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ORIGINAL ARTICLE

Low-intensity laser therapy efficacy evaluation in FVB mice subjected to acute and chronic arthritis

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Abstract Rheumatoid arthritis, an autoimmune inflammation, has a high prevalence in the population, and while therapy is available, it required often injection of drugs causing discomfort to patients. This study evaluates the clinical and histological effect of low-intensity laser therapy (LILT) as an alternative treatment, in a murine model of acute and chronic inflammation. FVB mice received either a Zymosan A injection into one knee joint inducing acute inflammation, followed after 15 min or 24 h by LILT or a collagen bovine type II injection emulsified in "Freund's Complete Adjuvant" to induce chronic arthritis, followed at 4 weeks with multiple LILT sessions. LILT mediated by either 660, 808, or 905 nm and tissue response was evaluated based on clinical symptoms and histological analysis of inflammatory infiltrate and damage to the articular surfaces. LILT can be effective in elevating clinical symptoms, so Kruskal-Wallis testing indicated no significant differences between knees affected by acute arthritis and treated once with LILT and an injured knee without treatment ($p > 0.05$) for 660 and 808 nm with some improvements for the 905-nm LILT. Mice receiving two treatments for acute arthritis showed exacerbation of inflammation and articular resorption following therapy with a 660-nm continuous laser ($p < 0.05$). For chronic inflammation, differences were not noted between LILT treated and untreated injured knee joints ($p > 0.05$). Among the lasers, the 905 nm tends to show better results for anti-inflammatory effect in acute arthritis, and the 660 nm showed better results in chronic arthritis. In conclusion, LILT wavelength selection depends on the arthritis condition and can demonstrate anti-inflammatory effects for chronic arthritis and reduced resorption area in this murine model.

Keywords Inflammation · Lilt · Histology · Immunohistochemistry · Repair

Introduction

The etiology of rheumatoid arthritis is still not well understood, so one agrees that it is an autoimmune inflammation that affects the synovial fluid, articular surface, and underlying bone, and if left untreated or if treatment fails may exhibit extra-articular manifestations. Early diagnosis and medication with drugs such as methotrexate and corticosteroids, among others, and monitoring of the progression are keys to change the course of the disease and prevent articular destruction [1]. However, these drugs cause immune suppression and lead to an increased occurrence of opportunistic infections such as tuberculosis, and thus alternative treatment methods are desirable [2].

Low-intensity laser therapy (LILT) is a potentially simple and low-cost standalone therapy alternative and it could assist conventional treatments [3]. LILT was shown to enable anti-inflammatory and analgesic effects and promote tissue repair [4, 5]. Upon absorption, the photon's energy by a target chromophore initiates photochemical processes, which in turn can activate signaling pathways promoting morphological differentiation, cell proliferation, and tissue neoformation and revascularization leading to a reduction of edema [5]. However,

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the irradiance (mW cm^{-2}), radiant exposure (J cm^{-2}), and preferred wavelength [nm], within the tissue's "transparent" window from 630 to 1300 nm, are still hotly debated. Additionally, photon delivery can be in either a continuous (CW) or pulsed mode, and the frequency of treatment sessions is providing additional parameters for treatment optimization.

LILT was also shown to modulate the acute inflammatory response by reduction of prostaglandins and inhibition of cyclooxygenase locally, driven in part by short-term augmentation of iNOS expression. Conversely, the literature shows treating chronic inflammation with high radiant exposure or high irradiance resulted in the abrogation of an anti-inflammatory effect. Instead, a worsening of the condition, including articular surface destruction, was observed [6].

In rheumatoid arthritis patients, blocking the receptor activator of nuclear factor kappa-B ligand (RANKL) using osteoprotegerin (OPG) altered peri-articular bone loss whereby tartrate-resistant acid phosphatase (TRAP) activity increases damaged chondrocytes. Hence, over-expression of these biomarkers represents extended joint damage and bone erosion in later-stage rheumatoid arthritis. Osteocalcin (OCN) is an osteoblast-derived protein and suspected in inflammation regulation. All biomarkers are used extensively in clinical diagnosis.

This study investigates effects post-LILT, mediated by one of three wavelengths, in pre-clinical models of both acute and chronic arthritis by evaluating clinical, histological, and anatomical biomarker previously associated with inflammation and the development of rheumatoid arthritis.

