in vivo *28*: 1101-1106 (2014)

Healing Effects and Superoxide Dismutase Activity of Diode/Ga-As Lasers in a Rabbit Model of Osteoarthritis

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Abstract. Background/Aim: Osteoarthritis is a major cause of pain and disability in joints. The present study investigated the effects of differences of wavelengths and continuous versus pulsed delivery modes of low-level laser therapy (LLT) in a rabbit model of osteoarthritis. Comparison of the healing effects and superoxide dismutase (SOD) activity between therapy using diode and Ga-As lasers was our primary interest. Materials and Methods: Simple continuous wave (808-nm diode) and super-pulsed wave (904-nm Ga-As) lasers were used. Osteoarthritis was induced by injecting hydrogen peroxide into the articular spaces of the right stifle in rabbits. The rabbits were randomly assigned to four groups: normal control without osteoarthritis induction (G1), osteoarthritis-induction group without treatment (G2), osteoarthritis induction with diode irradiation (G3), and osteoarthritis induction with Ga-As irradiation (G4). Laser irradiation was applied transcutaneously for 5 min every day for over four weeks, starting the first day after confirmation of induction of osteoarthritis. The induction of osteoarthritis and effects of LLT were evaluated by biochemistry, computed tomography, and histological analyses. Results: The SOD activity in G3 and G4 rabbits at two and four weeks after laser irradiation was significantly higher than that of G1 animals ($p<0.05$). However, there was no significant difference between G3 and G4 animals. Moreover, there were significant differences at two and four weeks between the control and osteoarthritis-induction groups, but no significant difference between G3 and G4 in the computed tomographic analyses and histological findings. Conclusion: These results indicate that diode and Ga-As lasers are similarly effective in healing and inducing SOD activity for LLT applications in a rabbit model of OA.

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Key Words: laser, diode, Ga-As, osteoarthritis, superoxide dismutase.

Osteoarthritis is the most common joint disorder affecting the aging population. It is typically a slowly progressive degenerative disease characterized by damage of the joint cartilage (1). Several treatment options are available to reduce pain, increase function, and reduce symptoms of osteoarthritis. These include non-invasive treatments such as weight control and drug therapy (2). Low-level laser therapy (LLT) is as an alternative treatment for osteoarthritis, but its efficacy needs further supporting evidence. Although the exact mechanisms underlying the development of osteoarthritis on the cellular level, leading from the presence of risk factors to cartilage degeneration, have not been elucidated, current studies focus on oxidative stress as one of the factors involved in the pathogenesis of osteoarthritis (3).

Free radicals that induce oxidative stress are a major cause of cell membrane destruction and tissue damage. They are key in the intermediary metabolism of various biological and pathological conditions (4, 5). Although studies have evaluated different types of physical therapy in joint inflammatory disorders, only a few investigations have assessed the efficacy of LLT in osteoarthritis. This study investigated and compared effects of LLT mediated by different wavelengths and continuous *versus* pulsed delivery modes on osteoarthritis and superoxide dismutase (SOD) activity in a rabbit model of osteoarthritis.

Materials and Methods

Animals and osteoarthritis induction. Thirty-two male New Zealand white rabbits, three months old and weighing 3-3.5 kg, were used for this study. The rabbits were randomly assigned to four groups: normal control group without osteoarthritis induction (G1), osteoarthritis induction without treatment (G2), osteoarthritis induction with simple continuous wave laser (808-nm diode) irradiation (G3), and osteoarthritis induction with super-pulsed wave laser (904-nm Ga-As) irradiation (G4), with eight rabbits in each group. Osteoarthritis was induced by injecting 0.5 ml of 4% hydrogen peroxide (HP) into the articular spaces of the right stifle joint in restrained rabbits, three times weekly for four weeks, using a 23-gauge needle. This study protocol was approved by the Institutional Animal Care and Use Committee of Chungbuk National University (2012-112).

