Lasers Med Sci (2013) 28:1495–1502
DOI 10.1007/s10103-013-1265-x

ORIGINAL ARTICLE

The effects of minimally invasive laser needle system on suppression of trabecular bone loss induced by skeletal unloading

Chang-Yong Ko · Heesung Kang · Yeonhang Ryu · Byungjo Jung · Hyunsoo Kim · Daewon Jeong · Hong-In Shin · Dohyung Lim · Han Sung Kim

Received: 10 September 2012 / Accepted: 2 January 2013 / Published online: 17 January 2013

Abstract This study was aimed to evaluate the effects of low-level laser therapy (LLLT) in the treatment of trabecular bone loss induced by skeletal unloading. Twelve mice have taken denervation operation. At 2 weeks after denervation, LLLT (wavelength, 660 nm; energy density, 3 J/cm^2) was applied to the right tibiae of six mice (LASER) for 5 days/week over 2 weeks by using a minimally invasive laser needle system (MILNS) which consists of a 100 μm optical fiber in a fine needle (diameter, 130 μm). Structural parameters and histograms of bone mineralization density distribution (BMDD) were obtained before LLLT and at 2 weeks after LLLT. In addition, osteocyte, osteoblast, and osteoclast populations were counted. Two weeks after LLLT, bone volume fraction, trabeculae number, and trabeculae thickness were significantly increased and trabecular separations, trabecular bone pattern factor, and structure model index were significantly decreased in LASER than SHAM ($p<0.05$). BMDD in LASER was maintained while that in SHAM was shifted to lower mineralization. Osteocyte and osteoblast populations were significantly increased but osteoclast population was significantly decreased in LASER when compared with those in SHAM ($p<0.05$). The results indicate that LLLT with the MILNS may enhance bone quality and bone homeostasis associated with enhancement of bone formation and suppression of bone resorption.

Keywords Trabecular bone loss · Minimally invasive laser needle system · Bone quality · Bone formation · Bone resorption

Introduction

Skeletal unloading, such as immobilization and microgravity, causes a rapid and marked bone loss that is associated with a malfunction of bone homeostasis (decreased bone

Chang-Yong Ko and Heesung Kang contributed equally to this work.

C.-Y. Ko
Research Team, Korea Orthopedics and Rehabilitation Engineering Center, Incheon 403-712, Republic of Korea

H. Kang · Y. Ryu · B. Jung · H. S. Kim (✉)
Department of Biomedical Engineering and Yonsei-Fraunhofer medical device lab, Yonsei University,
Yonseidae-gil 1, Maeji, Heungup,
Wonju, Gangwon-Do 220-710, Republic of Korea
e-mail: hanskim@yonsei.ac.kr

H. Kim · D. Jeong
Department of Microbiology and the Aging-associated Vascular Disease Research Center, College of Medicine, Yeungnam University, Daegu 705-717, Republic of Korea

H.-I. Shin
IHBR, Department of Oral Pathology, School of Dentistry, Kyungpook National University, Daegu 700-412, Republic of Korea

D. Lim
Department of Mechanical Engineering, College of Engineering, Sejong University, Seoul 143-747, Republic of Korea

Present Address:
H. Kim
Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

Present Address:
Y. Ryu
Korean National Evidence-based Healthcare Collaborating Agency, Seoul 110-450, Korea

formation and increased resorption) [1–3]. Although pharmacological therapies have been widely used to address such a bone loss, they may induce undesirable side effects because they do not target a specific site of bone loss [4]. Therefore, nonpharmacological therapies, such as physical stimulations like whole body vibration, ultrasound stimulation, and laser irradiation have been suggested [5–9].

Several studies showed that laser irradiation has positive effects on bone regeneration, although the mechanism for effects of laser on bone is not clear. Previous in vitro studies have shown that laser irradiation regulates the proliferation and differentiation of osteoblasts [10–14]. In contrast, laser irradiation reduces the RANKL/OPG mRNA ratio in osteoblasts, thus indicating inhibition of osteoclast differentiation [14]. Therefore, laser irradiation influences bone homeostasis [15]. Moreover, laser irradiation exhibits biomodulation effects by improving mitochondrial activity through an increase in the mitotic process and activation of ATP production [15].

Although several studies have applied a laser irradiation to treatment of bone loss, the results in animals have remained controversial. Renno et al. showed the enhancement of strength in femur of osteopenic animals at 2 months after laser irradiation (830 nm, 100 W/cm^2, 120 J/cm^2) [6, 7]. Diniz et al. suggested the possibility of laser therapy (830 nm, 50 mW/cm^2, 4 J/cm^2) as an adjuvant of bisphosphonate for the treatment of bone loss [16]. However, Muniz Renno et al. reported that there was no difference in bone strength and physical properties of rats with bone loss treated with a combination of laser therapy (830 nm, 100 W/cm^2, and 120 J/cm^2) and exercise when compared with rats subjected to exercise alone [17]. In most of the previous studies, laser was indirectly irradiated to bone via skin surface. This methodology caused a considerable loss of laser energy reaching bone during penetrating biological tissues by reflection and scattering on the boundary of different tissues [18–21].

