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Effects of low-level laser therapy on cartilage repair in an experimental model of osteoarthritis

Auswirkungen der Low-Level-Lasertherapie auf die Knorpelreparatur in einem experimentellen Osteoarthritis-Modell

Abstract

Objective: The aim of this study was to evaluate the effects of low level laser therapy (LLLT) on the degenerative process in the articular cartilage after an anterior cruciate ligament transection (ACLT) model in rats.

Methods: Eighty male rats (Wistar) were divided into four groups: 1.) intact control group (CG), 2.) injured control group (ICG), 3.) injured laser-treated group at 10 J/cm^2 (L10) and 4.) injured laser-treated group at 50 J/cm^2 (L50). Animals were divided into 2 subgroups, with different periods of sacrifice (5 and 8 weeks post-surgery). The ACLT was used to induce osteoarthritis (OA) in the knees of the rats. LLLT started 2 weeks after the surgery and it was performed for 15 and 30 sessions, respectively using a 685-nm laser, at 10 and 50 J/cm^2. Qualitative and semi-quantitative histologic, morphometric and immunohistochemistry analyses were performed.

Results: Initial signs of tissue degradation could be observed 5 weeks post-ACLT, evidenced by the decrease of proteoglycan concentration and increase in cartilage thickness of the ICG. After 8 weeks post-surgery, analysis showed a progression of the degenerative processes in the ICG revealed by the increased cellularity and higher TNF-α, IL1-β and MMP-13 immunoexpression. LLLT was able to modulate some of the aspects relating to the degradative process, such as biomodulation of the number of chondrocyte proliferation, prevention of proteoglycan loss, and decrease of MMP-13 immunoexpression.

Conclusion: This study showed that the 685-nm laser irradiation, especially at 10 J/cm^2, prevented features related to the articular degenerative process in the knees of rats.

Keywords: articular cartilage; osteoarthritis; laser therapy; proteoglycan.

Zusammenfassung

Ziel: Das Ziel dieser Studie war es, die Effekte der Low-Level-Lasertherapie (LLLT) auf den degenerativen Prozess im Gelenkknorpel von Ratten nach vorderer Kreuzbanddurchtrennung zu evaluieren.

Methoden: Achtzig männliche Wistar-Ratten wurden in vier Versuchsgruppen unterteilt: 1.) intakte Kontrollgruppe (CG), 2.) verletzte Kontrollgruppe (ICG), 3.) verletzte Laser-behandelte Gruppe bei 10 J/cm^2 (L10) und 4.) verletzte Laser-behandelte Gruppe bei 50 J/cm^2 (L50). Die Tiere wurden in zwei Untergruppen aufgeteilt und entweder 5 oder 8 Wochen nach der Operation eingeschläfert. Die vordere Kreuzbanddurchtrennung wurde verwendet, um in den Kniegelenken der Ratten Osteoarthritis (OA) zu induzieren. Die LLLT begann 2 Wochen nach der Operation und wurde für 15 bzw. 30 Sitzungen bei 10 und 50 J/cm^2 mit einem 685 nm-Laser durchgeführt. Qualitative und semi-quantitative histologische, morphometrische und immunhistochemische Analysen wurden durchgeführt.

Ergebnisse: Erste Anzeichen von Gewebeabbau wurden 5 Wochen nach der vorderen Kreuzbanddurchtrennung beobachtet und durch die Abnahme der Proteoglycan-Konzentration und die Erhöhung der Knorpeldicke in der verletzten Kontrollgruppe (ICG) belegt. Acht Wochen nach der Operation zeigte sich in der ICG ein Fortschreiten der degenerativen Prozesse durch eine erhöhte Zellularität und eine höhere TNF-α-, IL1-β- und MMP-13-Immunexpression. Mittels LLLT war es möglich, einige der mit dem Abbauprozess in Zusammenhang stehende Aspekte, wie die Proliferationsrate der Chondrozyten, die Vermeidung des Proteoglycan-Verlustes und die Abnahme der MMP-13-Immunexpression zu modulieren.

Fazit: Die vorliegende Studie hat gezeigt, dass eine 685 nm-Laserbestrahlung, vor allem bei 10 J/cm^2, Vorgänge verhindern kann, die zu degenerativen Prozessen in den Kniegelenken von Ratten führen.

Schlüsselwörter: Gelenkknorpel; Osteoarthritis; Lasertherapie; Proteoglycan.

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1   Introduction

Osteoarthritis (OA) is a degenerative joint disease characterized by progressive destruction of articular cartilage and reactive new bone formation at the joint surface and surrounding areas [1, 2]. It is the most frequent cause of disability in the United States and afflicts both non-weight-bearing and load-bearing joints, especially in the medial compartment of the knee [3]. The degenerative changes are thought to be due to cartilage metabolic dysfunction in joints that support body weight [3–5]. OA leads to joint pain and dysfunction and it affects more people than any other joint disease. In most instances, articular degeneration develops in the absence of an identifiable cause. However increasing age, excessive joint loading and joint abnormalities increase the risk of developing OA [6].

