Original Article

pISSN, eISSN 0125-5614
M Dent J 2019; 39 (2) : 143-152

Effect of different wavelength of LEDs on osteoblast-like cell cultured in 3D collagen type I scaffold

Tatsanee Tanglitanont[1], Rochaya Chintavalakorn[2], Peerapong Santiwong[2], Anak Khantachawana[3], Kwanchanok Viravaidya-Pasuwat[4]

[1] *Private Clinic, Bangkok, Thailand.*
[2] *Department of Orthodontics, Faculty of Dentistry, Mahidol University.*
[3] *Department of Mechanical Engineering and Biological Engineering Program, King Mongkut's University of Technology Thonburi.*
[4] *Department of Chemical Engineering and Biological Engineering Program, King Mongkut's University of Technology Thonburi.*

Objectives: To investigate the effect of 4 different wavelengths of light-emitting diodes (LEDs) irradiation on biological response of osteoblast cells (MC3T3-E1) cultured in 3 dimension (3D) collagen type I scaffold.

Materials and Methods: The MC3T3-E1 were cultured on 3D type I collagen scaffolds and irradiated daily by LEDs light with wavelengths of 630, 680, 760 and 830 nm for 42 days at radiant exposure of 3.1 J/cm^2 (intensity 2 mW/cm^2). The 3D cultured were subjected to biological tests concerning cell proliferation by DNA assay, cell differentiation by alkaline phosphatase (ALP) activity and mineralization by calcium phosphate deposits at day 0, 7, 14, 21, 28, 35 and 42. The 3D cultured at day 42 was investigated by scanning electron microscope (SEM) to determine the effect of LEDs on cell formation. The mineralization at day 42 in the 3D cultured was evaluated from elemental analysis to determine the ratio of calcium and phosphorus of mineralized granule.

Results: Statistical analysis revealed a significantly higher rate of cell proliferation ($p<0.05$) in all irradiated cultures in comparison with the controls. The 630 and 680 nm groups yielded a higher number of cells than the 760 and 830 nm ($p<0.05$). Cell differentiation, obtained from ALP activity, was increased significantly after 680, 760 and 830 nm irradiation ($p<0.05$) but decreased after 630 nm irradiation. However, only 680 nm group had significantly greater mineralization than controls ($P<0.001$) at the end of the experimental period.

Conclusions: The results demonstrate that osteoblastic-liked cells respond to LEDs irradiation differently depending on wavelengths, from 630 to 830 nm, in proliferation and differentiation. To enhance bone mineralization, 680 nm peak irradiated is more effective than those of 630, 760 and 830 nm.

Keywords: calcification, differentiation, LEDs, light-emitting diodes, MC3T3-E1 Cell, mineralization

How to cite: Tanglitanont T, Chintavalakorn R, Santiwong P, Khantachawana A, Viravaidya-Pasuwat K. Effect of different wavelength of LEDs on osteoblast-like cell cultured in 3D collagen type I scaffold. M Dent J 2019; 39: 143-152.

Introduction

Photobiomodulation is a non-pharmacological therapeutic resource that is clinically accepted as an adjunctive tool in regenerative medicine and dentistry. Photobiomodulation uses low level light irradiated (LLLI) by a laser or LEDs to provide visible or near-infrared light to modulate cell response. LLLI was reported to be able to modulate inflammation and edema, relief pain, promote cell proliferation, and healing of hard and soft tissue lesion [1-4]. The acceleration of bone regeneration has recently attracted major interest in the field of tissue engineering because it helps facilitate a bone fracture healing processes such as accelerate bone regeneration in midpalatal suture after in surgical assisted rapid maxillary expansion (SARME) and rapid maxillary expansion (RME)

Correspondence author: Rochaya Chintavalakorn
Department of Orthodontics, Faculty of Dentistry, Mahidol University
6 Yothi Road, Ratchathewi, Bangkok 10400, Thailand
Tel: 022007817-8 Email: dr.krittika@gmail.com
Received : 28 June 2019 Accepted : 19 July 2019

[5, 6] promote alveolar bone repair after tooth extraction [7], assist osseointegration of implants by enhance the functional attachment of titanium implants to bone and promotes bone healing and mineralization [8], and assist bone remodeling during orthodontic movement of teeth [9-11]. With regard to bone synthesis, photobiomodulation was reported to increase osteoblast activity and decrease osteoclast number, by inducing alkaline phosphatase (ALP), osteopontin, and bone sialoprotein expression [12]. However some study reported that low level light therapy (LLLT) with GaAlAs laser had no effect on the rate of orthodontic tooth movement [13, 14].

The LLLT wavelengths using for photobiomodulation are in the range of 630 904 nm [9, 10, 13-18]. It was reported that different wavelength, visible red and invisible infrared rang (IR), affects the cell with different primary mechanism. Visible light was reported to induce photochemical reaction in the mitochondria, while the IR involves in photophysical reaction in cell membrane. However, both wavebands increase cell proliferation through the activation of the mitochondrial respiratory chain resulting in the increase of reactive oxygen species (ROS), and adenosine triphosphate (ATP) or cyclic AMP, and finally initiating a signaling cascade which promotes cellular proliferation [19]. According to the action spectra for biological responses such as the stimulation of growth, and DNA and RNA synthesis, several wavelengths are suggested to be optimal for LLLT, at around 400, 620, 680, 760, and 820 nm [20]. Some of these peaks are identifiable with cytochrome C oxidase in the mitochondria, which is a photoacceptor responsible for various cellular responses. According to the biphasic dose response or Arndt-Schulz curve, LLLT showed different modes of cell reaction at different levels of energy density both in vitro and in animal experiments [21]. It was reported that laser therapy significantly increased the bone nodules in a dose-dependent manner, and that cell proliferation and alkaline phosphatase (ALP) activity were higher in the early stages of cellular proliferation [22].

