Lasers in Medical Science (2019) 34:55–60
https://doi.org/10.1007/s10103-018-2586-6

ORIGINAL ARTICLE

Effects of Nd:YAG low-level laser irradiation on cultured human osteoblasts migration and ATP production: in vitro study

Yuji Tsuka[1] · Ryo Kunimatsu[1,2] · Hidemi Gunji[1] · Kengo Nakajima[1] · Aya Kimura[1] · Tomoka Hiraki[1] · Ayaka Nakatani[1] · Kotaro Tanimoto[1]

Received: 20 March 2018 / Accepted: 8 July 2018 / Published online: 12 July 2018

Abstract

Low-level laser therapy has become one of the fastest growing fields of medicine in recent years. Many in vivo and in vitro studies have shown that laser irradiation activates a range of cellular processes in a variety of cell types and can promote tissue repair. However, few in vitro experiments have evaluated the effects of laser irradiation on cells in real time. The purpose of this study was to examine the effects of neodymium-doped yttrium aluminum garnet (Nd:YAG) laser irradiation on the migration of cultured human osteoblasts. A dedicated 96-well plate was used, and confluent cultures of the human osteoblast-like cell line, Saos-2, were injured with a wound maker. The wounded cells were then exposed to the Nd:YAG laser (wavelength of 1064 nm) for 60 s at 0.3 W (10 pps, 30 mJ). The total energy density was about 10.34 J/cm^2. Images of the wounds were automatically acquired inside the CO_2 incubator by the IncuCyte ZOOM™ software. In addition, after laser irradiation, the production of adenosine triphosphate (ATP) was measured using the CellTiter-Glo™ Luminescent Cell Viability Assay. Migration of cells from the border of the original scratch zone was accelerated by laser irradiation. In addition, compared with the control group, significant enhancement of ATP production was observed in the irradiated group. The present study showed that Nd:YAG laser irradiation (wavelength of 1064 nm, 0.3 W, 10 pps, 30 mJ, 10.34 J/cm^2, irradiation time 60 s) may contribute to the regeneration of bone tissues owing to enhanced osteoblast cell migration.

Keywords Nd:YAG laser · Wound healing · Migration · Osteoblasts

Introduction

In recent years, lasers have been widely used in the medical field for diagnosis and other purposes, and their effectiveness and efficacy have been reported. Lasers have different characteristics and can be used for various purposes depending on wavelength. Wavelengths around 800 nm are high in tissue permeability and can be used for pain relief [1]. Wavelengths near 3000 nm are high in surface absorbency and can be used for cutting hard tissue [2].

Currently, surface absorption (CO_2 lasers and Er:yttrium aluminum garnet (YAG) lasers) and tissue-penetrating types of lasers (He:Ne lasers, Nd:YAG lasers, and diode lasers) are used in the medical and dental fields. When incision and hemostasis of soft tissue are required, high-power energy lasers are used. In contrast, low-level laser (light) therapy (LLLT) is useful for periodontal treatment [3, 4], hypersensitivity procedures [5, 6], and treatment of temporomandibular disorders [7]. Previously, procedures involving LLLT, which uses visible light (400–700 nm), were performed by the irradiation of injury sites with low-level or low-powered lasers to accelerate cellular processes, leading to better healing and decreased inflammation and pain [8–12].

In addition, near-infrared (NIR) light and visible red (VR) light with a wavelength of 700–1100 nm show lower water absorption and higher absorption by hemoglobin. In addition, because NIR light has higher tissue penetration compared to

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10103-018-2586-6) contains supplementary material, which is available to authorized users.

✉ Ryo Kunimatsu
ryoukunimatu@hiroshima-u.ac.jp

[1] Department of Orthodontics, Applied Life Sciences, Hiroshima University Institute of Biomedical & Health Sciences, Hiroshima, Japan

[2] Department of Orthodontics and Craniofacial Developmental Biology, Hiroshima University Graduate School of Biomedical Sciences, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553, Japan

VR light [13–16], clinical applications of NIR light are attracting attention. However, the action and mechanisms of NIR and VR light on osteoblasts remain unclear.

Recently, in vitro studies on cells of different origins using a wavelength spectrum of NIR or VR light (600–1100 nm) have shown modulation of cellular processes such as the production of ATP [14–20], cyclic adenosine monophosphate (CAMP) [18–20], and matrix metalloproteinase activity [20–22].

