



Irradiation by high-intensity red light-emitting diode enhances human bone marrow mesenchymal stem cells osteogenic differentiation and mineralization through Wnt/ β -catenin signaling pathway

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Abstract

Photobiomodulation therapy (PBMT) using a light-emitting diode (LED) has been employed for various photomedicine studies. The aim of this study was to determine the effects of a high-intensity red LED on the proliferation and osteogenic differentiation of human bone marrow mesenchymal stem cells (BMSCs) and the related mechanism. BMSCs were subjected to high-intensity red LED (LZ1-00R205 Deep Red LED) irradiations for 0 to 40 s with energy densities ranging from 0 to 8 J/cm². The distance from the LED to the cell layer was 40 mm. The spot size on the target was 4 cm². Cell proliferation was measured at 3, 24, 48, and 72 h. The effects of LED irradiation on osteogenic differentiation and mineralization were examined with a particular focus on the Wnt/ β -catenin signaling pathway. The high-intensity red LED irradiations did not alter BMSC proliferation after 72 h. LED exposure of 6 J/cm² (30 s) led to significant enhancements of osteogenic differentiation and mineralization. Additionally, the high-intensity LED irradiation induced activation of Wnt/ β -catenin. The effects of the high-intensity LED irradiation on BMSC osteogenic differentiation and mineralization were suppressed by treatment with the Wnt/ β -catenin inhibitor XAV939. $P < 0.05$ was considered significant. The results indicate that high-intensity red LED irradiation increases BMSC osteogenic differentiation and mineralization via Wnt/ β -catenin activation. Therefore, short duration irradiation with a portable high-intensity LED may be used as a potential approach in hard tissue regeneration therapy.

Keywords Bone regeneration · LED · Osteoblasts · Photobiomodulation therapy

Introduction

Human bone marrow mesenchymal stem cells (BMSCs) are consistently used in tissue engineering due to their multipotency [1]. The properties of BMSCs make them valuable for therapeutic purposes, such as the treatment of non-healing fractures, knee osteoarthritis, and Achilles tendinopathy [2–4]. In dentistry, the progression of inflammation caused by periodontal disease or peri-implantitis usually leads to the destruction of hard tissue. The destruction of the alveolar bone and periodontal ligament is one of the most

common causes of tooth loss [5–7]. To avoid tooth loss, the enhancement of the BMSC differentiation capacity is essential in regenerating the supporting alveolar bone [8].

Differentiation, the proliferation of BMSCs, and many other processes can be activated by the application of techniques such as photobiomodulation (PBM), electromagnetic fields, and ultraviolet light [9, 10]. Photobiomodulation therapy (PBMT) uses visible and near-infrared light and is considered a therapeutic advancement [11, 12]. It has been reported that bone exposure to heat at ≥ 47 °C leads to cell damage and bone resorption, whereas a temperature of ≥ 60 °C causes tissue necrosis [13, 14]. Considering that laser/biological tissue interactions are photothermal events, most lasers used on bone are usually harmful [15]. However, one of the most striking features of PBMT is that the effects are not mediated through thermal induction, but through a process referred to as “photobiomodulation” [12]. This principle implies that PBMT may be an effective method to regenerate hard tissue [16]. The

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LED applied as a kind of PBMT has the benefit of low cost and low risk, which has been introduced as a potential physical factor to stimulate proliferation and differentiation of various stem cell types [17–19]. A study has shown that low levels of LED at a wavelength of 620 nm enhance the proliferation and osteogenic differentiation of human umbilical cord mesenchymal stem cells [17]. However, few studies have focussed on effects of high-intensity LED on stem cells. High-intensity LED refers to the light-emitting diode with a large working power rating. Ordinary LED power is generally 0.05 W, while high-intensity LED power is greater than 1 W [20]. The high-intensity light sources must have a small apparent optical source size, a high light output, and an efficient optic design. Our research is focused on the exploration of the effect of high-intensity LEDs efficient for clinical application. Our previous study indicated that high-intensity LEDs could promote periodontal tissue regeneration [18]. Sequentially, the focus of this study is on the effects of the high-intensity red LED on the hard tissue regeneration.

In embryonic development, the Wnt/ β -catenin signaling pathway signaling is crucial to almost every process, especially in bone development [21]. In bone healing, β -catenin signaling also plays a central role in regulating the differentiation of pluripotent stem cells into osteoblasts [22]. Yang et al. showed that the Wnt signaling pathway is involved in strontium-induced osteogenic differentiation of mesenchymal stem cells [23]. Some studies have also shown that the Wnt signaling pathway can be activated by lasers or LEDs [24, 25]. Therefore, we hypothesized that high-intensity red LED may enhance the activity of BMSCs in hard tissue repair, such as increasing differentiation efficiency and mineralization by activating the Wnt/ β -catenin signaling pathway.

This study aimed to characterize the response of human BMSCs to high-intensity red LED irradiation by examining their proliferation, osteogenic differentiation, and mineralization. We also elucidated the underlying mechanism by analyzing key proteins in the Wnt/ β -catenin signaling pathway.

Materials and methods

Culture of human bone marrow mesenchymal stem cells

Human BMSCs were purchased from Promocell (Heidelberg, Germany) and cultured in mesenchymal stem cell growth medium with 10% supplementary mix in 75 cm² culture dishes at 37 °C with 5% CO₂. The culture medium was changed every 3 days. All experiments were performed using cells at passages 3–5.

