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The Effect of 635 nm Red Laser Irradiation on Proliferation of Bone Marrow Stem Cells

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Abstract

Photobiomodulation effects of Low-level light irradiation (LLLI) on regeneration have been reported in skin, nerve, and skeletal muscle tissues and bone. Bone Mesenchymal stem cells (BMSCs) are derived from bone marrow, which exhibited a fibroblast-like appearance, and could differentiate in vitro into different lineages. However, there is a reciprocal relationship between growth and osteogenic differentiation in MSCs. Therefore, it's important to investigate the effect of LLLI on BMSCs. The aim of our study was to investigate the proliferation effect of 635 nm red laser light on bone marrow MSCs with or without osteogenic supplements. Bone marrow was collected from the 4-week-old Sprague–Dawley rats femur and tibiae. MSCs with and without osteogenic supplements both were divided into three groups. A continuous 635 nm wavelength red light diode laser (a power output of 960 mW) was used in the study. The size of light spot was 35mm in diameter. Irradiation was performed every other day since the half of medium was changed to osteogenic differentiation media (ODM). The first irradiation day was set as 0 day. The duration of each irradiation for red light was calculated at 10 seconds for 1 J/cm^2, 20 seconds for 2 J/cm^2. Two of these groups were used as controls: MSCs incubated in DMEM without irradiation (control 1), MSCs incubated in ODM without irradiation (control 2). Cellular proliferation was evaluated by using WST-8. Cell viability was assessed with WST-8 kit at 2, 4, 6 and 8 days, respectively. At 4, 6 and 8 days, groups cultured with DMEM showed significantly higher viabilities than that in groups with ODM. In groups with DMEM, red light at all doses significantly stimulated cell viability as compared with the control 1. Groups irradiated at 1 and 2 J/cm^2 had more effective proliferation on 4 ($P < 0.01$) and 6 days ($P < 0.05$), when compared with the control 1. In groups with ODM, control 2 and the irradiated groups showed similar proliferation speeds. In conclusion, we can find that red light can promote proliferation of MSCs cultured in normal media, and suppress proliferation of MSCs cultured in ODM.

Keywords

Photobiostimulation, Mesenchymal Stem Cells (MSCs), Proliferation

1. Introduction

Photobiomodulation effects of Low-level light irradiation (LLLI) on regeneration have been reported in skin [1]

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[2], nerve [3], skeletal muscle tissues [4] and bone [5]. LLLI can be used as a efficiently tool for the preconditioning of bone marrow mesenchymal stem cells (MSCs), which derived from bone marrow and received wildly attentions in regeneration medicine. However, previous reports show different or conflicting results about photo-induced osteodifferentiation and proliferation. Oliveira *et al*. [6] showed that neither the MTT values nor mRNA expression of collagen I in the irradiated group differed significantly from those in the non-irradiated odontoblast-like cells. On the other hand, Ozawa *et al*. [7] reported that laser irradiation at an earlier stage of bone formation was more effective than irradiation at a later stage, and that stimulation of bone formation by laser was dependent on the total energy dose. Therefore, it's important to investigate the proliferation effect of 638 nm red laser light on bone marrow MSCs with or without osteogenic supplements.

2. Materials and Methods

2.1. Cell Culture

A 4-week-old Sprague-Dawley rat was sacrificed by neck dislocation. Bone marrow was washed out from the femur and tibiae with a needle, suspended in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, NY, USA), and centrifuged at 2000 rpm for 5 min. The marrow pellet was washed in phosphate-buffered saline (PBS), centrifuged at 1000 rpm for 10 min, and then resuspended in DMEM. Nucleated cells were isolated with a Percoll density gradient (Invitrogen) by centrifuging at 14,000 rpm for 12 min. The top 60% of the gradient was collected, and then washed with the complete culture medium containing 10% fetal bovine serum (FBS, Invitrogen), 100 U/ml penicillin (Sigma-Aldrich, MO, USA), 100 mg/ml streptomycin (Sigma-Aldrich), and 0.25 mg/ml amphotericin (Sigma-Aldrich). The cells were placed into T-25 tissue (Greiner, Frickenhausen, Germany) culture flasks at 37˚C in a 5% CO_2 atmosphere. . Nonadherent cells were removed by changing the medium after 24 hours. The culture medium was changed twice a week thereafter. For subculture, cells were detached with 0.25% trypsin (Amresco, OH, USA) and passaged at a ratio of 1:2 plates when cells grew to 80% - 90% confluence.

The cells were plated onto 96-well ELISA plates (Jet-Biofil, Guangzhou, China) at a density of 3×10^3 cells/well. After 24 hours incubation, the medium of half wells was changed to ODM (Cyagen biosciences, Guangzhou, China) which consisted of low glucose DMEM supplemented with 50 μg/ml ascorbicacid, 10^{-8} M dexamethasone, and 10 mM β-glycerolphosphate. The rest still cultured in DMEM. .

