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Research Article

Efficacy of Proliferation of HeLa Cells under Three Different Low-Intensity Red Lasers Irradiation

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This study was intended to compare the efficacy of proliferation of HeLa cells under three different low-intensity laser irradiation (LIL), that is, 633 nm, 658 nm, and 785 nm. The time-dependent responses of proliferation of HeLa cells after the red laser irradiation and the influence of fetal bovine serum (FBS) at 1%, 2%, 5%, or 10% on the proliferation of cells were also investigated. The results indicated that the proliferation of HeLa cells in 10% FBS was in proliferation-specific homeostasis (PSH) so that it was not modulated with LIL; the proliferation in FBS at 1%, 2%, or 5% was far from PSH so that it may be wavelength dependently modulated with LIL, and the maximum proliferation promotion was conducted with LIL at 633 nm amongst the three different LIL. It was concluded the wavelength-dependent photobiomodulation of LIL on proliferation of HeLa cells may be homeostatic.

1. Introduction

The interaction between low-intensity laser irradiation (LIL) and biological system or tissue has attracted much attention and motivated LIL therapy, an interdisciplinary branch of photomedicine in the past several decades, which involved the studies and applications of LIL in health care and disease treatment. This therapeutic approach has primarily been shown to be useful in the short-term treatment of acute pain caused by rheumatoid arthritis [1], osteoarthritis [2] and in the treatment of both acute and chronic neck pain [3], although it is still unclear how it works. Many kinds of photobiomodulation (PBM) may be involved when biological systems or cells are irradiated with LIL [4–6]. Amongst these effects, PBM on proliferation can happen to various types of cells, such as, fibroblasts, osteoblasts, lymphocytes, stem cells, endothelial cells, lung adenocarcinoma cancer cells, and HeLa cells. PBM on proliferation is the basis of LIL on wound healing, which has been explored and encouraged to be used as an alternative noninvasive method [7]. And researchers have made much progress for decades in the cellular and molecular mechanism of phototherapy or PBM [8–15], especially cellular responses to visible and near infrared radiation related to the mitogenic effects promoted by LIL, such as, absorption of light by mitochondrial enzymes [16], photon absorption by flavins and cytochromes in the mitochondrial respiratory chain affecting electron transfer [4], and photoactivation of calcium channels resulting in increased intracellular calcium concentration and cellular proliferation [17]. The mechanism underlying PBM is still elusive. In addition, not all of LIL studies supported the positive efficacy of PBM. For example, LIL from a gallium aluminum arsenide laser failed to increase proliferation, migration, or adhesion of cultured keratinocytes or fibroblasts [18]. LIL on wounds created in X-ray-treated skin failed to improve wound healing and inhibited healing with the increase of fluence [19]. These varied results prompt that many important factors, including laser parameters (e.g., wavelength, power density, fluence, or irradiation time) and cell types may influence significantly the stimulated effects.

Studies have found that red lights could modulate the maximum cell proliferation [16, 20]. However, few reports involved the comparison of cell proliferation modulated by different red lights in different environment conditions. This study was intended to investigate the efficacy of proliferation of HeLa cells in different concentrations of fetal bovine serum (FBS) under the irradiation of three red lasers

at 633 nm, 658 nm, and 785 nm, respectively. The time-dependent responses of proliferation of HeLa cells after LIL were also studied.

2. Materials and Methods

2.1. Cell Culture. The human cervical carcinoma HeLa cells were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin, in a humidified incubator with 5% CO_2 under 37°C condition. The cells were digested with 0.125% trypsin every three days and then subcultured into 100 mm culture dishes.