In order to evaluate the effect of laser irradiation on treatment of bone loss, quantitative study of bone quality, including bone microarchitecture and bone mineralization density distribution (BMDD), is required. However, there were few quantitative studies in which bone quality has been used as a parameter to investigate the effects of laser therapy on bone loss. Because material of identical bones is anisotropic and heterogeneous, the BMDD may be more effective to investigate bone material properties than bone mineral density or bone mineral content [1, 22, 23].

This study was aimed to investigate the feasibility of low-level laser therapy (LLLT) to prevent or treat trabecular bone loss induced by skeletal unloading. We already developed a minimally invasive laser needle system (MILNS) and utilized it to directly irradiate the bone surface percutaneously in order to minimize the loss of laser energy reaching the bone [24]. However, this study focused on the effects on cortical bone loss, but not the effects of trabecular bone loss. Furthermore, there were few longitudinal studies for the effects of a laser irradiation to treatment of bone loss despite the existing individual difference and/or variability in baseline values [5, 25–27]. Recently, a microcomputed tomography (micro-CT) has been known as "gold standard" for assessment of bone status [28], and the in vivo micro-CT has been widely used for longitudinal studies [1, 5, 8]. Therefore, in vivo micro-CT was utilized to perform longitudinal studies in microarchitectural properties and BMDD of trabecular bone. Additionally, quantitative study of the effects of laser therapy on the population of bone cells, namely, osteocytes, osteoblasts, and osteoclasts, was performed through histological analysis.

Materials and methods

Animal preparation

All procedures were performed according to a protocol approved by the Yonsei University of Animal Care Committee (YWC-P102). Twelve virginal ICR mice (6 weeks old, 24.2±0.8 g) were used. The mice were housed under standard conditions (room temperature, 23±2 °C; humidity, 50±10 %) with a 12-h light/dark cycle and allowed to move freely and feed on standard laboratory food and water ad libitum. The mice were subjected to sciatic neurectomy (denervation), which has been one of animal models for mimicking skeletal unloading [1–3], on the right hind limb to induce regional bone loss. Bone loss was verified by alterations in the trabecular bone microarchitecture (55.0 % decrease in bone volume fraction (BV/TV) after 2 weeks of denervation). The mice were then randomly allocated to two groups: LLLT treatment group (LASER; six mice) and non-LLLT group (SHAM; six mice).

Minimally invasive LLLT

Throughout this study, the MILNS previously developed by our group was used [24]. Briefly, a 130-μm-diameter fine needle was used to guide a 100-μm-diameter optical fiber. A diode laser (130 mW, 660 nm; No. ML101J27, ThorLabs, Newton, NJ) was used as a light source. The laser beam was collimated by a collimation lens and then focused onto the optical fiber by an objective lens. At the end of the optical fiber, the optical fiber jacket was removed and the optical fiber core was combined with the fine needle. The optical power output at the end of the fine needle from the diode laser was set to 10 mW just before the irradiation of the bone. The tibia was directly irradiated with the laser (660 nm, 10 mW) for 300 s (energy density, 3 J/cm^2). In each mouse in the LASER group, the right tibia was directly irradiated on bone surface percutaneously by the MILNS at 5 mm distance from the proximal

end of the tibia; this point was marked before the LLLT and re-marked every week using a permanent pen. The SHAM was stimulated by a fine needle without laser. Mice were immobilized by customized restrainer during the LLLT. Mice were irradiated 5 days/week for 2 weeks.

Structural parameter analysis

Tibiae of mice were scanned before LLLT and 2 weeks after LLLT with an in vivo micro-CT (Skyscan 1076, SKYSCAN N.V., Belgium) at a resolution of 18 μm^3 under gas anesthesia (O_2 with 2 % isoflurane).

To investigate the morphological characteristics of trabecular bone, structural parameters (bone volume fraction (BV/TV; in percent), trabeculae thickness (Tb.Th; in millimeters), trabecular separations (Tb.Sp; in millimeters), trabeculae number (Tb.N; 1/mm), trabecular bone pattern factor (Tb.Pf, 1/mm), and structure model index (SMI) were measured from two-dimensional images obtained by CT-AN 1.8.1.4 (SKYSCAN N.V., Belgium). The volume of interest of trabecular bone in metaphysis (1.8 mm in length) was then selected from a 1.8-mm length of bone, located 0.54 mm below the growth plate. In addition, the distribution of trabecular thickness was calculated. These measurements were followed as previous studies [1, 5, 28].

