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In Vitro and *In Vivo* Effects of Light Therapy on Cartilage Regeneration for Knee Osteoarthritis: A Systematic Review

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Abstract

Objective. To analyze the effects of light therapy (LT) on cartilage repair for knee osteoarthritis (OA) treatment. *Design*. The PubMed, Embase, Scopus, and Web of Science databases were searched up to August 31, 2020 to identify *in vitro* and *in vivo* studies that analyzed the effects of LT on knee cartilage for OA treatment. The study and sample characteristics, LT intervention parameters and posttreatment outcomes were analyzed. Risk of bias was assessed using the Risk of Bias Assessment for Non-randomized Studies (RoBANS) tool. *Results*. Three *in vitro* and 30 *in vivo* studies were included. Most studies were judged as high risk of performance and detection bias. Biochemical outcomes were analyzed for both i*n vitro* and *in vivo* studies, and histological and behavioral outcomes were analyzed for *in vivo* studies. LT reduced extracellular matrix (ECM) degradation, inflammation, and OA progression, promoting ECM synthesis. LT improved pain-like behavior in animal models, having no apparent effect on gait performance. There were conflicting findings of some of the biochemical, histological, and behavioral outcomes. *Conclusion*. The included studies presented different strategies and LT parameters. LT resulted in positive effects on cartilage repair and may be an adequate therapy for OA treatment.

Keywords

cartilage, knee, osteoarthritis, light therapy, laser

Introduction

Articular cartilage is composed of highly specialized cells, chondrocytes, that are sparsely distributed and have low replicative ability. The low replicative potential and the absence of vascular and neural support limit the repair process of the damaged cartilage.[1,2] Osteoarthritis (OA) is a degenerative joint disease with multifactorial etiology, being age, joint injury, trauma, and obesity the main predisposing risk factors.[3] The increased expression of inflammation mediators alters the cartilage homeostasis by favoring the catabolic activity of chondrocytes, resulting in cartilage matrix disruption and loss.[3,4] Contrary to others inflammatory arthritis diseases (e.g., rheumatoid arthritis), OA does not involve chronic systemic inflammation,[3] but has rather a joint-specific mechanism, leading to articular cartilage degeneration, subchondral bone remodeling, synovial thickening, and joint space narrowing.[4]

A variety of nonsurgical and surgical treatments are available for the management of OA. Light therapy (LT) is an option of nonsurgical treatment, which aims to promote cartilage tissue regeneration. The cellular mechanisms by which LT stimulates cells include light absorption by

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cytocrome *c* oxidase at mitochondria.[5,6] The activation of cytochrome *c* oxidase increases the calcium ions ($CA^{2}+$), oxygen reactive species, and adenosine triphosphate (ATP) production.[6] These molecules are involved in several intracellular signaling pathways that lead to gene transcriptions related to cell proliferation, protein synthesis, and inflammation decrease.[5,6] Despite the growing body of scientific evidence showing beneficial physiological effects,[7-10] LT has not been yet incorporated in clinical practice guidelines of OA treatment.[11-13]

A systematic review[14] from 2013 analyzed the effect of LT parameters on animal models, but it failed to comprehensively address the effects on cartilage regeneration. Since then, many *in vivo* studies have been published and most were included in another recent systematic review.[15] However, their analysis was limited to the grading of cartilage quality, and other important outcomes such as extracellular matrix (ECM) synthesis/degradation, inflammation markers, and behavioral and histological outcomes, were not evaluated. Their evaluation is important for a more comprehensive and in-depth understanding of the efficacy of LT in cartilage repair. This systematic review aims to summarize the cartilage regeneration outcomes of *in vitro* and *in vivo* studies after applying LT interventions (isolated and compared with control or other interventions).

Methods

This systematic review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyzes (PRISMA) guidelines.[16]

Search Strategy and Study Selection

The electronic databases PubMed, Embase, Scopus, and Web of Science were searched to identify original *in vitro* and animal (*in vivo*) studies that assessed the effects of LT on knee cartilage for OA treatment. Searches were performed from database inception up to August 31, 2020. The search strategy is presented in Supplementary Table S1. The reference list of the most relevant studies was also screened to identify any other potentially eligible studies.

All records were exported to an Excel file (Microsoft Office) and the duplicates were removed by the software filter and then manually verified. Two authors (SO and RA) independently screened all titles and abstracts of initially identified on the search. The full texts of the potentially eligible studies were extracted and evaluated by the same authors to further assess their eligibility. Two other reviewers (AL and OC) were consulted in case of disagreement. The inclusion criteria were (1) *in vitro* or animal (*in vivo*) studies, (2) studies that focused on the effects of LT on knee articular cartilage, and (3) studies that included *in vitro* or *in vivo* OA models. The exclusion criteria were (1) reviews or meta-analysis, conference proceedings, or case studies; (2) studies not written in English language; (3) studies that did not assess the effects of LT on chondrocytes activity; (4) *in vivo* studies that analyzed laser irradiation under arthroscopy; or (5) studies that used an inflammatory arthritis model (e.g., rheumatoid arthritis).

Risk of Bias Assessment

The risk of bias of the included studies was assessed using the Risk of Bias Assessment tool for Non-Randomized Studies (RoBANS). The RoBANS is a validated tool to assess the risk of bias of nonrandomized studies.[17] It contains 6 domains for risk of bias comprising the "Selection of Participants," "Confounding Variables," "Exposure of Measurement," "Blinding of Outcome Assessment," "Incomplete Outcome Data," and "Selective Outcome Reporting." The criteria of each domain were adapted to the context of our systematic review, to specifically analyze the risk of bias arising from *in vitro* and *in vivo* studies. Two other domains—"Planning and Implementation of Interventions" and "Funding Bias"—were added to analyze other sources of bias that arise specifically from LT interventions. **Table 1** describes the criteria used to judge the risk of bias of each domain. Two authors (SO and RA) judged and classified the risk of bias of all included studies as low risk, high risk or unclear.

Data Collection, Extraction, and Statistics

All data were extracted from the included studies into a Microsoft Excel spreadsheet by 1 author (SO) and reviewed by 3 other authors (RA, AL, and OC). The data collected were the following: sample size and characteristics (cell and animal type, *in vitro* and *in vivo* OA models, animal race, gender, age, and weight, experimental groups, number of animals per group, and sample collection methods), study characteristics (year, study design, aim, measured variables, limitations, and general remarks), LT parameters (emitter type, wavelength, operating mode, frequency, duty cycle, pulse duration, power, power density, beam spot size, energy per point, total energy, energy density, irradiation time, treatment duration, application technique, irradiation area, and the number of points irradiated), biochemical and histological cartilage response outcomes. The biochemical outcomes describe the chondrocytes activity, ECM synthesis and/or degradation and the inflammatory activity. The histological outcomes comprise the effects on the quality of articular cartilage. In addition, behavioral outcomes, that analyze the pain-like behaviors and comprised the gait performance, weightbearing, and mechanical hyperalgesia analysis were collected.

Table 1. Domains and Description for the Appraisal of the Risk of Bias for *In Vitro* and *In Vivo* Studies Using Risk of Bias Assessment Tool for Non-Randomized Studies (RoBANS).

Domain	Description for *In Vitro* and *In Vivo* Studies
Selection of chondrocytes or animals	**Selection bias** caused by inadequate selection of cells and animal participants. In *in vitro* studies, selection of chondrocytes cells should be performed from commercially available cell lines or from cartilage samples collected from animals. In both cases, chondrocytes should be obtained from hyaline cartilage. Chondrocytes should be isolated from more than one animal with same characteristics (type, race, weight, and age) from the same anatomical site. Ideally, allocation of cells would be randomized. Chondrocytes phenotype after isolation protocol should be confirmed for specific chondrogenic surface markers (e.g., CD44, CD49, CD73, CD90, CD105, CD151, and CD166) and/or for chondrogenic markers (e.g., COL II, ACAN, SOX-9).[16-18] Control and intervention groups should be clearly defined. In *in vivo* studies, animal participants with same characteristics should be selected. Ideally, allocation of animals would be randomized. Controls and intervention groups should be clearly described.
Confounding variables	**Selection bias** caused by inadequate confirmation and consideration of confounding variables. For *in vitro* studies, cartilage should be collected from the same anatomical sites and isolated chondrocytes should have the same viability and count among groups. Studies should implement the same isolation protocol and same protocol for establishing the primary cell culture(s). The number of cell passages should be the same for all experimental groups and should not be too high, since chondrocytes lose their phenotype with increasing number of passages.[19] The culture medium volume should be the same for all wells among experimental groups to avoid different radiation scattering between groups. Same experimental conditions should be guaranteed for both control and intervention groups (e.g., humidity, CO_2 and temperature conditions). *In vivo* studies should be consistent regarding the animal model, race, weight and age, ratio of male/female among experimental and control groups and number of animals per group. Animal participants should be housed under the same conditions, light cycles time and temperature. The animal euthanasia and OA induction method should be clearly described and the same among groups. The day of OA induction should be clearly defined and the recovery time before interventions should be performed for all experimental and control groups.
Exposure of measurement	**Performance bias** caused by inadequate measurement of exposure. Measurement techniques should be adequate and well-established for the specific outcomes that studies are assessing, and their measurement protocol should be clearly described to allow for replication. Semiquantitative and/or qualitative analysis should be performed by two independent observers to ascertain interoperator reliability.
Blinding outcome assessment	**Detection bias** caused by inadequate blinding of outcome assessment. Outcome assessor and/or data analysist not blinded to group (i.e., intervention vs. control). For quantitative analyses, the blinding of outcome assessor and/or data analyst was not considered necessary. Otherwise (semiquantitative and qualitative analyses), blinding was required.