Compared to lasers, light-emitting diodes (LEDs) are light-weight, low heat generation and cost–effective. Although their output is not monochromatic as laser diode, they are quasimonochromatic, deliver output power over a few nanometers. Moreover, they can be made to produce multiple wavelengths and can be arranged in order to get large and flat arrays allowing treatment of a large area [23] Evidence showed that treatment with lasers and LEDs with the same irradiation parameters promotes similar photobiologic effects [24, 25]. Therefore, LEDs offer a cost-effective alternative to lasers for photostimulation. However, the contradictory results in the literatures may originated from the complexity of illumination parameters such as wavelength, power density, energy, time, and frequency of application in each study.

2D cell culture showed a limited cell growth which prevents a long term study and does not represent the physiological cell-cell and cell-extracellular matrix (ECM) interactions found in real tissues [26]. 3D cell culture was reported to be more accurately resemble the in vivo situation [27, 28]. The objective of this study was to compare the effect of visible red range (630 and 680 nm) and near infrared (760 and 830 nm) range on MC3T3-E1 cells cultured on 3D collagen I scaffold.

Materials and methods

Culturing and Cell seeding on 3D collagen scaffolds

The murine osteoblast-like cell line, MC3T3-E1 was cultured in complete medium consisted of α-MEM supplemented with 10% fetal bovine serum (FBS), 1% antibiotics cocktail (10,000 U/ml penicillin and 10,000 µg/ml streptomycin and 1% Amphotericin B (250 µg/ml). The cell was carried out in a humidified incubator at 37°C with 5% carbon dioxide (CO2). The culture medium was changed every 3-4 days.

To seed the scaffolds with cells, the collagen (Type I) scaffolds were punched into cylinder-shape with a 10 mm diameter and 2 mm thickness, and then sterilized with ethylene oxide. A confluent cell was trypsinized and re-suspended to a concentration of 7.316 x10^5 cells/ml. A cell suspension of 0.38 ml was then seeded onto the scaffolds. Five 48-well plates containing scaffolds were incubated for 24 hours to allow attachment of the cell to the scaffold. Then, the complete medium was replaced by 0.38 ml of the osteogenic medium, which composes of complete medium plus 100 nM dexamethasone, 10 mM β-glycerophosphate and 50 mg/ml ascorbic acid. The osteogenic medium was changed every other day.

Light irradiation

Three LEDs panels were designed and constructing by calculating the inter-distance space of LEDs with Mathemetica version 9.0.0 (Walfram Research, USA) to irradiate the equal energy over the 48-well plates placed in dark environment. The material used for the LEDs lamp was aluminum-gallium-arsenide (AlGaAs) and had an emission wavelength centered at 630, 680, 760 and 830 nm with the half width of 20, 25, 30 and 35 nm, respectively. To irradiate to the cell seeded scaffold, the LED panel with 1.5 cm inter-distance space of LED was fixed perpendicularly above each culture well-plate at a distance of 6 mm in a dark box to protect environment light during irradiation. The irradiance intensity of every LEDs panel was set to 2 mW/cm^2 by adjusting the voltage; operated at 27.5 V for 630 nm; 27 V for 680 nm; 17 V for 760 nm; and 13.7 V for 830 nm. The utilized energy density was 3 J/cm^2 which was regulated by the time period of 25 minute. The cultures were irradiated as daily dose for 42 days.

Biological assessments

The cell seeded scaffolds were compared among 5 conditions including control groups concerning cell proliferation, alkaline phosphatase (ALP) activity and ability to form calcium phosphate deposits at day 0, 7, 14, 21, 28, 35 and 42. At each time interval, the scaffolds were harvested and washed 5 times in PBS and homogenized in 30 mM sodium citrate-buffered saline solution (SSC) (pH 7.4) containing 0.2mg/ml sodium dodecylsulfate (SDS) at 37 °C for 1 hour to break the cell membrane in order to obtain cell lysate.

DNA analysis

100 µl of cell lysate, was transferred to 96-well plate and added with 100 µL Hoechst 33258 working solution (Sigma). DNA was measured using a fluorescence microplate reader with excitation filter 365 nm and emission filter 450 nm (Bio-Tek instruments, Abcoude, The Netherlands). DNA standard curve was also made from 10^5 to 10^6 cells/ml.

ALP activity

20 µl of cell lysate was transferred to 96-well plate and subsequently added with 100 ml of substrate solution (p-nitrophenyl phosphate). The plate was incubated for 15 min at 37 °C. The reaction was stopped by adding 80 µl of stop solution (0.02 M NaOH). The plate was read at 405 nm (Bio-Tek instruments, Abcoude, The Netherlands).

Calcium content

Calcium content in the samples was measured by the ortho-cresolphthalein complexone (OCPC) method and was measured for absorbance at 570 nm (Bio-Tek instruments, Abcoude, The Netherlands) [29].