Current methods of treatment are based in the early or intermediate stages on conservative symptomatic treatments such as nonsteroidal anti-inflammatory drugs and physical rehabilitation, and in the later stages, on arthrodesis or total-joint arthroplasty [7]. However, due to the high prevalence of OA in society associated with the limited regenerative capacity of cartilage, it is clear that it is important to develop innovative clinical approaches and reliable techniques to stimulate growth of new tissue to treat degenerative diseases and trauma [8].

In this context, a significant body of evidence has now been accumulated which demonstrates that low-level laser therapy (LLLT) is not only effective in reducing post-injury inflammatory processes, but also in accelerating soft tissue healing and stimulating the formation of new blood vessels which can contribute to cartilage repair [9–12]. The action of LLLT is based on the absorption of the light by tissue which generates a series of modifications in cell metabolism. When LLLT is applied to tissue, the light is absorbed by chromophore

photoreceptors located in the cells. Once absorbed, the light can modulate cell biochemical reactions and stimulate mitochondrial respiration with the production of molecular oxygen and ATP synthesis [13]. These effects can increase the synthesis of DNA, RNA and cell-cycle regulatory proteins, therefore promoting cell proliferation [12, 13].

LLLT has a stimulatory effect on cartilage tissue and it can both increase cell proliferation and is effective in the treatment of cartilage damage in the presence of degenerative diseases [7, 8, 14]. In *in vitro* studies, Kushibiki et al. [15] and Wong et al. [8] demonstrated that LLLT stimulates the chondrocyte proliferation and increases the expression levels of chondrogenic mRNA in prechondrogenic cells. Furthermore, in arthritic-induced animal models, it was demonstrated that LLLT was able to decrease the level of cartilage damage and producing a better tissue structure, by biomodulating the inflammatory process and reducing swelling [14, 16–18]. Recently, Lin et al. [18] affirmed that 810-nm LLLT can improve cartilage structure, prevent articular cartilage degradation and decrease the expression of caspase-3 in the osteoarthritic knees of rats. Da Rosa et al. [12], comparing the effects of 660- and 808-nm lasers in an experimental model of OA, observed that laser therapy, especially at 808 nm, stimulated angiogenesis and reduced the formation of fibrosis. Although LLLT has positive effects on tissue regeneration, limited evidence is available which demonstrates the effects of this therapeutic approach on cartilage repair. Moreover, the use of a wide range of doses by different authors and the lack of standardized experimental conditions makes it difficult to compare published results. Therefore, before LLLT can be used with confidence as a treatment modality within the clinical setting, it is necessary to investigate the effects of this therapy at a cellular level to determine its safety and efficacy. Based on the stimulatory effects of LLLT on tissues, it was hypothesized that this therapy could modulate the degenerative process in the knees of induced osteoarthritis in rats, thus providing a treatment with additional advantages for clinical use. In this context, our aim was to investigate and to compare the effects of different laser parameters in the experimental model of OA in order to establish an ideal treatment protocol to be used in the clinical setting. To start this investigation, we performed an initial study exploring the effects of infrared laser irradiation at 830 nm on the degenerative process in the articular cartilage after an anterior cruciate ligament transection (ACLT) model in rats [19]. Based on the promising results found in this previous work [19], the present study intended to progress the investigation of the effects of other wavelengths (in this case a red wavelength – 685 nm) in the same experimental setting (OA model and methodology) to compare the results of both studies. Therefore the present study was performed at the same two different fluencies (10 J/cm^2 and 50 J/cm^2) on cartilage

damage in an OA experimental model in the knees of rats. Cartilage tissue response was evaluated both histologically and by an immunohistochemistry analysis after 5 and 8 weeks post-surgery.

2 Methods

2.1 Experimental groups

This study was approved and conducted in accordance with the Animal Care Committee guidelines of the Federal University of São Carlos (CEP 040/2010). Animals were maintained at 19–23°C on a 12:12 h light-dark cycle in the Animal Experimentation Laboratory of the Federal University of São Carlos. Rats were housed in plastic cages and had free access to water and standard food.

Eighty male Wistar rats (weighing 300±20 g, 12–13 weeks old) were randomly divided into 4 groups (n=20): 1.) intact control group (CG), 2.) injured control group (ICG), 3.) injured laser-treated group at 10 J/cm^2 (L10) and, 4.) injured laser-treated group at 50 J/cm^2 (L50). The animals were further divided into 2 subgroups, with different periods of sacrifice (5 and 8 weeks post-surgery).