BMDD analysis

It is very important to correct beam hardening error when measuring BMDD by in vivo micro-CT [29]. In a present study, beam hardening effect was corrected by flat-field correction before animal scanning and using correction parameters for beam hardening during the reconstruction procedure. Moreover, a beam filtration may be useful to reduce or remove the low-energy radiation during scanning [30]. Histogram of BMDD was calibrated using two phantoms (0.25 and 0.75 g/mm^3) before measurement. Here, X-ray attenuation coefficient was represented as mineralization because the latter was linearly calculated from the former.

Histological analysis

Mice were killed by cervical dislocation 2 weeks after LLLT. Right tibiae were extracted, and the other soft tissues were removed. The tibiae were fixed in 10 % formalin for 3 days. Next, they were decalcified with 10 % ethylene diamine tetra-acetic acid solution and embedded in paraffin blocks. Five-micrometer-thick sagittal sections of the trabecular in tibiae were then prepared. The sections were stained with hematoxylin–eosin for osteocyte ($1/mm^2$) and osteoblast (1/mm) counting. Osteoclast number (1/mm) was counted on tartrate-resistant acid phosphatase-stained sections. Methylene blue was used for background staining.

Statistical analysis

The structural parameters were analyzed using a two-way repeated-measures analysis of variance, with repeated-micro-CT scanning time as within subject factors (0 and 2 weeks) and a group as between group factors (LASER vs. SHAM). A Student's *t* test was performed to compare the relative variation (1 at 0 week) in structural parameters at 2 weeks after treatment and the number of bone cells between the LLLT and SHAM groups. All descriptive data are expressed as mean±standard error. Statistical analyses were carried out using SPSS 12.0 (SPSS Inc., USA). The significance level was set at $p<0.05$.

Results

Structural parameters

The structural parameters are shown in Table 1. BV/TV and Tb.N in LASER significantly decreased over time ($p<0.05$), whereas Tb.Pf and SMI significantly increased ($p<0.05$). However, in SHAM, BV/TV, Tb.Th, and Tb.N significantly decreased over time ($p<0.05$), whereas Tb.Sp, Tb.Pf, and SMI significantly increased ($p<0.05$). At 2 weeks, there were significant differences in all structural parameters between groups ($p<0.05$); LASER had a higher BV/TV, Tb.Th, and Tb.N and a lower Tb.Sp, Tb.Pf, and SMI compared with those of SHAM.

To compare the structural parameters between the groups, relative variations (based on 0 week before LLLT (the value at 2 weeks/that at 0 week and 1 at 0 week) were calculated (Fig. 1). However, the relative variations for BV/TV and Tb.N were 3.7- and 3.3-fold higher in LASER than SHAM ($p<0.05$), respectively. In LASER, Tb.Th and its distribution were maintained for 2 weeks ($p>0.05$, Fig. 2). However, the percent volumes of thinner trabeculae (0.018~0.088 mm) increased and those of thicker trabeculae (>0.088 mm) decreased over time in SHAM (Fig. 2). Two weeks after LLLT, the relative variation for Tb.Th was significantly higher in LASER than SHAM ($p<0.05$). However, the relative variation for Tb.Sp, Tb.Pf, and SMI was 0.8-, 0.7-, and 0.9-fold lower in LASER than SHAM (all $p<0.05$), respectively.

Such differences of structural parameters were shown in Fig. 3. The trabecular structure in the proximal metaphysis of tibia in LASER was maintained compared with that in SHAM, and new bone formation was observed in the LASER.

BMDD

Before LLLT, the two groups showed no difference in the BMDD (Fig. 4). At 2 weeks after LLLT, the BMDD was maintained in LASER but shifted to lower mineralization and slightly narrower in SHAM.

Table 1 Structural parameters before laser therapy and at 2 weeks after laser therapy

	BV/TV (%)		Tb.Th (mm)		Tb.Sp (mm)		Tb.N (1/mm)		Tb.Pf (mm)		SMI	
	0 week	2 weeks	0 week	2 weeks	0 week	2 weeks	0 week	2 weeks	0 week	2 weeks	0 week	2 weeks
SHAM	8.60±1.08	1.09±0.17*	0.08±0.00	0.07±0.00*	0.43±0.02	0.64±0.04*	1.030.11	0.15±0.02*	21.25±1.42	37.87±1.13*	2.58±0.06	3.03±0.03*
LASER	9.28±0.74	4.82±0.68*, **	0.08±0.00	0.08±0.00**	0.41±0.03	0.49±0.04**	1.10±0.09	0.59±0.08*, **	20.46±0.85	27.49±1.06*, **	2.56±0.04	2.84±0.03*, **

Results are represented as mean±standard error

*$p<0.05$ (vs. 0 week); **$p<0.05$ (vs. SHAM)