Scanning electron microscopy (SEM) observation

At day 42, the 3D cell seeded scaffold was harvested and rinsed with PBS for 5 min and then fixed with 2.5 % glutaraldehyde solution in PBS at 4 ºC for 1 hour. After that, the scaffold was washed with PBS for 10 min and immersed in 1% osmium tetroxide for 1 hour at room temperature for post-fixation. After washing with PBS, samples were

subsequently dehydrated by serial dilution of ethanol aqueous solutions and then dried in a critical point dryer. After coating with layer of gold, SEM was performed with beam energies of 6 to 25 kV. Moreover, energy dispersive spectrometry (EDS) analysis (Oxford Instruments, USA) was also performed to determine the chemical composition of calcium nodule in the ECM.

Statistical analysis

All data were expressed as means ± standard deviation, and analyzed by a Kruskal-Wallis test and Mann-Whitney test for pair-comparison. A statistical threshold of $p<0.05$ was used to indicate whether there were statistical significances among different groups. The analyses were performed with SPSS 20.0 software (SPSS, Chicago, IL).

Results

Effect of LED on cellular proliferation

The proliferation rate was measured through the cellular DNA content at different culture time and presented as a proliferation profile of MC3T3-E1 in 3D collagen scaffold in figure 1. Overall, LLLR produced a statistically significant increase in MC3T3-E1s proliferation ($p<0.05$) compared to control cells at culture time. In controlled group, cell numbers increased rapidly from day 0 (24 hours after cell seeding), and then reached a stationary phase at day 14. However, all irradiated group showed delay during the peak of cell proliferation (day 21).

At day 1 and 7, the mean proliferation rate of MC3T3-E1 in irradiated groups was enhanced 1.24-1.40 times in relation to control group with statistically different ($p<0.001$). However, there were no statistically significant differences among irradiated groups for these periods at all wavelength. After day14 and day 21, there were statistically significant differences among the irradiated groups. At day 14, the 630 nm and 680 nm groups presented higher proliferation rate than those of 760 nm and 830 nm groups. At day 21, the cell growth was enhanced 1.70 times by 630 nm LED, 1.82 times by 680 nm LED, 1.36 times by 760 nm LED, and 1.30 times by 830 nm LED in relation to control group. After 21 days, the proliferation rate of all cultures decreased with the significantly higher rate in the irradiated groups when compared to the control ($p<0.001$) till the end of the experiment. Only 680 nm group showed the significantly difference when compared to the other irradiated groups ($p<0.05$)

Effect of LED on cellular differentiation

The alkaline phosphatase (ALP) activity, a marker of early osteoblast differentiation, was investigated by quantitative analysis and results were shown in figure 2. At day 1, there were no statistically significant differences between non-irradiated and irradiated groups. The irradiated group present the maximum value that significantly greater (1.41 to 1.47 folds) than the control on day 14 ($p<0.05$) and decreased. However, it is still higher than control during the next 2 weeks except the 630 nm group that presented lower value than controlled group ($p<0.001$). Among irradiated groups, the 680 nm, 760 nm and 830 nm groups presented a significantly higher ALP activity than 630 nm group. On day 21, control group reached the highest value. On day 28, ALP activity started to decreased through day 42, except for control group and 760 nm groups that showed rebound value of on day 42.

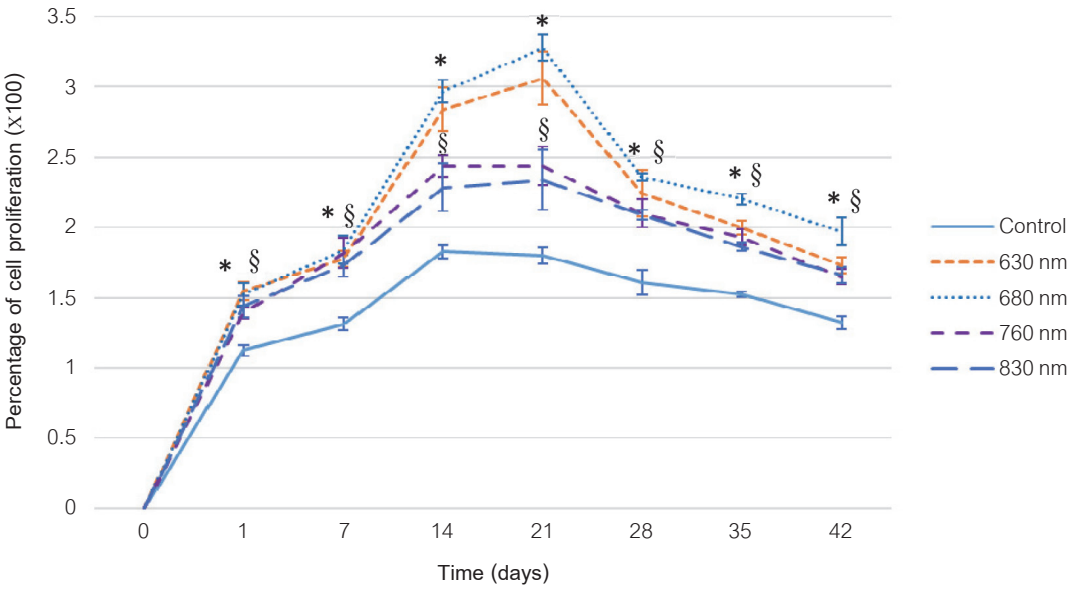


Figure 1 The effect of the wavelength of LED irradiation on the percentage of MC3T3-E1 cell proliferation when compare to control. (* p<0.05 630 nm and 680 nm, § p<0.05 630 nm and 680 nm compare to control)