2.2. Lasers Irradiation. HeLa cells at 1.5×10^4 cells/mL were seeded in 96-well microplates using plain DMEM supplemented with 1%, 2%, 5%, and 10% FBS, respectively (total of nine plates in each group). The plates were maintained in the humidified incubator with 5% CO_2 and 95% air at 37°C for 24 hours. The three red lasers used in this study were 633 nm He-Ne laser (Melles Griot, USA), 658 nm diode laser (LQC. Newport, USA), and 785 nm diode laser (LQC. Newport, USA), respectively. The laser power density was 10 W/m^2 and its irradiation time was 100 s. After irradiation, the cells were returned to the incubator. The control groups without LIL were exposed to the same environmental and stress conditions, such as, temperature, humidity, and FBS concentrations. The cell viability of each group was tested at the time of 24, 48, and 72 h, respectively after LIL turning off. All of the measurements were carried out at least in triplicate. In addition, in order to determine a better LIL dose, four different irradiation fluences 100, 300, 600, and 1000 J/m^2 of 633 nm He-Ne laser irradiation were chosen. The laser power density was 10 W/m^2 and the irradiation time was 10, 30, 60, and 100 s, respectively. The cells were cultured in plain DMEM supplemented with 5% FBS. The other procedures were performed according to the above method.

2.3. MTT Assay for Cell Proliferation. MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to analyze the cell proliferation. It is a laboratory test and standard colorimetric assay for measuring cellular viability and proliferation. Each well of 96-well microplate was added 20 μL of MTT solution (5 mg/mL sterile PBS) and incubated in dark environment at 37°C for 4 hours. Then the cultured medium with the MTT solution was removed and 150 μL/well of dimethylsulfoxide (DMSO) was added to dissolve the formazan crystals. The absorbance was measured via a multimode reader fluorimeter (Mithras LB 940, Germany) and the excitation wavelength was 490 nm. The MTT absorbance value was proportional to the number of viable cells and the cell proliferation.

2.4. Statistical Analysis. Data was given in the format of mean ± standard error. Student's t-test method was used to evaluate the statistical difference. The statistical value $P < 0.05$ means significantly statistical difference and $P < 0.01$ means obviously statistical difference.

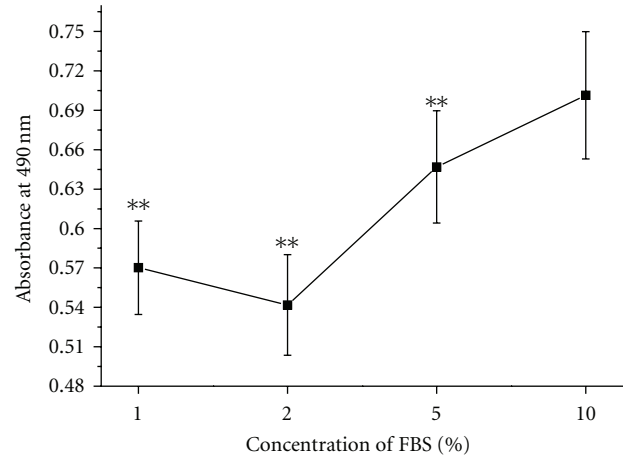

Figure 1: The cell proliferation of HeLa cells in different FBS concentrations without laser irradiation. Error bars were from three independent experiments. $^{**}P < 0.01$.

3. Results

The proliferation of cells varied with FBS concentrations. Figure 1 gave the relationship between HeLa cell viability and FBS concentrations (1%, 2%, 5%, and 10%). Herein, the cell viability was given in the format of absorbance. HeLa cells viability was nonlinearly dependent on FBS concentration. The lowest of cell viability was at 2% FBS. When the FBS concentration was more than 2%, cell viability increased with FBS concentration, and it reached the highest at 10% FBS. The cell growth in 1%, 2%, 5% FBS was significantly lower than that in 10% FBS, respectively ($P < 0.01$).

The proliferation response to laser irradiation fluence of 100, 300, 600, and 1000 J/m^2 was shown in Figure 2. The measurements were carried out at 24, 48, and 72 h after LIL. The cell viability was given in the ratio of the value of LIL group to that of the control group. As shown in Figure 2, the irradiation fluence influenced the proliferation response of HeLa cells. The cell viability increased with LIL fluence, and LIL group of 100, 300, 600 J/m^{2}did not differ statistically from that of 1000 J/m^2 ($P > 0.05$). The values of all the LIL groups were higher than the ones of their control groups, that is, the relative viability was higher than 1. Moreover, the proliferation of HeLa cells at 48 h was the most obvious one. Therefore, the fluence of 1000 J/m^2 was chosen in the following experiments.