2.2 Anterior cruciate ligament transection

The animals were submitted to general anesthesia induced by intra-peritoneal injection of xylazin (Syntec®, 20 mg/kg body weight) and ketamine (Agener®, 40 mg/kg body weight) and subjected to ACLT of the left hind paw. The left knee was shaved and sterilized. The joint cavity was approached by a lateral parapatellar incision, the patella was dislocated and the anterior cruciate ligament was transected. The anterior drawer test was performed by the surgeon and an observer to verify the success of the surgery procedure [19, 20]. The incision was closed in layers and antiseptically treated. The animals were further observed for signs of pain, infection and proper activity. The rats were given the appropriate postoperative care and allowed free activity in individual cages.

2.3 Laser treatments

Laser treatment started 2 weeks after surgery and it was performed for 15 and 30 sessions, for each subgroup, using the following protocol: 5 consecutive days of irradiation at intervals of 2 days, for 3 and 6 weeks, respectively. LLLT was applied at 2 points (on the medial and lateral sides of the joint), using the punctual contact technique. A low-energy gallium-aluminum-

arsenate (GaAlAs) laser (Thera Lase; DMC, São Carlos, São Paulo, Brazil) was used at 685 nm in continuous wave mode, with a 0.028 cm^2 spot area, a power output of 30 mW, and fluencies of 10 J/cm^2 (irradiation time of 10 s, energy per point 0.3 J) and 50 J/cm^2 (irradiation time of 47 s, energy per point 1.4 J). On the respective days, the animals were euthanized individually by carbon dioxide asphyxia. Knee joints were removed for analysis.

2.4   Sample preparation

After harvesting, the specimens were fixed in 4% formaldehyde for 2 days, followed by decalcification in 4% EDTA. The specimens were divided into two pieces, using a blade at the mean point between both condyles and perpendicular to the articular surface. Samples were embedded in paraffin blocks and histological sections were prepared. Thin sections (6 µm) were prepared in the sagittal plane, starting from the medial margin of the joint using a microtome (Leica RM2145, Germany). Samples were stained with hematoxylin and eosin (HE; Merck, Germany), Safranin-O (Merck, Germany) and Picro-sirius Red (Merck, Germany). Moreover, an additional 3 sections were prepared for immunohistochemical analysis.

2.5   Histological descriptive analysis

Histopathological alterations in the articular cartilage were evaluated by 2 blinded observers. For descriptive analysis, the samples were stained with HE to evaluate cartilage structure, the number of cells and cellular organization. The specimens were examined using light microscopy (magnification, 100×) (Leica Microsystems AG, Wetzlar, Germany).

2.6   Semi-quantitative analysis

A modified Mankin questionnaire [21] was used as a histopathological grading system to assess the cartilage damage (Table 1) using the HE and Safranin-O stained samples for the cellularity and proteoglycans analyses, respectively.

The Mankin questionnaire is a widely used score which works as a grading system to evaluate the progression of OA and it was first presented by Mankin et al. [22]. This system is based on a score that evaluates cellular changes and architectural modifications (erosion, vessel penetration through tidemark) in the presence of OA. Although in the following years this method was adapted and modified by some researchers, it is still one of the most current methods for OA histopathologic assessment [23].

At least 3 sections from each specimen were examined using light microscopy (magnification, 100×) (Leica Microsystems AG, Wetzlar, Germany). Two experienced observers performed the scoring in a blinded manner.

2.7   Morphometric analysis

The morphometric study was carried out using one randomized slide stained with HE. The cartilage thickness and number of chondrocytes in each area were quantitatively scored using the computer-based image analysis software (AxioVision 3.1; Carl Zeiss, Oberkochen, Germany). To count the number of chondrocytes, 3 areas of 80.000 μm^2, at the anterior, central and posterior region of each slide were chosen. Within each area, cells were marked and the chondrocytes' average was calculated. Thickness was also measured in 3 regions, one central and 2 lateral (300 mm left and right from the first region) from the subchondral bone to articular surface [21]. Two experienced observers performed the scoring in a blinded manner [21].

2.8   Collagen analysis

Histological sections stained for the Picro-sirius polarization method were viewed under polarized light (Leica, Germany) to assess the collagen organization in the cartilage tissue [24–27]. This method allows an indirect evaluation of the stage of bone matrix organization based on the birefringence of the collagen fiber bundles after staining with Picro-sirius Red.

Similarly to the morphometric analysis, 3 areas of 80.000 μm^2 (anterior, central and posterior region) of each slide were chosen to quantify the amount of collagen using the software Image J (Version 1.45; National Institutes of Health, Bethesda, USA). By means of the software it is possible to quantify the brightness of birefringence by calculating the intensity in "pixels" as the color of collagen fibers stained with Picro-sirius Red and viewed with polarized light depends upon fiber thickness: if the fiber thickness increases, the color changes from green to yellow to orange to red.

Consequently, in each field, an indirect evaluation of the total collagen fibers' organization based on the birefringence of the collagen fiber bundles after staining with Picro-sirius Red was performed. Two experienced observers performed the scoring in a blinded manner [28].