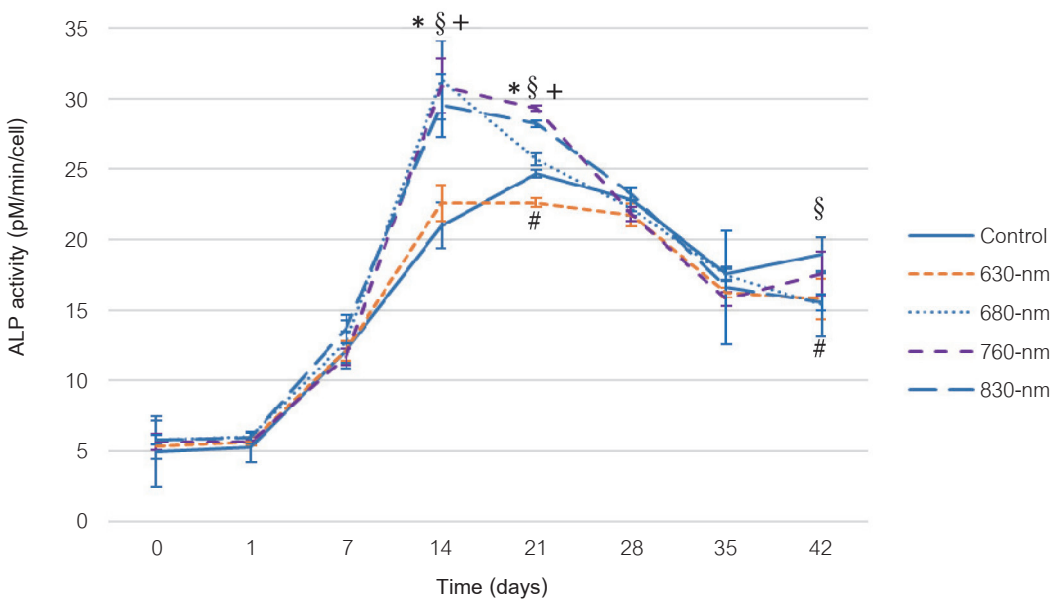


Figure 2 The effect of the wavelength of LED irradiation on the alkaline phosphatase (ALP) activity of MC3T3-E1s when compare to control. (# p<0.05 630 nm compare to control, * p<0.05 680 nm compare to control, § p<0.05 760 nm compare to control, + p<0.05 830 nm compare to control)

Effect of LEDs on cellular mineralization

In additional to the ALP that is an early maker in the osteoblastic differentiation, the calcium deposition is a late stage marker of bone formation. The cellular mineralization was investigated by determines the total calcium content and shown in figure 3. After irradiation 24 hour, the calcium content of light stimulated cultures and control cultures were lower and did not differ significantly. At day 7 and 14 marked accumulation of calcium was detected with higher value in irradiated groups than controlled group that indicate a mineral formation. After 14 days, the calcium content of 760 nm and 830 nm groups was similar to controls and then significantly decreases to lower amount than control at day 28. The 630 nm group showed a significantly increase from day 14 to 28 ($p<0.001$) but no statistically significant different can be found after 35 and 42 days of incubation while 680 nm group revealed a significant enhancement until the late stages of culture ($p<0.001$).

The significant increase in the ALP levels during the second week suggested that more cells were shifting to a differentiated stage. Formation of mineral deposits occurred following the maximal ALP activity, earlier and greater in 680 nm cultures. Comparison of the results presented in figures 2 and 3 showed that ALP activity increased and rapidly decreased significantly at day 14 accompanied with the high level of calcium content 1 week after that. This finding suggests the effectiveness of LEDs at 680 nm in enhancing early onset of bone formation.

Effect of LEDs on microstructure of 3D structure

Microstructure of scaffolds up to day 42 of all sample were observed by SEM and shown in figure 4. The scaffold had an interconnected porous structure with the pore size range of 50-150 μm and the porosity around 96%. The cultured scaffold both controlled and irradiated group that were shown in figure 5 demonstrated that cell grown on scaffold surface were flattened with dendritic formation. The ECM bridging the scaffold pores forming the ECM network within the porous structure. Cells appear to adhere to the scaffold through three dimensional extensions, gradually filling in pores as proliferation proceeds.

