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REVIEW ARTICLE

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Effects of photobiomodulation therapy on regulation of myogenic regulatory factor mRNA expression in vivo: A systematic review

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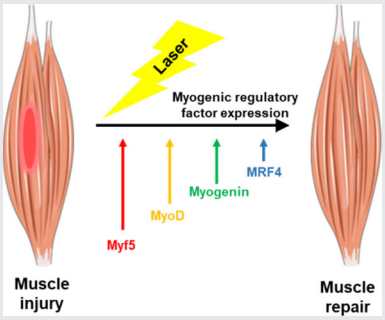
Abstract



Non-invasive promotion of myogenic regulatory factors (MRFs), through photobiomodulation therapy (PBMT), may be a viable method of facilitating skeletal muscle regeneration post-injury, given the importance of MRF in skeletal muscle regeneration. The aim of this systematic review was to collate current evidence, identifying key themes and changes in expression of MRF in in vivo models. Web of Science, PubMed, Scopus and Cochrane databases were systematically searched and identified 1459 studies, of which 10 met the inclusion criteria. Myogenic determination factor was most consistently regulated in response to PBMT treatment, and the expression of remaining MRFs was heterogenous. All studies exhibited a high risk of bias, primarily due to lack of blinding in PBMT application and MRF analysis. Our review suggests that the current evidence base for MRF expression from PBMT is highly variable. Future research should focus on developing a robust methodology for determining the effect of laser therapy on MRF expression, as well as long-term assessment of skeletal muscle regeneration.

KEYWORDS
myogenic regulatory factor, photobiomodulation therapy, systematic review

1 | INTRODUCTION

Musculoskeletal injuries affect one-in-four adults within the United Kingdom every year, leading to significant pain, performance detriment and reduced quality of life

Abbreviations: LLLT, low level laser therapy; HLLT, high level laser therapy; MRF, myogenic regulatory factor; MRF4, myogenic regulatory factor 4; Myf5, myogenic factor 5; MyoD, myogenic differentiation factor 1; PBMT, photobiomodulation therapy; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; SC, satellite cells.

[1]. To facilitate the healing and regeneration of these damaged tissues, electrotherapeutic modalities are often employed by rehabilitation therapists to assist in this endeavour [2]. Photobiomodulation therapy (PBMT) is one treatment solution available, which is non-invasive and has shown positive results in facilitating skeletal muscle recovery [3]. Following damage, skeletal muscle goes through a highly controlled and tightly regulated process to regenerate damaged muscle fibres. The inflammatory cascade activated in response to injury, results in the activation and propagation of myogenic stem cells, termed satellite cells (SCs) [4]. From these SCs, myogenic progenitor cells (MPCs) are formed [5]. Myogenic regulatory factors (MRFs) are essential in the control and regulation of MPCs commitment to the myogenic lineage and also the processes of determination and differentiation [6]. The result is the differentiation of myoblasts that will regenerate the damaged tissue, ultimately with the aim of restoring contractile function [7] as shown in Figure 1. The MRFs consist of myogenic determination factor 1 (MyoD), myogenic factor 5 (Myf5), myogenin and MRF4, which are all able to induce myoblastic traits within non-myogenic cell lineages and fibroblasts [9]. Each of the MRFs play a key temporal role: while SCs are activated and proliferating, they express MyoD and Myf5, and then later myogenin as the terminal differentiation factor [10]. The expression of MRF4 remains high at the end of regeneration and also during SC return to quiescence [5]. Once the regeneration of the tissue is complete, the progeny of the SCs return to quiescence to maintain the SC pool, and myostatin is expressed, inhibiting excessive myoblast proliferation and maintaining homeostasis within the tissue [11, 12]. In some cases of significant injury, disease or mutations within these genes, MRFs may not be sufficiently expressed; therefore, muscle regeneration is severely limited or prevented altogether [13]. Furthermore, limited or no expression of MRFs can lead to potentially excessive scar formation and limited tissue recovery [14].

To promote skeletal muscle regeneration, accelerate the healing process and minimize excessive scar tissues formation, PBMT has been indicated as a viable non-invasive intervention [3]. Given the fundamental role MRFs play in the regeneration of skeletal muscle, it is necessary to characterize their expression in response to PBMT. Such evidence could be used to optimize treatments in terms of dose, timing and duration.

