated in a semidark room from underneath the dishes for 2.5,7.5,12.5 sec respectively. The beam was kept perpendicular to each well completely covering the area of a single well (0.8 cm diameter) to result in fluences (Energy densities) of 0.5, 1.5, 2.5 J/cm^2. The exact output power and the power penetrating the culture dish was checked with a power meter (Ophir Nova) before irradiation.

Cell viability

Evaluation of cell toxicity and survival of cells after radiation of different laser energy densities was evaluated after 24 hrs using MTT assay.

In this test the cells were placed in an incubator for 24 hrs. Then, 10 ml of MTT solution and 90 ml of αMEM containing 10% FBS were added to each well and placed in incubator for 3-4 hrs at 37˚C. At this stage, Formazan crystals were visible by inverted microscopy in living cells. After dissolving formazan crystals by adding dimethyl sulfoxide, light absorption was read by the ELISA reader (BioTek EL x 808 microplate reader, Germany) at a wavelength of 540-690 nm. The results were reported as percentages.

Cell proliferation

In order to investigate the cell proliferation, 5000 cells were plated in 96 well plates 24 and 48 h after laser radiation the samples were stained with Trypan blue and counted under an optical microscope in a Neubauer chamber and the ratio of their increase was reported numerically in comparison with the beginning of the study.

Statistical analysis

Values are expressed as mean ± SD. Data were analyzed using Student's t-test and two-way ANOVA with Tukey's post-hoc test. The density and time were considered as independent variables. All statistical analyses were performed at a significance level of 0.05 using R software, version 3.3.3 (An open source programming environment for data analysis) and SPSS. version 16.

Results

Stem cell surface markers

Flow cytometric evaluation of the PDL stem cells showed that the extracted cells were positive for CD105 and CD90 and negative for CD45 indicating their mesenchymal nature (Figure 1).

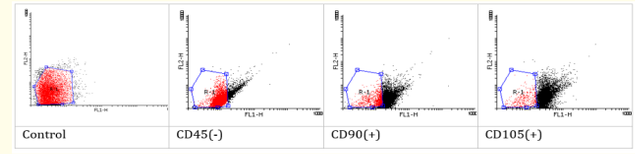

Figure 1: Flowcytometric analysis results for cell surface biomarkes of CD105, CD90, CD45 on the extracted stem cells. Black dots indicate positive biomarker expression and red dots indicate negative expression of the biomarkers.

PDLsc and Gingival fibroblast cell viability

In the HGF group there was a higher mean percentage of viability after 24 hrs in 2.5J/cm^2 and 0.5J/cm^2 irradiations. The PDLsc showed an increased viability percentage after 24 hrs in the 2.5j/cm^2 irradiated group.

Two way ANOVA showed no statistically significant effect of Energy density and time on PDLsc viability results (P>0.05). Also, in the HGFcell group, the results of this test showed that only time has a significant effect (P<0.05). Student test was used to compare the effect of different energy densities with control. There was only a statistically meaningful increase in the 0.5j/cm^2 irradiated HGF cell group compared to control, P=0.017 (Table 1).

			Energy density			P-value		
	Time	Control	0.5	1.5	2.5	C[a].0.5	C.1.5	C.2.5
PDLsc	0	100	100	100	100	-	-	-
	24	100	101.60 ± 21.49	104.10 ± 15.92	122.73 ± 15.92	0.875	0.595	0.065
HGF	0	100	100	100	100	-	-	-
	24	100	128.01 ± 15.99	110 ± 22.47	124.94 ± 26.24	*0.017	0.291	0.101

Table 1: Cell viability results compared with control 24 hours after laser irradiation with different energy densities.

[a] control, * Significant.

PDLsc and Gingival fibroblast cell Proliferation

An increase in proliferation was observed in all groups after 24 hrs. The highest mean amount of proliferation was observed in the 2.5J/cm^2 irradiated groups after 24 hrs in both cell types. In the PDLsc group this increase was statistically significant (P=.0001). However, we could see a reduction in proliferation after 48 hrs in all groups. Also when compared with the control using an Student t-test, the increase in proliferation was only statistically significant in the PDLsc group irradiated with an energy density of 2.5j/cm^2 at 24 hrs (p=0.005). No positive effect was found in the HGF groups (Table 2).

			Energy Density			P value		
	Time	Control	0.5	1.5	2.5	C[a].0.5	C.1.5	C.2.5
PDLsc	0	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	-	-	-
	24	1.09 ± 0.08	1.21 ± 0.18	1.17 ± 0.12	1.37 ± 0.16	0.211	0.293	*0.005
	48	1.59 ± 0.24	0.82 ± 0.11	0.95 ± 0.08	1.02 ± 0.12	*0.001	*<0.001	*0.001
HGF	0	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	-	-	-
	24	1.65 ± 0.24	1.55 ± 0.19	1.46 ± 0.24	1.62 ± 0.25	0.749	0.935	0.596
	48	2.36 ± 0.12	1.18 ± 0.04	1.04 ± 0.15	1.16 ± 0.21	*<0.001	*<0.001	*<0.001

Table 2: Results of cell proliferation 24 and 48hours after laser irradiation with different energy densities compared with control non irradiated cells.