(continued)

Table 1. (continued)

Domain	Description for *In Vitro* and *In Vivo* Studies
Incomplete outcome data	**Attrition bias** caused by inadequate handling of incomplete data outcome. Missing data in >5% of outcome variables.
Selective outcome reporting	**Reporting bias** caused by selective outcome reporting. Based on reporting of the collected/assessed outcomes and multiple subgroup analyses.
Planning and implementation of interventions*	**Performance bias** caused by inadequate planning and implementation of interventions. LT should be performed by the same operator and parameters should be clearly described to allow for replication. The application mode such as distance to cells/skin, scanning or skin contact method, angle of light source should be clearly described. Type of light source, operating mode (continuous or pulsed) and number of actuators should be defined in each experimental group. LT parameters should be stated, as well as the number of LT sessions and the number of irradiated points. A temperature control should be performed during interventions since LT should not induce a temperature rise in tissues or cells.[4,20] Previous calibration and/or power parameters control during experiments should be performed. In *in vitro* studies, the radiation scattering between wells in same well plate must be considered during irradiation. Blinding of personnel or testing source (cells/animals) is not possible. In these interventions, the LT parameters are pre-determined, the personnel who applies the intervention (LT) cannot change the intervention or affect the outcomes. Thus, we did not judge performance bias related to blinding of personnel or testing source.
Funding bias[a]	**Funding bias** caused due to financial sponsoring or conflict of interest. Conflict of interest from study authors and/or sponsoring of industry.

CD44, CD49, CD73, CD90, CD105, CD151, and CD166, antigen molecules at cells surface; COL II, collagen type II; ACAN, aggrecan; SOX-9, SRY-box transcription factor 9; OA, osteoarthritis; LT, light therapy.

[a]Domains added to the validated RoBANS tool to adjust to the context of this systematic review.

The median, 25% and 75% percentiles, and minimum and maximum values were calculated for each LT parameter.

Results

Search Strategy

The PRISMA flowchart search can be found in **Fig. 1**. From the initial 1049 records, identified 33 studies (3 *in vitro*[18-20] and 30 *in vivo*[7-9,21-47]) met the eligibility criteria and were included in this systematic review.

Risk of Bias

In Vitro Studies. The judgment of risk of bias for each *in vitro* study and a summary for each domain is displayed in **Figure 2a**. The "Selection of Cells" domain presented unclear risk of bias in 2 *in vitro* studies[19,20] that did not report the animal characteristics from which the cartilage samples were obtained. The "Confounding Variables" domain was judged as low risk of bias for all *in vitro* studies. Although, the culture medium volume was not provided in 2 studies[19,20] and the influence of the culture medium volume in varying levels of irradiation could not be assessed. The "Planning and Implementation of Interventions," "Exposure of Measurement," and "Blinding Outcome Assessment" domains were judged as high risk of bias due to an inadequate measurement of semiquantitative or qualitative outcomes. The "Incomplete Outcome Data" domain was judged as unclear risk for all *in vitro* studies because these studies did not report the sample size.

In Vivo Studies. The judgment of risk of bias for each *in vivo* study and a summary for each domain is displayed in **Fig. 2b**. The "Selection of Animals" domain was judged as unclear risk in 40% of *in vivo* studies because animal age, sex, or weight were not reported, precluding the evaluation of the potential risk of differences between groups. The "Planning and Implementation of Interventions" domain was judged as high risk of bias for all studies because the studies did not control for the temperature before, during and after the LT intervention. The temperature control is an important factor since the observed outcomes may result from thermic effects and not from the application of light by itself.[48,49] All studies also failed in consistently reporting all the LT parameters. The "Exposure of Measurement" domain was judged as high risk of bias in 43% of studies as these studies did not include 2 independent observers for semiquantitative and/or qualitative analysis. In the absence of those independent operators, blinding should have been implemented to avoid detection bias, which was only performed by 53% of studies as represented by "Blinding Outcome Assessment" domain. The "Selective Reporting of Outcomes" domain was judged as high risk of bias in 13% of studies as these studies did not report the results of all measured outcomes. The "Funding Bias" domain was also judged as high risk of bias in 13% of studies due to the lack of reporting of potential conflict of interest.

In Vitro Studies

Study Characteristics. All *in vitro* studies were based on monocultures experiments. Two studies (67%) used chondrocytes isolated from the knee cartilage of New Zealand white rabbits, which were further expanded *in vitro*. One study (33%) used human chondrocytes cell lines. Only 1 study (33%) conducted experiments in an *in vitro* OA model, which consisted in the administration of recombinant human interleukin-1β (IL-1β) to stimulate the inflammatory environment that occurs naturally in knees with OA.

LT Parameters. All studies reported the wavelength, operating mode, power output, energy density, irradiation time, treatment duration, and irradiation area parameters. The light stimulus was used in pulse mode in 1 study (33%) and continuous in the remaining 2 studies (67%). The median laser wavelength was 632.8 nm (range, 632.8-910.0 nm), with a median power output of 7 mW (range, 2.5-10.0 mW), and a median energy density of 4.0 J/cm^2 (range, 2.50-5.87 J/cm^2) for a median duration of 390 seconds (range, 180-660 seconds) with a median irradiating area of 0.91 cm^2 (range, 0.785-9.6 cm^2). Supplementary Table S2 reports the LT parameters used in each study. Supplementary Table S3 summarizes the descriptive statistics of the reported LT parameters.

Biochemical Outcomes. The *in vitro* studies only reported biochemical outcomes (Supplement Table S4), including the chondrocytes activity (67%, $k = 2$), ECM synthesis and/or degradation (100%, $k = 3$) and the expression of inflammatory markers (67%, $k = 2$). **Table 2** presents the study design and outcomes reported from each *in vitro* study.

The chondrocyte proliferation and viability measured by MTS[19] [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy phenyl)-2-(4-sulfophenyl)-2H-tetrazolium] and XTT[20] [2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide] assays were increased after LT, which were time and dosage dependent.

At the ECM, the matrix proteins collagen type II (COL II) and aggrecan (ACAN) expressions were increased after LT treatment measured by polymerase chain reaction (PCR[19]) and immunocytochemistry.[20] One study[19] reported no significant differences in glycosaminoglycans (GAGs) stained by Alcian blue during the treatment duration but revealed a significant difference at the post-treatment follow-up (until day 12 posttreatment). The same study[19] reported a downregulation of collagen type