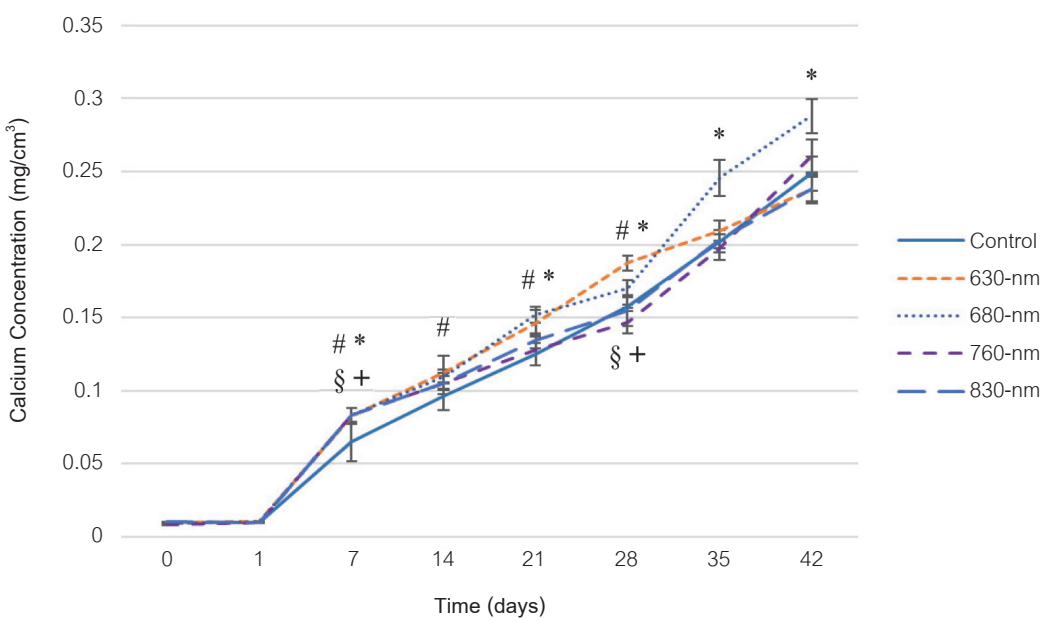


Figure 3 Calcium content of MC3T3-E1 after light irradiation when compare to control. (# $p<0.05$ 630 nm, * $p<0.05$ 680 nm, § $p<0.05$ 760 nm, and + $p<0.05$ 830 nm compare to control)

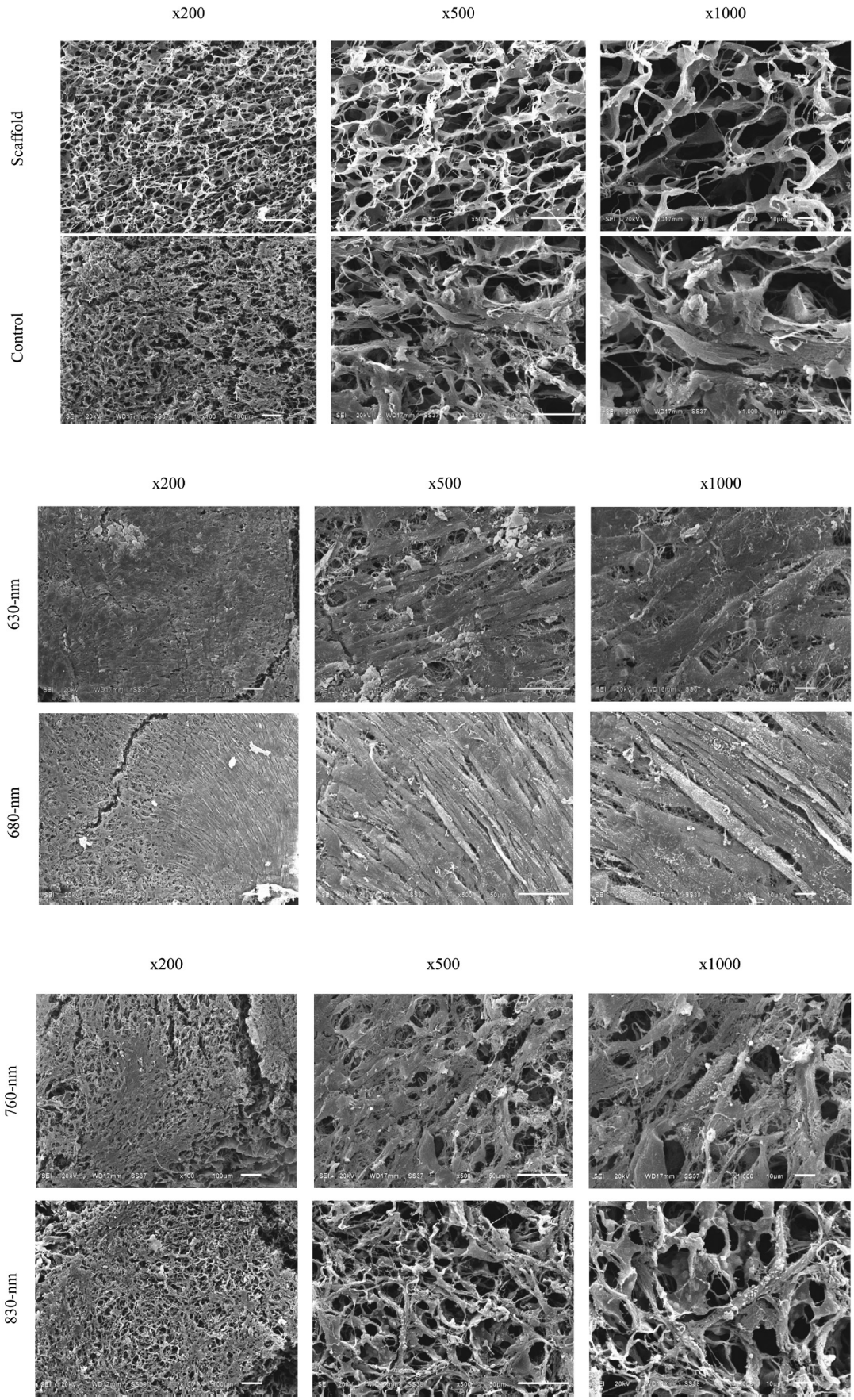


Figure 4 Morphology of MC3T3-E1 cells cultured under different wavelength of LED irradiation for 42 days.