Irradiation procedure

This study employed red LED prototype emitter (LZ1-00R205 Deep Red LED, LedEngin, Santa Clara, CA, USA) that emits red light specifically at 600 to 700 nm wavelengths with a peak at 650 nm. The intensity of radiant energy was confirmed by a power meter (Nova II; Ophir, North Andover MA, USA). The intensity at light source was 1100 mW/cm². The distance from the LED to the cell layer was 40 mm, while the spot size was 4 cm². And the intensity at well level was 200 mW/cm² [26]. Radiant exposure can be calculated by multiplying intensity by the exposure time. The total radiant exposures were 2, 4, 6, and 8 J/cm² for 10 s, 20 s, 30 s, and 40 s with continuous output. The experiment involved five groups: the control group (0 J/cm²); 2 J/cm² group, 4 J/cm² group, 6 J/cm² group, 8 J/cm² group. At the appropriate time point (see each assay below), irradiation was performed in the dark for only once. Over these irradiation periods, there were no detectable temperature changes compared to the controls.

Cell proliferation assay

BMSCs were plated in normal culture medium (Dulbecco's modified Eagle's medium with 10% fetal bovine serum (FBS), 500 U/mL penicillin, 500 g/mL streptomycin, and 25 g/mL amphotericin B) at a density of 2×10^3 cells/well in 96-well cell culture plates (Costar Stripwell plate, Corning, NY) which could be detached. In a previous study [27], cells were seeded in disassembled wells of cell culture plates, and each well was irradiated separately. After 24 h of incubation, irradiations were delivered in the dark and the cells were allowed to recover for 3, 24, 48, or 72 h. The MTT (3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, yellow tetrazole) assay utilizes the colorimetric reaction of the insoluble formazan dye associated with the reduction of the tetrazolium salt, MTT. MTT assay is used as a measure of cell proliferation. The number of viable cells at each time point was determined by measuring the amount of formazan generated in four wells per group using Cell Count Reagent SF (Nacalai Tesque, Kyoto, Japan). The formazan concentration was determined by absorbance at 450 nm. Data were analyzed using Soft Max[®] Pro Microplate Data Acquisition and Analysis software (Molecular Devices, Sunnyvale, CA, USA).

Alkaline phosphatase activity assay

Human BMSCs were seeded into 24-well plates at a density of 4×10^4 cells/well and cultured to confluence in a normal culture medium. The culture medium was then replaced with osteogenic medium containing 50 μ g/mL L-ascorbic acid 2-phosphate, 10 mM β -glycerophosphate, and 10 nM dexamethasone. After 12 h, the cells were subjected to irradiation.

After 1 or 2 weeks, the cells were washed and fixed and stained with alkaline phosphatase (ALP) with an Alkaline Phosphatase Staining Kit (Cosmo Bio, Tokyo, Japan). For measurement of ALP activity, cells were washed with phosphate-buffered saline (PBS) and lysed in 0.2% Triton X-100, and the ALP activity was quantified by one-step p-nitrophenyl phosphate (pNPP, Thermo Fisher Scientific, Rockford, IL, USA). ALP activity was normalized to the quantity of DNA. DNA content was measured by a DNA assay kit (Thermo Fisher Scientific). Data were analyzed using SoftMax Pro software.

Mineralization assay

Calcium depositions in the extracellular matrix were observed by alizarin red S staining. Following the same seeding, growth, and irradiation procedures described for ALP, BMSCs were cultured in osteogenic differentiation medium for 2 and 3 weeks. The cells were then washed with PBS and fixed in 70% ethanol for 10 min at -20°C . BMSCs were stained with a solution of 1% alizarin red S (Wako Pure Chemical Industries, Tokyo, Japan) for 3 min at room temperature and then washed with PBS. Extracellular calcium depositions were measured after dissolving precipitates with 10% formic acid. The amount of calcium was measured using a calcium detection kit (Wako Pure Chemical Industries), following the manufacturer's instructions. Absorbance was measured at 610 nm using a multi-microtiter reader.

Osteocalcin ELISA

The sandwich enzyme immune assay used in this study was specific for human osteocalcin and measured its levels directly in cell osteogenic culture supernatants after 2 and 3 weeks of culture following the same seeding, growth, and irradiation procedures described for ALP. Osteocalcin (OCN) levels in the culture supernatant were quantified with an OCN detection kit (GLA-type Osteocalcin EIA kit, Takara Bio, Shiga, Japan), following the manufacturer's instructions.

Quantitative real-time polymerase chain reaction

Gene expression changes of Runx2 and collagen type I alpha 1 (COL1A1) were determined by quantitative real-time polymerase chain reaction (PCR) in osteogenic cultures grown for 1 and 2 weeks. Total RNA was isolated using an RNeasy Mini Kit (Qiagen, Venlo, Netherlands), and 10 μL RNA from each sample was reverse transcribed into cDNA using PrimeScript RT reagent kit (Takara, Shiga, Japan). All real-time PCR assays were performed using the TaqMan Real-Time PCR assay system (Thermo Fisher Scientific) following the manufacturer's instructions. Gene expression levels were calculated

using the $\Delta\Delta\text{Ct}$ method [28] and normalized to GAPDH expression.