2.2. Procedure of Irradiation

A laser with a continuous wavelength of 635nm (a power output of 38mW) was used in this study. The diameter of light spot is 7 mm. At cell-layer level, the power density measured by a power meter was 6.67 mW/cm^2. Because the biostimulation of once irradiation could continue for 48 hours [8], irradiation was performed every other day since the half of medium was changed to ODM. The first irradiation day was set as 0 day. Total energy corresponding to 10 sec exposure was 1 J/cm^2, 40 sec exposure was 4 J/cm^2. Two of these groups were used as controls: MSCs incubated in DMEM without irradiation (control 1), MSCs incubated in ODM without irradiation (control 2). Non-exposed cells were maintained outside the incubator under the same conditions as the exposed cells.

2.3. Cell Proliferation Assays

Cell viability was assessed with WST-8 kit (Beyotime Inst Biotech, China) at 2, 4, 6 and 8 days, respectively. At the indicated time, WST-8 was added to the cells, according to the manufacturers' instructions, and incubated for 1 hour. OD450, the absorbance value at 450 nm, was read in an ELX 800 universal microplate reader (Bio-Tek Instruments, VT, USA). The value is directly proportional to the number of viable cells in a culture medium and the cell proliferation.

2.4. Statistical Analysis

Results are presented as means ± S.D. of three independent experiments. Statistical significance was determined by analysis of variance (ANOVA), and P values of <0.05 were considered significant.

3. Result

As shown in **Figure 1**, viable cell numbers increased rapidly from 0 day (24 hours after cell seeding) to 4 days, and then reached a stationary phase by 6 days. Similar cell growth curves were observed in every group throughout the cell-culture period. At 4, 6 and 8 days, groups cultured with DMEM showed significantly higher viabilities than that in groups with ODM. In groups with DMEM, red light at all doses significantly stimulated cell viability as compared with the control 1. Groups irradiated at 1 and 2 J/cm^2 had more effective proliferation, as higher OD_{450} was observed on 4 ($P < 0.01$) and 6 days ($P < 0.05$), when compared with the control 1. In groups with ODM, control 2 and the group irradiated at 1 J/cm^2 showed similar proliferation speeds (**Figure 2**). Red light at 2 J/cm^2 significantly inhibited cell viability as compared with the control 2 ($P < 0.05$).

4. Discussion

In our study, the results of WST-8 confirmed that red laser also was able to stimulate proliferation of bone marrow MSCs cultured in normal media. However, red light slowed down cellular proliferation of MSCs cultured in media with osteogenic supplements. A possible explanation is that a reciprocal relationship between growth and osteogenic differentiation is apparent in MSCs [9]. Genes involved in the production and deposition of the extracellular matrix are expressed during the proliferative period, and the synthesis of an organized bone-specific extracellular matrix contributes to the shutdown of proliferation [10].

We distinguished the role of red laser irradiation in photoinduced osteogenic differentiation via investigating the cellular proliferation effects of 635nm laser on bone marrow MSCs cultured in two different biological systems. Irradiated MSCs in two different *in vitro* environments showed different bio-reactions. Different energy

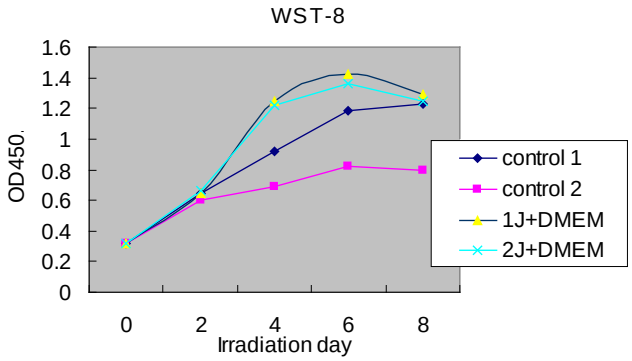

Figure 1. Cell growth curves of MSCs cultured in DMEM. MSCs in DMEM showed a statistical increase of viability at 4, 6, and 8d, as compared to the control 2. Final saturation densities did not show statistical differences among DMEM groups, whereas they were statistically higher than control 2. Groups treated with LLLI showed a statistical increase of viability at 4, 6d, as compared to the control 1.

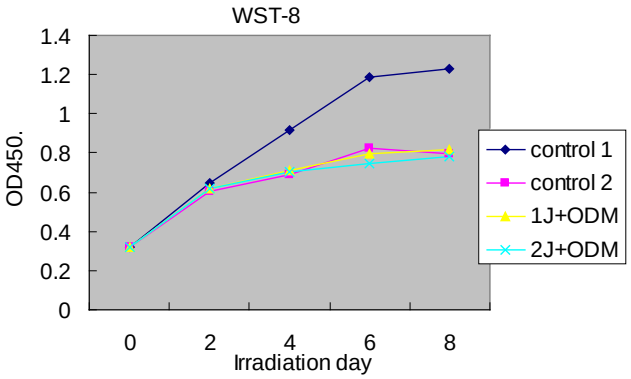

Figure 2. Cell growth curves of MSCs cultured in ODM. Final saturation densities did not show statistical differences among ODM groups, whereas they were statistically lower than control 1.

densities promote proliferation of MSCs in normal media, while it decelerates proliferation of MSCs in media with osteogenic supplements. Our findings may provide appropriate strategies for the preconditioning of MSCs *in vitro* prior to transplantation.

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