In the field of clinical dentistry, the Nd:YAG laser is used most widely for soft tissue applications, and several studies have underscored its beneficial effects in the treatment of periodontal disease and peri-implant regions [23]. However, the molecular mechanisms of Nd:YAG lasers with a wavelength of 1064 nm are not fully understood. Thus, the purpose of this study was to examine the effects of Nd:YAG laser irradiation on the migration of cultured human osteoblasts.

Materials and methods

Cell culture

Cells of the human-derived osteoblast-like cell line, Saos-2 (Riken, Tsukuba, Japan), were cultured in 100-mm tissue culture dishes in Dulbecco's Modified Eagle Medium (Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS; Bioserum, Melbourne, Victoria, Australia) and 1% antibiotic-antimycotic mixture (Invitrogen Corporation, Carlsbad, CA) and then incubated at 37 °C in a humidified atmosphere of 95% air and 5% CO_2.

Cell plating and scratching

Saos-2 cells were seeded at a density of 2×10^3 cells/well in an ImageLock TM® 96-well plate with a surface area of 0.32 cm^2 per well (Essen BioScience, Tokyo, Japan). After reaching confluence, the FBS concentration in the medium was gradually decreased to 0% as described above. After 12 h in 0% FBS, a uniform and reproducible scratch was made in all wells of a 96-well plate using WoundMaker™ (Essen BioScience). After creating the scratch, the medium was aspirated, and the well was washed with fresh medium to remove the cells from the scratched area.

Laser irradiation

An Nd:YAG laser (Inpulse, Incisive, Richmond, CA) with a wavelength of 1064 nm was used in this study. The guide light is a diode laser with a wavelength of 630–680 nm. A 0.32-mm optical fiber was used as the tip of the Nd:YAG laser. The specifications of the Nd:YAG laser are shown in Table 1. After scratching, the Nd:YAG laser was used to irradiate the cells for 60 s at an output of 0.3 W (10 pps, 30 mJ). Irradiation was performed by setting the area of the irradiation field to 1.74 cm^2. The total energy density was about 10.34 J/cm^2. The laser handpiece was fixed with a stand, and the distance from the bottom of the culture dish to the tip was about 2.4 cm. The beam divergence angle of the Nd:YAG laser is 27°; however, this is a theoretical figure, and the actual arriving energy may be lower. Cells that were scratched but not irradiated with the laser were used as the control group.

Table 1 The specifications of the Nd:YAG laser

Wavelength (nm)	1064
Operating mode	Pulsed
Frequency (Hz)	10
Pulse duration (us)	100
Duty cycle	0.001
Power	30 mJ/100 μs
Average power (W)	0.3

Analysis of scratched and irradiated cultures

We analyzed the cultures using the IncuCyte ZOOM™ live-cell imaging system (Essen BioScience). The cells were photographed automatically at the same place each time every 4 h until 25 h after laser irradiation. The imaging system measures the closed area of the scratch in real time and automatically calculates the cell area, scratch width, and cell density percentage at each time point. The control group consisted of eight wells, and the laser irradiation group consisted of four wells.

Effects of Nd:YAG laser irradiation on ATP production by human osteoblasts

Saos-2 cells were seeded at a density of 2×10^3 cells/well in 96-well plates. After reaching 60% confluence, the FBS concentration was reduced gradually to 0% as described above. Cells were incubated in 0% FBS for 12 h followed by Nd:YAG laser irradiation. At 24 h after laser irradiation, CellTiter-Glo™ luminescent cell viability assays (Promega, Madison, WI) and a GloMax® detection system luminometer (Promega) were used to determine ATP levels by luminescence.

Statistical analysis

The mean of the three experiments was calculated and was considered to represent the values of each experimental parameter. Data from three independent experiments are presented as the mean and standard deviation. One-way analysis of variance was used for group comparisons. The differences between the control group and each experimental group were

analyzed using Dunnett's test. P values < 0.05 were considered statistically significant.

Results

Cell imaging

Cell images were obtained with the IncuCyte ZOOM™ live-cell imaging system every 4 h (Fig. 1). More than 1 h after laser irradiation, more cells were observed in the laser irradiation group than in the control group.

Cell migration

Relative wound density, wound confluence, and wound width values as analyzed by IncuCyte ZOOM™ are shown in Figs. 2, 3, and 4, respectively. Regarding the relative wound density, migration of the cells from the border of the original scratch zone was accelerated by laser irradiation (Fig. 2). Wound confluence was enhanced by laser irradiation of the cells compared to the non-treated control group (Fig. 5). Measurement of wound widths indicated that wound closure was promoted in an irradiation time-dependent manner (Fig. 4).