0258-851X/2014 $2.00+.40

Laser irradiation. The rabbits in G1 and G2 did not undergo irradiation of the stifle joint. For the rabbits in G3, diode irradiation was applied transcutaneously for 5 min every day, using a DVL-20 Diode Laser System (Asuka Medical Inc., Kyoto, Japan) for four weeks, starting the first day after confirmation of osteoarthritis induction. This diode laser operates in the near-infrared spectrum at a continuous wavelength of 808 nm, with an output power of 1 W. Ga-As laser irradiation in G4 animals was applied transcutaneously for 5 min every day, using a SCAN-BIO LASER (TS-1003A, TMC, Seoul, Korea) for four weeks, starting the first day after osteoarthritis continuation. This system operates in the near-infrared spectrum at a super-pulsed wavelength of 904 nm, with an output power of 1 W. The machine was set at 100% duty cycle and 2,500 Hz frequency. The total transmitted energy of both lasers to the stifle joint was 6 J.

SOD activity analyses. Blood was collected from the ear vein of restrained rabbits at two and four weeks after laser irradiation. Plasma was obtained by centrifugation of blood at 3000× *g* at 4˚C for 20 min for analysis of the anti-oxidant enzyme SOD. The amount of protein was quantified using the modified Bradford method (6), and the total protein quantity was obtained from each sample. SOD activity was measured using the standard sample provided in the kit to obtain a standard curve, and the percentage inhibition calculated from each sample compared with the standard curve to obtain the calculated value (SOD units) which was then used to calculate SOD unit/g total protein as a measure of SOD activity.

Computed tomography (CT). Both stifles of all rabbits were examined in the craniocaudal and mediolateral projections during the experimental periods. The results were evaluated using the following parameters: increased synovial mass, altered thickness of the joint space, subchondral bone opacity change, subchondral bone cyst formation, altered perichondral bone opacity, perichondral bone proliferation, intra-articular calcified bodies, and mineralization of joint soft tissues (7).

Gross appearance. After euthanasia, the stifle joints of all rabbits were disarticulated, and both femur and tibia were dissected free of muscle and soft tissue at two and four weeks after laser irradiation. Gross appearances of the distal femur and proximal tibia were recorded by digital camera to evaluate changes in cartilage surface. A simple gross morphological grading system was used to evaluate cartilage degradation (8) (Table I).

Histological analysis. Histological evaluation was performed at two and four weeks after laser irradiation. The separated end of the femur was fixed in 10% formalin and was decalcified in a decalcifier (Shandon TBD-1; Thermo, Waltham, MA, USA) for five days. The samples were embedded in paraffin in the usual manner. Serial 4-μm-thick sections were stained with hematoxylin and eosin. A modified Mankin's semiquantitative scoring system (9) was used to quantify changes within the cartilage sections (Table II).

Statistical analysis. The SOD activity is expressed as the mean±standard deviation (SD). The groups were compared using Student's *t*-test and one-way ANOVA to determine statistical significance. Null hypotheses of no difference were rejected if *p*-values were less than 0.05.

Table I. *Scoring of gross morphology in the stifle joint.*

Gross morphology	Score
Non-specific change	0
Subchondral lesion change (reddening, subchondral opaque)	1
Articular cartilage erosion	2
Erosion to subchondral bone	3

Results

SOD activity. The SOD activity in G1 animals was significantly lower than in G2 ones ($p<0.05$). With time, SOD activity decreased in animals of G2, G3, and G4. The SOD activity after laser irradiation in G2, G3 and G4 animals was significantly higher than that in G1 ($p<0.05$). However, there was no significant difference among the groups (Figure 1).

Computed tomography. A smooth normal contour and a bony trabecular shadow were seen on the articular surface of the stifle in normal G1 animals. In G2 rabbits, the contour of the lateral femoral condyle was irregular and joint effusion was observed. The overall damage in G3 and G4 rabbits at four weeks after laser irradiation was slightly improved, but the joint effusion persisted. There was no significant difference between G3 and G4 animals (Figure 2).

Gross findings of the stifle joint. Inflammation and swelling were observed in the right stifle joints of rabbits injected with HP compared to normal healthy rabbits. Grossly, the articular surface was even, smooth and clear, and viscous synovial fluid was seen in G1 animals not injected with HP (Figure 3). In G2 animals, the articular surface was irregular, the lateral femoral condyle slightly eroded and the synovial fluid was less translucent. The degree of deformity in G2 rabbits was higher than that in other groups (Figure 4). The degree of deformity of the articular surface in G3 and G4 animals was lower than in those of G2, although there was no significant difference between G3 and G4 rabbits.