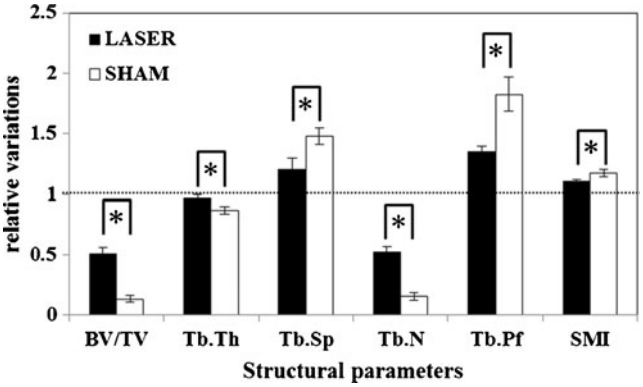

Fig. 1 Relative variations of structural parameters in tibia proximal metaphysis based on 0 week at the start of treatment on trabecular bone. *Dotted line* indicates relative variation (*1*) at the start of treatment. Results are represented as mean±standard error, *$p<0.05$

Histology

Two weeks after LLLT, the density of osteocytes (1/mm^2) and the number of osteoblasts (1/mm) in the LASER (436.0 ±18.2 and 48.0±5.4) was significantly higher than those in the SHAM (280.9±27.5 and 32.3±1.2; $p<0.05$, $p<0.05$; Fig. 5a, b). In contrast, the number of osteoclasts (1/mm) in LASER (2.5±0.2) was significantly lower than that in the SHAM (4.1±0.4) ($p<0.05$; Fig. 5c). More quantitative and thicker trabeculae were observed in LASER than in SHAM (Fig. 5d).

Discussion

Several studies suggested that laser irradiation may be effective for healing or regeneration of defective bones [10–15]. However, the reported effects of laser irradiation on bone loss have been diverse widely [6, 7, 16, 17]. A marked loss of laser energy reaching bone during penetrating biological tissues may be responsible. In order to minimize the loss of laser energy reaching bone, therefore, this

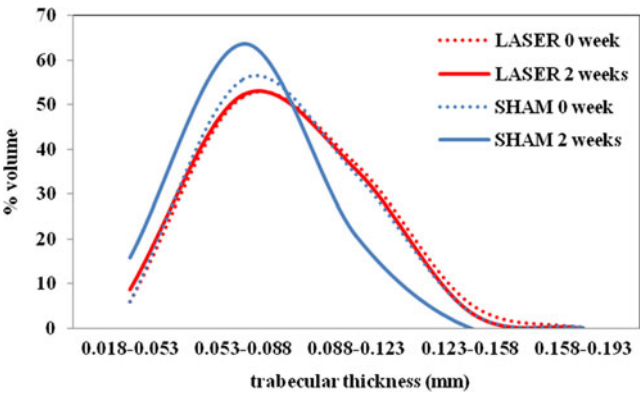

Fig. 2 Distribution of trabecular thickness

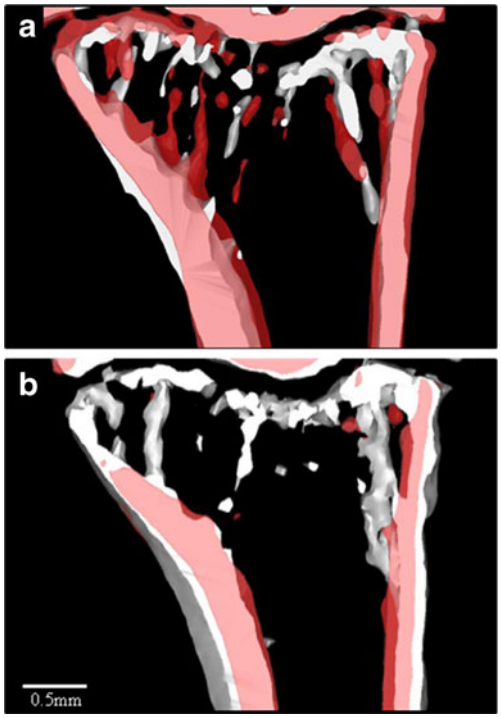

Fig. 3 Overlaid comparison of overlaid bone structure before and after minimally invasive LLLT: **a** LASER and **b** SHAM (*white*, 0 week and *red*, 2 weeks)

study used the MILNS for the treatment or prevention of bone loss induced by skeletal unloading.