2.9 Immunohistochemistry

Paraffin was removed with xylene from serial sections, rehydrated in graded ethanol, and then pre-treated in a microwave oven with 0.01 M citric acid buffer (pH 6) for three cycles of 5 min each at 850 W for antigen retrieval. The material was pre-incubated with 0.3 % hydrogen peroxide in phosphate-buffered saline (PBS) solution for 5 min for inactivation of endogenous peroxidase and then blocked with 5% normal goat serum in PBS solution for 10 min. The specimens were then incubated with primary antibodies: tumor necrosis factor (TNF-α) (polyclonal rabbit anti-rat, ab6671; abcam, Cambrige, MA, UK), interleukin-1 (IL-1β) (polyclonal rabbit anti-rat, sc-7884; Santa Cruz Biotechnology, California, USA) and metalloproteinase 13 (MMP-13) (polyclonal rabbit anti-rat, ab75606; abcam, Cambrige, MA, UK). The tissue sections were deparaffinized, rehydrated, and incubated with prepared 30% hydrogen peroxide diluted in PBS for 30 min. This was followed by application of biotin-labeled secondary antibody (ABC kit Cat. No. PK-6200; Vector Laboratories, Burlingame, CA, USA) at 1:5 dilution for 30 min. Colorimetric detection followed with a diaminobenzidine substrate (DAB substrate kit Cat. No. SK-4100; Vector Laboratories, Burlingame, CA, USA) and HE. For a negative control, the primary antibody was omitted and PBS was applied alone. Digital images of the 100× magnification were captured by optical microscopy. Brown marked cells was considered positive for TNF-α, IL1-β and MMP-13 expression.

2.10 Immunohistochemical semi-quantitative analysis

TNF-α, IL-1β and MMP-13 immunoexpression was analyzed by the percentage of immunopositive cells in control and experimental animals. A total of 1000 cells were evaluated in 5 fields at 400× magnification. These values were used as labeling indices.

2.11 Statistical analysis

The normality of the distribution of all variables was verified using the Shapiro and Wilk's W-test. For the variable that exhibited normal distribution, comparisons among the groups were made using one-way analysis of variance (ANOVA) with post hoc Tukey's test. For the variable that exhibited non normal distribution, the Kruskal-Wallis test was used. STATISTICA version 7.0 was used to carry out the statistics analysis. Values of $p<0.05$ were considered statistically significant.

3   Results

3.1   General findings

Neither postoperative complications nor behavioral changes were observed. The rats returned rapidly to their normal diet and showed no loss of weight during the experimentation. None of the animals died during the experiment and no infection in the surgical site was observed.

3.2   Descriptive analysis

Histopathological analysis revealed that after 5 weeks, the intact control group 'CG' presented normal tissue structure, with absence of fibrillation and moderate presence of chondrocytes organized in a parallel arrangement in the superficial region and in columns in the intermediate region (Figure 1A). The injured control group 'ICG' presented initial signs of degradation, with a slight fibrillation and irregularities in the articular surface and moderate presence of chondrocytes cells, with a slight disorganization of the cell columns (Figure 1C). In the laser-treated group, at 10 J/cm^2 (L10), histology revealed a slight fibrillation at the articular surface, with moderate presence of cells in a normal orientation (Figure 1E). Similar findings were observed in the laser-treated groups, at 50 J/cm^2 (Figure 1G).

After 8 weeks, CG presented normal structure of cartilage (Figure 1B). ICG showed intense fibrillation, surface irregularities and hypercellularity with disorganization of the chondrocytes (Figure 1D). In the L10 group, a slight presence of fibrillation and irregularities were observed with moderate presence of cells in a similar orientation compared to the intact group (Figure 1F). Furthermore, the L50 group presented slight disorganization of the columns of chondrocytes with intense presence of cells and moderate presence of fibrillation in the articular surface (Figure 1H).

3.3   Semi-quantitative analysis

Five weeks post-surgery, statistical analysis revealed that similar findings were found for all groups in the semi-quantitative evaluation of cellularity (Figure 2A). With increasing time, 8 weeks after post-surgery, the number of cells in the injured control group 'ICG' was statistically increased compared to the intact control group 'CG' (Figure 2A). Laser treatment, at both fluencies, was able to modulate cell proliferation in response to the degenerative process (p=0.00899 and p=0.00767 for L10 and for L50 vs. CG, respectively).

The results of the proteoglycans analysis showed that in the first period analyzed, the injured groups (treated and non-treated: ICG, L10 and L50) showed a significant loss of proteoglycan

compared to CG (Figure 2B). Eight weeks post-surgery, the loss of proteoglycan presented by ICG was significantly lower compared to CG (p=0.00036). Statistical analysis revealed that laser treatment, at 10 J/cm^2, presented a similar amount of proteoglycan compared to intact animals (CG) (p=0.23). Furthermore, LLLT, at 50 J/cm^2 was not able of preventing proteoglycan loss and showed no difference compared to ICG (p=0.094) (Figure 2B).

