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LITERATURE REVIEW

Effects of Low-Level Laser Therapy on Skeletal Muscle Repair

A Systematic Review

ABSTRACT

Alves AN, Fernandes KPS, Deana AM, Bussadori SK, Mesquita-Ferrari RA: Effects of low-level laser therapy on skeletal muscle repair: a systematic review. Am J Phys Med Rehabil 2014;93:1073–1085.

A review of the literature was performed to demonstrate the most current applicability of low-level laser therapy (LLLT) for the treatment of skeletal muscle injuries, addressing different lasers, irradiation parameters, and treatment results in animal models. Searches were performed in the PubMed/MEDLINE, SCOPUS, and SPIE Digital Library databases for studies published from January 2006 to August 2013 on the use of LLLT for the repair of skeletal muscle in any animal model. All selected articles were critically appraised by two independent raters. Seventeen of the 36 original articles on LLLT and muscle injuries met the inclusion criteria and were critically evaluated. The main effects of LLLT were a reduction in the inflammatory process, the modulation of growth factors and myogenic regulatory factors, and increased angiogenesis. The studies analyzed demonstrate the positive effects of LLLT on the muscle repair process, which are dependent on irradiation and treatment parameters. The findings suggest that LLLT is an excellent therapeutic resource for the treatment of skeletal muscle injuries in the short-term.

Key Words: Laser Therapy, Injury, Repair, Skeletal Muscle, Sports

Muscle injuries are a common occurrence and can have a major impact on the performance of athletes and amateur sports enthusiasts.[1–3]

The skeletal muscle regeneration process involves three major phases that function in a coordinated, interrelated fashion (degeneration/inflammation, repair/fibrosis, and remodeling), which ideally lead to the structural and functional recovery of the injured muscle.[4] The initial inflammatory phase involves the influx of cytokines, which promote chemotaxis, muscle fiber necrosis (myonecrosis), the removal of necrotic tissue by macrophages, a local increase in vascularity, and the activation of myogenic precursor (stem) cells, which are denominated satellite cells.[4–6] These cells then proliferate and differentiate into myoblasts, which subsequently fuse damaged muscle fibers to form new functional fibers.[5–7] The process ends with the maturation of new fibers, the contraction and reorganization of scar tissue, and the functional recovery of the injured muscle.[1]

Although skeletal muscle has considerable regenerative capacity, the process is slow and often results in some degree of functional impairment and increased susceptibility to further injury.[1,8,9] Therefore, standardized therapeutic approaches that can accelerate and enhance the muscle repair process are extremely important.

In recent years, low-level laser therapy (LLLT) has emerged as an effective alternative for the treatment of muscle injuries and has become increasingly common in clinical practice, especially in the fields of physical therapy and sports medicine as well as different fields of traditional medicine, demonstrating therapeutic effects on different types of biologic tissues.[10] Studies have reported positive effects on tendinopathy,[11,12] osteoarthritis,[13,14] arthritis,[15] wound healing,[16] back pain,[17,18] neck pain,[19] and peripheral nerve injury.[20,21] Moreover, positive effects have been reported in the treatment of muscle injuries, such as the modulation of inflammatory response and myonecrosis,[22–24] growth factors,[25–27] the proliferation of satellite cells and fibroblasts,[28–31] remodeling of extracellular matrix (ECM),[22,27,32,33] and angiogenesis.[22,32]

Despite the benefits reported in experiments conducted in vitro, studies conducted on different animal models, and a number of clinical trials, divergent findings have been described. This is mainly because of the variability in the irradiation parameters and treatment protocols used.

The aim of the present article was to perform a review of the literature to demonstrate the most current applicability of LLLT for the treatment of skeletal muscle injuries, addressing different lasers, irradiation parameters, and treatment results in animal models.

METHODS

Searches were performed in the PubMed/MEDLINE (Medical Literature Analysis and Retrieval System Online), SCOPUS, and SPIE Digital Library databases for original articles regarding the effects of LLLT on skeletal muscle regeneration in experimental models published in English from January 2006 to August 2013. The Medical Subject Headings and SCOPUS were used to find additional key words related to *lasers*, *laser therapy*, *low-level laser therapy*, *low intensity laser therapy*, *phototherapy*, or *photobiomodulation*; *repair* or *regeneration*; and *skeletal muscle*. The bibliographies of all retrieved articles were also examined to identify additional studies (Fig. 1). Two reviewers then independently applied the predetermined eligibility criteria to the full text of the studies retrieved.