Effects of Nd:YAG laser irradiation on ATP production by human osteoblasts

The effect of Nd:YAG laser irradiation on ATP synthesis by Saos-2 cells was measured using the CellTiter-Glo™ Luminescent Cell Viability Assay (Fig. 5). ATP synthesis was significantly increased in the laser-irradiation group compared to the control group.

Discussion

Several reports on NIR lasers have shown promotion of bone formation by a variety of methods. Son et al. [24] revealed that LLLT with a diode laser with a wavelength of 808 nm accelerates the bone healing process in early stages of bone formation in vivo. In addition, our recent study revealed that 910-nm laser irradiation of periodontal tissue leads to metabolic activation, which ultimately increases the rate of tooth movement in rats [25]. An in vitro study showed that NIR laser irradiation with a wavelength of 910 nm enhances cell proliferation and migration of human gingival epithelial cells [26]. In addition, we also showed that NIR laser irradiation (910 nm, 2.85–17.1 J/cm^2) enhances cell proliferation and migration of mouse calvaria osteoblast cells [27]. On the other hand, Ninomiya et al. [28] reported that Nd:YAG laser irradiation with a wavelength of 1064 nm accelerates bone formation in the femur metaphysis. We also showed that Nd:YAG laser irradiation affects bone metabolism during tooth movement in vivo [29]. Thus, we hypothesized that Nd:YAG laser irradiation with a wavelength of 1064 nm, which is within the NIR range, may affect osteoblast metabolism.

A few experiments using a wound healing test have been conducted to examine the influence of Nd:YAG laser irradiation in vitro, but no experiments have evaluated its influence on cells in real time. Thus, here we investigated the influence of an Nd:YAG laser, which has not been extensively studied, on the migration of bone lineage cells in vitro using WoundMaker™ and IncuCyte ZOOM™. WoundMaker™ is different from conventional wound makers because it allows scratches to be made very accurately. The IncuCyte ZOOM™ system is a live-cell imaging and analysis platform that enables quantification of cell behavior over time by automatically gathering and analyzing images around the clock. The system provides insight into active biological processes in real time, which is not possible using single- or end-point

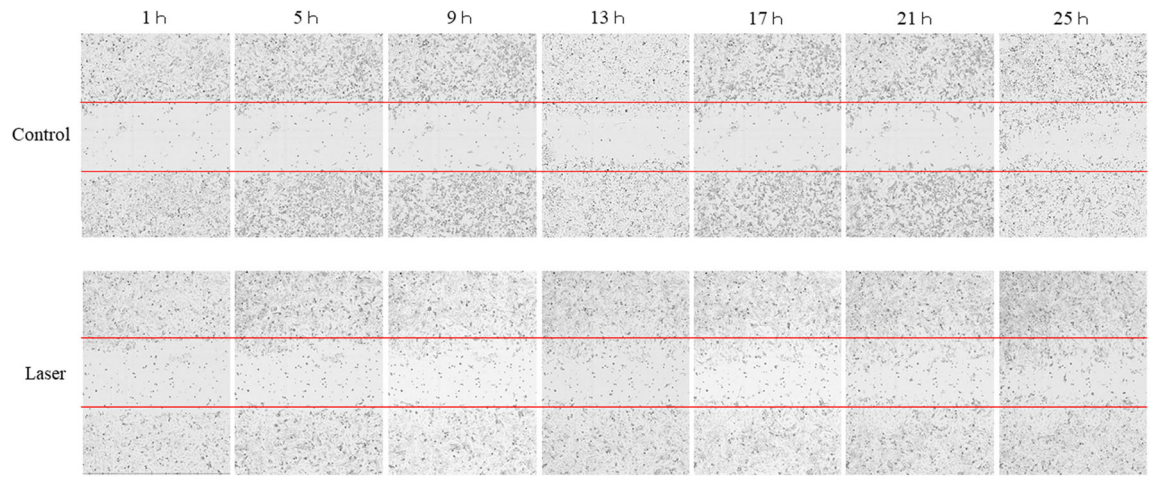

Fig. 1 Cell imaging of human osteoblasts after Nd:YAG laser irradiation. Cell images were acquired with the IncuCyte ZOOM™ live-cell imaging system every 4 h. More cells were observed in the laser irradiation group than in the control group