Materials and methods

Animals

Here, FVB male mice ($n = 72$) were used, as previous bioluminescence imaging (BLI) of acute iNOS expression post-LILT, establishing iNOS as a potential LILT modifiable biomarker [3, 4] employed the same strain. Mice weighing approximately 25 g were obtained from the Princess Margaret Cancer Centre Animal Facility, Toronto, Canada. Animals were housed five per cage, with free access to water and food, in a temperature (23 ± 1 °C)- and 12/12 h light/dark cycle-controlled environment. The study was conducted by ethical standards for the use and handling of animals and approved by the Ethics Committee on Animal Care, University Health Network, Toronto, Canada.

Initial experiments quantified the articular surface resorption in the acute model showing an increase of articular surface resorption over the first 3 days post-inflammation induction. The data showed rapid onset of surface damage over the first 3 days without further intervention.

Euthanasia was by cervical dislocation under deep isoflurane anesthesia. Left limbs were collected for qualitative

and quantitative histology and immunohistochemistry analysis, to assess the presence of inflammatory infiltrate and to determine LILT mediated modifications to the articular surface integrity.

Experimental groups

Mice were divided into three main groups comprising two acute and one chronic arthritis LILT treatment plan.

Group G1 ($n = 24$) consisted of mice with acute arthritis treated once according to the protocol of Moriyama et al. [4] 15 min after Zymosan A injection, followed 24 h later by euthanasia. Group G2 ($n = 24$) comprised mice with acute arthritis treated twice, 15 min after induction and 24 h later with euthanasia at 48 h. Group G3 ($n = 24$) consisted of mice with chronic arthritis and treated three times a week for 4 weeks, totaling 12 LILT sessions. Euthanasia was performed 24 h after the last treatment. Mice in all three groups were divided into the following subgroups containing six animals each: A—no LILT treatment, B—660 nm continuous wave LILT, C—808 nm continuous wave LILT, and D—LILT employing a 905 nm pulsed laser.

Right knees of G1-A, G2-A, and G3-A mice were also removed and processed for microscopic analysis as non-inflamed controls.

Inflammation induction

Chronic arthritis was induced as described by Baddack et al. [7]. A solution containing 50 µg mBSA and 100 µg bovine collagen type II (MD Bioproducts, Zurich, Switzerland) in 50 µL of PBS was prepared and emulsified with 50 µL FCA. Finally, 10 µL of the emulsion was injected into the left knees by Hamilton syringe (Hamilton Co, Sigma, Germany).

For induction of acute arthritis, 30 mg of Zymosan A (Sigma, Germany) was dissolved in 1 mL saline, and 10 µL of this solution was injected into the knee as described above.

Anesthesia of the animals was induced by 5% and maintained 1–2% isoflurane in air. Sterile gauze soaked in saline 0.9% placed on the animal's eyes prevented corneal drying. After reaching a deep anesthesia plane, a 5-mm skin incision was made by the left knee joint enabling visualization of the joint, followed by injection of 10 µL of either FCA or Zymosan A solution under the patella. The skin was properly closed and sutured with silk thread. Animals were observed daily to assess discomfort and mobility. After the predetermined periods listed above, LILT of the injured knee commenced.

Lasers and irradiation

The 660 and 808 nm were assembled by the biophotonics group at the Princess Margaret Cancer Centre, both capable

of providing up to 300 mW to the tissue surface via multimode optical fibers equipped with a micro-lens producing a flat top beam. The 905-nm laser was manufactured by Theralase Inc. (Toronto, Ontario, Canada) and delivered an average power of 60 mW in 200 ns pulses at 10,000 Hz repetition rate in free space. Both light sources were placed perpendicular and 2.5 mm from the tissue surface resulting in a spot size of 7 mm (area 0.38 cm^2) thus encompassing the entire knee joint. Illuminations were performed only from one side due to the limited thickness of a murine joint. In all cases, the irradiance on the skin was limited to 25 mWcm^{-2} (9.5 mW total power) for 200 s to achieve a radiant exposure of 5 Jcm^{-2} (1.9 J).

Histological processing

After removal of the soft tissue, the articular region was separated carefully from the lower limb, and the fragments were immersed in fixative for 24 h and decalcified in 0.5 M EDTA, exchanging the solutions every 2 days. After the decalcification period (15 to 30 days), the acid was neutralized over 24 h in a 5% sodium sulfate solution. Subsequently, dehydration of specimens occurred in ascending series of alcohols: 70 (overnight), 80, 85, 90, 95, and 100% (2 h at each concentration). Once completed, the bone blocks were placed in equal parts of alcohol and xylene (overnight) and diaphanized in xylene with three changes every 2 h, and finally embedded in paraffin.