The studies selected for analysis were included in the review when meeting the following criteria:

(1) Articles published between January 2006 and August 2013
(2) Animal studies involving experimental models of muscle injury
(3) Studies that describe or allowed the calculation of the following dosimetric parameters: wavelength (lambda) in nanometers, power in milliwatts or watts, beam spot size in square centimeters, power density in watts per square centimeter, energy density in joules per square centimeter, frequency in hertz, pulse in nanoseconds or duty cycle of device in percentage, treatment time in seconds, dose per treatment session in joules, and onset and frequency of treatments. Total energy (joules), energy density (joules per square centimeter), and irradiation time were obtained using traditional formulas.[34]

Studies were excluded on the basis of the following criteria:

(1) In vitro studies or studies involving human subjects
(2) Case reports or review studies
(3) Noninclusion of an injury group without treatment
(4) Irradiation not performed in contact mode (energy lost); power and other parameters not

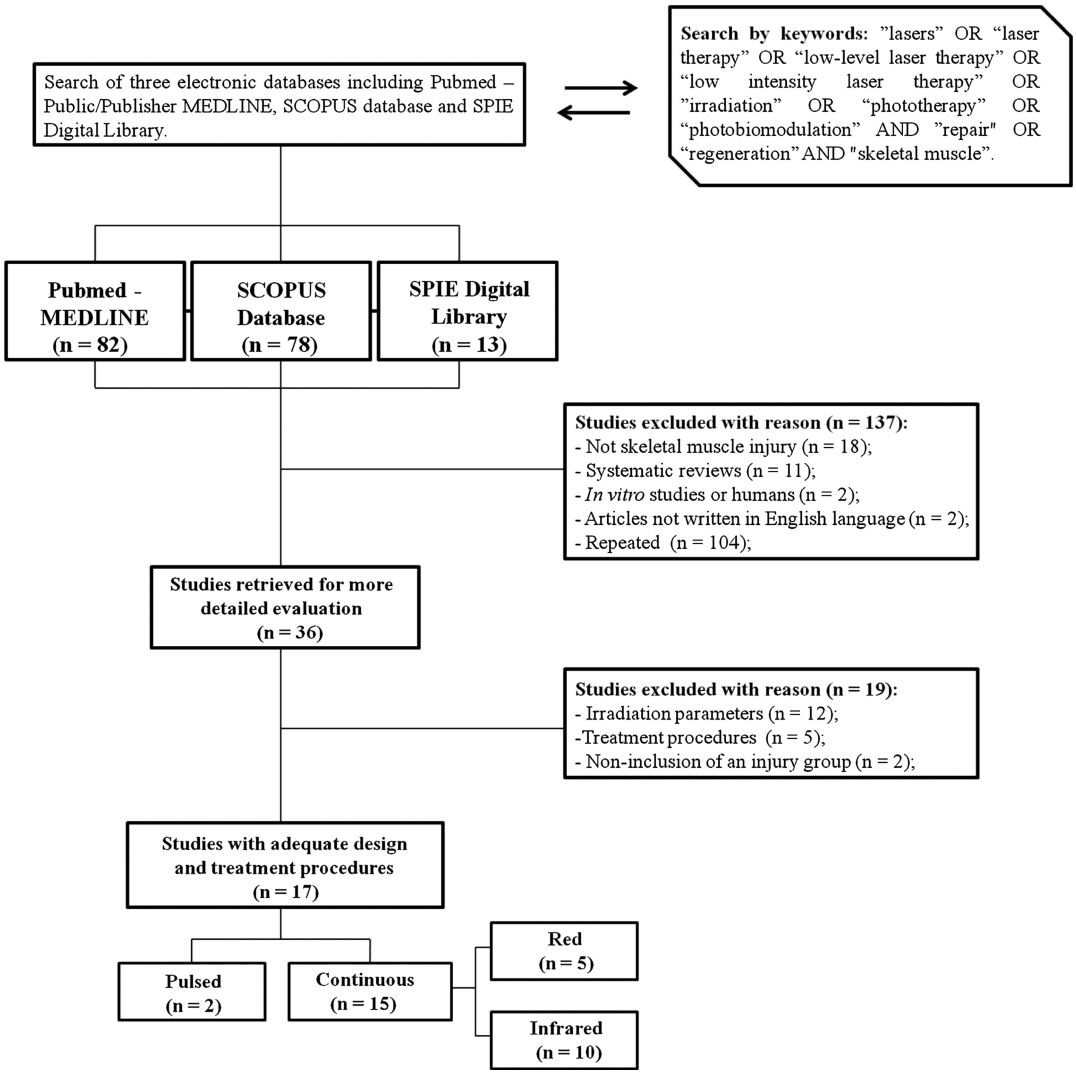

FIGURE 1 *Flow diagram of study selection procedure.*

described, rendering the determination of the real parameters impossible

RESULTS

The search identified 173 potentially relevant studies. On the basis of an analysis of the abstracts, 137 articles were excluded for the following reasons: nonuse of an experimental model of muscle injury (n = 18), systematic reviews (n = 11), in vitro studies (n = 2), studies not written in English (n = 2), and duplications (same study in more than one database; n = 104). Thirty-six studies were then selected for the full-text analysis, 19 of which were excluded because of the nondescription of irradiation parameters (n = 12), nondescription of treatment procedures (n = 5), and noninclusion of an injury group not submitted to laser irradiation (n = 2). Thus, 17 articles were included for the critical evaluation of the effectiveness of LLLT in the treatment of muscle injuries, 15 of which used laser irradiation in the continuous mode (red spectrum [n = 5; Table 1] and infrared spectrum [n = 10; Table 2]) and 2 of which used pulsed mode (Table 3).