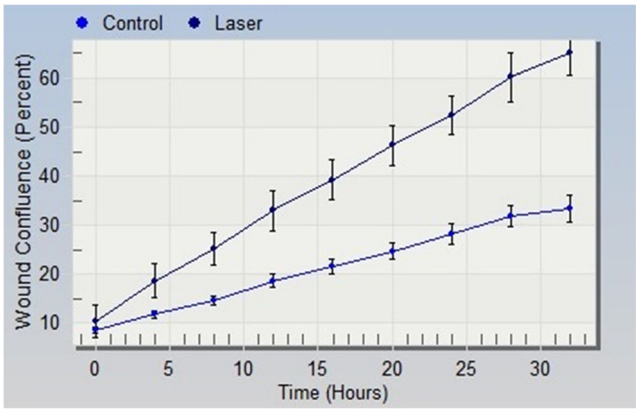

Fig. 2 Wound density analyzed by IncuCyte ZOOM™ after the wound healing test. Relative wound density was analyzed by IncuCyte ZOOM™. Migration of cells from the border of the original scratch zone was accelerated by laser irradiation compared with the control

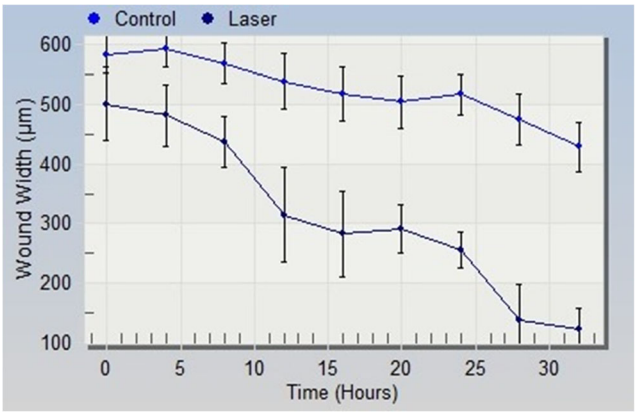

Fig. 4 Wound width analyzed by IncuCyte ZOOM™ after the wound healing test. Measurement of the wound width indicates that wound closure was promoted in an irradiation time-dependent manner compared with the non-treated control

measures alone. Experiments using this system have been published in over 1000 articles in recent years, and the system has been shown to be extremely reliable [30–32].

In the present study, the wound width was decreased by treatment with Nd:YAG laser irradiation at a dose of 10.34 J/cm^2 compared with the untreated control, suggesting that Nd:YAG laser irradiation enhances osteoblast cell migration. In addition, relative wound density and confluence were increased by Nd:YAG laser irradiation (1064 nm, 0.3 W, 10 pps, 30 mJ, 10.34 J/cm^2) compared with the control. Further, ATP production was increased following irradiation with the 1064-nm Nd:YAG laser, suggesting that Nd:YAG laser irradiation enhances human osteoblast cell proliferation.

Several reports [33–36] have been published on the effects of Nd:YAG laser irradiation on cell proliferation and differentiation. The results differ depending on the output number, the amount of energy, the method of irradiation, and the irradiation time. Chellini et al. reported no change in Saos-2 cell counts between laser and control groups 4 h after Nd:YAG laser irradiation (1064 nm, 1 or 1.4 W, 20 mJ, 1.5 J/cm^2). However, a significant increase in cell counts was observed in the laser irradiation group compared with the control group at 48 h after Nd:YAG laser irradiation. Laser irradiation has also been shown to lead to significant production of type I collagen [33].

In a previous in vitro study, mouse calvaria osteoblast cell proliferation was significantly suppressed by 13.6, 11.2, and 7.5% in Nd:YAG laser-irradiation groups treated with energy intensities of 1.5, 3, and 5 J/cm^2 (power output 0.75 W, 15 pps, 4–12 s), respectively, compared to that of controls. Regarding osteogenic differentiation, Nd:YAG laser irradiation at intensities of 1.5, 3, and 5 J/cm^2 enhanced bone formation by mouse calvaria osteoblasts [34]. The present study revealed that cell proliferation was significantly increased by Nd:YAG laser irradiation (0.3 W, 10 pps, 30 mJ, 10.34 J/cm^2) after 1 day, as seen with the ATP luciferase assay. In addition, laser irradiation at a dose of 10.34 J/cm^2 induced Saos-2 cells to