3.4   Morphometric analysis

At 5 weeks, similar findings were observed for all groups in the morphometric analysis of cellularity (Figure 3A). After 8 weeks, the degenerative process in the cartilage tissue continued its development in the non-treated injured (ICG) animals, revealed by the statistical difference between the both control groups CG and ICG (p=0.00365). Laser therapy at both fluencies, modulated cell proliferation, although no statistical difference could be observed between the treated groups and the intact control group 'CG' (p=0.0747 and 0.098 for L10 and L50, respectively) (Figure 3A). In addition, all injured groups (treated and non-treated: ICG, L10 and L50) showed a significantly higher cartilage thickness compared to CG, for both periods analyzed (Figure 3B).

3.5   Collagen fibers

Similar findings were found for all groups in the first and the second period analyzed (5 and 8 weeks post-surgery) for the evaluation of collagen fibers (Figure 4).

3.6   Immunohistochemistry

At 5 weeks post-surgery, the statistical analysis showed that similar findings were presented for all groups in TNF-α, IL1-β and MMP-13 immunoexpression (Figure 5A–C).

With increasing time post-surgery (8 weeks), all injured groups (ICG, L10 and L50) presented statistically higher immunoexpression of TNF-α and IL1-β compared to the intact control group 'CG' (Figure 5A and B). Moreover, in the second experimental period, MMP-13 immunoexpression of ICG was statistically higher compared to the CG and laser-treated groups, at both fluencies (Figure 5C).

4   Discussion

The present study investigated the effect of the 685-nm laser, used at two different fluencies (10 and 50 J/cm^2) on cartilage repair using an experimental model of OA in the knees of rats. The results demonstrated that initial signs of tissue degradation could be observed 5 weeks post-ACLT, with a decrease of proteoglycan concentration and an increase in cartilage thickness of the ICG. At this set point, laser therapy did not have any effect on the degenerative process. After 8 weeks post-surgery, analysis showed a progression in the degenerative process in the injured non-treated groups, revealed by an increase of cellularity and proteoglycan, increased thickness and higher TNF-α, IL1-β and MMP-13 immunoexpression. Laser therapy was able to modulate some of the aspects related to the degradative process, such as the decrease in the number of chondrocytes, prevention of proteoglycan loss and a decrease in MMP-13 immunoexpression.

The high prevalence of degenerative diseases (including OA), highlighted the importance of the development of improved therapeutic strategies [3, 12]. In this context, experimental and clinical studies have investigated the effects of LLLT on cartilage in the presence of OA [17, 18, 29]. Some authors have demonstrated that laser therapy is able to reduce the formation of fibrosis, to stimulate neoangiogenesis and produce anti-inflammatory effects in experimental OA induced in rats [12, 30]. Also, clinical studies showed that LLLT is able to improve joint function and to stimulate pain relief in patients with OA of the knees [31].

A series of molecular and cellular modifications is associated with the progression of OA, including the increased metabolic rate of chondrocytes and accelerated cell proliferation in an attempt to recover the damaged tissue [31]. This fact leads to an abnormal increase in the number of cells, cellular disorganization and cell death by apoptosis [23, 32–34]. Also, proteoglycan loss in the cartilage is another abnormality often observed in degenerative processes and appears before macroscopic signs of deterioration of the articular surface [35]. In the present study, LLLT (especially at 10 J/cm^2) biomodulated cell metabolism and prevented proteoglycan loss, may positively interfere with the delay of the evolution of the degenerative process in articular cartilage. These results are in agreement with Gottlieb et al. [36] and Lin et al. [37], who also observed an increased amount of proteoglycan and chondroitin sulfate in osteoarthritic cartilage of rabbit and rats, respectively.

Similarly, the increase of thickness of articular cartilage is related to the initial phase of cartilage injury and it is marked by the action of inflammatory cytokines and increased chondrocyte metabolic rate to try to prevent the degradation of the tissue [38–40]. In the

present study, LLLT was not able to modulate the increase of cartilage thickness related to the process of OA, at both periods analyzed. This phenomenon may be related to the higher metabolism found in the chondrocytes in the laser-treated animals, which could result in an increased synthesize extra-cellular matrix thereby providing better structural organization of the tissue. Bayat et al. [7] reported, in a morphometric analysis of thickness in an experimental model of OA in rabbits, a higher thickness in the laser-treated animals compared to the control group.

Several authors have affirmed that OA articular cartilage displays fewer organized networks and that collagen content falls in advanced OA compared to early OA articular cartilage, especially type II [21, 28, 41–43]. In this study, the Picro-sirius polarization method was not able to show any collagen modification by comparing the results found in the intact and injured animals. It could be that the experimental periods analyzed were insufficient to induce changes in the collagen fibers of articular cartilage.