Microscopic analysis

Five (5 μm) thick sections from paraffin-embedded samples were obtained in semi-serial form, covering the entire joint and stained with hematoxylin-eosin and Masson trichrome for the imaging of the articular surface a ×5 objective lens in an optical microscope (AxioImager Z2 Zeiss, Germany). Histological sections along the articular surfaces were selected randomly to quantify the absorptive region based on statistical planning. Quantification used Stereological software (StereoInvestigator, MBF Bioscience, USA) for of the resorbed articular surface (μm^2) by imaging a 150-μm-long edge grid over the area of interest by the Cavalieri method [8]. The resorbed area is calculated by the sum of the grid points over the demarcated articular surface area multiplied by the section thickness (5 μm). This volume was divided by the total articular volume obtained similarly providing the fraction of resorption in each knee.

Immunohistochemistry for bone resorption factors

After being deparaffinized, the histology sections in xylene and hydration in descending ethanol series antigen retrieval was performed by immersion of the histology slides in *Diva Decloaker®* solution (Biocare Medical, CA, USA) inside a pressurized chamber (*DecloakingChamber®* BiocareMedical,

CA, USA) at 95 °C for 10 min. After washing with phosphate-buffered saline solution (PBS), the histology slides were immersed in 3% hydrogen peroxide for 1 h to block endogenous peroxidase and treated with 3% bovine serum albumin for 12 h to block non-specific binding sites. Slides from all experimental groups were divided into four lots and incubated with one of goat anti-rat OCN (osteocalcin), goat anti-rat OPG, goat anti-rat RANKL, and goat anti-rat TRAP (all from Santa Cruz Biotechnology, CA, USA), indicating damage to the cortical bone. Primary antibodies were diluted in PBS plus 0.1% Triton X-100 (PBS-TX) and stored for 24 h in a humid chamber. Staining of the sections used the Universal Labeled Dako (HRP) Streptavidin-Biotin Kit® (Dako Laboratories, CA, USA). After washing, sections were incubated with biotinylated secondary antibody for 2 h, washed and treated with streptavidin conjugated with horseradish peroxidase (HRP) for 1 h. After three washes in PBS-TX, the primary antibodies were revealed using chromogen 3,3′-diaminobenzidine tetrahydrochloride (DAB Chromogen Kit®, Dako Laboratories, CA, USA). Following a series of PBS washes, the sections were counterstained with Harris hematoxylin. Sections without the primary antibodies served as negative controls. Histological and staining procedures followed either universally accepted standard or particular manufacturer's recommendation.

Bright field images of histological slides were obtained using an optical microscope (Optiphot-2, Nikon, Japan) and evaluated by an investigator blinded to the LILT conditions. Immunostaining produced a brownish stain in the cytoplasm of cells and the extracellular matrix. The entire length of the articular surface was measured at ×100 magnification. A semi-quantitative analysis was performed using five sections, where a score criterion according to Faria et al. [9] was employed with 0 = absence of, 1 = low, 2 = moderate, 3 = high, 4 = extremely high levels of immunostaining.

Statistical analysis

The data were submitted to Homogeneity Normality test. G1 and G3 presented normal distribution and were submitted to ANOVA and Tukey post-test. G2 presented non-normal distribution and was submitted to the Kruskal-Wallis and Dunn post-test. All executed in SPSS Statistics 20 with $\alpha = 0.05$.

Results

Clinical observations

Clinically, mice with acute arthritis showed no clinical signs or symptoms characteristic for inflammation for the first 4 h. Conversely, animals with chronic arthritis showed significant clinical signs of inflammation in the left knee 2 weeks after FCA administration. The joints

presented with an increased diameter, due to swelling in the articular region, averaging 8 ± 2.4 mm, which is twice the normal (right) diameter of 4 ± 1.7 mm.

Also, some animals in group 3 had difficulty in ambulation following injection of FCA, and the injured limb showed intense redness (Fig. 1). These clinical symptoms improved after treatment with LILT, especially using the 905 nm pulsed laser based on visual evaluation by a single observer blinded to the treatment conditions.