The studies included in this review used different irradiation parameters to treat muscle injuries (Tables 1–3). Moreover, a wide variety of experimental models were used to experimentally induce muscle injury, the most common of which was cryoinjury (n = 11). In all experimental models, the skeletal muscle evaluated was the only site studied, the most common of which was the tibialis anterior (TA) muscle (n = 16). All samples were collected from experimental models involving laboratory rats (Wistar and Sprague-Dawley).

DISCUSSION

Muscle injuries constitute a major problem faced by high-performance athletes and amateurs in

TABLE 1 Selected studies that used laser in continuous mode (red spectrum)

Authors	Muscle and Animal	Injury Model	Wavelength, nm	Power, mW	Beam Spot, cm^2	Irradiation Time per Points, secs	Energy Density, J/cm^2	Total Energy per Treatment, J	Single or Multiple Points	Onset and Frequency of Treatment	Periods Evaluated	Results
Rodrigues et al.[35]	TA (Wistar rat)	Cryoinjury	660	20	0.04	20	10	0.4	Single point	48 hrs postinjury, 5 times per week	7, 14, and 21 days	↑ Inflammatory cells at 7 days (only with 2 J)
				40		50	50	2				↓ Inflammatory infiltrate at 14 and 21 days (both doses) ↑ Organization of muscle structure at 21 days (both doses, mainly with 2 J) ↓ COX-2 mRNA at 7, 14, and 21 days (both doses) ↓ Myogenin and VEGF mRNAs at 7 days (both doses) ↑ VEGF mRNA at 14 and 21 days (both doses, mainly with 2 J) ↑ MyoD mRNA at 7, 14 (only with 2 J), and 21 days (both doses) ↑ Myogenin mRNA at 21 days (only with 0.4 J)
Fernandes et al.[23]	TA (Wistar rat)	Cryoinjury	660	20	0.04	10	5	1.6	Multiple points (8)	24 hrs postinjury, 3 times per week	1, 7, and 14 days	↓ IL-1β mRNA at 7 days
Mesquita-Ferrari et al.[25]	TA (Wistar rat)	Cryoinjury	660	20	0.04	10	5	1.6	Multiple points (8)	24 hrs postinjury, 3 times per week	1, 7, and 14 days	↓ TNF-α mRNA at 1 and 7 days ↓ TGF-β mRNA at 7 days
De Souza et al.[32]	TA (Wistar rat)	Cryoinjury	660	20	0.04	10	5	1.6	Multiple points (8)	4 hrs postinjury, 3 times per week	1, 7, 14, and 21 days	↓ Myonecrosis at 7 days ↑ Angiogenesis at 7 days ↑ Deposition of collagen types I and III at 7 days
Baptista et al.[33]	TA (Wistar rat)	Cryoinjury	660	20	0.04	10	5	1.6	Multiple points (8)	24 hrs postinjury, 3 times per week	1, 7, 14, and 21 days	↑ Immunostaining of collagen type IV at 7 days

TABLE 2 Selected studies that used laser in continuous mode (infrared spectrum)