Cytochrome c oxidase, which resides within the mitochondria, is theorized to be the primary mechanism by which PBMT laser medium interacts with the targeted tissues [15]. This chromophore is sensitive to wavelengths within the red to infrared spectrum (λ660-1100 nm) [16]. When exposed to laser light at the appropriate wavelength, the chromophore's respiratory chain activity is promoted, enabling an increase in the cellular energy currency of adenosine triphosphate (ATP), facilitating an increased propagation and differentiation of myogenic cells [17, 18]. Although there is evidence for the differentiation of myogenic cells, little attention has been paid to the expression of MRFs in response to different parameters of PBMT. Reports suggest that PBMT has mixed results on healing different tissue types, possibly as a result of incorrect dosing [16]. Insufficient tissue irradiation can lead to no benefit, while excessive irradiation can lead to an inhibitory effect on tissue healing [19].

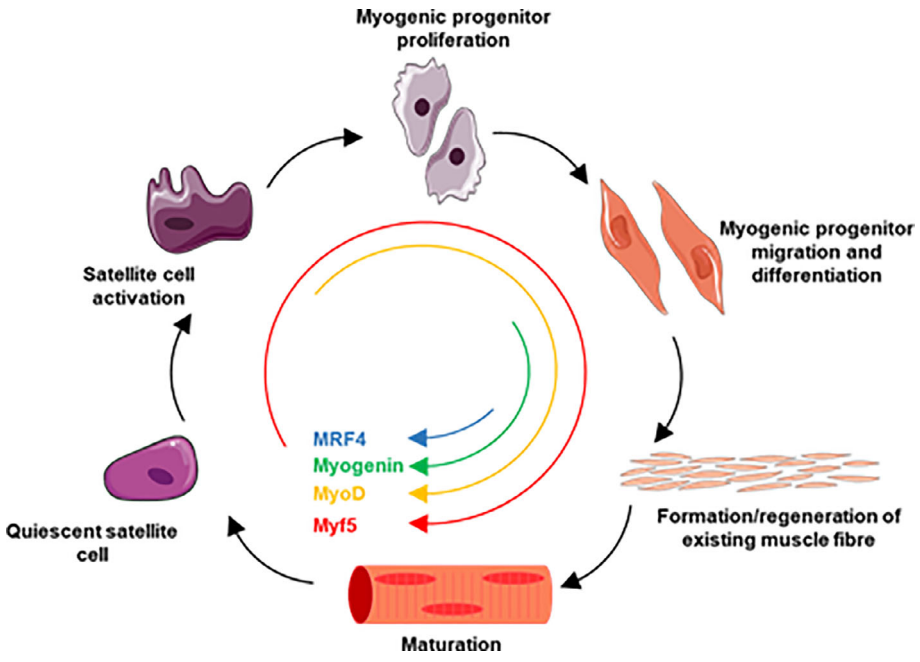


FIGURE 1 Timeline of the myogenic regulatory factor expression pathway, adapted from Zanou and Gailly [8]. Upon initial injury, the quiescent satellite cells express myogenic factor 5 (Myf5), proliferating into myoblasts, followed by myogenic determination factor (MyoD) expression. As the myotube begins formation, myogenin is expressed, leading to the fusion of myoblasts. Finally, MRF4 promotes myotube maturation and myofibre organization

Likewise, unoptimized dosimetric parameters can lead to poor or insignificant outcomes, with heterogeneous treatment dosages leading to varied results [3, 20].

Unoptimized treatment parameters, such energy-per-point (Joules) or wavelength (nanometres), have been shown to lead to ineffective treatment outcomes, making it essential to establish an effective PBMT dosage [19]. Previous systematic reviews have sought to analyse the effect of PBMT on the entire inflammatory and regenerative process but did not review the effect of PBMT on MRF regulation in greater detail [3, 21]. Given that no standardized protocol exists for promoting MRF upregulation, the collation of current evidence will be a key step towards establishing optimal dosimetric parameters to control these important factors necessary for muscle regeneration. Many of the studies in this area are largely animal-based and although these are critical in refining treatment parameters for human trials to demonstrate clinical applicability, the methodological quality in these studies often shows wide variation and applicability in human populations [22]. Through a controlled systematic review of the literature, it will be possible assess the findings of the existing studies in terms of quality and the potential for impact in humans.

To this end, this systematic review will analyse the current literature on MRF expression in response to PBMT within animal populations, intending to identify any optimal treatment parameters that may exist. The aim is that this study will facilitate further work in human-based research by demonstrating the potential efficacy of PBMT in animal models.

2 | METHODS

The review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [23].

2.1 | Study criteria

2.1.1 | Inclusion and exclusion criteria

The inclusion and exclusion criteria for this review are summarized in Table 1 and detailed below.