Histological analysis

For acute arthritis, the fraction of resorbed articular surface almost doubled at 48 h post-induction if left untreated. The largest mean fraction area of resorbed articular surface was noted at the end of the chronic inflammation model. The untreated control group (G1-A) showed articular surface deviating from its normal appearance including visible resorption in the articular capsule at 13.5%. For groups G1-B and G1-C mice treated with continuous wave LILT, these features appeared similar and not statistically different (Table 1). In the acute group, treated 15 min after the induction of arthritis, all groups presented lesion similar to the injured and untreated group (G1-A) showing limited effectiveness of LILT in the acute inflammation situation. However, a small number of histological sections presented a lesser degree of resorption than the untreated G1-A group. Group 1D, treated with a pulsed 905-nm laser, presented qualitatively with improved
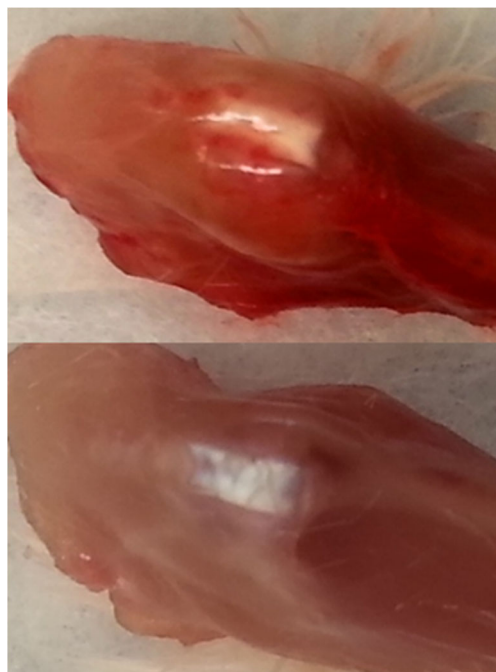

Fig. 1 Comparison of a left limb with chronic inflammation revealing intense redness (top) and a right limb without inflammation of the same mouse (bottom)

Table 1 The articular surface resorption [%] in lesioned animals submitted to different treatments

Subgroup	G1[a] (1 session)	G2[b] (2 sessions)	G3[a] (12 sessions)
A	11.67 (6.15)*	23.00 (11.87; 40.79)	40.67 (15.53)***
B	34.83 (17.36)*	30.00 (18.51; 39.49)**	16.83 (11.39)***
C	12.13 (14.85)	15.00 (2.89; 30.78)	21.00 (14.04)
D	19.00 (15.54)	12.50 (4.67; 17.33) β	26.67 (11.55)
P	0.037	0.029	0.029

A no treatment, *B* LILT using a 660 nm continuous wave laser, *C* LILT using an 808-nm continuous wave laser, *D* LILT using a 905-nm pulsed laser

*Significant difference ($p = 0.046$); **significant difference ($p = 0.044$); ***significant difference ($p = 0.026$)

[a] Mean (standard deviation), ANOVA and Tukey post-test

[b] Median and confidence interval, Kruskal-Wallis test and Dunn post-test

histological features. Statistical differences between the control and 660 were found ($p = 0.046$) (Fig. 2).

Histological findings in twice treated acute arthritis (G2) showed an increased degree of resorption and destruction of the articular capsule, with the capsule being absent in some sections (Fig. 3d). In particular, 660 nm showed a statistically significant increase in median surface resorption, whereas 808 and 905 nm indicated a reduction in the median resorbed articular surface area, which was statistically significant only for the 905 nm mediated LILT.

The main articular degradation was observed in the control group with chronic inflammation (G-3A), see (Fig. 4a), demonstrated by a high degree of surface resorption. Qualitatively, the left knees from G-3B (660 nm CW) and G-3C (808 nm CW) animals presented similar features as knees from G-3D (905 nm pulsed laser) mice, showing different histological features. The articular surface was mostly continuous, resorption was less pronounced than in the control group (G-3A), and the articular capsule was mostly intact (Fig. 4b–d). Animals receiving the 660 nm mediated LILT presented with a significant decrease of the resorbed area ($p = 0.026$).

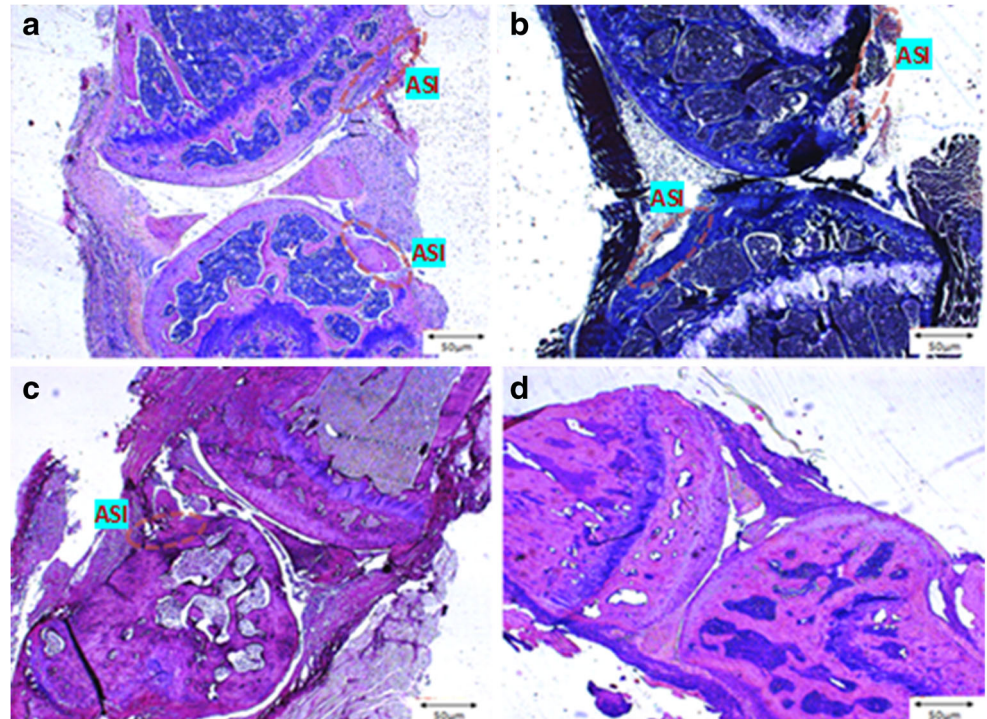## Immunostaining for OCN, OPG, RANKL, and TRAP