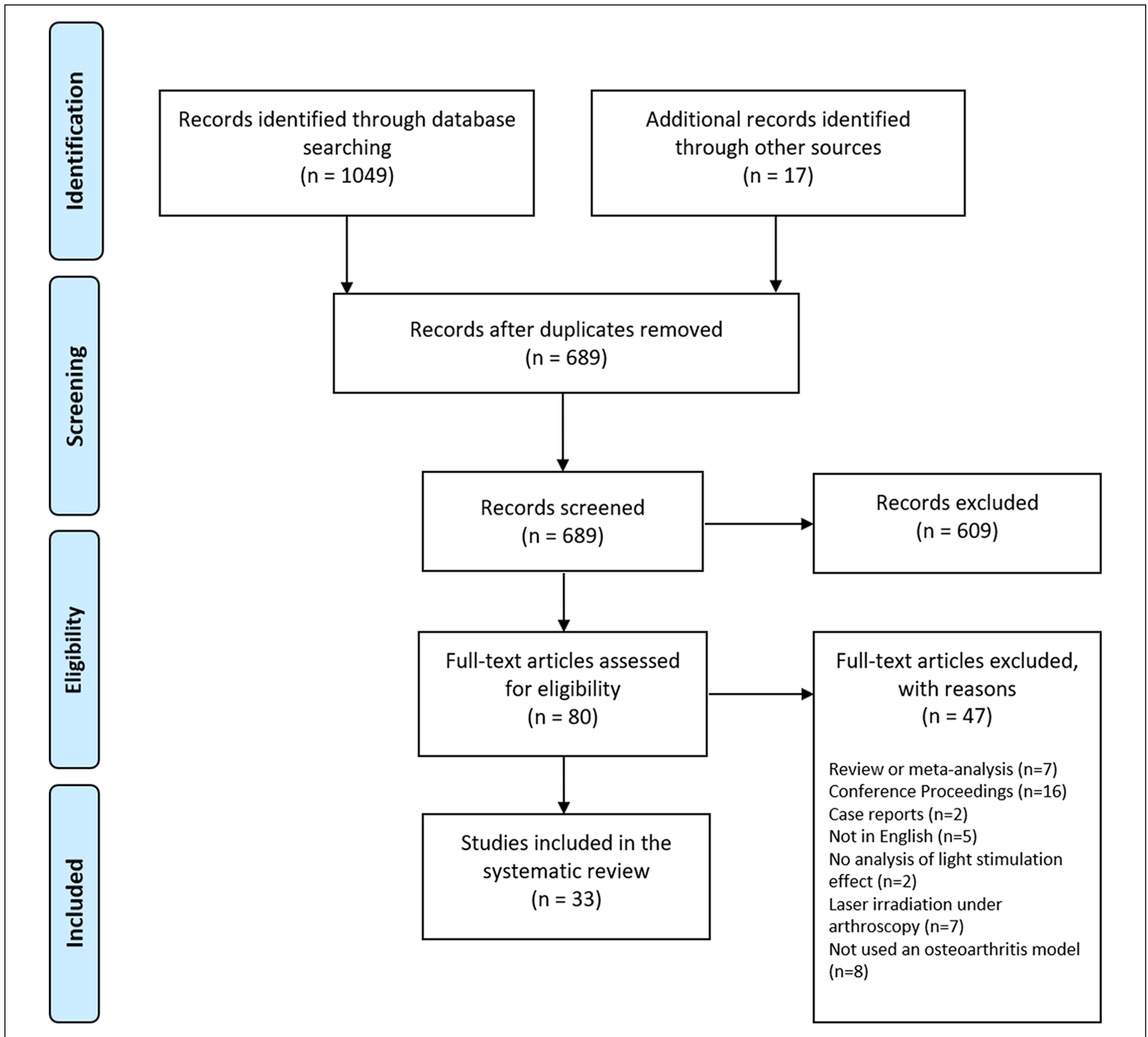

Figure 1. Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) flowchart of included and excluded studies.

I (COL I) and an increase in transcription factor SOX-9 expression (measured by PCR and Western blot).[19] After the light exposure, another study[18] demonstrated a significant decline in the expression of matrix metalloproteinase (MMP) MMP-1 and MMP-3, in contrast to the expressions of MMP-9 and MMP-13 that were not significantly altered (measured by PCR and Western blot).[18] Conversely, a significant decrease in the expression of MMP-13 (measured by PCR and Western blot) after LT was reported in other study.[19]

The inflammation analyzes revealed a significant downregulation of inflammatory markers such as IL-1β, IL-6, and TNF-α (measured by PCR),[18,19] with or without the administration of IL-1β to the cell culture medium.[18]

In Vivo *Animal Studies*

Study Characteristics. A total of 1,400 animals were in 30 *in vivo* studies, distributed in 2 to 9 experimental groups, containing 5 to 20 animals per group. The most common animal model was rat (80%, $k = 24$), of which 83% were Wistar rats. Male animals were more commonly used (73%), with only 2 studies including females (7%) and 3 studies including both genders (10%). The rat models ($n = 1071$) were

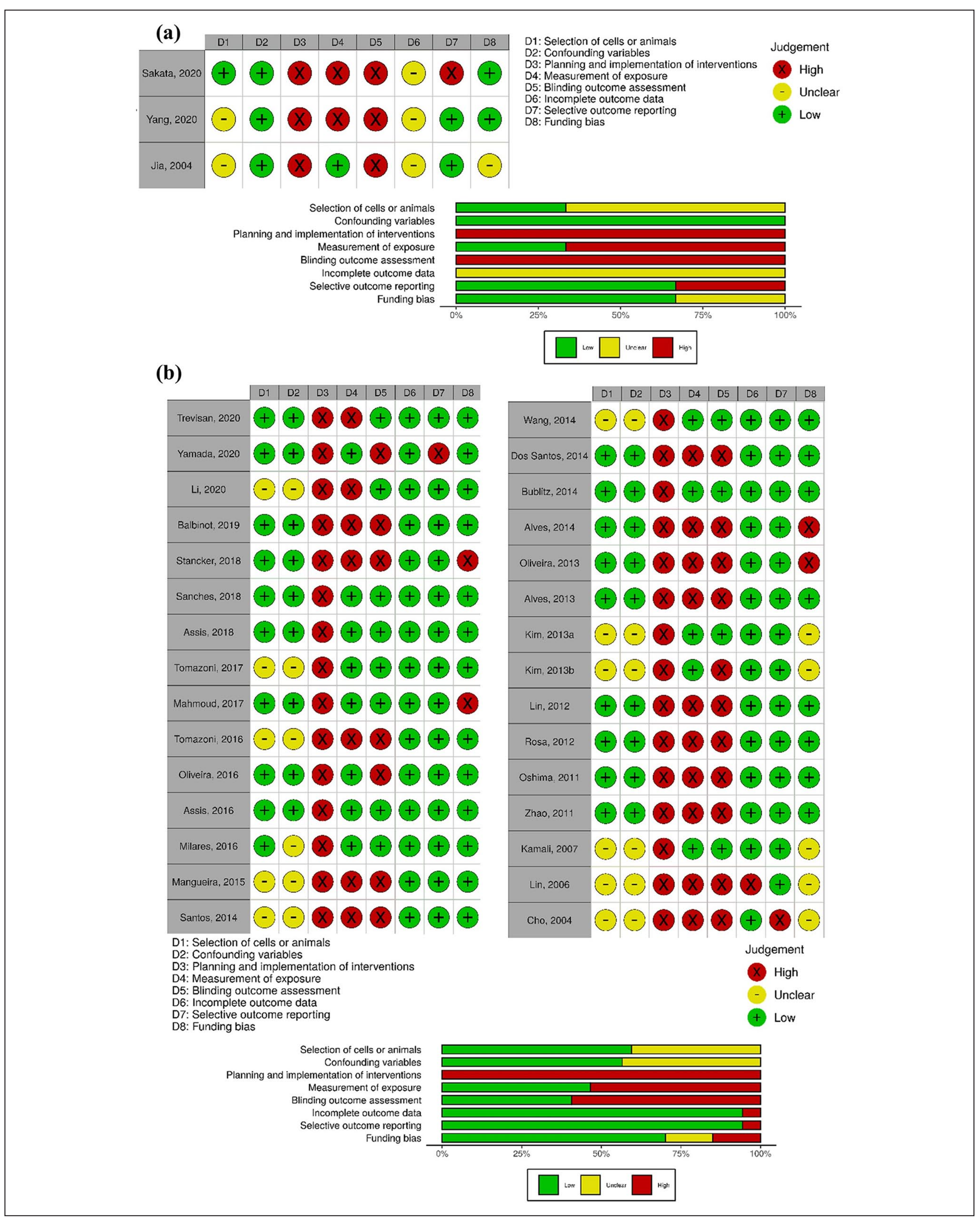

Figure 2. Risk of bias plots. Traffic lights and weight summary plots for (**a**) *in vitro* studies and (**b**) *in vivo* studies.

Table 2. Study Design and Reported Biochemical Outcomes for *In Vitro* Studies.