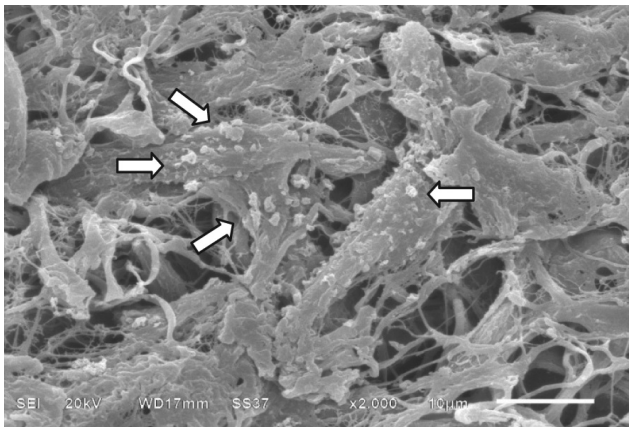
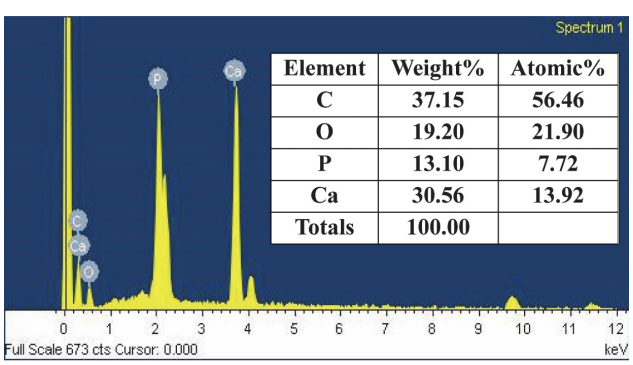


Figure 5 Mineralization deposits of 3D collagen scaffold seeded with MC3T3-E1s.

The cell distribution on the top of the scaffold after irradiation appears to be denser in 630 nm and 680 nm group than 760 nm and 830 nm group. The 630 nm and 680 nm irradiation groups showed sheet-like material and fibril which represented ECM development on the collagen scaffold with dense regular layer of flat and spinocellular cells and relatively little ECM on the collagen scaffold. The cells exhibited the contact with each other and adhering firmly to the matrix.

The round granule depositions, which indicate the mineralization, were found in all groups as observed in figure 5. These results were supported by the amplified mineralization evident from the Energy Dispersive Spectroscopy (EDS) analyses that indicated the component of calcium, phosphorus carbon, and oxygen in round granule as shown in figure 6. EDS analysis revealed that mineral granule was calcium phosphate.



Element	Weight%	Atomic%
C	37.15	56.46
O	19.20	21.90
P	13.10	7.72
Ca	30.56	13.92
Totals	100.00	

Figure 6 SEM micrographs and diagramed illustrations of EDS analysis performed 42 days after seeding of MC3T3-E1 on 3D collagen scaffold.

Discussion

Biochemical mechanisms underlying positive effects of LLLI has not been completely understood [30]. Karu et al. also suggested that wavelength, dose, and intensity played an important role on the cell respond [31]. In the case of LLLI laser, it was reported that cultured cells were affected by wavelength, dose and intensity [30].

This study investigated the effects of LEDs wavelengths (630 nm, 680 nm, 760 nm and 830 nm) on the proliferation, differentiation and mineralization of osteoblast-like cell lines in 3D type I collagen scaffold. It was found that LEDs irradiation can stimulate osteoblast-like proliferation. All irradiation wavelengths showed significantly higher proliferation rate than the control. In addition, 680 nm irradiation showed the highest proliferation rate about 1.54 folds higher than non-irradiated proliferation rate. The higher cell proliferation of the LEDs groups can be caused by the activation of the mitochondrial respiratory chain and the initiation of cellular signaling by LLLI as reported by previous study [20]. The visible LLLI seems to have more effect on the activation of the mitochondrial respiratory chain [19] than IR counterpart, as evidenced by a higher proliferation observed in 630 nm and 680 nm than 760 and 830 nm. Our finding is consistent with the previous report that the visible, infrared, and ultraviolet (UV) LLLI from laser light can promote the cell proliferation. The visible spectrum, ranging from 600–700 nm, showed the highest effect [32]. This suggested that the LEDs are as effective as the laser for using in the light source for the LLLI process. Moreover, the magnitude of the LLLI effect on cell proliferation depends on the physiological state of the cell at the time of irradiation [22]. Khalid et al. suggested that to obtain the maximum proliferation of cells, cell cultures should be 20% confluent at the time of irradiation to obtain the maximum yield from LLLI [33]. The energy density should keep between 0.05 and 10 J/cm^2 for bio-stimulation to prevent bio-inhibitory effects that may occur when the energy density above 10 J/cm^2.

The effect of irradiation on cell differentiation was observed by monitoring ALP activity, a marker of normal bone cell activity. ALP activity was increased

in MC3T3-E1 after 680 and 830 nm LEDs irradiation at a dosage of 3 J/cm^2. This increase suggested that the irradiation may stimulate early osteoblast maturation. The results are consistent with those observed by Stein et al. who also found an increased in osteoblast ALP activity after 830 nm laser irradiation [34]. By contrast, Khadra et al. reported a GaAlAs laser LLLI which was irradiated only one time where they did not observe any cell differentiation effect after treatment. This suggested that the prolong irradiation is necessary for the LLLI process [35].