Negative controls did not show any brown staining, demonstrating the specificity of the primary antibodies. The pattern of OCN, OPG, RANKL, and TRAP immunostaining resulted in comparable results between most groups. Groups G1-C and G1-D (Fig. 5b, d) showed lower immunostaining than G2-C and G2-D did (Fig. 5a, c), which exhibited a moderate to dense immunostaining pattern. The similar immunostaining pattern was noted for joints from group G2-B animals (Fig. 5f). For Group 3 mice, G3-D showed sparse (Fig. 5e),

Fig. 2 Comparison of staining between different treatment regimes in group 1 acute arthritis animals. Shown are the left knees of animals for **a** G1-A, **b** G1-B, **c** G1-C, and **d** G1-D treatment groups. All micrographs are shown at ×50 magnification. *Red circles* show articular surface injury (ASI)
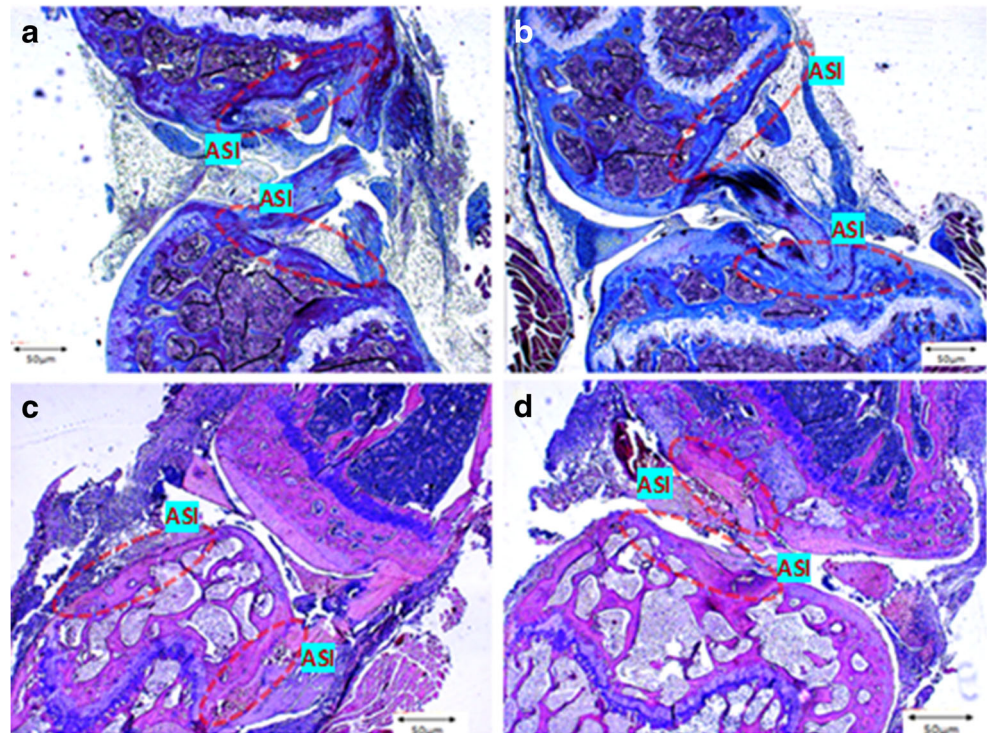
G3-C moderated (Fig. 5h), and group G3-B extremely dense immunostaining (Fig. 5g), respectively. The quantity of OCN, OPG, RANKL, and TRAP proteins as presented by the brown color stain appears qualitative to be mostly independent of the remaining articular surface.