Skeletal unloading for 2 weeks resulted in significant bone loss, consistent with previous studies [1–3]. Tibiae with skeletal unloading-induced bone loss were treated with a LLLT system. During the experiments, progressive bone loss occurred in both LASER and SHAM. However, the magnitude or rate of bone loss had a significant difference between LASER and SHAM. At 2 weeks after LLLT, the decrement rates of BV/TV, Tb.N, and Tb.Th and the increment rates of Tb.Sp and Tb.Pf were restrained in LASER when compared with

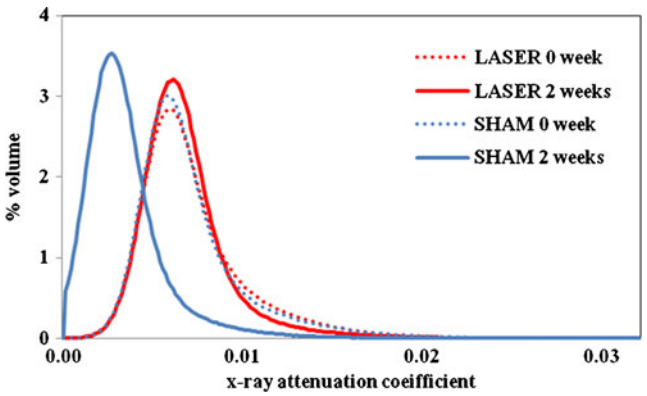

Fig. 4 Histogram of bone mineralization density distribution in tibiae trabecular bone

those in SHAM. These results means that the continuous progress of perforation, thinning, and loss of connectivity of bone induced by skeletal unloading might be diminished by LLLT, suggesting that this treatment modality might improve trabecular bone microarchitectural properties. Moreover, the BMDD in LASER was maintained, whereas that in SHAM was shifted to lower mineralization. These results indicated that LLLT might enhance bone mineralization and suppress alterations in bone turnover, thus preventing alterations in bone homeostasis [22, 23]. In addition, these differences in BMDD might support the above mentioned results of trabecular bone microarchitectural properties. Bone structural properties are strongly correlated to the bone mechanical characteristics [31]. It is also well known that bone mineralization is one of the important determinant factors that influence bone mechanical characteristics [23]. The bone stiffness and strength were decreased at lower mineralization rate while they were increased at higher mineralization rate [32]. As a result, these results suggest that the minimally invasive LLLT with the MILNS might enhance bone qualities such as bone structural properties and bone mineralization, and therefore, leads to reduction in fracture risks.

Skeletal unloading induces alterations in osteoblastogenesis [33, 34] that results in the reduction of lifespan, number, and function of osteoblasts [34, 35], and the increased osteoblast apoptosis [33, 34], leading to decrease in bone formation. Skeletal unloading also induces osteocyte apoptosis and osteoclast recruitment, resulting in an increase in bone resorption [36]. These alterations in bone homeostasis induce a significant bone loss. In this study, a significant bone loss was confirmed during skeletal unloading. Moreover, we also observe an increase in a degree of bone loss overtime (55.0 % decrease in BV/TV after 2 weeks of denervation (before LLLT) and 86.3 % decrease in BV/TV after 4 weeks of denervation (2 weeks after LLLT)). This bone loss is consistent with previous studies [1–3]. Osteocytes are the mechanosensory cells of bone [36, 37]. They also regulate the activities of osteoblasts and osteoclasts, leading to bone loss or gain according to the external stimuli [38]. An increase in osteocyte apoptosis, which occurs after skeletal unloading [36], induces bone loss and bone fragility [39]. In addition, a previous study showed that the rate of remodeling was negatively correlated with the number of osteocytes, particularly more number of osteocytes results in suppression of bone remodeling [40]. Two weeks after LLLT, more osteoblasts and osteocytes were observed in LASER than SHAM. In contrast, the number of osteoclasts was more in SHAM than LASER. Our results suggest that the LLLT might effectively prevent osteoblast and osteocyte apoptosis associated with the suppression of osteoclast