Authors	Muscle and Animal	Injury Model	Wavelength, nm	Power, mW	Beam Spot, cm^2	Irradiation Time per Points, secs	Energy Density, J/cm^2	Total Energy per Treatment, J	Single or Multiple Points	Onset and Frequency of Treatment	Periods Evaluated	Results
Alves et al.[22]	TA (Wistar rat)	Cryoinjury	780	40	0.04	10	10	3.2	Multiple points (8)	2 hrs postinjury, once per day	1, 3, and 7 days	↓ Inflammatory infiltrate and myonecrosis at 1 day ↑ Angiogenesis at 3 and 7 days ↑ Immature muscle fibers at 7 days ↑ MMP-2 gelatinase activity at 7 days ↑ Collagen organization and distribution at 7 days
Assis et al.[27]	TA (Wistar rat)	Cryoinjury	808	30	0.00785	47	180	1.4	Single point	Immediately postinjury, once per day	4 days	↓ Injured area ↑ MyoD, myogenin, and VEGF mRNAs ↓ TGF-β1 mRNA ↓ Deposition of collagen type I
Brunelli et al.[36]	TA (Wistar rat)	Cryoinjury	780	20	0.04	20	10	0.4	Single point	48 hrs postinjury, 5 times per week	7, 14, and 21 days	↓ Inflammatory infiltrate at 7 days (both doses) ↑ Organization of muscle fibers at 7 and 14 days (both doses)
				40		50	50	2				↓ Injured area at 7 days (both doses) ↑ Immunostaining of MyoD at 7 days (both doses)
De Almeida et al.[24]	TA (Wistar rat)	Trauma	810	100	0.028	10 30 90	35.71 107.14 321.43	1 3 9	Single point	1 hr postinjury, once per day	6, 12, and 24 hrs	↓ COX-2 and TNF-α mRNAs at 6, 12, and 24 hrs (all doses) ↓ Damage and morphologic changes caused by the injury (all doses)
De Paiva Carvalho et al.[37]	TA (Wistar rat)	Strain	810	100	0.028	30	107.14	3	Single point	1 hr postinjury, once per day	3 and 6 hrs	↓ COX-1 and COX-2 mRNA at 3 and 6 hrs ↓ Plasma levels of PGE_2 at 3 and 6 hrs ↓ SFI at 6 hrs
Vatansever et al.[26]	TA (Wistar rat)	Cryoinjury	830	30	0.0028	29	30	0.87	Single point	24 hrs postinjury, once per day	7 days	↑ MyoD and VEGF mRNAs
Assis et al.[38]	TA (Wistar rat)	Cryoinjury	808	30	0.00785	47	180	1.4	Single point	Immediately postinjury, once per day	4 days	↓ Lipid peroxidation (TBARS) and nitrotyrosine formation ↓ Production of nitric oxide ↓ Protein expression of iNOS, TNF-α, and IL-1β. ↓ NF-κβ and COX-2 mRNAs ↑ SOD mRNA

(Continued on next page)

TABLE 2 *(Continued)*

Authors	Muscle and Animal	Injury Model	Wavelength, nm	Power, mW	Beam Spot, cm^2	Irradiation Time per Points, secs	Energy Density, J/cm^2	Total Energy per Treatment, J	Single or Multiple Points	Onset and Frequency of Treatment	Periods Evaluated	Results
Ramos et al.[39]	TA (Wistar rat)	Strain	810	100	0.028	10	35.71	1	Single point	1 hr postinjury, once per day	6 and 12 hrs	Fatigue outcome: ↑ Peak force % of maximal contraction at 12 hrs (dose of 1 and 3 J) ↑ Time to decrease to 50% of maximal contraction at 6 hrs (all doses)
						30	107.14	3				↑ Time to decrease to 50% of maximal contraction at 12 hrs (only with 9 J)
						60	214.29	6				↑ AUC % at 6 hrs (except dose of 6 J)
						90	321.43	9				↑ AUC % at 12 hrs (all doses) ↓ SFI at 12 hrs (except dose of 9 J)
Rennó et al.[40]	TA (Wistar rat)	Cryoinjury	830	30	0.028	47	50	1.41	Single point	24 hrs postinjury, once every 48 hrs	13 days	↓ Damage and morphologic changes caused by the injury ↓ Immunostaining of COX-2
Cressoni et al.[31]	TA (Wistar rat)	Surgical induction	785	75	0.3	36	3	2.7	Multiple points (3)	24 hrs postinjury, once every 48 hrs	2, 4, and 8 days	↓ Polymorphonuclear leukocytes and mononuclear cells at 2, 4, and 8 days ↑ Fibroblasts at 8 days