Results concerning ALP activity showed that levels of the enzyme were low in the first week but increased significantly during the second week, reaching maximal levels around day 14 in LEDs irradiation and at day 21 in non-irradiated cultures, then decreased after 3rd week to the same level as the control. This suggested that LEDs irradiation showed a transient effect on ALP activity. The significant increase in the ALP levels during the second week suggested that the cells were shifting to differentiated stage. Formation of mineral deposit occurred following the maximal ALP activity.

The morphology of the 3D cultured cell of LEDs LLLI group showed a higher density than non-irradiated group. The scaffold that was exposed to 630 nm and 680 nm showed higher cell density than those of 760 nm and 830 nm. The SEM images were in good agreement with proliferation rate where a higher proliferation was observed in 630 nm and 680 nm. The EDS analysis showed the calcium to phosphorous ration of 1.8 which slightly higher than the mineralized bone. This ratio indicated the formation of hydroxyapatite which is the main mineral component of calcified bone matrix.

This study revealed that specific LEDs wavelength effects on biological action. Some devices combine a red with a near infrared wavelength on the basis that the combination of two wavelengths can have additive effects and allow the device to be more broadly utilized to treat more diseases with one device [36]. Further study is suggested in order to address the effective parameters of LLLI on bone regeneration in clinical setting including treating with 2 or more combination wavelength.

Conclusion

The present study showed that osteoblast-like cells responded to LEDs irradiation is a wavelength-dependent. The specific LEDs wavelength has its own specific ability on biological action. Our study shown that LEDs can enhance cell proliferation at all wavelength. Visible red range (630 and 680 nm) showed a higher proliferation than near-infrared range (760 and 830 nm) after day 7. For enhancing bone mineralization and calcification, LEDs irradiation at the 680 nm group is more effective than those of 630 nm, 760 nm and 830 nm. For the differentiation, 680 nm, 760 nm and 830 nm showed early differentiation at first 2 weeks, all groups showed the same ALP activity level at the end of the study. Further studies are necessary to investigate the most effective parameters of LLLI on bone regeneration in clinical setting.

References

1. Silva Junior AN, Pinheiro AL, Oliveira MG, Weismann R, Ramalho LM, Nicolau RA. Computerized morphometric assessment of the effect of low-level laser therapy on bone repair: an experimental animal study. ***J Clin Laser Med Surg***, 2002; 20: 83-7.
2. Nissan J, Assif D, Gross MD, Yaffe A, Binderman I. Effect of low intensity laser irradiation on surgically created bony defects in rats. ***J Oral Rehabil***, 2006; 33: 619-624.
3. Almeida-Lopes L, Rigau J, Zangaro RA, Guidugli-Neto J, Jaeger MM. Comparison of the low level laser therapy effects on cultured human gingival fibroblasts proliferation using different irradiance and same fluence. ***Lasers Surg Med***, 2001; 29: 179-84.
4. Gaida K, Koller R, Isler C, Aytekin O, Al-Awami M, Meissl G, et al. Low Level Laser Therapy--a conservative approach to the burn scar? ***Burns***, 2004; 30: 362-7.
5. Angeletti P, Pereira MD, Gomes HC, Hino CT, Ferreira LM. Effect of low-level laser therapy (GaAlAs) on bone regeneration in midpalatal anterior suture after surgically assisted rapid maxillary expansion. ***Oral Surg Oral Med Oral Pathol Oral Radiol Endod***, 2010; 109: 38-46.
6. Cepera F, Torres FC, Scanavini MA, Paranhos LR, Capelozza Filho L, Cardoso MA, et al. Effect of a low-level laser on bone regeneration after rapid maxillary expansion. ***Am J Orthod Dentofacial Orthop***, 2012; 141: 444-50.