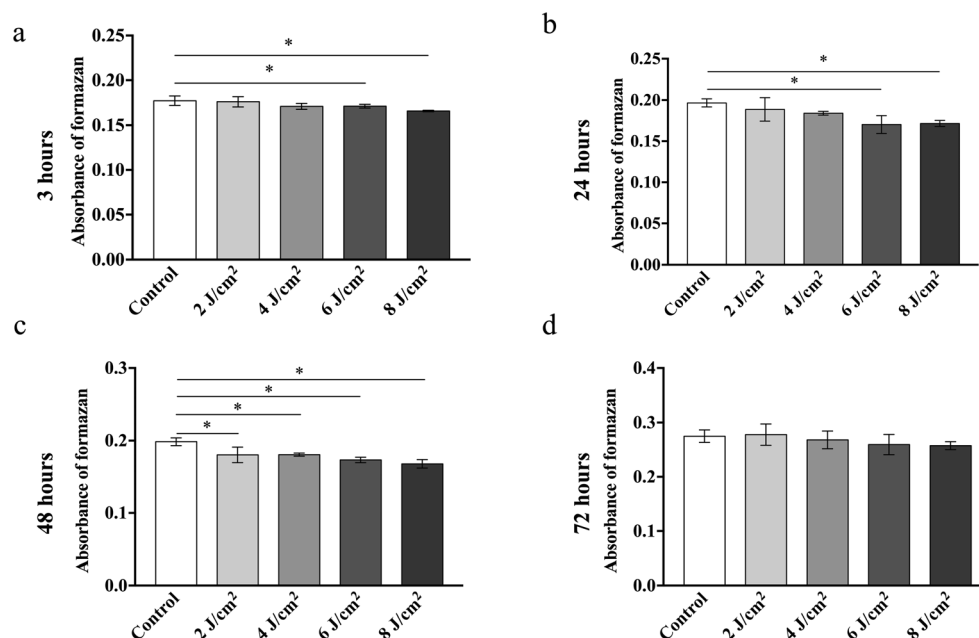
Western blotting

The optimal LED irradiation energy density was determined to be 6 J/cm^2 by previous assays. BMSCs were seeded at a density of 8×10^4 cells/well into 12-well plates in normal culture medium and cultured for 2 days. The cells were then serum starved in osteogenic medium for 12 h, subjected to irradiation, and cultured for 0, 30, or 60 min. Total protein was extracted using RIPA buffer containing a protease inhibitor cocktail (Thermo Fisher Scientific). The total protein concentration was determined using a BCA Protein Assay kit (Thermo Fisher Scientific). Protein samples were electrophoresed on 12.5% sodium dodecyl sulfate gels (Nacalai Tesque) and transferred onto polyvinylidene difluoride membranes (Nacalai Tesque). The membranes were treated with blocking solution, Blocking One (Nacalai Tesque), and incubated overnight at 4°C with primary antibodies (Cell Signaling Technology, Danvers, MA) against GSK-3 β , phospho-GSK-3 β , and β -catenin. Membranes were washed with tris buffered saline with 0.1%-detergent (TTBS) (10 \times) (pH 7.4), nuclease and protease tested and incubated with secondary antibodies for 3 h at room temperature. Immunoreactive bands were visualized using a chemiluminescence kit (Nacalai Tesque). Signals were analyzed with a ChemiDoc MP System (Bio-Rad, Berkeley, CA, USA). After treatment with XAV939 at three concentrations (0.1, 1, and 10 μM), western blotting was performed to determine the optimal concentration of the Wnt/ β -catenin inhibitor XAV939.

Immunofluorescence staining

BMSCs were seeded in 24-well plates at 4×10^4 cells/mL in 1000 μL normal culture medium. After culturing of 1 day for cell adhesion, the cells were serum starved in osteogenic medium for 12 h and then subjected to irradiation at the optimal LED energy density of 6 J/cm^2 . At 2 h before irradiation, cells were treated with the Wnt/ β -catenin inhibitor XAV939 (10 μM) (Sigma-Aldrich, Saint Louis, MO, USA). After irradiation, the cells were fixed with 4% paraformaldehyde, washed with PBS, and permeabilized with 0.5% Triton X-100 diluted in PBS. Blocking was performed with a 3% bovine serum albumin-phosphate buffer solution (Sigma-Aldrich) diluted in PBS and then incubated overnight at 4°C with diluted primary rabbit antibodies against GSK-3 β , phospho-GSK-3 β , and β -catenin. Immunofluorescence staining was performed using Alexa Fluor 488[®] (Thermo Fisher Scientific). Nuclei were stained using DAPI (Dojindo Laboratory, Kumamoto, Japan). Images were captured using a confocal laser scanning microscope (Zeiss International,

Fig. 1 Effect of high-intensity LED irradiation on the proliferation of BMSCs. Energy densities ranged from 0 to 8 J/cm². The proliferation of BMSCs was suppressed depending on the energy density at **a** 3 h, **b** 24 h, and **c** 48 h, and no significant difference was observed at **d** 72 h. * $P < 0.05$ versus control



Optical and Optoelectronic Technology, Oberkochen, Germany).

Wnt/ β -catenin inhibition

To determine whether the Wnt/ β -catenin signaling pathway was involved in the LED-induced BMSCs osteogenic differentiation and mineralization process, the effects of LED irradiation on immunofluorescence staining, osteogenic differentiation, and mineralization were examined after treatment with XAV939 at the optimal concentration.

Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics Ver. 17 (IBM, Chicago, IL, USA). One-way analysis of variance followed by Tukey's post hoc test was used to determine significance. $P < 0.05$ was considered as significant.

Results

Cell proliferation

The proliferation of BMSCs in normal culture medium after irradiation at energy densities ranging from 0 to 8 J/cm² was measured at 3, 24, 48, and 72 h.

At 3 h and 24 h, BMSC proliferation was suppressed at doses of 6 J/cm² and 8 J/cm², respectively, as compared with the control group (Fig. 1a, b). At 48 h, the proliferation of BMSCs was suppressed at all energy density levels (Fig. 1c). However, no significant differences were observed in

any energy densities at 72 h (Fig. 1d). $P < 0.05$ was considered statistically significant.

Osteogenic differentiation and mineralization

Intracellular ALP production and extracellular calcium deposition were significantly enhanced by 6 J/cm² (30 s) LED irradiation (Fig. 2a–d). OCN secretion was also significantly increased at 2 and 3 weeks by 6 J/cm² irradiations (Fig. 2e). Next, gene expression levels of Runx2 and COL1A1 were assessed by quantitative real-time PCR. Runx2 and COL1A1 mRNA was significantly enhanced by LED exposures of 6 J/cm² at 1 and 2 weeks compared with the control group (Fig. 2f, g). $P < 0.05$ was considered statistically significant.

Irradiation activates the Wnt/ β -catenin signaling pathway

To investigate how irradiation promotes osteogenic differentiation of BMSCs, we determined whether irradiation

Fig. 2 Effects of LED irradiation on osteogenic differentiation and mineralization. **a** ALP staining of BMSCs after LED irradiation (0–8 J/cm²) and cultured for 1 and 2 weeks. **b** The intensity of formed calcified nodules was determined by alizarin red staining at 2 and 3 weeks. **c** ALP activity was measured at 1 and 2 weeks. The amount of ALP was normalized to the amount of DNA to normalize ALP activity. **d** Extracellular calcium deposition was measured at 2 and 3 weeks. **e** Effect of LED irradiation on the production of osteocalcin (OCN) at 2 and 3 weeks. Gene expression of **f** Runx2 and **g** COL1A1 gene at 1 and 2 weeks after irradiation as determined by quantitative real-time PCR. * $P < 0.05$ versus control; scale bar = 200 μ m