AUC, area under the curve; iNOS, inducible nitric oxide synthase; NF-κβ, nuclear factor kappa B; SFI, Sciatic Function Index; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances.

various sports.[3] Football (soccer) is one of the most practiced sports modalities in the world, and it is estimated that one-third of injuries that occur during the practice of this sport are muscle related.[3,43] Such injuries often result in an inability to participate in training and competitions, compromised athletic performance, and an increased susceptibility to recurrent injuries.[1,8,9] The best treatment of muscle injuries has not yet been clearly defined. Moreover, treatment protocols vary considerably depending on the severity of the injury.[1,44,45] Therefore, standardized therapeutic approaches that can accelerate and enhance the muscle repair process are extremely important.

LLLT has emerged as an option for the treatment of muscle injuries. This noninvasive, low-cost, easy-to-administer form of therapy can help reduce the use of medications, which are often associated with side effects. In contrast, no side effects have been reported for LLLT. In this review, all articles reported beneficial effects of this resource in the treatment of acute muscle injuries, such as modulation of the inflammatory process,[22–24,35,36,41] the stimulation of new blood vessels,[22,32] remodeling of the ECM,[22,32,33] and stimulation of the proliferation and differentiation of satellite cells.[26,27,35,3] Thus, the present review is of paramount importance to the choice of irradiation and treatment parameters, allowing a better understanding of the mechanisms involved.

Among the articles included in this review, considerable heterogeneity was found regarding the irradiation and treatment parameters as well as the methods used to measure the results. Furthermore, the different experimental models of muscle injury used, such as cryoinjury (n = 11), trauma (n = 3), surgical induction (n = 1), and excessive stretching (n = 2), hinder the comparison of the results because of variations in the extent of different types of injury.[46] For example, cryoinjury causes localized muscle damage and inflammation, with the destruction of local vessels and nerves, resulting in partial neurovascular injury and fibrosis at the site.[47,48] In contrast, stretching causes muscle damage and inflammation that can extend throughout the epimysium, resulting in bleeding between the fascia and the muscle,[49] and often affects components of the myotendinous junction.[50]

The conservative management of muscle injuries is commonly accepted.[8] The most often used therapeutic resources for conservative treatment are cryotherapy,[51,52] nonsteroidal anti-inflammatory drugs or corticosteroids,[52,53] massage,[54] hyperbaric oxygen therapy,[55] therapeutic ultrasound,[56] shock

TABLE 3 Selected studies that used laser in pulsed mode

Authors	Muscle and Animal	Injury Model	Wavelength, nm	Power, mW / Pulse, ns: Frequency, Hz	Beam Spot, cm^2	Irradiation Time per Points, secs	Energy Density, J/cm^2	Total Energy per Treatment, J	Single or Multiple Points	Onset and Frequency of Treatment	Periods Evaluated	Results
De Almeida et al.[41]	TA (Wistar rat)	Trauma	904	60	0.0364	17	27.47	1	Single point	1 hr postinjury, once per day	6 hrs	↓ Protein expression of TNF-α, IL-1β, and IL-6 (only 1-J dose)
				200		50	82.42	3				
				700		100	164.84	6				
						150	247.25	9				
Silveira et al.[42]	Gastrocnemius (Wistar rat)	Trauma	904	40	0.10	12.5	5	2.5	Multiple points (5)	2 hrs postinjury, 3 times in the first day (2, 12, and 24 hrs) and each 24 hrs	5 days	↓ Serum CK activity ↓ TBARS levels ↓ Superoxide anion production ↓ Activity of SOD ↓ Hydroxyproline content
				60								
				9500								

CK, creatine kinase; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances.

wave therapy, and electrical stimulation.[2,3] With regard to LLLT, the choice of irradiation and treatment parameters, such as wavelength, power output, beam area, total energy, irradiation time, frequency of treatment, mode of application, and the onset of treatment, are crucial to achieving positive effects in the treatment of muscle injuries. Unfortunately, few studies adequately describe all the information essential to the reproducibility and the reliability of the findings.[57]

In the present review, the main criteria that led to the exclusion of articles were an inadequate description of the beam area, which is critical to determining the energy delivered to the tissue, and the nondescription of the onset of treatment after the induction of injury. These points are extremely important to the study of the muscle repair process because the administration of light at different times can exert an influence on each stage of the repair process by modulating different cell types and events that have a direct effect on the results.[58] This is seen clearly in the comparison of two studies that used the same irradiation parameters but initiated treatment at different times. Baptista et al.[33] began treatment 24 hrs after injury induction and found no differences in histologic features, whereas De Souza et al.[32] initiated treatment 4 hrs after injury induction and found both a reduction in myonecrosis and an increase in the number of blood vessels after 7 days.