Discussion

The laser and light biomodulation literature, including LILT and low-level laser therapy (LLLT), bears extensive examples for efficient pain and inflammation reduction, stimulation of

Fig. 3 Comparison of staining between different treatment regimes in group 2 acute arthritis animals with repeat LILT. Shown are the left knees of animals for **a** G2-A, **b** G2-B, **c** G2-C, and **d** G2-D. All micrographs are shown at ×50 magnification. *Red circles* show discontinuity of the articular capsule

Fig. 4 Comparison of staining between different treatment regimes in group 3 chronic arthritis animals. Shown are the left knees of animals for **a** G3-A, **b** G3-B, **c** G3-C, and **d** G3-D. Images were collected at ×50 magnification. *Red circles* show articular surface injury (ASI)
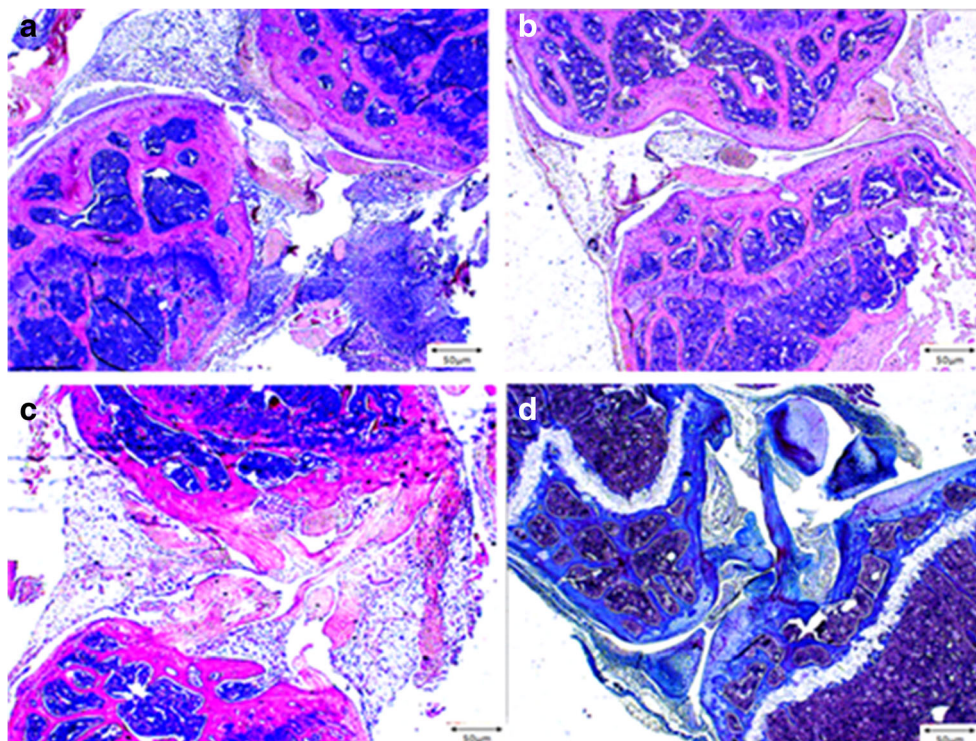
collagen generation, acceleration of healing, and other arthritis-relevant outcome metrics [10]. It was shown that longer wavelengths tend to result in improved clinical and physiological biomarkers [11, 12]. The irradiance and radiant exposures quoted in these manuscripts are comparable to those used here. These techniques have been employed to treat different pathologies, such as osteoporosis, muscle injuries, and arthritis. A wide range of inflammation applications, LILT wavelength, and treatment parameters was employed using subjective effect measures, such as pain scores. However, studies evaluating anatomical changes are not widely available [10, 13, 14], and the fact that LILT and LLLT experiments often presented with incomplete reporting of the treatment parameters inhibits these treatment options to enter mainstream medicine.

While the changes in knee diameter and pain are rapid, so possibly transient responses, the amount of resorbed articular surface and changes in bone osteolysis is a better indicator for long-term joint health effects. Subchondral bone osteolysis is indicated by biomarkers including OCN, OPG, RANKL, and TRAP expression and presents clinically more relevant measures of progressing rheumatoid arthritis. OCN is produced by osteoblasts, associated with bone formation, whereas OPG, RANKL, and TRAP are associated with osteoclasts and are associated with bone resorption and hence present an indicator for inflammation-induced bone destruction early in the disease process.

The LILT treatment parameters selected here were reported previously for pre-clinical studies, whereby in particular

positive outcomes, such as reduction of the inflammatory process and tissue matrix degradation [10–12], have been reported.