First Author, Year	Operating Mode and Treatment Duration	Study Design	Biochemical Outcomes		
			Chondrocytes Proliferation and Activity	ECM Synthesis/Degradation	Inflammatory Markers
Sakata, 2020[18]	Pulsed mode once for the duration of 4 or 12h	Human articular chondrocyte-knee (NHAC-Kn) cell line treated with recombinant human IL-1β	NA	↓ MMP-1 and ↓ MMP-3 after both 4 and 8 J/cm^2 No effect on MMP-9 and MMP-13 after both 4 and 8 J/cm^2 (gene expression by RT-PCR, protein expression by Western Blot and ELISA)	↓ IL-1β, ↓ IL-6, ↓ TNF-α for both 4 and 8 J/cm^2 (gene expression by RT-PCR and protein expression by Western blot)
Yang, 2020[19]	Continuous, 8 min daily for 1, 3 and 5 days	Chondrocytes isolated from New Zealand white rabbits' cartilage and expanded C28/I2 Human Chondrocyte Cell Line for gene expression analysis only	↑ Chondrocytes viability after 8 min of LT (MTS assay) No effect after 11 and 13 min of LT exposure ↑ Viable cells (live and dead assay)	No effects on GAGs production (Alcian blue assay) until 6 days but were higher than control in posttreatment period ↑ Matrix deposition (safranin O staining) ↓ COL I for all time points and ↑ COL II after 5 days (protein expression by western blot) ↑ ACAN, ↑ COL II, ↑ SOX-9 after 5 days and ↓ COL I after 3 and 5 days (gene expression by RT-PCR) ↓ MMP-13 after IL1-β and LT stimulation (protein expression by western blot and gene expression by RT-PCR)	↓ IL1-β after LT and ↓ TNF-α after IL1-β and LT stimulation (protein expression by Western blot and gene expression by RT-PCR)
Jia, 2004[20]	Continuous, 3 times at 24-hour intervals.	Chondrocytes isolated from New Zealand rabbits´ cartilage and expanded	↑ Chondrocytes viability after irradiation at 4 J/cm^2 Irradiations at 1, 2, 3 J/cm^2 did not improve this outcome After 5 and 6 J/cm^2, the viability decreased (XTT assay)	↑ GAGs expression intensity (Toluidine-blue staining) ↑ COL II expression intensity (immunocytochemistry)	NA

COL I and II, collagen type I and II; MMP, matrix metalloproteinase; ACAN, aggrecan; SOX-9, SRY-box transcription factor 9; GAGs, glycosaminoglycans; IL, interleukin; TNF-α, tumor necrosis factor-α; RT-PCR, reverse transcription polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; NA, not applicable.

1.5 to 3 months old and weighed 150 to 350 g, whereas the rabbit models (n = 267) were 4 to 15 months old and weighed between 2,000 and 4,500 g.

The *in vivo* studies described different models of experimental OA induction, being the most common the anterior cruciate ligament transection (ACLT) in the knee (k = 11, 37%), followed by intra-articular injections of papain solution (k = 9, 30%) or monoiodoacetate (MIA) (k = 5, 17%). After the animal sacrifice, most studies collected the knee joints (k = 22, 73%). The study design and outcomes reported for *in vivo* studies are presented in **Table 3**.

LT Parameters. The light stimulus was used in continuous mode in more than half of the studies (70%, k = 21). The median wavelength was 808 nm (range, 630-904 nm), at a median power of 50 mW (range, 30-60,000 mW), a median power density of 1,700 mW/cm^2 (range, 0.4-3570 mW/cm^2), and a median energy density of 50 J/cm^2 (range, 2-1,500 J/cm^2) for a median irradiation time of 40 seconds (range, 10-900 seconds). Most commonly, the treatment duration was 3 times a week (90%, k = 27) with a median number of sessions of 15 (range, 1-32) by skin contact (83%, k = 25) in a median of 2 (range, 1-2) points within the joint. Irradiation area was the less reported parameter, being only provided in 3 studies (10%). Supplementary Table S2 reports the LT parameters used in each *in vivo* study and Supplementary Table S3 summarizes the descriptive statistics for the reported LT parameters.

Biochemical Outcomes. The chondrocytes proliferation was not analyzed in any of the *in vivo* studies, but more than half of the studies evaluated the ECM synthesis/degradation and inflammatory markers (Supplementary Table S4).

The ECM synthesis and/or degradation after LT varied across the included studies. The expressions of COL II (measured by immunoexpression,[21] Western blot,[22] picrosirius red staining,[31,36] and PCR[34,43]), GAGs (measured by toluidine blue staining[7]), and ACAN (measured by PCR[34]) increased significantly following LT. However, there was one study that revealed no effect on COL II and ACAN expressions (measured by immunoexpression[32] and PCR,[43] respectively). The MMP-13 expression after LT showed conflicting results; it was significantly downregulated in 5 studies (measured by PCR[22,28,34] and immunoexpression[29,33]) and its expression was not affected in 4 studies (measured by immunoexpression[30,35,37] and PCR[43]). The MMP-3 analysis also showed a significantly decreased expression (measured by immuoexpression[8] and PCR[28]) or no effect (measured by PCR[43]) after LT. Other MMPs such as MMP-1, MMP-2, and MMP-9 were significantly reduced following LT (measured by PCR[22,34] and Western blot[36]). The LT also significantly increased the expression of the tissue inhibitors of metalloproteinases TIMP-1 and TIMP-2 (measured by PCR[22,34]).

The pro-inflammatory markers also showed conflicting evidence across the included studies. The most reported markers were IL-1β and TNF-α. These 2 markers were consistently and significantly decreased after LT (measured by enzyme-linked immunosorbent assay [ELISA][8,9,25-27,39], PCR,[22,25,33,34,38,43] and imunoexpression[29]). Similar trends were observed for expressions of IL-6 (measured by ELISA[8,9,25,39] and PCR[22,25,33,38]) and caspase-3 (measured by immunoexpression[29,41]), whereas the anti-inflammatory marker IL-10 significantly increased after light treatment (measured by PCR[22,33]). In contrast, some studies reported no effect on the expressions of IL-1β (measured by immunoexpression[32,35,37] and PCR[43]), IL-6 (measured by ELISA[26]), TNF-α (measured by ELISA[26] and immunoexpression[32,37]), IL-10 (measured by ELISA[9,26]), and caspase-3 and caspase-8 (both measured by immunoexpression[30,41]). The LT exposure promoted a significant decrease in total number of inflammatory cells, namely neutrophils and macrophages (quantified by ELISA[9,28] and differential cell counting[33,38]). On the contrary, another study reported no effect on total number of inflammatory cells as measured by immunoexpression.[42] LT also reduced oxidation (measured by lipid oxidation assay[9]) and astrogliosis (measured by immunoexpression[7]), which is associated with central inflammation in the spinal cord.[7]

Histological Outcomes. The histological outcomes were assessed through the grading of OA, morphometric analysis, and cartilage organization (Supplementary Table S4). The LT significantly decreased the stage of OA, as graded by the Osteoarthritis Research Society International (OARSI) or OARSI modified score,[8,21,29,30] Mankin,[26,41,44] or other score systems.[34,43,46] Only 2 studies[23,37] reported no effect on OA grading after LT.

The LT interventions resulted in conflicting morphometric findings. While some studies showed significant decrease in chondrocytes density[23,30,32,37] and increase in cartilage thickness,[29,30] other studies[35,45] reported no statistical differences on these 2 outcomes. The most commonly reported histological effects of LT were the slowing down of chondrocytes proliferation (in number and organization), fewer signs of fibrillation and less irregularities in articular cartilage.[8,21,23,24,26,28,29,32,34-37,41,46] Three studies[33,36,38] detected local inflammatory signs after the induction of OA in, and 1 study[42] reported the formation of epithelium and new blood vessels after LT.

Behavioral Outcomes. The pain-like behavior was assessed by gait performance, weight distribution in each hind limb (weightbearing) and mechanical hyperplasia analysis (Supplementary Table S4). The LT did not influence the

Table 3. Study Design and Biochemical, Histological, and Behavioral Outcomes for *In Vivo* Studies.