The proliferation responses of HeLa cells in 1%, 2%, 5%, and 10% FBS to 633 nm, 658 nm, and 785 nm irradiation at 1000 J/m^2 were given in Figures 3, 4, and 5, respectively. The assessments were carried out at 48 h after laser irradiation. Control groups without LIL were also investigated at the same time. The proliferation difference in 5% FBS was significant between LIL groups of 633 nm ($P < 0.01$), 658 nm ($P < 0.05$), and 785 nm ($P < 0.01$) and their corresponding control groups, respectively. The proliferation difference in 1% FBS was also significant between control group and LIL group of 785 nm ($P < 0.01$). For the other concentrations of FBS, the LIL group did not differ statistically from

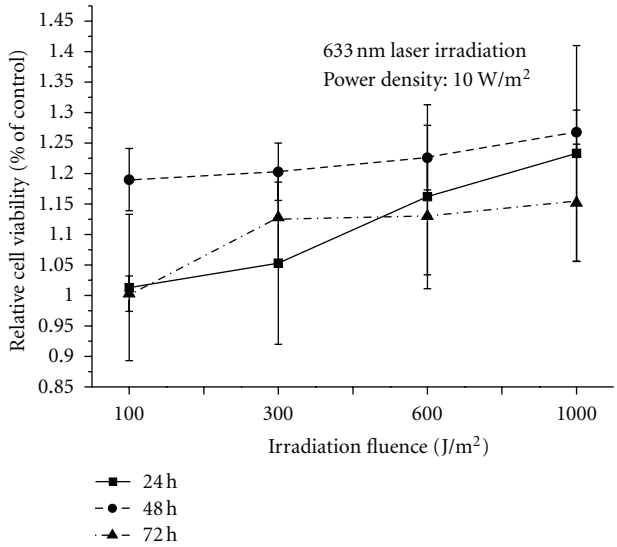

Figure 2: The viability of HeLa cells irradiated with LIL at 633 nm in 5% FBS. The laser power density was 10 W/m^2. Error bars were from three independent experiments.

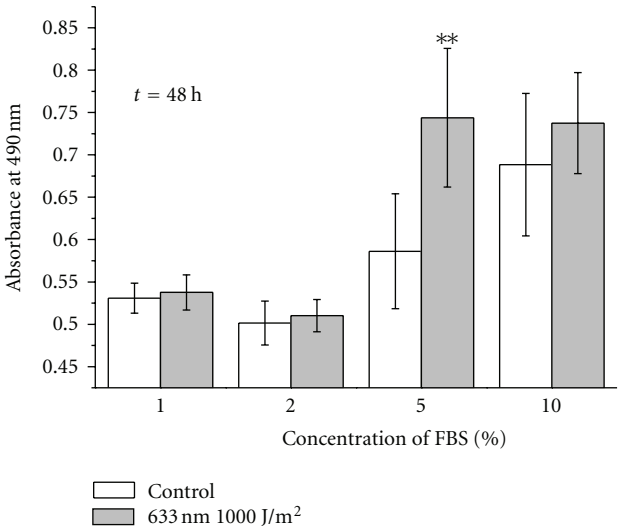

Figure 3: The proliferation of HeLa cells in different FBS concentrations (laser wavelength $\lambda = 633$ nm, energy density 1000 J/m^2, irradiation time 100 s). The absorbance value was assessed at 48 h after laser irradiation. Error bars were from three independent experiments, $^{**}P < 0.01$.