TABLE 1 Summary of inclusion and exclusion criteria applied to assessed articles

Inclusion criteria	Exclusion criteria
In vivo animal studies	Fatigue-recovery, in vitro or human studies
Studies investigating myogenic regulatory factors	Studies with no English option
Therapeutic laser applied to at least one group	Studies not relating to therapeutic laser
Dosimetric parameters stated	No control and/or injury-control groups
Induced muscle damage from trauma	Muscle damage induced by venom or toxins
Studies relating to skeletal muscle	No animal welfare statement
Original studies from peer-reviewed journals	Induced comorbidities, eg, diabetes
Quantitative studies using qRT-PCR	Studies using immunohistochemistry

Abbreviation: qRT-PCR, quantitative reverse transcription-polymerase chain reaction.

2.1.2 | Studies

All research articles included for the review were required to fit within the 'Populations, Intervention, Control and Outcome' framework. Articles that did not fit were excluded from this review. The review included experimental studies and randomized control studies. The eligible research had to include an injury control group (ICG), be accessible as full-text and be in English. Review studies and case reports were excluded.

2.1.3 | Populations

Trials that included any animal model were included. Models had to be healthy with no induced comorbidities, for example, diabetes. Any muscle damage needed to be induced by cryoinjury, incision, contusion, or exercise exertion, with studies being excluded if damage was induced by venom or toxins. Damage had to be applied only to skeletal muscle, and studies were omitted if lesions were in other tissue types, for example, skin, bone, cartilage or nerve. Fatigue recovery models, in vitro cell cultures and human studies were excluded, as were any studies that did not declare an animal welfare statement.

2.1.4 | Interventions

Trials that were selected had to include exposure to a form of PBMT within the red to infrared spectrum (λ660-1100 nm), either from a laser or an LED light source. The minimum dosimetric parameters required for inclusion included wavelength (nm), optical power output (mW), beam size, treatment

fluence (J/cm^2) and treatment frequency. Studies were excluded if dosimetric parameters could not be calculated from the other given variables.

2.1.5 | Outcome measures

Articles were included if the primary outcome measures included the mRNA expression of any form of MRFs by quantitative reverse transcription-polymerase chain reaction (qRT-PCR), specifically MyoD, myogenin, Myf5 and MRF4. Immunohistochemistry studies were excluded.

2.2 | Search methods

2.2.1 | Electronic databases

Research articles were searched for in the Web of Science, PubMed, Scopus and Cochrane databases in October 2021, by a single investigator.

2.2.2 | Search terms

Due to the breadth of nomenclature within PBMT research, a search string was put together to cover all terms used to describe PBMT: 'class III laser' OR 'class 3 laser' OR 'low level laser therapy' OR 'LLLT' OR 'LILT' OR 'class IV laser' OR 'class 4 laser' OR 'high level laser therapy' OR 'HLLT' OR 'HILT' OR 'photobiomodulation' OR 'phototherapy' AND 'muscle' OR 'myocyte' OR 'myoblast' AND 'regeneration' OR 'repair' OR 'myogenesis'.

2.2.3 | Reference searching

Reference lists for all papers that met the study's inclusion criteria were screened to identify any additional studies which were not present during the initial literature search.

2.3 | Study selection

Throughout the literature search, all prospective studies were uploaded to EndNote X9.2 to be collated, with duplicates removed. Article titles and abstracts were reviewed for their eligibility. If the abstract was not sufficient to make a judgement or was unclear, the full-text was analysed to determine the research's eligibility. Each piece of eligible research was screened through full-text analysis and then referred to as 'eligible' or 'excluded'. The search results and reasons for subsequent exclusions are presented in the PRISMA flow diagram (Figure 2).

2.4 | Data extraction

Population size, trauma type, treatment variables, analysis time points, MRF expression assessment methods and results (including *P* values, where reported) were collated into a Microsoft Excel spreadsheet, which is summarized in Tables 2–4.

2.5 | Data synthesis

Given the considerable heterogeneity within extracted data, all results were reported narratively as opposed to statistical meta-analysis.

2.6 | Assessment of methodological quality

Studies underwent a risk of bias analysis, employing the 'SYstematic Review Centre for Laboratory animal Experimentation' tool (SYRCLE) to analyse the relevant methodological domains for quality and risk of bias for each study [34]. Every study was manually assessed and curated for each domain based on the information provided by the article, which was subsequently graded. Each of the SYRCLE domains are: 'sequence generation', 'baseline characteristics', 'allocation concealment', 'random housing', 'blinding (performance bias)', 'random outcome assessment', 'blinding (detection bias)', 'incomplete outcome data', 'outcome reporting', 'other forms of bias'.

Each study was read and each of the above domains were scrutinized and graded 'unclear', 'low' and 'high' depending on the apparent level of bias and whether sufficient information was available to make a judgement (Table 5).

3 | RESULTS

The results of this systematic review show a broad array of outcomes from a mixture of treatment parameters. This concurs with previous findings in this field by Alves et al. and Teles et al., who stated that variety in dosage parameters leads to a difference in outcomes [3, 21].