First Author, Year	Operating Mode Treatment Duration (No. of Sessions)	Experimental Design	OA Model	Animal Type Gender Animal Race Age (months) Weight (g)	Biochemical Outcomes		Histological Outcomes			Behavioral Outcomes
					ECM Synthesis/ Degradation	Inflammatory and Pain Markers	Osteoarthritis Grade	Morphometric Analysis	Cartilage Organization	
Trevisan, 2020[21]	Continuous 3 times per week (12)	G1: OA (n = 10) G2: OA + LT (n = 10)	ACL transection	20 Rats Male Wistar 2 months 150 g	↑ COL II **[G2 vs G1]** (immunoexpression) ↑ TGF-β (growth factor) **[G2 vs. G1]** (immunoexpression)	NA	↓ OARSI score **[G2 vs. G1]**	NA	Abnormal chondrocyte orientation and proliferation. Few irregularities and fibrillation after LT.	No effect on gait performance
Yamada, 2020[9]	Pulsed 3 times per week (8)	G1: Saline injection (n = 10) G2: Saline + LT at 18 J/cm^2 (n = 10) G3: OA (n = 10) G4: OA + LT at 6 J/cm^2 (n = 10) G5: OA + LT at 18 J/cm^2 (n = 10)	Intra-articular injection of MIA	50 Rats Male Wistar 3 months 250 g	NA	↓ Lipid oxidation **[G4/G5 vs. G2]** in spinal cord and **[G4 vs. G2]** in blood serum ↓ Protein carbonyl **[G5 vs. G2]** in spinal cord ↑ SOD activity **[G4/G5 vs. G2]** in brainstem ↓ Nonprotein thiol **[G4/G5 vs. G2]** in blood serum ↑ Nonprotein thiol **[G4/G5 vs. G2]** in brainstem ↓ MPO activity **[G5 vs. G2]** in intra-articular lavage ↑ MPO activity **[G4/G5 vs. G2]** in blood Serum ↓ IL-1β, TNF-α, IL-6 **[G5 vs. G2]** No differences in IL-10 (protein expression by ELISA)	NA	NA	NA	↑ Mechanical Hyperplasia ↑ Spontaneous pain **[G5 vs. G2]**
Li, 2020[8]	NR daily (7)	G1: Saline injection (n = 8) G2: OA (n = 8) G3: OA + LT (n = 8) G4: OA + sham LT (n = 8)	Intra-articular injection of MIA	32 Rats Male Sprague-Dawley NR 220-250 g	↓ MMP-3 **[G3 vs. G2/G4]** (immunoexpression)	↓ TNF-α, ↓ IL-1β, ↓ IL-6 **[G3 vs. G2/G4]** (protein expression by ELISA)	↓ OARSI score **[G3 vs. G2/G4]**	NA	LT inhibited cartilage destruction and proteoglycan loss.	↑ Mechanical hyperalgesia ↑ Weightbearing **[G3 vs. G2/G4]**
Balbinot, 2019[7]	Continuous Daily (15)	G1: OA (n = 9) G2: OA + LT (n = 10)	Intra-articular injection of MIA	19 Rats Male Wistar 3 months 355 ± 22 g	↑ PGs ↑ Chondrocyte content **[G2 vs. G1]** (toluidine blue staining)	↓ Reactive astrogliosis in spinal cord (immunoexpression) **[G2 vs. G1]**	NA	↑ Chondrocyte content **[G2 vs. G1]** (toluidine blue staining)	NA	No effect on gait performance; ↑ Weightbearing; ↑ Mechanical Hyperplasia **[G2 vs. G1]**
Stancker, 2018[22]	Continuous Daily (7)	G1: Culture medium injection (n = 10) G2: OA (n = 10) G3: OA + LT (n = 10) G4: OA + ADSCs (n = 10) G5: OA + ADSCs + LT (n = 10)	Intra-articular injection of papain solution	50 Rats Male Wistar 3 months 250-300 g	↑ COL II **[G3/G4 vs. G2]** **[G5 vs. G2/G4]** ↓ MMP-2 and ↓ MMP-13 **[G3/G4/G5 vs. G2]** No differences among treated groups. (Protein expression by Western blot) ↓ MMP-1, ↓ MMP-2, ↑ TIMP-1 and ↑ TIMP-2 **[G3/G4 vs. G2]** **[G5 vs G2/G3/G4]** (Gene expression by PCR)	↓ IL-1-β and ↓ TNF-α **[G3/G4 vs. G2]** **[G5 vs. G2/G4]** ↓ IL-6 **[G3/G4 vs. G2]** **[G5 vs. G2/G3/G4]** ↑ IL-10 **[G3 vs. G2]** **[G5 vs. G2/G4]** (Gene expression by PCR)	NA	NA	NA	NA

(continued)

Table 3. (continued)

First Author, Year	Operating Mode Treatment Duration (No. of Sessions)	Experimental Design	OA Model	Animal Type Gender Animal Race Age (months) Weight (g)	Biochemical Outcomes		Histological Outcomes			Behavioral Outcomes
					ECM Synthesis/ Degradation	Inflammatory and Pain Markers	Osteoarthritis Grade	Morphometric Analysis	Cartilage Organization	
Sanches, 2018[23]	Continuous 3 times a week (29)	G1: OA (n = 10) G2: OA + LT (n = 10) G3: OA + CS/GL (n = 10) G4: OA + LT + CS/GL (n = 10)	ACL transection	40 Rats Male Wistar 2 months 150 g	↑ COL II **[G4 vs. G1]** No differences among treated groups. (immunoexpression)	↓ IL-1β and ↑ IL-10 **[G4 vs. G1]** No differences among treated groups. (immunoexpression)	↓ OARSI score **[G3/G4 vs. G1]** No differences among treated groups	↓ Density of chondrocytes **[G2/G3/G4 vs. G1]** No differences among treated groups. No difference in cartilage thickness	Degradation, fibrillation, hypercellularity and chondrocytes disorganization among treated groups.	NA
Assis, 2018[24]	Continuous NR	G1: OA (n = 10) G2: OA + aerobic exercise (n = 10) G3: OA + aquatic exercise (n = 10) G4: OA + aerobic exercise + LT (n = 10) G5: OA + aquatic exercise + LT (n = 10)	ACL transection	50 Rats Male Wistar 1.5 months 150 ± 11.2 g	No effect on COL I ↑ COL II **[G2/G3/G4/G5 vs. G1]** No differences among treated groups. (immunoexpression)	↑ IL-10 **[G2/G3/G4/G5 vs. G1]** ↑ TGF-β **[G2/G4 vs. G1]** No differences among treated groups. (immunoexpression)	↓ OARSI score **[G2/G3/G4/G5 vs. G1]** No differences among treated groups.	NA	Abnormal chondrocyte orientation and proliferation, few irregularities and fibrillation among treated groups.	NA
Tomazoni, 2017[25]	Continuous 3 times a week (24)	G1: No intervention (n = 6) G2: OA (n = 6) G3: OA + exercise (n = 6) G4: OA + NSAID (n = 6) G5: OA + LT (n = 6) G6: OA + exercise + NSAID (n = 6) G7: OA + exercise + LT (n = 6) G8: OA + NSAID + LT (n = 6) G9: OA + exercise + LT + NSAID (n = 6)	Intra-articular injection of papain solution	54 Rats Male Wistar NR 200-250 g	NA	↓ IL-1β **[G5 vs. G2/G3/G4/G6G9]** **[G7 vs. G6/G9]** No effect on IL-6 and TNF-α (gene expression by PCR) ↓ IL-1β **[G4/G5/G7/G8 vs. G2]** **[G5 vs. G2/G7]** **[G7 vs. G6/G9]** ↓ IL-6 **[G3/G4/G5/G6/G7/G8/G9 vs. G2]** **[G5/G7 vs. G4/G6]** ↓ TNF-α **[G3/G4/G5/G6/G7/G8/G9 vs. G2]** **[G5/G7 vs. G3/G6]** (Protein expression by ELISA)	NA	NA	NA	NA
Mahmoud, 2017[26]	Pulsed 2-3 times a week (10)	G1: OA (n = 6) G2: OA + US (n = 6) G3: OA + LT (n = 6)	Injection of saline solution of Lev	18 Rats Male Albino 3 months 150-200 g	NA	↓ IL-1β **[G2/G3 vs. G1]** ↓ IL-6 **[G2 vs. G1]** ↑ IL-10 and ↓ INF-γ **[G2 vs. G1/G3]** No effect on TNF-α (Protein expression by ELISA)	NA	↓ Mankin scores **[G2/G3 vs. G1]**	Similar histological evidences among treated groups: smooth surface without irregularities.	↑ Knee maximum extension **[G2/G3 vs. G1]**
Tomazoni, 2016[28]	Continuous 3 times a week (24)	G1: No intervention (n = 6) G2: OA (n = 6) G3: OA + LT (n = 6) G4: OA + exercise (n = 6) G5: OA + Diclo (n = 6) G6: OA + exercise + LT (n = 6) G7: OA + Diclo + LT (n = 6) G8: OA + exercise + Diclo (n = 6) G9: OA + exercise + LT + Diclo (n = 6)	Intra-articular injection of papain solution	54 Rats Male Wistar NR 200-250 g	↓ MMP-3 **[G3/G5/G7/G9 vs. G2]** No differences among treated groups. ↓ MMP-13 **[G3 vs. G2/G7/G6]** **[G4/G5/G8/G9 vs. G2/G7]** (gene expression by PCR)	↓ Inflammatory cells number **[G3/G5 vs. G2/G4/G6/G7/G8/G9]** (intra-articular wash counting) ↑ MPO activity **[G3/G6 vs. G2/G4/G5/G7]** (synovial supernatant quantification)	NA	NA	LT and exercise alone promoted a homogeneous chondrocytes distribution. Remaining treated groups presented chondrocytes distributed heterogeneously.	NA

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Table 3. (continued)