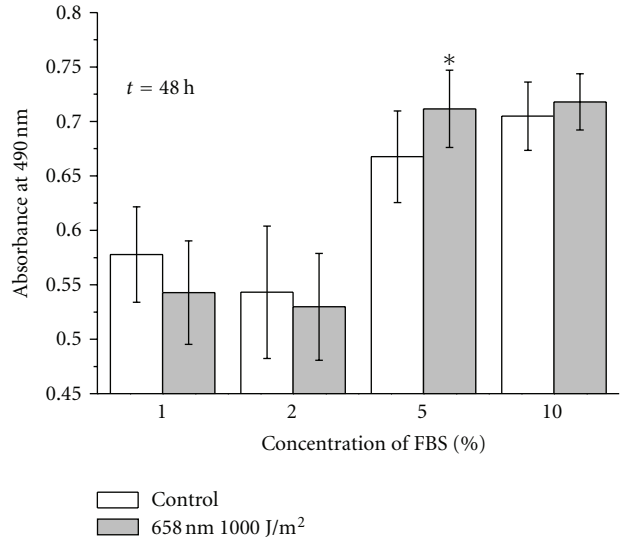

Figure 4: The proliferation of HeLa cells in different FBS concentrations (laser wavelength $\lambda = 658$ nm, energy density 1000 J/m^2, irradiation time 100 s). The absorbance value was assessed at 48 h after laser irradiation. Error bars were from three independent experiments, $^{*}P < 0.05$.

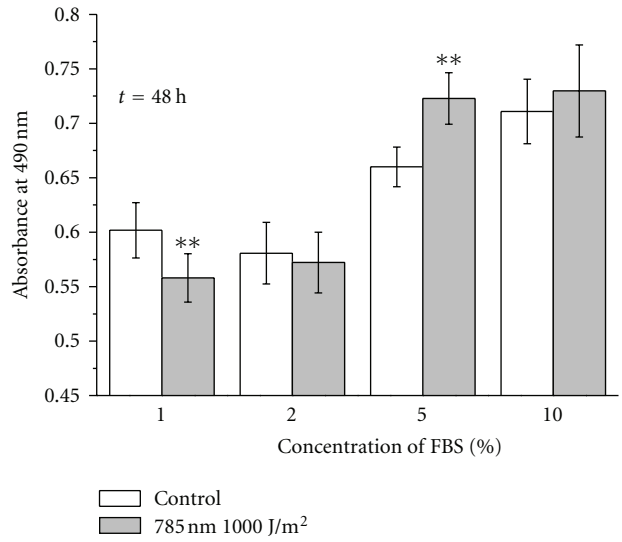

Figure 5: The proliferation of HeLa cells in different FBS concentrations (laser wavelength $\lambda = 785$ nm, energy density 1000 J/m^2, irradiation time 100 s). The absorbance value was assessed at 48 h after laser irradiation. Error bars were from three independent experiments, $^{**}P < 0.01$.

the respective control group ($P > 0.05$). Furthermore, for the control groups, the cell growth in 5% FBS was significantly lower than that in 10% FBS ($P < 0.01$). When the cells were irradiated with LIL, the cell growth in 5% FBS did not differ statistically from that in 10% FBS ($P > 0.05$). This means that LIL completely recovered the proliferation of cells in 5% FBS.

The time-dependent responses of proliferation of HeLa cells in 5% FBS after the three red laser irradiation at 633 nm, 658 nm, and 785 nm were compared in Table 1, in which the cell viability of control groups was 100%. The cell viability was assessed at 24 h, 48 h, and 72 h after lasers irradiation. Obviously, the proliferation of HeLa cells was wavelength-dependent, and the response of proliferation to LIL was obviously time-dependent. At 24 h, 48 h and 72 h after laser irradiation, the proliferation difference was significant between LIL groups of 633 nm and 658 nm ($P < 0.01$) or 785 nm ($P < 0.05$), but there was no significant difference between LIL group of 658 nm and 785 nm ($P > 0.05$). The three red laser irradiations might promote cells proliferation,

Table 1: The time-dependent responses of proliferation of HeLa cells in 5% FBS after three red lasers stimulation (Laser energy density and its irradiation time were 1000 J/m^2 and 100 s, resp.).

Time	Wavelength		
	633 nm	658 nm	785 nm
24 h	1.230 ± 0.082^a	1.034 ± 0.022^c	1.064 ± 0.067^a
48 h	1.269 ± 0.110^a	1.049 ± 0.083^b	1.105 ± 0.071^a
72 h	1.140 ± 0.094^c	1.003 ± 0.030^c	1.014 ± 0.054^c

Notes: The values were the ratio of laser irradiation group to control group (%) ($^aP < 0.01$; $^bP < 0.05$; $^cP > 0.05$).

respectively, and all the three proliferation peaks were at 48 h after laser irradiation.