Histological findings. In the control group G1, the normal articular surface was smooth and the matrix and chondrocytes were organized into superficial, mid, and deep zones (Figure 5). In G2 rabbits, the articular cartilage was less cellular and chondrocytes were scattered, with the disappearance of cell nuclei in most layers due to necrosis. These changes were more significant at four weeks and the articular surface was very irregular (Figure 5). The histological features in G3 and G4 animals included focal swelling of cartilage matrix and cartilage hypertrophy. The

Table II. *Gross morphological scoring of articular surface in the stifle joint.*

Structure	Cellularity	Matrix staining	Tidemark integrity	Score
Smooth surface/normal	Normal arrangement	Normal staining	Normal and intact	0
Roughened surface/single crack or area of deamination	Clustering in superficial layer or loss of cells up to 10%	Slight loss of stain	Disrupted	1
Multiple cracks/moderate delamination	Disorganisation or loss of up to 25%	Moderate loss of stain	X	2
Fragmentation in cartilage or severe delamination	Cell rows absent or loss of to 50%	Severe loss of stain	X	3
Loss of fragments	Very few cells present	No stain present	X	4
Complete erosion to tidemark	X	X	X	5
Erosion beyond tidemark	X	X	X	6

necrotic changes of chondrocytes in G3 and G4 animals were less than in G2 (Figure 5). Modified Mankin's scores clearly showed that the structural and cellular changes in the osteoarthritis-affected cartilage markedly increased compared to control cartilage. In addition, the score in G2 was significantly higher than in other groups (p<0.05). However, there was no significant difference between G3 and G4 (Table III).

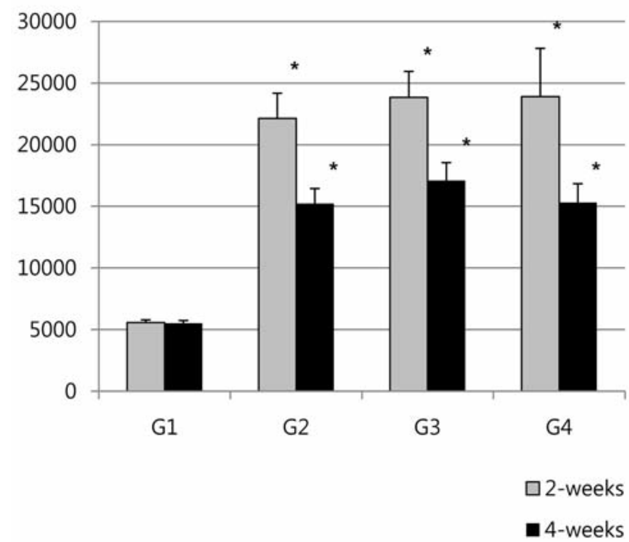

Figure 1. *Superoxide dismutase (SOD) activity after diode (G3) and Ga-As (G4) laser irradiation to the stifle joint. G1: Normal control without osteoarthritis induction, G2: Osteoarthritis induced group without treatment, G3: Diode laser irradiated group, G4: Ga-As laser irradiated group. *p<0.05, Statistically different compared to control G1.*

Discussion

Analyses of the biochemical and histomorphometric measures demonstrated the efficacy of LLT in osteoarthritis treatment. Our study comparing diode and Ga-As lasers found both modes of operation to be equally effective, with no statistically significant difference between lasers. Osteoarthritis is the most common form of arthritis, and is typically a degenerative disease in which the joint cartilage gradually wears away. The prevalence of osteoarthritis increases significantly in advanced age. Despite strong efforts to regenerate the joint cartilage, osteoarthritis is a progressive and irreversible disease.

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species (ROS) and the antioxidant defense system. It is implicated in various pathological conditions including cancer, atherosclerosis, rheumatoid arthritis, osteoarthritis, fibromyalgia, and osteoporosis (10,11). Osteoarthritis occurs in many joints including the knee and hip, and oxidative stress plays an important role in pathogenesis of osteoarthritis (10). HP induces oxidative changes in the articular capsule and produces activated oxygen in the form of $^{\bullet}OH$ and $O_2^{\bullet-}$ due to continuous reaction by superoxide (12). Under normal conditions, chondrocytes exist in an avascular environment with low oxygen supply and display a metabolism adapted to anaerobic conditions. In pathological conditions, changes in the oxidative stress status in the synovial fluid causes pathological acceleration of tissue metabolism and prolonged abnormal transformations in the joint (3, 13). In the present study, the SOD activity was significantly higher in the groups with osteoarthritis compared to the normal group due to the effect of free radicals formed by the introduction of HP. This result was similar to those of published studies (14). SOD is the primary enzyme that controls the biological effects of ROS. The action of SOD is to catalyze the dismutation of superoxide into oxygen and HP. This antioxidant enzyme responds to increased oxidative stress and acts as a ROS scavenger. Therefore, the level of antioxidant enzyme activity reflects the antioxidative status of the antioxidant defense system.