Additionally, although OA is not conventionally considered as an inflammatory disease, a series of inflammatory mediators are expressed during the disease progression [10, 44]. Many biochemical pathways are activated within joint tissues during the course of OA, especially IL-1β and TNF-α. These cytokines induce chondrocytes to produce proteases to drive catabolic pathways, inhibit matrix synthesis and promote cellular apoptosis [44]. Also, the degradation of articular cartilage matrix is related to the increase of collagenases and matrix metalloproteinase, mainly MMP-1, -8 and -13 [45–47]. Increased expression of inflammatory markers was found in the control animals, mainly at 8 weeks, indicating the progression of the course of the disease and the development of the inflammatory process. Laser therapy was able to modulate the expression of MMP-13, which corroborates with many authors who affirm that LLLT has been found to produce anti-inflammatory effects in a variety of disorders [48]. The anti-inflammatory effects of LLLT on degenerative processes has been demonstrated by a number of several authors who observed a decrease in prostaglandin E2 and TNF expression after LLLT in OA experimental models [48].

The existence of window specificity at certain wavelengths and energy dosages are well-known, and this supports the importance of studies comparing the effects of different parameters of irradiation on tissue stimulation [11, 33]. A previous study published by our research group [19] using 830 nm at fluencies of 10 J/cm^2 and 50 J/cm^2 showed that 830-nm LLLT was not able to decrease the degenerative process measured by Mankin score and prevent the increase of cartilage thickness related to the degenerative process. Furthermore, the biomodulation of the expression of IL1-β, TNF-α, and MMP-13 did not show any effect.

The group irradiated with 830 nm and 50 J/cm^2 revealed a lower amount of collagen type 1 [19].

The results of the present study show that treatment using the 685-nm laser at both fluencies produced morphologic modifications in cartilage tissue which in the long term could result in tissue repair and functional recovery. In this context, laser therapy with 685 nm might be a promising alternative to improve the healing of cartilage tissue in degenerative conditions. As this study was limited to a relatively short-term evaluation of the performance of LLLT (under optimal conditions to provide full control over the degenerative processes) information about the long-term performance of this therapeutic modality remains to be provided which is still not available.

5   Conclusion

In conclusion, 685-nm laser light, at 10 J/cm^2 and 50 J/cm^2, had a positive effect on the degenerative processes in the knee cartilage of OA-induced animals. Consequently, this data highlights the potential of the use of this therapy to improve the biological performance for cartilage regeneration applications. Further long-term studies should be carried out to provide additional information concerning the later stages of interaction between LLLT and severe OA.

Conflict of interest: The authors have no competing financial interests.