4. Discussion

LIL may modulate the cell proliferation, which was dependent on many factors, such as, laser wavelength, dose, or intensity as well as FBS concentrations. Among them, the FBS concentration was the key factor. It could be used as a mean to produce different cell growth states. In this study, 1%, 2%, 5%, and 10% FBS were chosen and PBM on cell proliferation was investigated and compared under these nutritional conditions. Cells in 10% FBS were in normal culture state, while cells in 1%, 2%, 5% FBS were in nutritional stress. Many studies have found no PBM on proliferation in 10% FBS, so that it was stated that the proliferation in 10% FBS may be in proliferation-specific homeostasis (PSH) which is a negative feedback to maintain proliferation at its local peak [21]. There was no PBM on proliferation in PSH, but there was PBM on proliferation far from PSH. It was supported in this study. There was no significant PBM on HeLa cells proliferation in 10% FBS. All the three concentrations of FBS, 1%, 2%, and 5%, inhibited proliferation in comparison with proliferation in PSH, respectively. This means that the concentrations of FBS, 1%, 2%, and 5%, can reduce the cell growth rate and make the cell far away from PSH. As the results shown, there may be PBM on proliferation in FBS at 1% and 5%, respectively. LIL at 785 nm inhibited the proliferation in 1% FBS, but all the three kinds of LIL completely recovered 5% FBS-inhibited proliferation in comparison with the proliferation in PSH. In other words, the proliferation may be in PSH in 10% FBS or in 5% FBS under LIL. This is a redundant phenomenon. LIL might promote proliferation in 5% FBS through redundant pathways, which will be further studied [22]. The result was in accordance with the finding of Almeida-Lopes et al. [23] and Tagliani et al. [24]. However, it should be pointed out that 1% and 2% FBS were so low that it can not maintain proliferation and may cause some harmful cell stress. In this case, cells did not produce a satisfactory proliferation response to laser irradiation or may cause other stimulation effects. In our study, we found that LIL didn't promote the proliferation of HeLa cells in 1% and 2% FBS, and LIL at 785 nm inhibited the proliferation in 1% FBS. The reason may be that the LIL at 785 nm might promote other cellular functions except proliferation, such as, in G0 phase so that the proliferation was inhibited. All in all, cellular proliferation in FBS at 1%, 2%, or 5% are far from PSH, so that it can be modulated with LIL. This was also a support to the viewpoint of homeostatic PBM [21].

This study compared the proliferation of HeLa cells modulated by three different LIL at 633 nm, 658 nm, and 785 nm. Among them, the best proliferation effect was at 633 nm. The results indicated that laser wavelength was an important factor that influenced the PBM. This may be due to different wavelength absorption by cellular chromophores, which modulated cell functions differently. The suitable wavelength at 633 nm may be absorbed well by the photoreceptor, such as, chromophores in cytochrome c oxidase or porphyrins, which could lead to second messenger activity resulting in functional changes and cell proliferation. The other possible reason was in relation to the type of lasers. The 633 nm laser used in this study was gas laser, which has longer coherence length and more obvious biological effects than the diode laser (658 nm, 785 nm) [25]. Our results agreed with Moore's work [16]. It is a possible reason why 633 nm He-Ne laser has been widely used in wound healing in clinical application.

5. Conclusions

The proliferation of HeLa cells in 10% FBS was in PSH so that it was not modulated with LIL. The proliferation in FBS at 1%, 2%, or 5% was far from PSH so that it may be wavelength dependently modulated with LIL. The maximum proliferation promotion was conducted with LIL at 633 nm amongst the three kinds of LIL, 633 nm, 658 nm, and 785 nm. This study provided helpful experimental data and shed new light on the research of LIL therapy and its mechanism.

Authors' Contribution

H. Q. Yang and Y. H. Wang contributed equally to this work.

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