First Author, Year	Operating Mode Treatment Duration (No. of Sessions)	Experimental Design	OA Model	Animal Type Gender Animal Race Age (months) Weight (g)	Biochemical Outcomes: ECM Synthesis/ Degradation	Biochemical Outcomes: Inflammatory and Pain Markers	Histological Outcomes: Osteoarthritis Grade	Histological Outcomes: Morphometric Analysis	Histological Outcomes: Cartilage Organization	Behavioral Outcomes
de Oliveira, 2016[27]	Continuous NR	G1: No intervention ($n = 18$) G2: OA ($n = 18$) G3: OA + LT ($n = 18$)	Intra-articular injection of papain solution	54 Rats Male Wistar 3 months 250-300 g	NA	↓ TNF-α **[G3 vs. G2]** (Protein expression by ELISA)	NA	NA	NA	↑ Mechanical hyperalgia **[G3 vs. G2]**
Assis, 2016[29]	Continuous 3 times a week (24)	G1: No intervention ($n = 10$) G2: OA ($n = 10$) G3: OA + aerobic exercise ($n = 10$) G4: OA + LT ($n = 10$) G5: OA + aerobic exercise + LT ($n = 10$)	ACL transection	50 Rats Male Wistar 1.5 months 150 g	↓ MMP-13 **[G3/G4/G5 vs. G2]** No differences among treated groups. (immunoexpression)	↓ IL-1β **[G3/G4/G5 vs. G2]** No differences among treated groups. ↓ Caspase-3 [G3/G4 vs. G2] [G5 vs. G2/G3/G4] (immunoexpression)	↓ OARSI score [G3/G4/G5 vs. G2] No differences among treated groups.	No differences in chondrocytes density ↑ Cartilage thickness **[G3/G4/G5 vs. G2]** No differences among treated groups.	Few signs of fibrillation and irregularities, moderate number of chondrocytes and organization among treated groups.	NA
Milares, 2016[30]	Continuous 3 times a week (24)	G1: OA ($n = 10$) G2: OA + exercise ($n = 10$) G3: OA + LT ($n = 10$) G4: OA + exercise + LT ($n = 10$)	ACL transection	40 Rats Male Wistar 1.5 months 150 g	↓ MMP-13 **[G4 vs. G1]** No differences among treated groups. (immunoexpression)	↓ IL-1β and ↓ Caspase-3 **[G2/G4 vs. G1]** No differences among treated groups. (immunoexpression)	↓ OARSI score [G2/G3/G4 vs. G1] No differences among treated groups.	↓ Chondrocytes density and ↑ Cartilage thickness **[G2/G3/G4 vs. G1]** No differences among treated groups.	Less tissue degradation, marks of fibrillation and irregularities, and chondrocytes organization among treated groups.	NA
Mangueira, 2015[31]	Continuous NR	G1: Saline injection ($n = 9$) G2: OA ($n = 9$) G3: OA + LT at 660 nm ($n = 10$) G4: OA + LT at 780 nm ($n = 10$)	Intra-articular injection of collagenase	36 Rats Male Wistar NR 220-260 g	↑ COL III area **[G3 vs. G2/G4]** ↑ COL II area **[G3/G4 vs. G2]** (Picrosirius red staining)	NA	NA	NA	NA	NA
Dos Santos, 2014[32]	Continuous NR	G1: No intervention ($n = 5$) G2: OA ($n = 5$) G3: OA + PMT at 2 J ($n = 5$) G4: OA + LT at 4 J ($n = 5$)	Intra-articular injection of papain solution	20 Rats Male Wistar 3 months 250-300 g	NA	↓ Neutrophils and ↓ Macrophages **[G3/G4 vs. G2]** **[G3 vs. G4]** (Intra-articular wash counting) ↓ IL-1β and ↑ IL-10 **[G3 vs. G2]** No differences among treated groups. ↓ TNF-α **[G4 vs. G2/G3] [G3 vs. G2]** ↓ IL-6 **[G4 vs. G3]** (Gene expression by PCR)	NA	NA	Similar histological evidences among treated groups: low intensity acute inflammation and normal articular surface.	NA
Wang, 2014[34]	Continuous 3 times a week (6, 12, 18, or 24)	G1: OA ($n = 80$) G2: OA + LT ($n = 80$)	ACL transection	160 Rabbits NR New Zealand 6 months 3500± 800 g	↑ COL II, ↑ ACAN, ↑ TGF-β, ↑ MMP-1 and ↑ MMP-13 after 8 weeks **[G2 vs. G1]** ↑ TIMP-1 and ↑ MMP-3 after 6 and 8 weeks **[G2 vs. G1]** No effect on IGF-1, BMP-2 and BMP-7 (gene expression by PCR)	↓ IL-1β after 6 and 8 weeks **[G2 vs. G1]** (gene expression by PCR)	↓ OA score after 6 and 8 weeks **[G2 vs. G1]**	NA	LT improved cartilage damage and erosion at all locations.	↑ Weight bearing after 6 and 8 weeks **[G2 vs. G1]**

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Table 3. (continued)

First Author, Year	Operating Mode Treatment Duration (No. of Sessions)	Experimental Design	OA Model	Animal Type Gender Animal Race Age (months) Weight (g)	Biochemical Outcomes		Histological Outcomes			Behavioral Outcomes
					ECM Synthesis/ Degradation	Inflammatory and Pain Markers	Osteoarthritis Grade	Morphometric Analysis	Cartilage Organization	
Dos Santos, 2014[33]	Continuous 5 times a week (15 or 30)	G1: Control ($n = 20$) G2: OA ($n = 20$) G3: LT at 10 J/cm^2 ($n = 20$) G4: LT 50 J/cm^2 ($n = 20$)	ACL transection	80 Rats Male Wistar 3 months 300 ± 20 g	No differences in COL II organization and intensity ↓ MMP-13 after 8 weeks **[G3/G4 vs G2]** No differences among treated groups (immunoexpression)	No effect on TNF-α and IL-1β (immunoexpression)	NA	↓ Cell number after 8 weeks **[G3/G4 vs. G2]** No differences among treated groups No effect of cartilage thickness	G4 showed chondrocytes disorganization and intense presence of cells, while G3 presented slight fibrillation and moderate presence of cells after 8 weeks of LT.	NA
Bublitz, 2014[35]	Continuous 5 times per week (15)	G1: OA ($n = 10$) G2: LT at 10 J/cm^2 ($n = 10$) G3: LT at 50 J/cm^2 ($n = 10$)	ACL transection	30 Rats Male Wistar 3 months 300 ± 20 g	No effect on MMP-13 expression (immunoexpression)	No effect on IL-1β expression (immunoexpression)	NA	No effect on chondrocytes number. ↓ Cartilage area **[G2/G3 vs. G1]** ↓ PGs reduction score **[G2/G3 vs. G1]** (safranin O staining) No differences among treated groups	G2 presented more tissue and chondrocytes organization with no fibrillation in comparison to G1 and G2. G3 presented tissue disorganization in comparison to G2, but better organized than G1.	NA
Alves, 2014[36]	Continuous 3 times a week (4, 7, or 10)	G1: No intervention ($n = 15$) G2: OA ($n = 15$) G3: LT at 50 mW ($n = 15$) G4: LT at 100 mW ($n = 15$)	Intra-articular of papain solution	60 Rats Male Wistar 3 months 250-300 g	↓ COL I and ↑ COL II **[G3/G4 vs G2]** (Picrosirius red staining) ↓ MMP-2 and ↓ MMP-9 after 7 and 14 days **[G3/G4 vs G2]** ↓ MMP-2 and ↓ MMP-9 after 21 days **[G3 vs G2]** (Protein expression by Western blot) No differences among treated groups	NA	NA	NA	After 21 days, G3 showed tissue repair, but fewer fibroblast. G4 presented a thick synovial membrane and tissue repair.	NA
Oliveira, 2013[37]	Continuous 5 times a week (15 or 30)	G1: No intervention ($n = 20$) G2: OA ($n = 20$) G3: LT at 10 J/cm^2 ($n = 20$) G4: LT at 50 J/cm^2 ($n = 20$)	ACL transection	80 Rats Male Wistar 3 months 300 ± 20 g	No effect on MMP-13 ↑ COL I after 5 weeks **[G3 vs. G2]** ↓ COL I after 8 weeks **[G3/G4 vs. G2]** (immunoexpression) No differences among treated groups	No differences in TNF-α and IL-1β (immunoexpression)	No effect on Mankin score	↓ Chondrocytes number after 8 weeks **[G3/G4 vs. G2]** No differences among treated groups No effect on cartilage thickness.	After 8 weeks, G3 showed a better tissue organization compared with G2, moderate number of cells, slight fibrillation and irregularities. G4 exhibited more disorganized tissue compared to G3, moderate presence of chondrocytes and fibrillation.	NA

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Table 3. (continued)