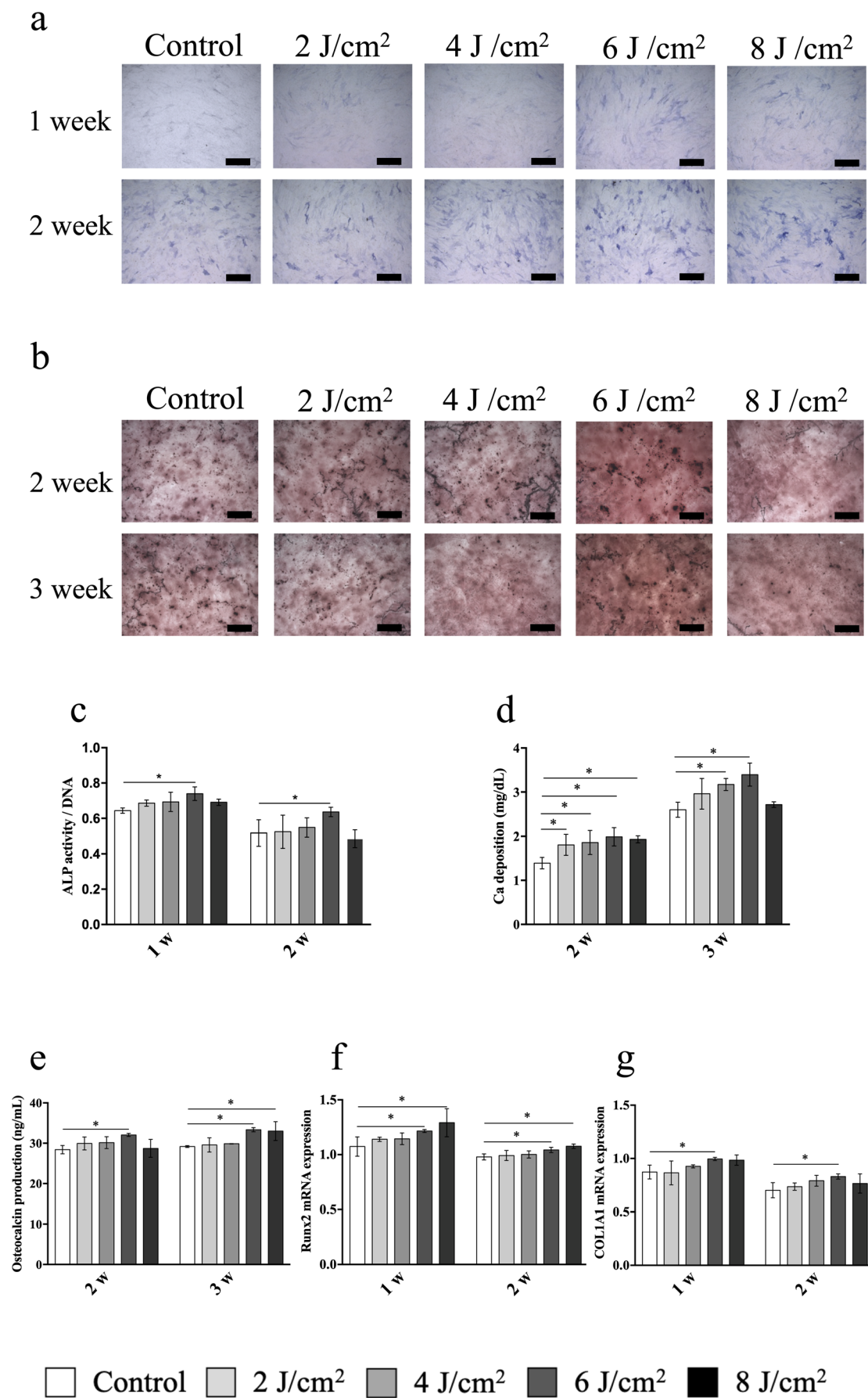
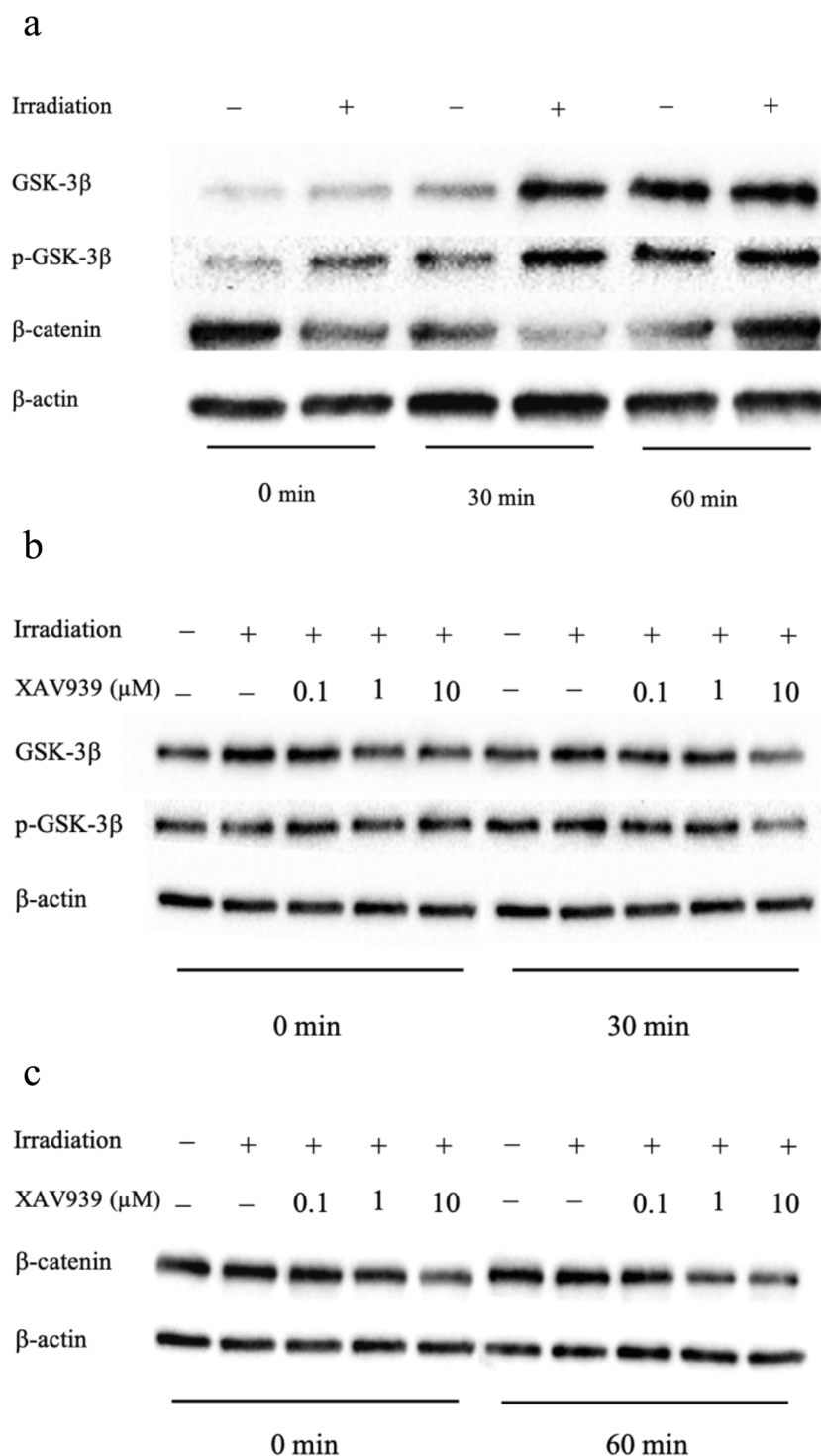


Fig. 3 Effects of LED irradiation on the Wnt/ β -catenin signaling pathway in BMSCs irradiated for 0, 30, 60, and 90 min. **a** BMSCs were irradiated at an energy density of 6 J/cm², and protein expression of GSK-3 β , phospho-GSK-3 β , and β -catenin was evaluated by immunoblotting. **b** Effect of XAV939 (0.1, 1, and 10 μ M) on LED irradiation-stimulated activation of the GSK-3 β and phospho-GSK-3 β for 0 and 30 min. **c** Effect of XAV939 (0.1, 1, and 10 μ M) on LED irradiation-stimulated activation of the β -catenin for 0 and 60 min