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Legends

Figures
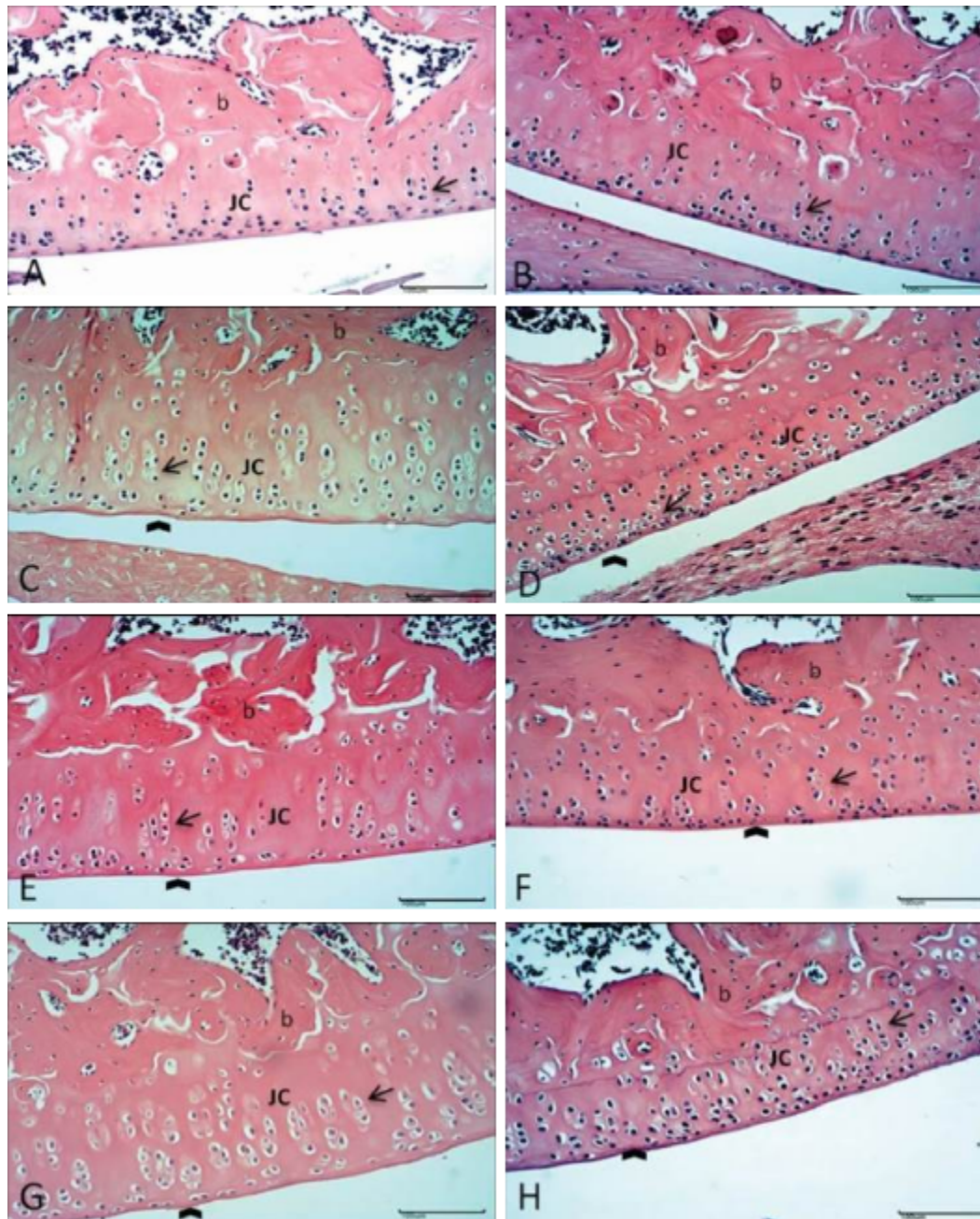
Figure 1   Representative photomicrographs from the experimental groups: Intact control group (CG) after 5 weeks (A) and 8 weeks (B), injured control group (ICG) without laser treatment 5 weeks (C) and 8 weeks (D) post-surgery, injured laser-treated group at 10 J/cm^2 (L10) 5 weeks (E) and 8 weeks (F) post-surgery, and injured laser-treated group at 50 J/cm^2 (L50) 5 weeks (G) and 8 weeks (H) post-surgery. Organization of chondrocytes (arrow), fibrillation and irregularities (arrowhead), joint cartilage (JC) and subchondral bone (b). Scale bar= 100 µm.
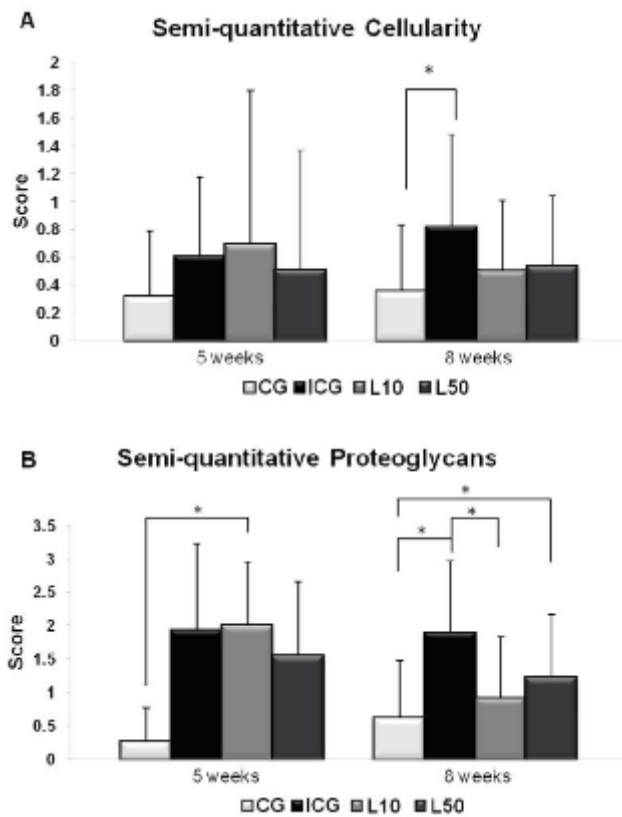
Figure 2   Results of the semi-quantitative analysis of cellularity (A) and of the semi-quantitative analyses of proteoglycans (B). CG: Intact control group, ICG: Injured control group, L10: Injured laser-treated group at 10 J/cm^2, L50: Injured laser-treated group at 50 J/cm^2. *$p<0.05$.
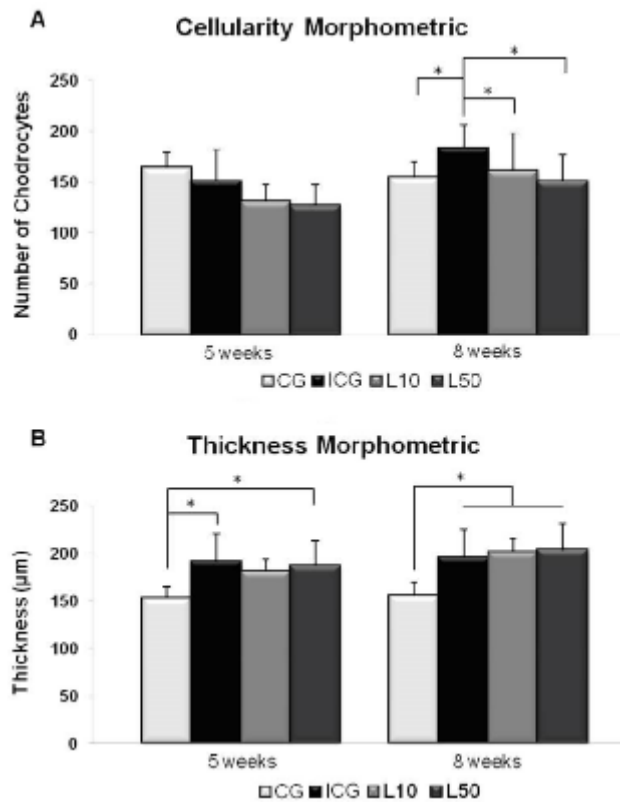
Figure 3 Results of the morphometric analysis of cellularity (A) and thickness (B). CG: Intact control group, ICG: Injured control group, L10: Injured laser-treated group at 10 J/cm^2, L50: Injured laser-treated group at 50 J/cm^2. *$p<0.05$.
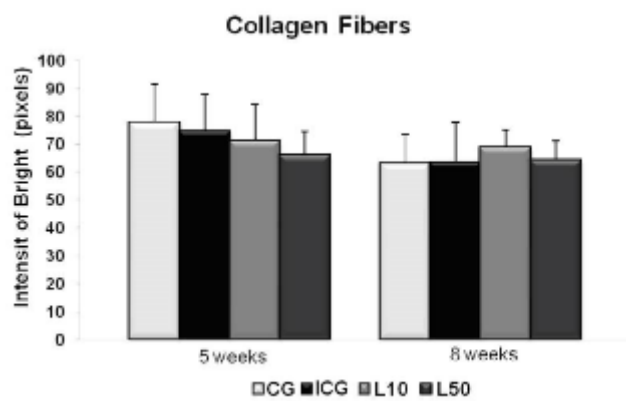