First Author, Year	Operating Mode Treatment Duration (No. of Sessions)	Experimental Design	OA Model	Animal Type Gender Animal Race Age (months) Weight (g)	Biochemical Outcomes		Histological Outcomes			Behavioral Outcomes
					ECM Synthesis/ Degradation	Inflammatory and Pain Markers	Osteoarthritis Grade	Morphometric Analysis	Cartilage Organization	
Alves, 2013[38]	Continuous Once	G1: No intervention ($n = 15$) G2: OA ($n = 15$) G3: LT at 50 mW ($n = 15$) G4: LT at 100 mW ($n = 15$)	Intra-articular injection of papain solution	60 Rats Male Wistar 3 months 250-300 g	NA	↓ IL-1β and ↓ IL-6 **[G3 vs. G2]** ↓ TNF-α **[G4 vs. G2/G3]** (gene expression by PCR) ↓ Neutrophils **[G3/G4 vs. G2]** ↓ Macrophages **[G3 vs. G2/G4]** (Intra-articular wash counting)	NA	NA	G3 presented tissue with fibroblast and discrete inflammatory cells, while G4 showed acute inflammatory infiltrate, red blood cells and hyaline material presence.	NA
Kim, 2013[39]	Pulsed 5 times per week (15)	G1: Saline injection ($n = 10$) G2: OA ($n = 10$) G3: LT ($n = 10$)	Intra-articular injection of MIA	30 Rats Male Sprague-Dawley NR 150-160 g	NA	↓ TNF-α, ↓ IL-1β and ↓ IL-6 **[G3 vs. G2]** (protein expression in blood by ELISA)	NA	NA	NA	NA
Kim, 2013[40]	Pulsed 5 times per week (15)	G1: Saline injection ($n = 10$) G2: OA ($n = 10$) G3: LT ($n = 10$)	Intra-articular injection of MIA	30 Rats Male Sprague-Dawley NR 150-160 g	NA	NA	NA	NA	NA	↑ Mechanical hyperplasia after 14 and 21 days ↑ Weightbearing after 21 days **[G3 vs. G2]**
Lin, 2012[41]	Continuous 5 times a week (10)	G1: No intervention ($n = 8$) G2: OA ($n = 8$) G3: LT ($n = 8$)	ACL transection	24 Rabbits male/female (50%-50%) New Zealand 3 months 2,000-2,500 g	NA	↓ Caspase-3 **[G3 vs. G2]** No effect on Caspase-8 (immunoexpression)	↓ Markin score **[G3 vs. G2]**	NA	After LT, the tissue presented a smoother surface with slight disorganization.	NA
Da Rosa, 2012[42]	Continuous Daily (7, 14, or 21)	G1: OA ($n = 12$) G2: LT at 660 nm ($n = 12$) G3: LT at 808 nm ($n = 12$)	Intra-articular injection of papain solution	36 Rats Male Wistar 3 months 250-300 g	NA	No effect on the number of inflammatory cells. (immunoexpression)	NA	NA	↑ Newly formed vessels after 7 days **[G2 vs. G1]** ↓ Fibrosis intensity after 14 days **[G2/G3 vs. G1]** ↓ Fibrosis intensity after 21 days **[G3 vs. G1]** G3 presented better results regarding the formation of epithelium and new blood vessels.	NA

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Table 3. (continued)

First Author, Year	Operating Mode Treatment Duration (No. of Sessions)	Experimental Design	OA Model	Animal Type Gender Animal Race Age (months) Weight (g)	Biochemical Outcomes		Histological Outcomes			Behavioral Outcomes
					ECM Synthesis/ Degradation	Inflammatory and Pain Markers	Osteoarthritis Grade	Morphometric Analysis	Cartilage Organization	
Oshima, 2011[43]	Pulsed 5 times a week (25)	G1: OA (n = 7) G2: LT (n = 7)	ACL transection	14 Rabbits Female New Zealand 9-15 months 3,500-4,500 g	↑ COL II **[G2 vs. G1]** (gene expression by PCR) No effect on ACAN, MMP-3, and MMP-13	↓ TNF-α **[G2 vs. G1]** (gene expression by PCR) No effect on IL-1β	↓ OA grade **[G2 vs. G1]** (Gross appearance analysis)	NA	NA	NA
Zhao, 2011[44]	NR Every other day (15)	G1: OA (n = 10) G2: OA + sham LT (n = 10) G3: Laser 10.6 μm + 650 nm (n = 10) G4: LT at 650 nm (n = 10) G5: Laser 10.6 μm (n = 10)	Naturally after extreme exercise	50 Mice male/female (50%-50%) C57 Black 5 months 20-25 g	NA	NA	↓ Mankin score **[G3 vs. G2]**	NA	NR	NA
Kamali, 2007[45]	Pulsed Twice per week (8, 16, or 32)	G1: Control (n = 6-8) G2: LT (n = 6-8)	Osteo-chondral defect (5 × 4 mm)	41 Rabbits NR Dutch White 4 months 2000 ± 300 g	NA	NA	NA	No differences in cartilage thickness. ↑ Cartilage stiffness (biomechanical analysis) after 8 weeks **[G2 vs. G1]**	NA	NA
Lin, 2006[46]	Continuous 3 times a week (24)	G1: Early-stage OA; (n = 3-4) G2: Intermediate-stage OA (n = 3-4) G3: Late-stage OA (n = 3-4) G1s, 2s, 3s: LT (n = 3-4) G1c, 2c, 3c: Control (n = 3-4)	Intra-articular injection of papain solution	78 Rats Female Wistar NR 320-350 g	NA	NA	↓ OA grade **[G1s vs. G1c]** **[G2s vs. G2c]**	NA	G1s and G1c showed fibrillation. G2s and G2c presented chondrocyte enlargement. G3s and G3c exhibited deep fibrillation and partial to total cartilage loss.	NA
Cho, 2004[47]	Pulsed Daily (15 or 30)	G1: OA (n = 5) G2: LT for 2 weeks (n = 5) G3: LT for 4 weeks (n = 5) G4: OA for 2 weeks without LT (n = 5) G5: OA for 4 weeks without LT (n = 5)	Intra-articular injection of H_2O_2	28 Rabbits NR New Zealand 10 months 2,500-3,000 g	NA	NA	NA	NA	After 4 weeks of LT, chondrocytes disorganization was observed.	NA

NR, not reported; NA, not applicable; G, group; LT, light therapy; OA, osteoarthritis; ACL, anterior cruciate ligament; MIA, monosodium iodoacetate; Lev, levofloxacin; COL I, II, and III, collagen type I, II, and III; MMP, matrix metalloproteinase; TIMP, tissue inhibitors of metalloproteinases; IL, interleukin; TNF-α, tumor necrosis factor-α; INF-γ, interferon gamma; ACAN, aggrecan; PGs, proteoglycans; OARSI, Osteoarthritis Research Society International; PCR, polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; ADSCs, adipose-derived stem cells; US, ultrasounds; NSAID, nonsteroidal anti-inflammatory drug; Diclo, sodium diclofenac; SOD, superoxide dismutase; MOP, myeloperoxidase.

gait performance[7,21] or maximum knee extension.[26] Weight-bearing and mechanical hyperplasia were significantly increased after LT.[7-9,34,40,50]

Discussion

The main findings of this systematic review are that LT promoted ECM synthesis and lowered pain and inflammation in *in vitro* and *in vivo* studies, suggesting a potential to slow down OA and cartilage degeneration.

The majority of the studies were judged as high risk of performance and detection bias. The dosage calculation was inconsistent since some authors considered the beam surface,[21,22] while others assumed an irradiated surface area,[7,43] and roughly one third of studies[8,26,39,41,43-47,51] failed to report all LT parameters. Those factors preclude direct comparisons among studies. Lack of temperature control during stimulation also contributed for the high risk of performance bias. More than half of the studies did not measure the qualitative and/or semiquantitative data adequately, lacking 2 independent observers[7,8,18,19,21,22,28,31-33,36-38,41-44,46,47] or blinding of the observers.[7,9,20,22,27,28,31-33,36-38,40-44,46,47] These qualitative and semiquantitative analyses are more prone to errors as they estimate the concentrations and do not provide an accurate quantification. The use of distinct techniques may also diagnose differently the outcomes. One study did not find statistically significant differences in gene expression of IL-1β by PCR after LT, but on the protein expression (ELISA assay) analysis differences were detected.[25] Therefore, it is also advisable to complement qualitative analysis with a proper quantitative measurement of the outcomes.[8,22,25,26,28,32,34,38,39,43,50]

Many research models of OA have been explored to study the disease and its effect on the whole joint. The included *in vitro* studies used monolayer cultures of chondrocytes from primary sources. Other *in vitro* models such as cartilage explants,[52] 3-dimensional culture[53] or co-culture with other cell types also implicated in the disease[54] were not explored in the context of light stimulation. The application of those models could also contribute with relevant insights about the *in vitro* effects of LT. *In vivo* models allow a more in-depth study of OA disease in the whole joint, as well as the effects of time, motion, and weightbearing. The included *in vivo* studies used small animals, most induced OA by surgical procedures (e.g., ACLT) or by chemical injections (e.g., papain and MIA). The surgical OA models promote joint destabilization which, consequently, results in OA, enabling a more comprehensive study of cartilage degeneration and its progression.[21,23,24,29,30,32,34,35,37,41,43] The chemically induced models focus mainly on the inflammation and pain mechanisms.[7-9,22,25,27,28,33,36,38-40,42,46] Thereby, *in vitro* and *in vivo* research models are significant to elucidate the LT effects on OA, contributing to the growing understanding of this therapy, before translating into clinical studies.