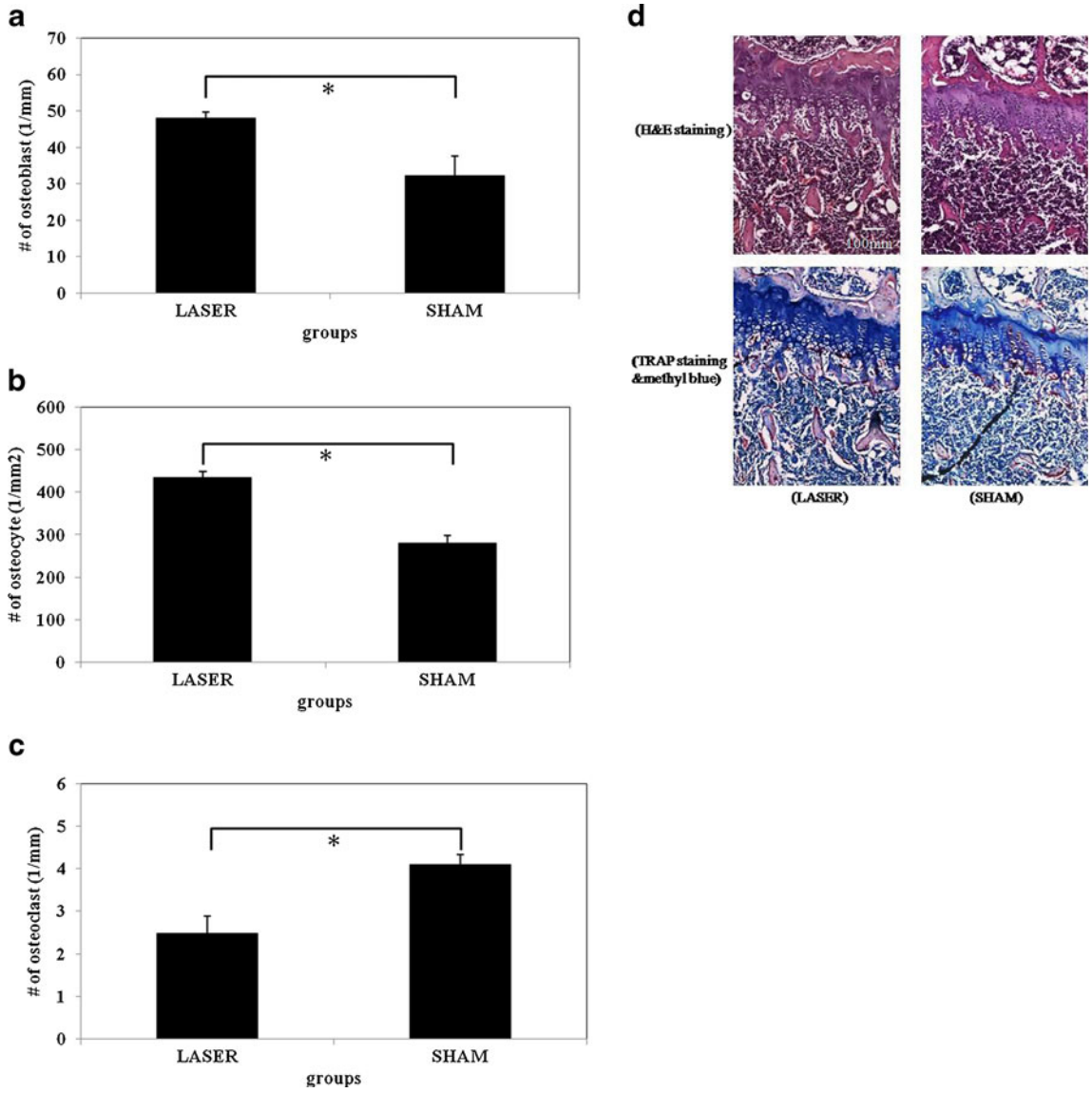

Fig. 5 Histology results; **a** the number of osteoblast, **b** the density (1/mm^2) of osteocytes, **c** the number of osteoclast, and **d** magnified images of a histological section (×40). Results are represented as mean±standard error; *$p<0.05$

recruitment, thereby diminishing the progress of bone loss and fragility. Moreover, the LLLT might suppress alterations in bone turnover, which was supported by the result that BMDD was maintained in LASER.

In previous studies, laser was irradiated on skin surface to treat bone loss. Therefore, this methodology caused a significant loss of laser energy in tissue [18–21]. When a relatively low amount of laser energy reaches bone, laser irradiation may not positively affect bone healing [18, 41, 42]. Although some studies have suggested the use of high-intensity laser in order to overcome this limitation [7, 18], serious biological tissue damage has been induced [43, 44]. In this study, the MILNS was directly applied in order to overcome these limitations of previous studies. Even if the bone was irradiated with a low-intensity laser (3 J/cm^2 of energy density) for a short duration (300 s), an effective prevention of bone loss was achieved.

Conclusions

This study suggested that the laser therapy, particularly using the MILNS, diminished the continuous progress of bone loss and weakness through the enhancement of bone qualities and bone homeostasis. Therefore, the laser therapy may contribute to a reduction of fracture risk due to bone loss. To the best of our knowledge, this study may prove valuable as the first trial to investigate the effects of LLLT with the MILNS on the prevention and treatment of trabecular bone loss induced by skeletal unloading.

Acknowledgments This research was supported by the Leading Foreign Research Institute Recruitment Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (MEST; 2010-00757).