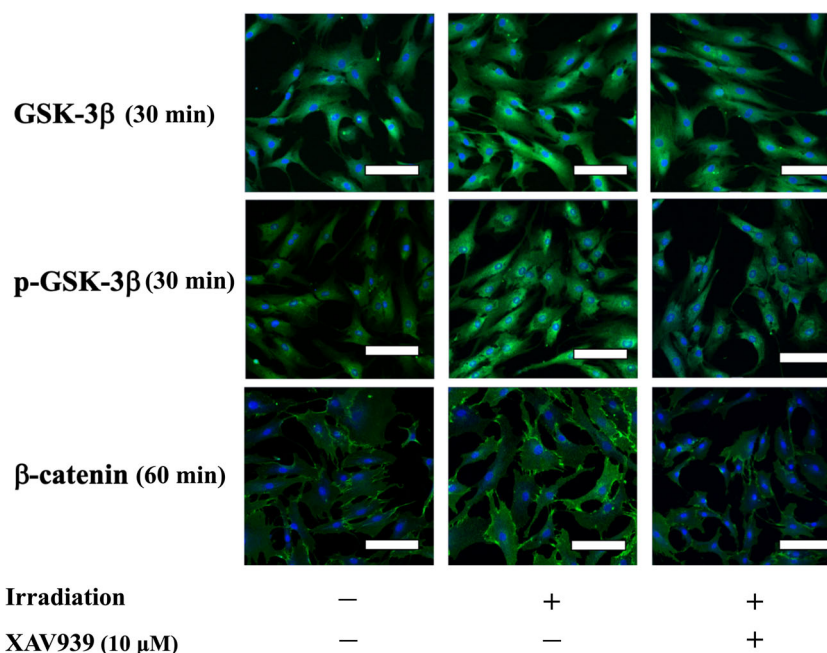


stimulated the Wnt/ β -catenin signaling pathway in BMSCs. In osteogenic differentiation medium, the levels of GSK-3 β and phospho-GSK-3 β were upregulated in irradiated cells at 30 min, and the levels of β -catenin in irradiated cells were upregulated at 60 min compared to the control (Fig. 3a).

Involvement of Wnt/ β -catenin signaling pathway

Finally, to investigate the effect of Wnt/ β -catenin under LED irradiation, irradiated BMSCs were cultured in osteogenic differentiation medium with Wnt/ β -catenin inhibitor XAV939. In western blots, the levels of GSK-3 β and

Fig. 4 Effect of XAV939 (10 μ M) on LED irradiation-stimulated activation of the Wnt/ β -catenin signaling pathway. The fluorescence intensities of GSK-3 β and phospho-GSK-3 β were reduced at 30 min, and that of β -catenin was reduced at 60 min (green fluorescence). Scale bar = 100 μ m



phospho-GSK-3 β were decreased after XAV939 treatments (1 and 10 μ M) at 30 min (Fig. 3b). Moreover, the levels of β -catenin were decreased after XAV939 treatments (1 and 10 μ M) at 60 min (Fig. 3c). In fluorescence staining, the fluorescence intensity of GSK-3 β , phospho-GSK-3 β was inhibited after XAV939 treatment (10 μ M) at 30 min and β -catenin was inhibited after XAV939 treatment (10 μ M) at 60 min (Fig. 4). We also found that XAV939 treatments (1 and 10 μ M) significantly decreased ALP production, mineralization and OCN secretion (Fig. 5a–e). Runx2 and COL1A1 gene expression was increased by LED irradiation after treatment with XAV939 (1 and 10 μ M) at 1 week, which eventually decreased after 2 weeks (Fig. 5f, g). $P < 0.05$ was considered as significant.

Discussion

The results of this study indicated that irradiation by a high-intensity red LEDs had no significant effect on proliferation of human BMSCs after long-term culture, but significantly enhanced the osteogenic differentiation and mineralization of human BMSCs via the Wnt/ β -catenin signaling pathway.

Some studies have shown that the proliferation of stem cells is promoted by lasers [29–31]. Our previous study also indicated that high-intensity red LED irradiation promotes the proliferation of human periodontal ligament stem cells. The study suggested that increasing the ATP concentration in PDLSCs induces intracellular signaling to promote proliferation [18]. Therefore, it appears reasonable to assume that LED light would affect the proliferation of BMSCs. However, in

this study, LED did not exert a significant effect on BMSC proliferation after 72 h; in fact, proliferation decreased within the initial 48 h. It has been reported that the proliferation of murine bone marrow cells is not significantly different compared with the non-irradiated group; however, 647 nm red light enhanced osteogenic differentiation [32]. Peng et al. indicated that 620 nm noncoherent red light decreased cellular proliferation, but it enhanced osteogenic differentiation [27]. The likely reason is that there is a negative correlation between proliferation and osteogenic differentiation in bone marrow stem cells. During cell proliferation, genes involved in the production and deposition of extracellular matrices are expressed then organized bone-specific extracellular matrix is synthesized, which blocks cell proliferation [33]. Therefore, it appears that the significant increase in osteogenic differentiation and mineralization following irradiation was not a consequence of the general increase in cell number, but rather to an increase in the percentage of cells showing mineralization [27].