The use of LT resulted in biochemical-induced effects on articular cartilage. The *in vitro* studies demonstrated that the LT yielded a positive biochemical effect in cartilage, including an increase of ECM synthesis (COL II, ACAN, and GAGs) and a downregulation of ECM proteases (MMPs) and inflammatory markers (IL-1β, IL-6, and TNF-α).[18-20] The LT produced a continuing effect on chondrocytes activity that persisted up to 12 days after treatment.[19] The i*n vivo* studies showed that LT stimulated ECM matrix production while inhibiting its degradation.[7,8,21,22,28,29,32,34,36,43] There was however 1 study reporting no effect in any of the matrix proteins[32] and4 other studies[30,35,37,43] reporting no effect on MMPs expression. The COL I expression, which is commonly seen in fibrocartilage, was unaffected or decreased,[24,36,37] suggesting that the LT is promoting a hyaline-like cartilage regeneration.[1] The effects of LT on inflammation markers were inconsistent. While most studies found a significant decrease on the expression of inflammatory markers,[8,9,22,25-30,33,34,38,39,41,43] other studies did not find any effect.[32,35,37,42] These findings combined suggest that LT can slow down the cartilage degeneration and has a potential to modulate the OA-derived joint inflammation, but the effects are variable.

Cartilage degeneration is characterized by chondrocytes hypertrophic proliferation and differentiation, resulting in chondrocytes apoptosis and cartilage replacement by bone.[55] The use of LT improved cartilage quality as assessed by histological analysis and suggested a deceleration of cartilage degeneration.[8,21,24,26,28,34,41] Histologically, the included studies found a decrease of both OA progression[8,21,26,29,30,34,41,44] and chondrocytes density[23,29,30,32,37] with improved cartilage thickness.[29,30] Four studies did not show any effect in any of the histological outcomes, including OA progression, chondrocytes density or cartilage thickness.[23,35,37,45] These findings combined suggest that LT seem to have a significant effect in slowing down the OA progression.

The animal behavior after exposure to LT highlighted an analgesic effect as observed by weightbearing readaptation (distribution of weight across the hind paws) and mechanical hyperplasia (paw withdrawal in response to an increasing force).[7-9,34,40,50] Although no changes on gait performance were observed, a control group without induction of OA would be needed to confirm if the lack of differences mean the LT had no effect on gait patterns or if the induction OA did not result in gait impairments.[7,21] One study[46] used *in vivo* models with different OA stages and demonstrated that LT stimulated cartilage regeneration only at early and intermediate stages of OA, which suggests that LT might be unable to delay OA progression at more advanced stages.

To interpret outcomes, it is important to understand how variables can interfere in those results and that physiological effects of LT are dose dependent. While a very low energy may not be sufficient to promote a cellular response, an excessively high energy will inhibit those effects.[5,6] Dose

dependency of LT was investigated in 2 studies,[19,20] which confirmed that longer exposure times or energy densities of LT did not result in better cellular viability. The influence of the LT dosages on articular cartilage repair was variable across the studies and remains inconclusive. While 1 study[33] reported that 71.4 J/cm^2 at 50 mW was better than 142 J/cm^2 at 50 mW in eliciting an anti-inflammatory response, another study[38] concluded that 142 J/cm^2 at 50 mW was more efficient in modulating all inflammatory markers than 142 J/cm^2 at 100 mW. Other studies[32,35,37] also compared lower energy densities (10 and 50 J/cm^2 at 30 mW) but no significant effect was observed in the inflammation process. Only 1 study[9] showed that 9 J/cm^2 at an average power of 40 mW enhanced the anti-inflammatory response in pulsed mode. The effect of different wavelengths was also investigated. Both *in vitro* and *in vivo* studies applied red and near-infrared light (600-1100 nm), which corresponds to a higher absorption by chromophores at cellular mitochondria.[56] However, while an wavelength of 660 nm was better in repairing cartilage than 780 nm,[31] a wavelength of 808 nm was more effective in stimulating angiogenesis than 660 nm.[42] Other studies applied higher wavelength values using carbon dioxide laser (10.6 μm) to stimulate knee "acupoints" for laser acupuncture, but only 2 studies[8,44] used this type of laser, limiting the conclusions. Finally, the included studies applied a wide range of other LT parameters (other than energy density, power, and wavelength), which hampers more direct comparisons between the studies and limits the critical rationale about the most appropriate dosage for knee cartilage repair.

The effects of LT combined with other conservative therapies was assessed in a few studies. When the LT was combined with different exercise modalities or topical use of nonsteroidal anti-inflammatory drugs (NSAIDs), there were no additional effects as compared with LT alone.[24,25,28-30] On the other hand, when combined with intra-articular injection of stem cells or with chondroitin and glucosamine sulfate, LT showed an enhanced therapeutic effect in the articular cartilage.[22,23]

The World Association for Laser Therapy (WALT) guidelines recommends minimum dosage values for the application of LT in the knee.[57,58] The WALT guidelines recommend a minimum of 4 J ± 50% energy per point, at a power of 5 to 500 mW, for 20 to 300 seconds, at 780 to 860 nm, when using GaAlAs lasers.[57] More than one-fourth of the studies did not follow those recommendations, applying lower values of energy per point—0.3 J and 1.4 J.[23,24,29,30,32,35,37] Some of these studies were previously highlighted for not showing any effects on ECM synthesis,[32] downregulation of MMPs[30,35,37] and inflammation markers,[32,35,37] and lack of histological improvements.[23,35,37] The administration of dosage values of LT below the therapeutic window may not be enough to trigger a cellular response. Most of the studies that used lower dosage values only observed significant differences in some of the outcomes (biochemical, histological, or behavioral) after more than 20 sessions of treatment.[23,24,29,30,32,37] The number of sessions of treatment appears to be related to energy dose, with lower dosage values requiring more sessions of LT to elicit therapeutic effects. Following the WALT guidelines is of upmost importance to ensure optimized results, to establish direct comparisons among studies and to standardize the LT dosages according to the diagnosis. However, the WALT guidelines are only valid for GaAs and GaAlAs lasers and their recommended values may not be applicable to other type of lasers, such as He-Ne,[19,34] InGaAlP,[42] and to LEDs (light-emitting diodes).[21] It is thus paramount and a priority to extend these guidelines to other types of lasers to allow researchers and clinicians to apply recommended dosages regardless of the type of laser utilized.

Some limitations of this systematic review should be highlighted. Our search strategy identified only a small number of *in vitro* studies, which limits our discussion on the *in vitro* effects of LT. Only *in vivo* studies that used OA models were included, excluding inflammatory arthritis and rheumatoid arthritis models, knowing that this may have restricted some studies to this analysis. However, the effect of LT on systemic inflammatory diseases was not within goal of this systematic review. The lack of consistent reporting of the same outcomes under the same testing conditions precluded the performance of a meta-analysis. Performance and detection bias were judged as high risk of bias for all studies, which limits the strength of the conclusions that can be made.

This systematic review aims to provide future directions in LT field. The outcomes tables (Tables 2 and 3, Supplementary Tables S2 and S3) summarized in this systematic review provide a useful source for comparison of different parameters and their findings. Researchers should report clearly all stimulation parameters and follow WALT guidelines to standardize the application of LT and to ensure a minimum therapeutic effect. Further efforts should focus on extending the current guidelines to other laser types and LEDs. The implementation of LT and techniques to measure the outcomes should also be improved in future studies by controlling the temperature during stimulation and complementing their qualitative analyses with quantitative measurements.

Conclusions

There was poor standardization of LT parameters, its application methods, and outcomes measured. Still, the *in vitro* and *in vivo* research models suggest that the use of LT may be considered as a nonsurgical treatment option on the management of knee OA, especially on early stages, since positive effects on ECM production, inflammatory response, deceleration of OA progression and pain-like behavior have

been demonstrated. In addition, future studies should comprehensively report the LT parameters and comply the WALT guidelines.

Author Contributions

All authors were involved in the idealization of the systematic review and contributed for the design. SO and RA screened the articles and full text articles. Conflicts were resolved by OC and AL. SO extracted data to Microsoft Excel spreadsheet and it was reviewed by RA, BBH, OC, and AL. SO and RA performed risk of bias assessment. S.O drafted the manuscript with input from all authors. BBH, FS, and JEM provided guidance and advice during all steps of the development of the systematic review. All authors have read and approved the final manuscript.

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Declaration of Conflicting Interests

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