Another important factor observed in this systematic review is that only one study correlated the biochemical/molecular findings with clinical/functional improvement. De Paiva Carvalho et al.[37] evaluated functional recovery using the walking track test outcome (sciatic function index) and found that LLLT (wavelength, 810 nm; power output, 100 mW; total energy, 3 J) was effective in improving functional recovery in association with a decrease in inflammatory markers (cyclooxygenase-1 [COX-1], COX-2, and prostaglandin E2 [PGE_2]) after 6 hrs. Ramos et al.[39] also used the walking track test outcome for functional assessment and analyzed three parameters of muscle fatigue to evaluate muscle performance: (1) the maximum force elicited at the onset of each of the six electrically induced tetanic contractions, (2) the time elapsed until a 50% reduction in the force of an electrically induced contraction in comparison with the force at the onset of contraction, and (3) muscle performance after electrically induced tetanic contraction. The authors found that LLLT (wavelength, 810 nm; power output, 100 mW) was effective in enhancing functional recovery and

muscle performance in the periods evaluated (6 and 12 hrs) but did not assess biochemical/molecular markers. Thus, there remains a gap in the correlation of biochemical/molecular findings with functional analysis. Future studies that examine both aspects could bring a better understanding of the relationship between biochemical makers and functional recovery, which is of considerable importance to the field of rehabilitation.

Effect of LLLT on Inflammation

The inflammatory phase is characterized by the degeneration of muscle fibers, the recruitment of neutrophils and macrophages to the injury site, as well as the production of inflammatory cytokines (tumor necrosis factor alpha [TNF-α], interleukin 1 beta [IL-1β], IL-6, and IL-8) and proteolytic enzymes responsible for the phagocytosis of cell dendrites and the spread of the inflammatory response.[2,59] This phase is also accompanied by an increase in the production of reactive oxygen species, such as superoxide, hypochlorous acid, and hydrogen peroxide, which may also be involved in the exacerbation of the inflammatory process, causing damage to healthy muscle fibers.[60,61] Thus, LLLT in this phase may enhance the tissue repair process, possibly through its ability to limit the inflammatory response and attenuate oxidative damage. Indeed, LLLT has been shown to be an extremely efficient therapeutic resource for the modulation of the inflammatory process after muscle injury, regardless of the characteristics of the laser device, such as operating mode (continuous or pulsed), type of emitter (Gallium Arsenide, Aluminum Gallium Arsenide, and Aluminum Gallium Indium Phosphide), and range of the light spectrum (red or infrared) (Tables 1–3).

Furthermore, LLLT has been shown to be more effective than the administration of the pharmacologic agent diclofenac sodium, which is a potent nonsteroidal anti-inflammatory drug that acts by blocking the pathway of COX inhibitors.[61] COX-1 and COX-2 are the most highly expressed members of the COX family in injured muscle.[62] COX-1 plays a constitutive role and synthesizes prostaglandins, which are important to homeostasis,[63] whereas COX-2 contributes to the production of PGE_2, which is related to pain and the recruitment of inflammatory neutrophils.[64,65] De Paiva Carvalho et al.[37] demonstrated that LLLT (wavelength, 810 nm; power output, 100 mW; total energy, 3 J) was as effective as the topical and the intramuscular administration of diclofenac sodium on the expression of COX-1 and COX-2 after 3 and 6 hrs and exhibited a better effect on the reduction of PGE_2 and the functional impairment index (walking track analysis) 6 hrs after the induction of stretch injury. De Almeida et al.[41] compared the effects of cryotherapy, the topical application of LLLT, and diclofenac sodium in an experimental model of muscle injury induced by acute trauma in rats and found that LLLT (wavelength, 904 nm; power output, 60 mW; total energy, 1 J) had a better effect on the reduction of proinflammatory cytokines (TNF-α, IL-1β, and IL-6) 6 hrs after induced injury in comparison with the other forms of treatment.