7. Mozzati M, Martinasso G, Cocero N, Pol R, Maggiora M, Muzio G, et al. Influence of superpulsed laser therapy on healing processes following tooth extraction. ***Photomed Laser Surg***, 2011; 29: 565-71.
8. Petri AD, Teixeira LN, Crippa GE, Beloti MM, de Oliveira PT, Rosa AL. Effects of low-level laser therapy on human osteoblastic cells grown on titanium. ***Braz Dent J***, 2010; 21: 491-8.
9. Cruz DR, Kohara EK, Ribeiro MS, Wetter NU. Effects of low-intensity laser therapy on the orthodontic movement velocity of human teeth: a preliminary study. ***Lasers Surg Med***, 2004; 35: 117-20.
10. Kawasaki K, Shimizu N. Effects of low-energy laser irradiation on bone remodeling during experimental tooth movement in rats. ***Lasers Surg Med***, 2000; 26: 282-91.
11. Kau CH, Kantarci A, Shaughnessy T, Vachiramon A, Santiwong P, de la Fuente A, et al. Photobiomodulation accelerates orthodontic alignment in the early phase of treatment. ***Prog Orthod***, 2013; 14: 30.
12. Saracino S, Mozzati M, Martinasso G, Pol R, Canuto RA, Muzio G. Superpulsed laser irradiation increases osteoblast activity via modulation of bone morphogenetic factors. ***Lasers Surg Med***, 2009; 41: 298-304.
13. Marquezan M, Bolognese AM, Araujo MT. Effects of two low-intensity laser therapy protocols on experimental tooth movement. ***Photomed Laser Surg***, 2010; 28: 757-62.
14. Takeda Y. Irradiation effect of low-energy laser on alveolar bone after tooth extraction. Experimental study in rats. ***Int J Oral Maxillofac Surg***, 1988; 17: 388-91.
15. Limpanichkul W, Godfrey K, Srisuk N, Rattanayatikul C. Effects of low-level laser therapy on the rate of orthodontic tooth movement. ***Orthod Craniofac Res***, 2006; 9: 38-43.
16. Youssef M, Ashkar S, Hamade E, Gutknecht N, Lampert F, Mir M. The effect of low-level laser therapy during orthodontic movement: a preliminary study. ***Lasers Med Sci***, 2008; 23: 27-33.
17. Habib FA, Gama SK, Ramalho LM, Cangussu MC, Santos Neto FP, Lacerda JA, et al. Laser-induced alveolar bone changes during orthodontic movement: a histological study on rodents. ***Photomed Laser Surg***, 2010; 28: 823-30.
18. Seifi, M., et al., Effects of two types of low-level laser wave lengths (850 and 630 nm) on the orthodontic tooth movements in rabbits. ***Lasers Med Sci***, 2007; 22: 261-4.
19. Gao X, Xing D. Molecular mechanisms of cell proliferation induced by low power laser irradiation. ***J Biomed Sci***, 2009; 16: 4.
20. Karu TI, Kolyakov SF. Exact action spectra for cellular responses relevant to phototherapy. ***Photomed Laser Surg***, 2005; 23: 355-61.
21. Sommer AP, Pinheiro AL, Mester AR, Franke RP, Whelan HT. Biostimulatory windows in low-intensity laser activation: lasers, scanners, and NASA's light-emitting diode array system. ***J Clin Laser Med Surg***, 2001; 19: 29-33.
22. Pinheiro AL, Gerbi ME. Photoengineering of bone repair processes. ***Photomed Laser Surg***, 2006; 24: 169-78.
23. Whelan HT, Smits RL, Jr., Buchman EV, Whelan NT, Turner SG, Margolis DA, et al. Effect of NASA light-emitting diode irradiation on wound healing. ***J Clin Laser Med Surg***, 2001; 19: 305-14.
24. Posten W, Wrone DA, Dover JS, Arndt KA, Silapunt S, Alam M. Low-level laser therapy for wound healing: mechanism and efficacy. ***Dermatol Surg***, 2005; 31: 334-40.
25. Vinck EM, Cagnie BJ, Cornelissen MJ, Declercq HA, Cambier DCJLiMS. Increased fibroblast proliferation induced by light emitting diode and low power laser irradiation. ***Lasers in Medical Science***, 2003; 18: 95-99.
26. Pampaloni F, Reynaud EG, Stelzer EH. The third dimension bridges the gap between cell culture and live tissue. ***Nat Rev Mol Cell Biol***, 2007; 8: 839-45.
27. Abramovitch-Gottlib L, Gross T, Naveh D, Geresh S, Rosenwaks S, Bar I, et al. Low level laser irradiation stimulates osteogenic phenotype of mesenchymal stem cells seeded on a three-dimensional biomatrix. ***Lasers Med Sci***, 2005; 20: 138-46.
28. Renno AC, McDonnell PA, Crovace MC, Zanotto ED, Laakso L. Effect of 830 nm laser phototherapy on osteoblasts grown in vitro on Biosilicate scaffolds. ***Photomed Laser Surg,*** 2010;28:131-3.
29. Chintavalakorn R, Tanglitanont T, Khantachawana A, Viravaidya-Pasuwat K, Santiwong P. The effect of light-emitting diode irradiation at different wavelengths on calcification of osteoblast-like cells in 3D culture. ***Conf Proc IEEE Eng Med Biol Soc***, 2015; 2015: 3513-6.
30. Huang YY, Chen AC, Carroll JD, Hamblin MR. Biphasic dose response in low level light therapy. ***Dose Response***, 2009; 7: 358-83.
31. Karu T. Molecular mechanism of the therapeutic effect of low-intensity laser radiation. ***J Lasers Life Sci***, 1988; 2: 53-74.
32. Wilden L, Karthein R. Import of radiation phenomena of electrons and therapeutic low-level laser in regard to the mitochondrial energy transfer. ***J Clin Laser Med Surg***, 1998; 16: 159-65.
33. AlGhamdi KM, Kumar A, Moussa NAJLims. Low-level laser therapy: a useful technique for enhancing the proliferation of various cultured cells. ***Lasers in Medical Science***, 2012; 27: 237-49.
34. Stein A, Benayahu D, Maltz L, Oron U. Low-level laser irradiation promotes proliferation and differentiation of human osteoblasts in vitro. ***Photomed Laser Surg***, 2005; 23: 161-6.
35. Khadra M, Lyngstadaas SP, Haanaes HR, Mustafa K. Effect of laser therapy on attachment, proliferation and differentiation of human osteoblast-like cells cultured on titanium implant material. ***Biomaterials***, 2005; 26: 3503-9.
36. Calderhead RGJLT. The photobiological basics behind light-emitting diode (LED) phototherapy. ***Laser Therapy***, 2007; 16: 97-108.