In the acute inflammation model, mice did not show clinical signs prior to LILT, possibly due to the short duration so that signs may have been masked by the physical injury related to the Zymosan A administration. Chemical or mechanical damage could have been the cause of increased iNOS expression previously observed [15]. The duration selected for the chronic inflammation model coincide with the anticipated microscopic alterations seen during the clinical manifestation of rheumatoid arthritis in mice, so the temporal evolution of this pathology is different in humans [16]. Inducing chronic arthritis by injection of FCA resulted in noticeable histological and clinical signs of increased nociception.

The presence of inflammation was evaluated by clinical indicators including mechanical articular problems, swollen joint, and reduced functional capacity [17]. Post-Zymosan A or FCA induction, mice presented with an increased knee diameter, indicative of edema. For chronic inflammation, animals also refrained from touching the ground with the left hindquarters when roaming, indicating discomfort and pain. The affected articulation presented redness due to an increase in local perfusion.

Da Rosa et al. used 660 and 808 nm-mediated LILT for the treatment of osteoarthritis and found positive outcomes in their 2012 study, whereby 808 nm stimulated more the angiogenesis process and reduced fibrosis formation [18]. The 660-nm continuous laser was reported to stimulate collagen
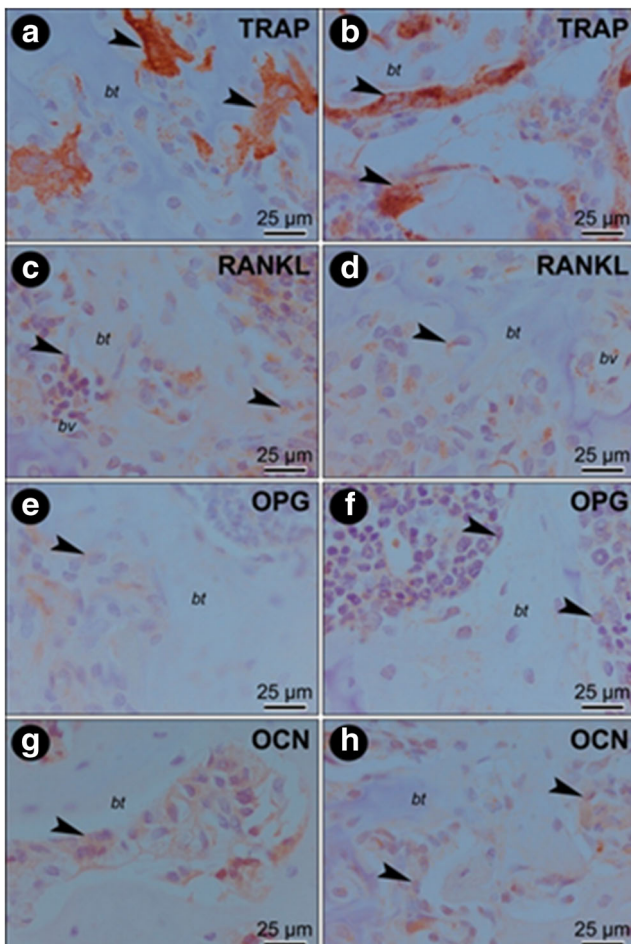
Fig. 5 Left knee immunostaining from different groups and subgroups. **a** G2-C. **b** G1-C. **c** G2-D. **d** G1-D. **e** G3-D. **f** G2-B. **g** G3-B. **h** G3-C. *Markers* identify bone resorption (arrowheads) *BV* blood vessel, *BT* Bone tissue

metabolism, accelerate wound healing, and increase local perfusion and is used widely in the therapeutic procedure for the reduction of pain and inflammation [19].

Histologically assessed tissue modulation following 660-nm-mediated LILT was less than for the other two wavelengths. Nevertheless, in chronic arthritis, 660-nm LILT eliminated clinical signs of discomfort and inflammation after a single application as previously reported. No significant differences to non-LILT-treated joints were evident. The articular surface presented at 24 h post-inflammation induction with significant resorption, and the articular capsule was absent or with morphological alterations in some animals. In situations of an acute inflammation treated either once or twice, LILT mediated by 660 nm did not improve in the inflammatory process and did not modify articular damage already present due to the inflammation.