As a technique involving a non-invasive contact medical device, LLT has a long record of successful clinical studies,

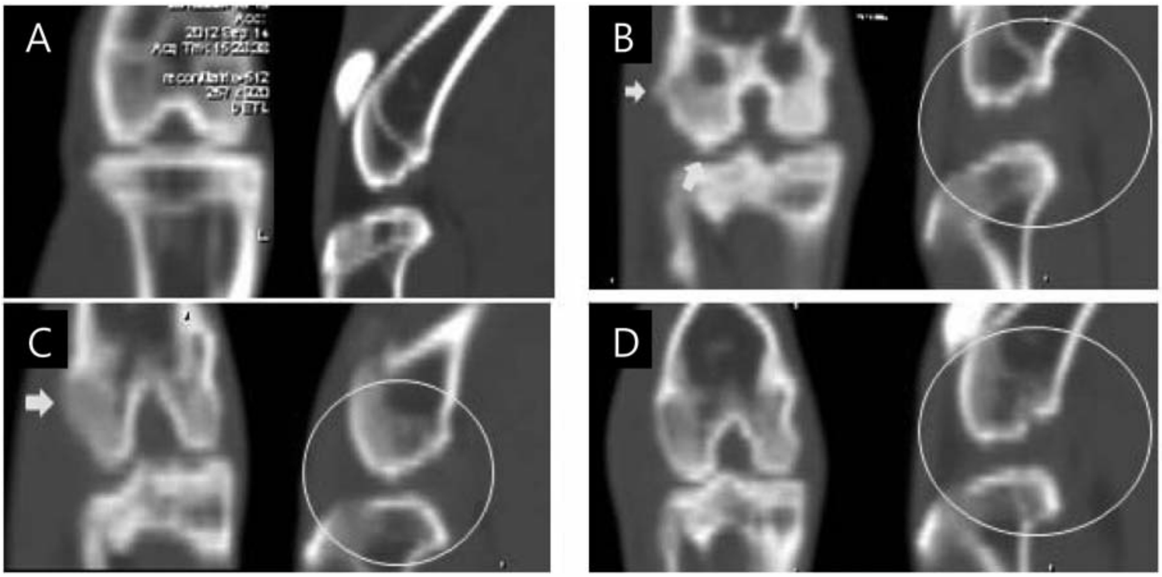

Figure 2. *Computed tomographs at 4 weeks after diode (C) and Ga-As (D) laser irradiation. Note damaged articular surface (arrow) and whitish joint effusion in articular space (circle). A: Normal control without osteoarthritis induction (G1), B: Osteoarthritis induced group without treatment (G2), C: Diode laser irradiated group (G3), D: Ga-As laser irradiated group (G4).*