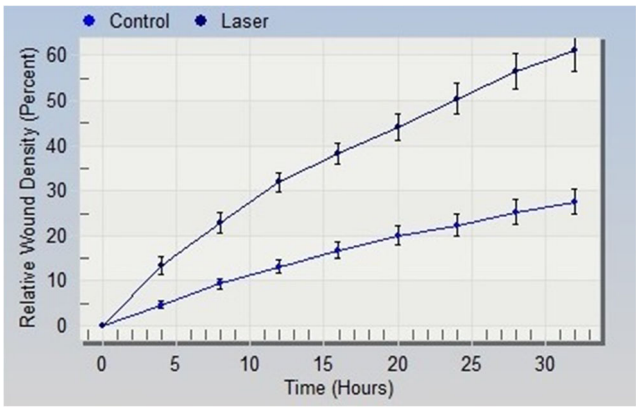

Fig. 3 Wound confluence analyzed by IncuCyte ZOOM™ after the wound healing test. Wound confluence, which was analyzed by IncuCyte ZOOM™, was enhanced by laser irradiation compared to non-treated control human osteoblasts

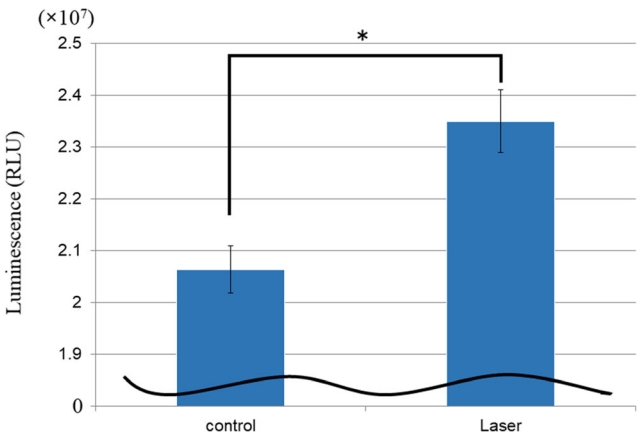

Fig. 5 Effects of Nd:YAG laser irradiation on ATP production by human osteoblasts. The effect of Nd:YAG laser irradiation on ATP synthesis by Saos-2 cells was measured using the CellTiter-Glo™ Luminescent Cell Viability Assay. ATP synthesis was significantly increased in the laser-irradiation group compared to the control group. *$P < 0.01$ vs. control

migrate more rapidly than nonirradiated control cells. The difference between the two results may be explained by the following two reasons. First, in the report by Kim et al., a mouse calvarial osteoblastic cell line was used, but in our experiments, we used a human osteoblastic osteosarcoma cell line. The difference in cell types may have caused the difference in the results. Second, in the report by Kim et al., high-power output (0.7 W) was used, whereas in the present study, low-power output was used (0.3 W). According to the Arndt-Schulz law, weak stimuli promote biological or physiological responses, and strong stimuli suppress these reactions or are harmful [35, 36]. Differences in the power number of Nd:YAG laser irradiation may have altered the metabolic capacity of the cells. Further studies are necessary to evaluate the optimal output numbers for promoting cell growth and bone differentiation.

On the other hand, some reports have examined high levels of radiation energy. Nd:YAG laser irradiation (0.5 W, 10 Hz, 50 mJ) at doses of 7.9 and 15.8 J/cm^2 promotes proliferation of human fibroblasts at 48 h compared to controls. In contrast, laser irradiation at doses of 2.6 and 5.3 J/cm^2 produces no change in cell proliferation in human gingival fibroblasts [37].

Another in vitro study reported that 1064-nm laser irradiation with 5 J/cm^2 has a favorable stimulatory effect on wounded cells, suggesting a possible mechanism in which the wavelength may potentially influence the cellular responses of wounded cells [38]. The results of our study confirmed that Nd:YAG laser irradiation (10.34 J/cm^2) of injured cells increased the cellular repair capacity. These results are similar to those reported by Evans et al. However, the results of various studies [33, 34, 37, 38] differ depending on the output number, amount of energy, pulse, irradiation time, etc. Further studies are needed to elucidate the optimal output number and associated details.

Conclusion

Nd:YAG laser irradiation under specific parameters (wavelength of 1064 nm, 0.3 W, 10 pps, 30 mJ, 10.34 J/cm^2, irradiation time 60 s) may induce a beneficial response as a biological defense mechanism following osteoblast cell damage.