808-nm-mediated LILT has been used to treat superficial injuries aided by its lower attenuation compared to red wavelength subcutaneous lesions to promote tissue regeneration

[20]. Dos Santos et al. evaluated the effect of 808-nm-mediated LILT at 2 or 4 J per rat joint, following papain-induced inflammation, causing an acute inflammatory process in the synovial fluid and joint gaps filled by fibrinous and hyaline material connected to the surface of the synovial membrane. Animals treated with LILT presented with less inflammation in the connective tissue of the synovial membrane. Articular surfaces and marrow spaces presented with normal features [21]. Similarly, Alves et al. found that 24 h after 808-nm-mediated LILT (4 J per joint), there was a significant reduction of inflammatory cells as well as an increased IL-1β and IL-6 messenger RNA (mRNA) expression.

Here, LILT mediated by 808 nm improved the observable clinical conditions of the animals; however, it was ineffective in controlling resorption of the articular surface. The histological observations were similar to the 660-nm laser for chronic inflammation. Sporadic improvements were seen only for group G3-C animals, and chronic inflammation when the articular surface and articular capsule were present with less resorption.

The 905-nm pulsed laser is widely used in various indications for LILT and surgical situations, so it can cause microscopic thermal effects as the maximum permissible exposure can be exceeded [19]. Here, clinical observations include reduced local inflammation, particularly immediately after the treatment, and histologically less articular resorption is noted for acute arthritis. For acute arthritis and animals in groups G1-D and G2-D, improvement of inflammation was most significant. Group G2-D mice that showed no clinical signs prior to treatment now reverted from touching the floor with the injured paws. Histologically, the articular surface presented similar or less resorption for acute arthritis than did the group receiving no treatment. For chronic arthritis, no statistical differences were found, and the 905-nm pulsed laser showed the greatest resorption. This condition can be explained by the biphasic dose-response hypothesis [22].

Prior publications showing an effect of LILT on iNOS expression in FVB mice, as quantified by bioluminescence [3, 5], suggested that upregulated iNOS result in faster NO production and hence faster resolution of the inflammation, evident also by a faster clearing of the inflammatory cells in the synovium [4].

This, in turn, should have resulted in lower articular surface resorption; however, the immune-histological data presented here suggests that 905 nm may penetrate deep enough into the subchondral bone to cause an upregulation of osteocalcin and osteopontin there, which may become therapy-limiting in this pre-clinical situation.

The osteoclast-associated RANKL, OPG, and TRAP are upregulated versus not-inflamed knee joints, whereby in particular, TRAP appears to be highly expressed for group G2 irradiated by 808 or 905 nm, indicative of damaged

chondrocytes, possibly reflecting the biphasic tissue response, now in previously undamaged cortical bone. RANKL expression appears to be more upregulated following 808-nm LILT compared to 905 nm and appears to be inversely related to the osteoprotegerin (OPG), so it is not clear if this is a coincidental finding. Activation of osteoblasts as measured by OCN appears to be best stimulated by 660-nm LILT, so it is very weak for the other protocols. These indicators of cortical bone damage and hence long-term damage to the bone demonstrated that assessment of LILT efficacy based purely on clinical parameters would be insufficient. Long wavelength with the ability of deep penetration into the bone appears to be required for long-lasting LILT effects in rheumatoid arthritis.

The fact that the quantity of OCN, OPG, RANKL, and TRAP proteins, as presented by the brown stain, appears to be qualitative independent of the remaining articular surface, and these markers may present independent, LILT-modifiable biomarkers, for both acute and chronic inflammation, permitting a more quantitative LILT evaluation in the future.

One caveat of the study is the euthanasia time point 24 h post-final LILT when particularly the acute inflammatory process may have dissipated preventing quantification of its clinical manifestation and few remaining histological and immunological alterations. The limited efficacy particularly of shorter wavelength-mediated LILT for acute inflammation does not lead to their recommendation in this setting. This is in stark contrast to chronic inflammation when particularly with longer wavelength beneficial, clinical, and histological effects were observed.

Conclusion

Based on statistical analysis and histological, clinical, and immunohistochemical observations in this experimental animal model, it is evident that LILT can promote analgesic and anti-inflammatory effects in both acute and chronic arthritis. In particular, this treatment can eliminate clinical signs and control articular resorption when using an appropriate laser for each tissue type. In contrast, LILT is largely responsible for exacerbating the local inflammatory response and causes clinical signs to begin to manifest. Therefore, LILT is dependent on the number of applications, wavelength, and forms of irradiation used and of the tissue and condition treated. For acute inflammation, a single treatment using lasers with an intermediate or long wavelength was sufficient to promote an anti-inflammatory effect and control of articular resorption; double treatment on sequential days in cases of acute inflammation is not beneficial. For chronic inflammation, a treatment plan comprising three sessions with an interval of 1 day between them was sufficient to eliminate the clinical signs and to control articular resorption.

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