Considering that regeneration of hard tissue is mainly enhanced by cell osteogenic differentiation [34], the present study investigated the effects of high-intensity LEDs on osteogenic differentiation and mineralization of human BMSCs. ALP, as a marker of osteogenic phenotype, symbolizes the activity of the hard tissue formation process [35]. The non-collagen OCN in the bone matrix is a late marker for osteoblast differentiation [36]. We measured ALP activity, the degree of ALP staining, and OCN secretion in this study and confirmed that the high-intensity LED affected the osteogenic differentiation of BMSCs throughout the early to late stages. Alizarin red staining is used to determine the presence of calcified nodules in the bone matrix [37]; the amount of

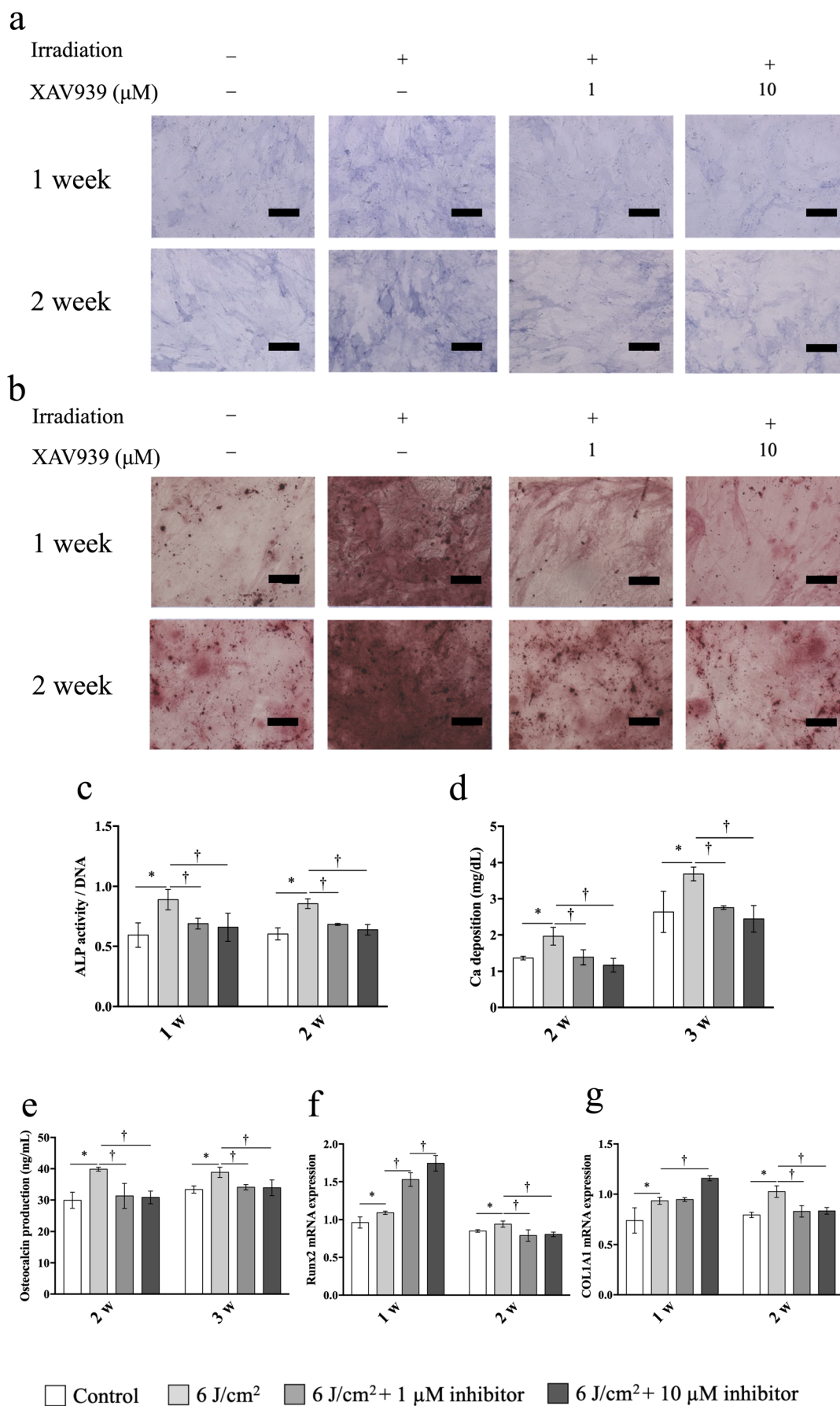


Fig. 5 Effect of XAV939 on LED irradiation-stimulated osteogenic differentiation and mineralization of BMSCs. **a–e** Treatment with 1 μ M and 10 μ M XAV939 reduced 6 J/cm² LED irradiation-induced ALP activity, mineralization, and OCN production. Gene expression of **f** Runx2 and **g** COL1A1 gene was only inhibited by XAV939 after culture for 2 weeks. * $P < 0.05$, control versus irradiation (6 J/cm²); † $P < 0.05$, irradiation (6 J/cm²) versus irradiation+XAV939; scale bar = 200 μ m

calcium was measured using a calcium quantification kit. The results of the current study suggest that high-intensity LED promotes the mineralization of BMSCs.