References

1. Ko CY, Seo DH, Kim HS (2011) Deterioration of bone quality in the tibia and fibula in growing mice during skeletal unloading: gender-related differences. J Biomech Eng 133(11):111003
2. Suzue N, Nikawa T, Onishi Y, Yamada C, Hirasaka K, Ogawa T, Furochi H, Kosaka H, Ishidoh K, Gu H, Takeda S, Ishimaru N, Hayashi Y, Yamamoto H, Kishi K, Yasui N (2006) Ubiquitin ligase Cbl-b downregulates bone formation through suppression of IGF-I signaling in osteoblasts during denervation. J Bone Miner Res 21(5):722–734
3. Ito M, Nishida A, Nakamura T, Uetani M, Hayashi K (2002) Differences of three-dimensional trabecular microstructure in osteopenic rat models caused by ovariectomy and neurectomy. Bone 30(4):594–598
4. Warden SJ, Bennell KL, Forwood MR, McMeeken JM, Wark JD (2001) Skeletal effects of low-intensity pulsed ultrasound on the ovariectomized rodent. Ultrasound Med Biol 27(7):989–998
5. Lim D, Ko CY, Seo DH, Woo DG, Kim JM, Chun KJ, Kim HS (2011) Low-intensity ultrasound stimulation prevents osteoporotic bone loss in young adult ovariectomized mice. J Orthop Res 29(1):116–125
6. Renno AC, de Moura FM, dos Santos NS, Tirico RP, Bossini PS, Parizotto NA (2006) Effects of 830-nm laser light on preventing bone loss after ovariectomy. Photomed Laser Surg 24(5):642–645
7. Renno AC, de Moura FM, dos Santos NS, Tirico RP, Bossini PS, Parizotto NA (2006) Effects of 830-nm laser, used in two doses, on biomechanical properties of osteopenic rat femora. Photomed Laser Surg 24(2):202–206
8. Woo DG, Ko CY, Kim HS, Seo JB, Lim D (2010) Evaluation of the potential clinical application of low-intensity ultrasound stimulation for preventing osteoporotic bone fracture. Ann Biomed Eng 38(7):2438–2446
9. Rubin C, Sommerfeldt D, Judex S, Qin YX (2001) Inhibition of osteopenia by low magnitude, high-frequency mechanical stimuli. Drug Discov Today 6(16):848–858
10. Fujihara NA, Hiraki KR, Marques MM (2006) Irradiation at 780 nm increases proliferation rate of osteoblasts independently of dexamethasone presence. Lasers Surg Med 38(4):332–336
11. Ozawa Y, Shimizu N, Kariya G, Abiko Y (1998) Low-energy laser irradiation stimulates bone nodule formation at early stages of cell culture in rat calvarial cells. Bone 22(4):347–354
12. Pinheiro AL, Gerbi ME (2006) Photoengineering of bone repair processes. Photomed Laser Surg 24(2):169–178
13. Stein A, Benayahu D, Maltz L, Oron U (2005) Low-level laser irradiation promotes proliferation and differentiation of human osteoblasts in vitro. Photomed Laser Surg 23(2):161–166
14. Xu M, Deng T, Mo F, Deng B, Lam W, Deng P, Zhang X, Liu S (2009) Low-intensity pulsed laser irradiation affects RANKL and OPG mRNA expression in rat calvarial cells. Photomed Laser Surg 27(2):309–315
15. Pires Oliveira DA, de Oliveira RF, Zangaro RA, Soares CP (2008) Evaluation of low-level laser therapy of osteoblastic cells. Photomed Laser Surg 26(4):401–404
16. Diniz JS, Nicolau RA, de Melo Ocarino N, do Carmo Magalhaes F, de Oliveira Pereira RD, Serakides R (2009) Effect of low-power gallium-aluminum-arsenium laser therapy (830 nm) in combination with bisphosphonate treatment on osteopenic bone structure: an experimental animal study. Lasers Med Sci 24(3):347–352
17. Muniz Renno AC, de Moura FM, dos Santos NS, Tirico RP, Bossini PS, Parizotto NA (2006) The effects of infrared-830 nm laser on exercised osteopenic rats. Lasers Med Sci 21(4):202–207
18. Luger EJ, Rochkind S, Wollman Y, Kogan G, Dekel S (1998) Effect of low-power laser irradiation on the mechanical properties of bone fracture healing in rats. Lasers Surg Med 22(2):97–102
19. Ninomiya T, Hosoya A, Nakamura H, Sano K, Nishisaka T, Ozawa H (2007) Increase of bone volume by a nanosecond pulsed laser irradiation is caused by a decreased osteoclast number and an activated osteoblasts. Bone 40(1):140–148
20. Ninomiya T, Miyamoto Y, Ito T, Yamashita A, Wakita M, Nishisaka T (2003) High-intensity pulsed laser irradiation accelerates bone formation in metaphyseal trabecular bone in rat femur. J Bone Miner Metab 21(2):67–73
21. Nissan M, Rochkind S, Razon N, Bartal A (1986) HeNe laser irradiation delivered transcutaneously: its effect on the sciatic nerve of rats. Lasers Surg Med 6(5):435–438
22. Roschger P, Paschalis EP, Fratzl P, Klaushofer K (2008) Bone mineralization density distribution in health and disease. Bone 42 (3):456–466
23. Boivin G, Farlay D, Bala Y, Doublier A, Meunier PJ, Delmas PD (2009) Influence of remodeling on the mineralization of bone tissue. Osteoporos Int 20(6):1023–1026
24. Kang H, Ko CY, Ryu Y, Seo DH, Kim HS, Jung B (2012) Development of a minimally invasive laser needle system: effects on cortical bone of osteoporotic mice. Lasers Med Sci 27(5):965–969
25. David V, Laroche N, Boudignon B, Lafage-Proust MH, Alexandre C, Ruegsegger P, Vico L (2003) Noninvasive in vivo monitoring of bone architecture alterations in hindlimb-unloaded female rats using novel three-dimensional microcomputed tomography. J Bone Miner Res 18(9):1622–1631
26. Ko CY, Jung YJ, Seo DH, Schreiber J, Lim D, Kim HS (2012) Trabecular Bone Loss in Lumbar Vertebrae and Tibiae following Sciatic Nerve Injury: Correlation between Baseline Bone Quantity (BV/TV) and the Magnitude and Rate of Bone Loss. Int J Precis Eng Manuf 13(9):1705–1708
27. Ko CY, Jung YJ, Park JH, Seo DH, Han P, Bae K, Schreiber J, Kim HS (2012) Trabecular bone response to mechanical loading in ovariectomized Sprague–Dawley rats depends on baseline bone quantity. J Biomech 45(11):2046–2049
28. Bouxsein ML, Boyd SK, Christiansen BA, Guldberg RE, Jepsen KJ, Muller R (2010) Guidelines for assessment of bone microstructure in rodents using micro-computed tomography. J Bone Miner Res 25(7):1468–1486
29. Mulder L, Koolstra JH, Van Eijden TM (2004) Accuracy of microCT in the quantitative determination of the degree and distribution of mineralization in developing bone. Acta Radiol 45 (7):769–777
30. Meganck JA, Kozloff KM, Thornton MM, Broski SM, Goldstein SA (2009) Beam hardening artifacts in micro-computed tomography scanning can be reduced by X-ray beam filtration and the resulting images can be used to accurately measure BMD. Bone 45 (6):1104–1116
31. Hazenberg JG, Taylor D, Lee TC (2007) The role of osteocytes and bone microstructure in preventing osteoporotic fractures. Osteoporos Int 18(1):1–8
32. Ciarelli TE, Fyhrie DP, Parfitt AM (2003) Effects of vertebral bone fragility and bone formation rate on the mineralization levels of cancellous bone from white females. Bone 32(3):311–315
33. Basso N, Jia Y, Bellows CG, Heersche JN (2005) The effect of reloading on bone volume, osteoblast number, and osteoprogenitor characteristics: studies in hind limb unloaded rats. Bone 37 (3):370–378
34. Dufour C, Holy X, Marie PJ (2007) Skeletal unloading induces osteoblast apoptosis and targets alpha5beta1-PI3K-Bcl-2 signaling in rat bone. Exp Cell Res 313(2):394–403