Figure 4 Results of the collagen fiber evaluation. CG: Intact control group, ICG: Injured control group, L10: Injured laser-treated group at 10 J/cm^2, L50: Injured laser-treated group at 50 J/cm^2.
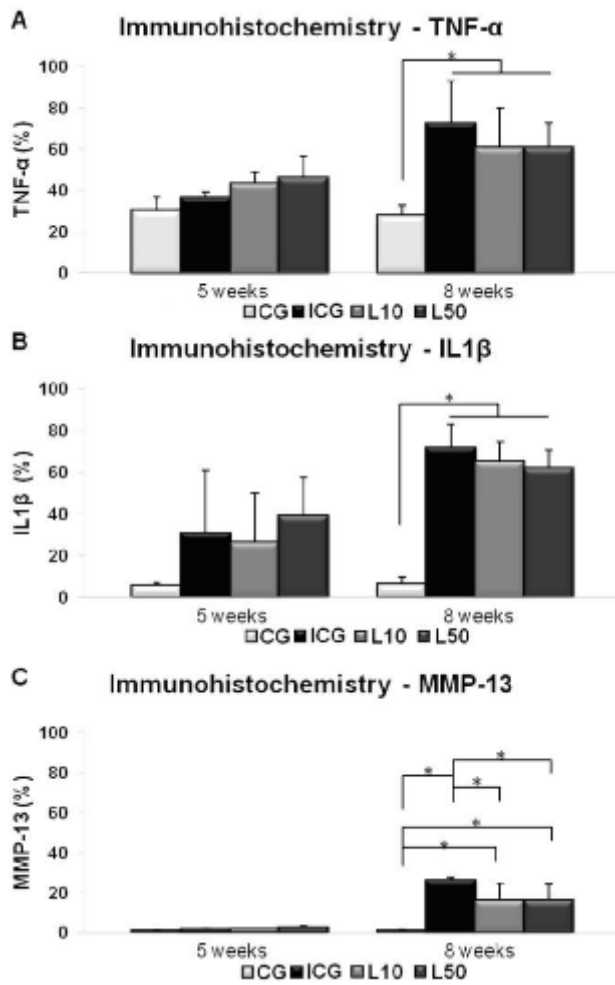
Figure 5 Results of the TNF-α (A), IL1-β (B) and MMP-13 expression (C), respectively. CG: Intact control group, ICG: Injured control group, L10: Injured laser-treated group at 10 J/cm^2, L50: Injured laser-treated group at 50 J/cm^2. *$p<0.05$.

Tables

Table 1 Modified Mankin score for evaluating the severity of cartilage damage.

	Score
Cellularity	
Normal	0
Hypercellularity	1
Severe hypercellularity	2
Hypocellularity	3
Safranin-O staining	
Normal	0
Slight reduction *up to half of a total area*	1
Slight reduction *on total area or total surface*	2
Severe reduction *up to half a total area*	3
Severe reduction *on total area or total surface*	4