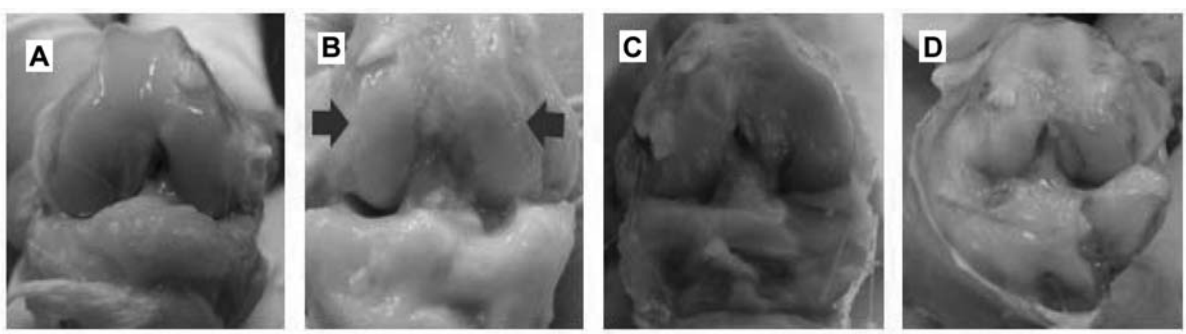

Figure 3. *Gross appearances of articular surface on distal femur and proximal tibia at 4 weeks after diode (C) and Ga-As (D) laser irradiation. Sham control group (A) showed no significant changes. Articular cartilage erosion, significant roughness and opaque change at the joint surface in B (non-irradiation) were observed (blue arrows). Subchondral reddening and subchondral opaque were observed in C and D. A: Normal control without osteoarthritis induction (G1), B: Osteoarthritis-induced group without treatment (G2), C: Diode laser irradiated group (G3), D: Ga-As laser irradiated group (G4).*

demonstrating medical efficacy and safety (15). LLT is effective for rheumatoid arthritis, but not for osteoarthritis (16). In the present study, although we noted beneficial effects of LLT on biochemical and pathophysiological changes, we were unable to detect statistically significant differences in the effects on osteoarthritis from diode and Ga-As laser exposure. We used equivalent total joules, power density, and wattage for both types of LLT regardless of the mode of delivery and wavelength. Several major types of lasers are commonly utilized for LLT. The Ga-As laser is most suitable for super-pulsing. The super-pulsing type of LLT was first developed for the carbon dioxide laser used in high-power tissue ablative procedures. The idea behind it was that by generating relatively short (millisecond) pulses, the laser media could be excited to higher levels than those normally possible in simple continuous wave mode where heat dissipation limits the maximum energy that can excite the media. Another type of pulsed light source used in LLT is the simple continuous wave laser that has a pulsed power supply generated by a laser driver containing a pulse generator (17). There is no consensus on the effects of different frequencies and pulse parameters on the physiology and therapeutic response of the various disease states that are often treated with laser therapy. Aside from

Table III. *Scoring of the structural and cellular changes after laser irradiation to the stifle joint.*

Group	Time after irradiation	
	2 Weeks	4 Weeks
G1	0.00±0.00	0.00±0.00
G2	3.25±0.95*[a,b]	3.75±0.50*[a,b]
G3	3.00±1.15*	2.050±0.57*
G4	3.01±0.95*	2.50±0.57*

G1: Normal control without osteoarthritis induction, G2: Oteoarthritis induced group without treatment, G3: Diode laser irradiated group, G4: Ga-As laser irradiated group. Data are the mean±SD (n=4). *Satistically significant difference by one-way ANOVA among groups ($p<0.05$); [a]statistical difference between G2 and G3; [b]statistical difference between G2 and G4.

safety advantages, pulsed light might simply be more effective than a simple continuous wave. The 'quench period' (pulse OFF times) reduces tissue heating, thereby allowing the use of potentially higher peak power densities than those that could be safely used in a simple continuous wave. Previous studies found Ga-As to be more effective than diode laser (18). However, Ga-As as a solo treatment may be less beneficial than diode in patients requiring nerve regeneration (19). Our study comparing Ga-As and diode lasers found both modes of operation to be equally effective, with no statistically significant difference between the irradiated groups.

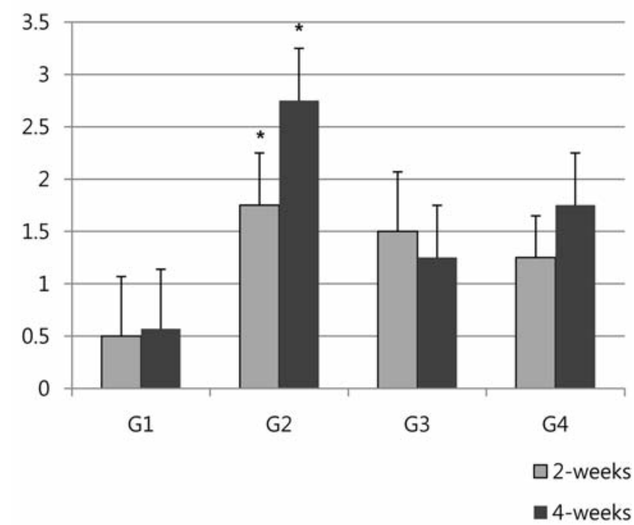

Figure 4. *Scores of the stifle morphology at 2 and 4 weeks after diode (G3) and Ga-As (G4) laser irradiation. G1: Normal control without osteoarthritis induction, G2: Osteoarthritis induced group without treatment, G3: Diode laser irradiated group, G4: Ga-As laser irradiated group. *Statistical significances were tested by one-way ANOVA among groups (p<0.05).*