35. Ahdjoudj S, Lasmoles F, Holy X, Zerath E, Marie PJ (2002) Transforming growth factor beta2 inhibits adipocyte differentiation induced by skeletal unloading in rat bone marrow stroma. J Bone Miner Res 17(4):668–677
36. Aguirre JI, Plotkin LI, Stewart SA, Weinstein RS, Parfitt AM, Manolagas SC, Bellido T (2006) Osteocyte apoptosis is induced by weightlessness in mice and precedes osteoclast recruitment and bone loss. J Bone Miner Res 21(4):605–615
37. Skerry TM (2008) The response of bone to mechanical loading and disuse: fundamental principles and influences on osteoblast/osteocyte homeostasis. Arch Biochem Biophys 473(2):117–123
38. Aarden EM, Burger EH, Nijweide PJ (1994) Function of osteocytes in bone. J Cell Biochem 55(3):287–299
39. Tomkinson A, Reeve J, Shaw RW, Noble BS (1997) The death of osteocytes via apoptosis accompanies estrogen withdrawal in human bone. J Clin Endocrinol Metab 82(9):3128–3135
40. Metz LN, Martin RB, Turner AS (2003) Histomorphometric analysis of the effects of osteocyte density on osteonal morphology and remodeling. Bone 33(5):753–759
41. David R, Nissan M, Cohen I, Soudry M (1996) Effect of low-power He–Ne laser on fracture healing in rats. Lasers Surg Med 19 (4):458–464
42. Reddy GK (2004) Photobiological basis and clinical role of low-intensity lasers in biology and medicine. J Clin Laser Med Surg 22 (2):141–150
43. Kreisler M, Daublander M, Willershausen-Zonnchen B, d'Hoedt B (2001) Effect of diode laser irradiation on the survival rate of gingival fibroblast cell cultures. Lasers Surg Med 28(5):445–450
44. Yamaguchi H, Kobayashi K, Osada R, Sakuraba E, Nomura T, Arai T, Nakamura J (1997) Effects of irradiation of an erbium: YAG laser on root surfaces. J Periodontol 68(12):1151–1155