During osteogenic differentiation progress, Runx2 is one of the essential transcription factors in the early stages [38]. Runx2 further promotes the differentiation of the cells into immature osteoblasts after differentiating into preosteoblasts [39]. The expression of Runx2 induces mesenchymal stem cells to commit to the osteoblastic lineage, differentiating into mature osteoblasts, and terminally differentiating into osteocytes [39]. COL1A1 gene encodes the pro- α 1 chains of type I collagen, which is found in most connective tissues, including cartilage, and plays an important role in bone formation [40]. Our results showed that the high-intensity LED irradiation enhanced the expression of transcription factors necessary for osteogenic differentiation and bone formation.

Canonical β -catenin-dependent Wnt pathway and the non-canonical β -catenin-independent Wnt pathway are two Wnt-mediated major molecular pathways. Both pathways are involved in regulating bone growth by increasing bone formation and promoting the differentiation of preosteoblasts [41–43]. The β -catenin signaling pathway is needed not only for osteogenesis but also for inhibition of bone formation [44]. Han et al. reported that Wnt/ β -catenin signaling is involved in hair growth-promoting effects of 655 nm red light and LED [24]. Hu et al. also confirmed that the Wnt pathway is activated in laser-induced choroidal neovascularization (CNV) models and plays a pathogenic role in CNV [25]. Zhang et al. conducted a study using C3H/HeJ mice and revealed higher expression levels of Lymphoid enhancer-binding factor 1 (Lef1) and β -catenin in the LLLT group, indicating a crucial role of the Wnt/ β -catenin signaling pathway in LLLT [45]. Therefore, we hypothesized that the LED light may activate the Wnt/ β -catenin signaling pathway. We further speculated that high-intensity red LED light could promote the osteogenic differentiation and mineralization of BMSCs by regulating the Wnt/ β -catenin pathway. When the Wnt/ β -catenin pathway is activated, GSK3 β is phosphorylated, and β -catenin accumulates in the cytoplasm and then translocates into the nucleus. Our results showed that irradiation with a high-intensity red LED enhanced the expression of GSK3 β , p-GSK3 β , and β -catenin that are involved in the Wnt/ β -

catenin signaling pathway. Furthermore, these effects were confirmed using XAV939, a small molecule inhibitor of the Wnt/ β -catenin pathway, which selectively inhibits β -catenin mediated transcription by suppressing poly ADP-ribosylation enzymes tankyrase 1 and 2 [46–48]. The results showed that both 1 and 10 μ M XAV939 inhibited the transmission of Wnt/ β -catenin signals, and the trend of the inhibitive effects was more obvious with the increase in the concentration of the inhibitor. Our results also showed that 1 and 10 μ M XAV939 inhibited the osteogenic differentiation and mineralization induced by the high-intensity LED. Although the LED increased Runx2 and COL1A1 gene expression after treatment with XAV939 for 1 week, it was decreased eventually after 2 weeks. We consider that this result was caused by XAV939 inhibiting the transmission of Wnt/ β -catenin signals to halt osteogenic differentiation at the initial stage. Therefore, XAV939 would promote BMSC differentiation into immature osteoblasts, thereby delaying hard tissue regeneration. The above results support that the hypothesis that high-intensity red LED activated the Wnt/ β -catenin signaling pathway that enhanced the osteogenic differentiation and mineralization of human BMSCs.

Cost-effective, low risk, and long life increase the clinical competitiveness of LEDs as a tool for tissue regeneration, and the convenience of operation should also be considered [15, 49]. The LED emitter used in this experiment had a high intensity (intensity 1100 mW/cm²), which means energy output can be completed efficiently. It has been shown that irradiation by a non-coherent red LED light with a wavelength of 620 nm and a intensity of 6.67 mW/cm² enhances osteogenic differentiation of rat mesenchymal stem cells by irradiating the cells for 600 s (4 J/cm²) every 48 h [27]. The high-intensity LED used in our study only required 30 s of exposure time to reach an optimum energy density of 6 J/cm², and irradiation was needed only once for the procedure. Such reductions of irradiation time and frequency make high-intensity red LEDs a promising clinical tool to promote hard tissue regeneration. Our experimental equipment design highly mimics clinical periodontal surgery. High-intensity LED was designed to be similar in size and weight as the dental LED for ease of manipulation and handling. Irradiation was performed from a distance of 40 mm, and the spot size was 4 cm² that would cover an area of two or three teeth. Literature estimates indicate that the penetration depth of laser radiation using wavelengths of 630 to 1100 nm may be up to 50 mm while the depth of 650 nm red LED penetration is about 2–3 mm [50, 51]. In order to make the red LED fully effective, mucoperiosteal flap can be opened to expose the bone surface during periodontal regenerative surgery. The red LED directly irradiates the bone surface and stimulates BMSCs. Adding only 30 s of irradiation can improve the regeneration ability of hard tissue to promote bone healing.

Conclusion

The present study demonstrates that irradiation with high-intensity red LEDs has no significant effect on proliferation of human BMSCs after long-term culture but enhances the osteogenic differentiation and mineralization of human BMSCs through the Wnt/ β -catenin signaling pathway. The limitation of this study is that the only meaningful results were achieved at the cellular level. Further studies are warranted from in vivo and clinical trials to verify these results.

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Author contributions All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Yaru Ruan, Hirohito Kato, Yoichiro Taguchi, Nobuhiro Yamauchi, and Makoto Umeda. The first draft of the manuscript was written by Yaru Ruan and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

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

This article does not contain any studies with human participants or animals performed by any of the authors.

Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

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