Acknowledgments This research was conducted at the Department of Orthodontics of Hiroshima University.

Role of funding source This work was supported by grants from the JSPS KAKENHI (numbers 16K11790, 16K20644, and 25862016).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Hudson DE, Hudson DO, Wininger JM, Richardson BD (2013) Penetration of laser light at 808 and 980 nm in bovine tissue samples. Photomed Laser Surg 31(4):163–168
2. Armengol V, Jean A, Rohanizadeh R, Hamel H (1999) Scanning electron microscopic analysis of diseased and healthy dental hard tissues after Er:YAG laser irradiation: in vitro study. J Endod 25(8): 543–546
3. Alzoman HA, Diab HM (2016) Effect of gallium aluminium arsenide diode laser therapy on Porphyromonas gingivalis in chronic periodontitis: a randomized controlled trial. Int J Dent Hyg 14(4): 261–266
4. Gündoğar H, Şenyurt SZ, Erciyas K, Yalım M, Üstün K (2016) The effect of low-level laser therapy on non-surgical periodontal treatment: a randomized controlled, single-blind, split-mouth clinical trial. Lasers Med Sci 31(9):1767–1773
5. García-Delaney C, Abad-Sánchez D, Arnabat-Domínguez J, Valmaseda-Castellón E, Gay-Escoda C (2017) Evaluation of the effectiveness of the photobiomodulation in the treatment of dentin hypersensitivity after basic therapy: a randomized clinical trial. J Clin Exp Dent 9(5):694–702
6. Femiano F, Femiano R, Lanza A, Lanza M, Perillo L (2017) Effectiveness on oral pain of 808-nm diode laser used prior to composite restoration for symptomatic non-carious cervical lesions unresponsive to desensitizing agents. Lasers Med Sci 32(1):67–71
7. Sayed N, Murugavel C, Gnanam A (2014) Management of temporomandibular disorders with low level laser therapy. J Maxillofac Oral Surg 13(4):444–450
8. Wagner VP, Curra M, Webber LP, Nör C, Matte U et al (2016) Photobiomodulation regulates cytokine release and new blood vessel formation during oral wound healing in rats. Lasers Med Sci 31(4):665–671
9. Suzuki R, Takakuda K (2016) Wound healing efficacy of a 660-nm diode laser in a rat incisional wound model. Lasers Med Sci 31(8): 1683–1689
10. Soares DM, Ginani F, Henriques ÁG, Barboza CA (2015) Effects of laser therapy on the proliferation of human periodontal ligament stem cells. Lasers Med Sci 30(3):1171–1174
11. Wu JY, Wang YH, Wang GJ, Ho ML, Wang CZ et al (2012) Low-power GaAlAs laser irradiation promotes the proliferation and osteogenic differentiation of stem cells via IGF1 and BMP2. PLoS One 7(9):e44027
12. Fabre HS, Navarro RL, Oltramari-Navarro PV, Oliveira RF, Pires-Oliveira DA et al (2015) Anti-inflammatory and analgesic effects of low-level laser therapy on the postoperative healing process. J Phys Ther Sci 27(6):1645–1648
13. Aoki A, Sasaki KM, Watanabe H, Ishikawa I (2004) Lasers in nonsurgical periodontal therapy. Periodontol 2000(6):59–97
14. Capon A, Mordon S (2003) Can thermal lasers promote skin wound healing? Am J Clin Dermatol 4:1–4
15. Hu WP, Wang JJ, Yu CL, Lan CC, Chen GS et al (2007) Helium-neon laser irradiation stimulates cell proliferation through photostimulatory effects in mitochondria. J Invest Dermatol 127(8):2048–2057
16. Sharma SK, Kharkwal GB, Sajo M, Huang YY, De Taboada L et al (2011) Dose response effects of 810 nm laser light on mouse primary cortical neurons. Lasers Surg Med 43(8):851–859
17. Walsh LJ (1997) The current status of low level laser therapy in dentistry. Part 2. Hard tissue applications. Aust Dent J 42(5):302–306
18. Karu T, Pyatibrat L, Kalendo G (1995) Irradiation with He-Ne laser increases ATP level in cells cultivated in vitro. J Photochem Photobiol B 27(3):219–223