Discussion

In the present study, we investigated the effect of a single session of low-power laser application of 980 nm laser with three densities of 0.5, 1.5 and 2.5 with an output power of 200 mw on proliferation and survival of stem cells derived from periodontal ligaments and gingival fibroblasts. According to the results, laser radiation had a positive stimulatory effect on the proliferation and viability of both cell types within 24 hrs, however, only the effect of 2.5 J/cm^2 and in PDSC group was statistically significant. This trend did not go on and after 48 hrs, a reduction in proliferation of both cell types compared to control groups was observed.

There are a few studies evaluating cellular effects of this wavelength in the literature and in most of them the laser parameters are differed from the present study.

In a study by Wang., *et al*. [4] a 980 nm and 810 nm wave length was studied on adipose stem cells. However they used a lower output power compared to us, according to their results both wavelengths of 810 and 980 nm with densities of 0.01-0.33 Juleson the proliferation and differentiation of stem cells derived from adipose tissue showed a bi-phasic response. Lower energy doses in both wavelengths lead to stimulation, but higher doses had inhibitory effects. They reported the most stimulatory effect was related to the wavelength of 980 nm with a density of 0.3 J/cm^2. This was similar to the increase we found in HGF viability after 24h with 0.5j/cm^2 (P=0.017). However, the wavelength of 810 nm had the highest effect at a higher energy density of 3 J/cm^2. This study had some other differences with ours in which laser irradiation was repeated five times and a pulse mode of irradiation was used.

The use of lasers in a continuous or pulsed mode may result indifferent outcomes in wound healing and tissue regeneration there for we should keep this in mind an important parameter when comparing different studies [15].

Crisan., *et al*. [11]. investigated the effects of two low-power diode lasers with two wavelengths of 830 and 980 nm, and an Er, YAG laser with a wavelength of 2,940 nm on the survival of human skin fibroblasts. Similar with the present study, they also evaluated irradiation was given as a single dose. However a pulse mode and with an energy density of 5.5 J/cm was applied in their study. According to their findings both two diode laser wavelengths had a stimulatory effect on human skin fibroblasts cell but he 2,940-nm wavelength irradiation resulted in apoptosis of the cells.

In Caccianiga`s study [9] a positive stimulatory effect was also observed, however unlike other studies they used a much higher energy density of 50 Jules and 5 times repetition of irradiation in order to evaluate the effect of a 980 nm diode laser on increasing the formation of the keratinization layer and found an increase in keratin synthesis. In this study, they stated that despite the high energy, they tried to manage the cell survival by preventing apoptosis in all specimens. For this, they used a special hand-piece, which results in optimal energy absorption by cell cultures that leads to inhibit the apoptotic effects. This hand-piece kept the energy characteristics unchanged at the distance of 0 to 100 cm, and imported a similar amount of energy at each point of an area of 1 cm^2. In fact, according to this hypothesis, they proceeded they used a strong and repeated stimulation to stimulate the cells to produce keratin.

Correct application of laser irradiation in clinical settings is also an important issue. The 980nm has become a popular adjunctive tool in dentistry and its low level therapy effects have been also of great interest. Ravi M., *et al.* [16] evaluated the 980 nm diode laser's effect on pain reduction and tissue response in vivo after periodontal surgery. Before tissue suture, a phototherapy with a density of 4 J/cm^2 was performed, in which the tissue response did not differ in the desired control and test group. This might be due to the fact that in clinical studies, because the radiation is done on tissue, instead of a cell layer, the amount of energy absorbed by the cells is different from in vitro and maybe a higher amount of energy density should have been tested.

Usumez also studied the effect of a 980 nm laser effect with a density of 8 J/cm^2 every day for four days, on the rate of mucositis recovery in an animal study. They found that the radiation had a stimulating effect on growth factor increase [17].

Previous studies using single session of laser irradiation have been conducted however the laser wave lengths and cell types used were different from this study they have also found appositive effect of a single session [3]. However it was clearly shown that laser therapy needs to be repeated and a single dose of the laser did not have a prolonged effect. Its seems to have a cumulative effect at each new dose applied as shown by Huang., *et al.* [18] and repetition of the irradiations my lead to different results.

A useful irradiation protocol has yet to be determined for application of 980 nm wavelength in bio-stimulation of oral tissues. Since cells do not similarly respond to laser radiation tissue and invitro culture medium and similar parameters will have different effects in two environments of "*in vivo*" and "*in vitro*" the need for more detailed clinical studies is also necessary to translate the in-vitro studied correctly to clinical use [17]. According to the results we found a single irradiation could only have a short time stimulatory effect. Repetition of irradiation and use of devices that can have much lower power out puts might result in different effects.

Conclusion

A single session of 980 nm laser application with the settings used in this study did not have a prolonged positive effect on HGF and PDLsc viability and proliferations and repeated sessions of irradiation seems to be necessary.

Conflict of Interest

This study was funded by Zahedan University of Medical Science's Oral and Dental research center. The authors declare that they do not have any kind of conflict of interest in this research.

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