However, it should be noted that the strong inhibition of factors involved in inflammation, such as proinflammatory cytokines, can be harmful to the regeneration of tissue because these factors stimulate proliferation and prevent the premature differentiation of myogenic precursor cells.[66] Nonetheless, studies using laser in the inflammatory phase have reported beneficial effects on the repair of muscle tissue.

Effects of LLLT on Myogenic Regulatory Factors

The activation of satellite cells occurs simultaneously to the inflammatory process. Although a small proportion undergoes self-renewal (i.e., a return to the quiescent state to replenish the satellite cell population), most contribute to the regeneration process and differentiate into myoblasts, which fuse to repair damaged fibers or form a new functional muscle fiber.[5,67,68] Satellite cells express myogenic regulatory factors (MRFs), which are composed of four members: Myf5, MyoD, myogenin, and MRF4. The primary MRFs are Myf5 and MyoD, which play roles in determining myogenesis and converting precursor cells into myoblasts. The secondary MRFs (myogenin and MRF4) are important to cell differentiation into myoblasts and myocytes for the formation of mature muscle fibers.[68] Thus, the modulation of MRFs can directly influence the muscle repair process. Recently, LLLT has shown promise in modulating MRFs regardless of the irradiation or treatment parameters.

Rodrigues et al.[35] report an increase in the expression of MyoD messenger RNA (mRNA) after 7, 14, and 21 days; a reduction in the expression of myogenin mRNA after 7 days; and an increase in myogenin mRNA after 21 days using red laser (wavelength, 660 nm; power output, 20 and 50 mW; total energy, 0.4 and 2 J) for the treatment of the TA muscle after cryoinjury. Using infrared laser

(wavelength, 830 nm; power output, 30 mW; total energy, 0.87 J), Vatansever et al.[26] found an increase in the expression of MyoD mRNA after 7 days. Brunelli et al.[36] found an increase in MyoD immunostaining after 7 days in the group treated with the laser (wavelength, 780 nm; power output, 20 and 50 mW; total energy, 0.4 and 2 J). Using LLLT (wavelength, 780 nm; power output, 30 mW; total energy, 1.4 J), Assis et al.[27] found increased expression of MyoD and myogenin mRNAs after 4 days.

Although these results are extremely interesting, further studies should be conducted to allow a better understanding of the effects of LLLT on the modulation of MRFs because it has been shown that cytokines, such as TNF-α, IL-1β, and IL-6, and some growth factors, such as transforming growth factor beta (TGF-β), fibroblast growth factor, and insulin-like growth factor–1, can either directly or indirectly influence the proliferation and the differentiation of satellite cells.[5]

Effects of LLLT on ECM Remodeling

ECM remodeling occurs throughout the repair process and is characterized by the synthesis and the degradation of proteins. Initially, fibroblasts migrate to the injury site, proliferate, and synthesize proteins, such as collagen types I, III, and IV; laminin; fibronectin; proteoglycans; and tenascin, thereby forming a new ECM.[59] The degradation of ECM proteins is mainly carried out by matrix metalloproteinases (MMPs), which selectively digest individual components of the ECM.[69,70] In skeletal muscle, the collagenases MMP-1, MMP-8, MMP-13, and MMP-18 have the ability to degrade interstitial collagen types I, II, and III, and the gelatinases MMP-2 and MMP-9 degrade denatured collagen types IV, VII, and X in many tissues.[70]

Gelatinases play important roles in muscle repair because the degradation of proteins that constitute the basement membrane facilitates the migration, the proliferation, and the fusion of myoblasts as well as the formation of new blood vessels.[70,71] However, the excessive accumulation of ECM proteins in muscle tissue leads to the development of the fibrous scar tissue.[72] The mechanical barrier created by the formation of fibrous scar tissue in skeletal muscle during the regeneration process hampers cell migration/fusion and limits vascular reperfusion, thereby contributing to slower tissue as well as a greater susceptibility to further muscle.[59,70,73]

The literature shows that the overproduction of TGF-β is a major factor that leads to the formation of fibrous tissue during the regeneration of muscle tissue in both animals and humans.[74,75] TGF-β plays a key role in the initiation of fibrotic cascades and the differentiation of satellite cells into myofibroblasts in injured muscle.[76] Moreover, TGF-β regulates the production of enzymes that degrade ECM components, such as collagenases and gelatinases, as well as the synthesis of enzymes that inhibit the degradation of ECM, such as tissue inhibitors of metalloproteinases and plasminogen activator inhibitor–1.[59,77]