19. Houreld NN, Masha RT, Abrahamse H (2012) Low-intensity laser irradiation at 660 nm stimulates cytochrome c oxidase in stressed fibroblast cells. Lasers Surg Med 44(5):429–434
20. Zungu IL, Hawkins Evans D, Abrahamse H (2009) Mitochondrial responses of normal and injured human skin fibroblasts following low level laser irradiation—an in vitro study. Photochem Photobiol 85(4):987–996
21. Abduljabbar T, Javed F, Kellesarian SV, Vohra F, Romanos GE (2017) Effect of Nd:YAG laser-assisted non-surgical mechanical debridement on clinical and radiographic peri-implant inflammatory parameters in patients with peri-implant disease. J Photochem Photobiol B Mar 168:16–19
22. Benedicenti S, Pepe IM, Angiero F, Benedicenti A (2008) Intracellular ATP level increases in lymphocytes irradiated with infrared laser light of wavelength 904 nm. Photomed Laser Surg 26(5):451–453
23. Wu JY, Chen CH, Wang CZ, Ho ML, Yeh ML et al (2013) Low-power laser irradiation suppresses inflammatory response of human adipose-derived stem cells by modulating intracellular cyclic AMP level and NF-κB activity. PLoS One 8(1):e54067
24. Son J, Kim YB, Ge Z, Choi SH, Kim G (2012) Bone healing effects of diode laser (808 nm) on a rat tibial fracture model. In Vivo 26(4): 703–709
25. Gunji H, Kunimatsu R, Tsuka Y, Yoshimi Y, Sumi K, et al. (2018) Effect of high-frequency near-infrared diode laser irradiation on periodontal tissues during experimental tooth movement in rats. Laser Sur Med : inpress
26. Ejiri K, Aoki A, Yamaguchi Y, Ohshima M, Izumi Y (2014) High-frequency low-level diode laser irradiation promotes proliferation and migration of primary cultured human gingival epithelial cells. Lasers Med Sci 29(4):1339–1347
27. Kunimatsu R, Gunji H, Tsuka Y, Yoshimi Y, Awada T, et al. (2018) Effects of high-frequency near-infrared diode laser irradiation on the proliferation and migration of mouse calvarial osteoblasts. Lasers Med Sci : inpress
28. Ninomiya T, Miyamoto Y, Ito T, Yamashita A, Wakita M et al (2003) High-intensity pulsed laser irradiation accelerates bone formation in metaphyseal trabecular bone in rat femur. J Bone Miner Metab 21(2):67–73
29. Tsuka Y, Fujita T, Shirakura M, Kunimatsu R, Su SC et al (2016) Effects of neodymium-doped yttrium aluminium garnet (Nd:YAG) laser irradiation on bone metabolism during tooth movement. J Lasers Med Sci 7(1):40–44
30. Sundaram GM, Common JE, Gopal FE, Srikanta S, Lakshman K et al (2013) 'See-saw' expression of microRNA-198 and FSTL1 from a single transcript in wound healing. Nature 495(7439):103–106
31. Li J, Hou B, Tumova S, Muraki K, Bruns A et al (2014) Piezo1 integration of vascular architecture with physiological force. Nature 13 515(7526):279–282
32. Sinha C, Arora K, Naren AP (2016) Methods to study Mrp4-containing macromolecular complexes in the regulation of fibroblast migration. J Vis Exp 19(111)
33. Chellini F, Sassoli C, Nosi D, Deledda C, Tonelli P, Zecchi-Orlandini S et al (2010) Low pulse energy Nd:YAG laser irradiation exerts a biostimulative effect on different cells of the oral microenvironment: "an in vitro study". Lasers Surg Med 42:527–539
34. Kim IS, Cho TH, Kim K, Weber FE, Hwang SJ (2010) High power-pulsed Nd:YAG laser as a new stimulus to induce BMP-2 expression in MC3T3-E1 osteoblasts. Lasers Surg Med 42:51
35. Tuner J (2010) The new laser Handbook. Preima books, Sweden. 528–565
36. Michael R Hamblin (2017) Handbook of low-level laser therapy, 8 Pan Stanford publishing Pte ltd Singapore 311–312
37. Gkogkos AS, Karoussis IK, Prevezanos ID, Marcopoulou KE, Kyriakidou K et al (2015) Effect of Nd:YAG low level laser therapy on human gingival fibroblasts. Int J Dent 25:8941
38. Evans DH, Abrahamse H (2008) Efficacy of three different laser wavelengths for in vitro wound healing. Photodermatol Photoimmunol Photomed 24:199–210