One of the limitations of the present study was that the irradiation parameters used were not the same. Although there were differences in the mode of operation and the wavelength used (the diode laser used 808-nm and the Ga-As laser used 904-nm), we found the effect of the diode laser

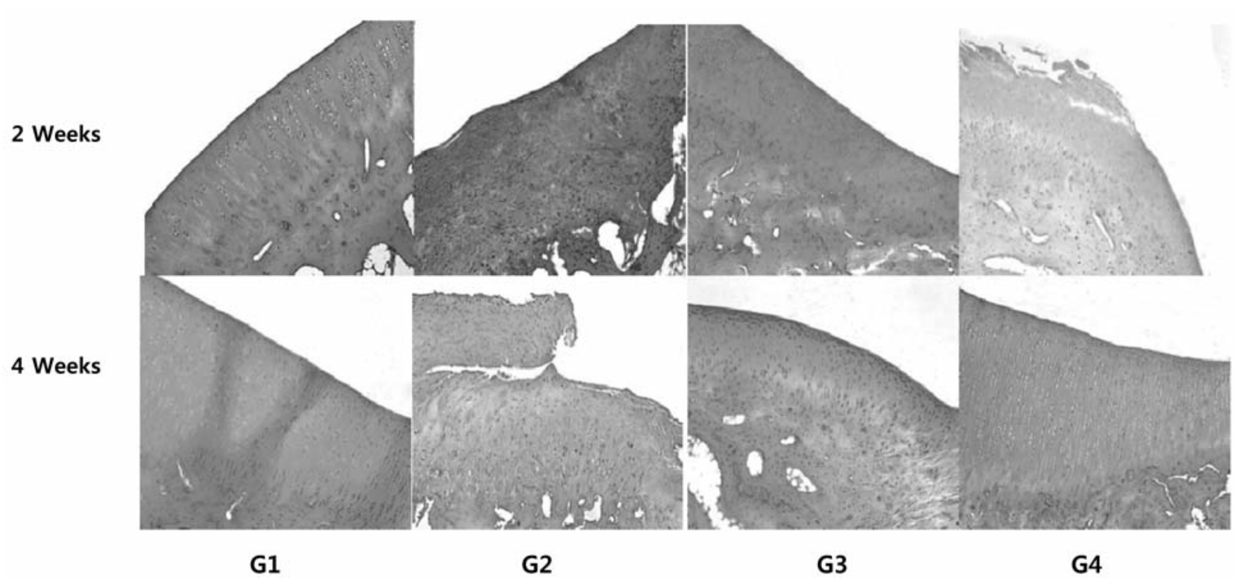

Figure 5. *Microphotos of cartilage obtained from distal femur of rabbits at 2 and 4 weeks after diode and Ga-As laser irradiation. G1: Normal control without osteoarthritis induction, G2: Osteoarthritis induced group without treatment, G3: Diode laser irradiated group, G4: Ga-As laser irradiated group. H&E stain, ×40.*

to be similar to that of Ga-As laser in osteoarthritis treatment. In addition, there was no significant difference in SOD activity between the two lasers. Therefore, both diode and Ga-As light can be used for LLT applications in osteoarthritis treatment.

In conclusion, LLT is effective in treating osteoarthritis based on SOD activity, CT, gross observation, and histopathology, after induction of osteoarthritis with a 4% HP injection in the stifle of normal healthy rabbits. No significant difference was seen between Ga-As and diode laser irradiation, but a significant improvement was seen in both LLT groups compared to the osteoarthritis will group. Considering the complex biology of LLT, there are likely several optimal sets of pulse parameters related to specific wavelengths, chromophores, and other optical properties of tissues. Further research is required to investigate LLT parameters such as wavelength, frequency, duty cycle, peak power, average power, peak power density, average power density, and energy density and their influence on osteoarthritis treatment.

Acknowledgements

This work was supported by a grant from the Next-Generation BioGreen 21 Program (PJ009744), Rural Development Administration, Republic of Korea.

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Received June 30, 2014
Revised August 5, 2014
Accepted August 7, 2014