Antifibrotic agents, such as suramin,[78,79] decorin,[80] and interferon gamma,[81] have been used to minimize the effects of TGF-β on the formation of fibrous tissue during the regeneration of skeletal muscle. However, some of these antifibrotic agents have severe side effects, which raises questions regarding the actual benefits of clinical use in humans.[82] LLLT has been shown to be effective in modulating the formation of fibrotic tissue during the repair of injured muscle tissue. Regardless of the irradiation and treatment parameters, LLLT has been shown to be effective in reducing the gene expression of TGF-β. Mesquita-Ferrari et al.[25] used red laser (wavelength, 660 nm; power output, 20 mW; total energy, 1.6 J) and found a reduction in the expression of TGF-β mRNA after 7 days. Assis et al.[27] found a reduction in the expression of TGF-β mRNA after 4 days using infrared laser (wavelength, 808 nm; power output, 30 mW; total energy, 1.4 J) on the TA muscle of rats in the repair process after cryoinjury.

In contrast, collagen deposition in muscle tissue has been shown to be dependent on irradiation and treatment parameters. De Souza et al.[32] and Baptista et al.[33] used LLLT in the red spectral range (wavelength, 660 nm; power output, 20 mW; total energy, 1.6 J) and found increased immunostaining of collagen types I, III, and IV after 7 days in the TA muscle of rats during the repair process after cryoinjury. Assis et al.[27] used infrared laser (wavelength, 808 nm; power output, 30 mW; total energy, 1.4 J) and found a reduction in the deposition of type I collagen after 4 days. Alves et al.[22] also used an infrared laser (wavelength, 780 nm; power output, 40 mW; total energy, 3.2 J) on the same experimental model and found an increase in the gelatinolytic activity of MMP-2 associated with an improvement in the organization and distribution of collagen fibers.

These findings demonstrate that the choice of irradiation parameters is crucial to achieving the desired effects during treatment with LLLT. However, the effects of LLLT on other growth

factors, such as connective tissue growth factor, platelet-derived growth factor, and myostatin, which are directly related to the development of fibrosis in muscle tissue, have not been studied.[59]

Effects of LLLT on Angiogenesis

The development of a new network of blood vessels (angiogenesis) at the injury site is extremely important to successful muscle regeneration. This process is regulated by vascular endothelial growth factor (VEGF), which exerts different effects on the vascular endothelium, including endothelial cell proliferation, the induction of rapid microvascular permeability, the survival of endothelial cells, the migration and adhesion of endothelial cells, and subsequent fusion between new vessels with preexisting movement.[83,84] Furthermore, it has been demonstrated that VEGF stimulates the expansion of the number of basal satellite cells adjacent to new capillaries, mainly because satellite cells reside in a juxtavascular niche. These findings demonstrate the dual (proangiogenic and promyogenic) functionality of VEGF.[85]

The results of the present review clearly show that LLLT is extremely effective in modulating the expression of VEGF mRNA and the consequent formation of new blood vessels during the repair of injured skeletal muscle. Rodrigues et al.[35] report that LLLT (wavelength, 660 nm; power output, 20 and 50 mW; total energy; 0.4 and 2 J) promoted a reduction in the expression of VEGF mRNA after 7 days and an increase after 14 and 21 days in the TA muscle during the repair process after cryoinjury. Vatansever et al.[26] found an increase in the expression of VEGF mRNA after 7 days using infrared laser (wavelength, 830 nm; power output, 30 mW; total energy, 0.87 J). Also using infrared laser (wavelength, 808 nm; power output, 30 mW; total energy, 1.4 J), Assis et al.[27] found an increase in the expression of VEGF mRNA after 4 days. Alves et al.[22] and De Souza et al.[32] demonstrated an increase in the number of blood vessels after 7 days using the same experimental model but different irradiation parameters.

It is noteworthy that some of these studies also found an increase in the expression of MyoD and myogenin mRNA associated with the increase in VEGF. These MRFs are commonly used as markers of satellite cell proliferation and differentiation.

CONCLUSIONS

The studies analyzed demonstrate the positive effects of LLLT on the muscle repair process, which are dependent on irradiation and treatment parameters. The findings suggest that LLLT is an excellent therapeutic resource for the treatment of skeletal muscle injuries in the short-term. However, the biologic mechanisms of action of LLLT have not yet been fully clarified, and further investigation should be conducted involving animal models and randomized human clinical trials.

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