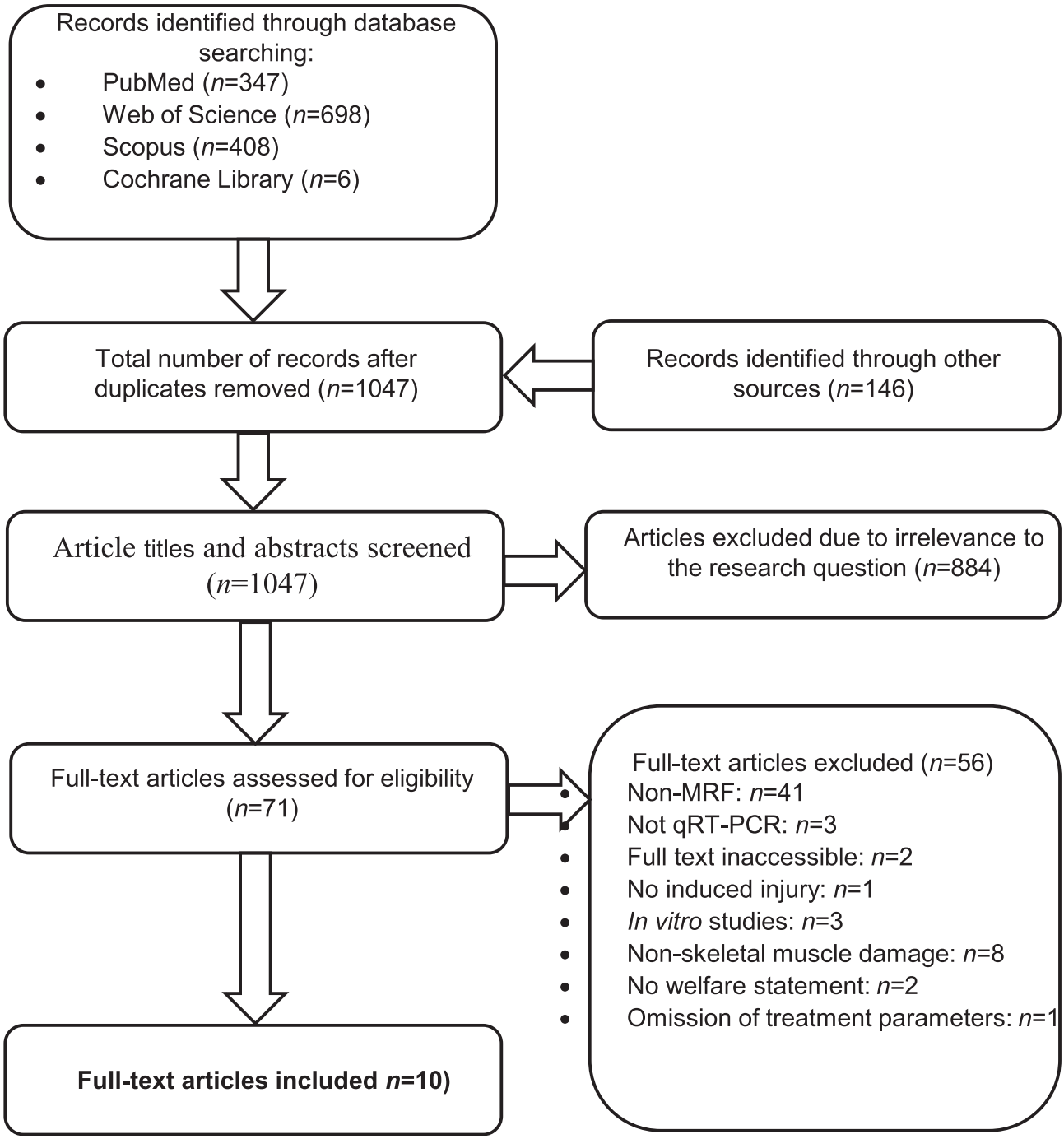


FIGURE 2 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram [23]. n = number of studies. 'MRF' = myogenic regulatory factors. 'qRT-PCR' = quantitative reverse transcription-polymerase chain reaction

3.1 | Myogenic determination factor

MyoD was measured by all of the 10 included studies. The frequency of results is summarized in Figure 3A. MyoD was predominately upregulated by PBMT over the assessment periods measured by all 10 studies, demonstrating a consistent and robust effect.

3.2 | Myogenic factor 5

Only two studies investigated Myf5 expression in response to PBMT [25, 32]. Morais et al. stated a significant increase of Myf5 mRNA expression was present following PBMT treatment at 4.9 J and 660 nm, but reported no inferential statistics to support this statement [25]. Only the treatment

TABLE 2 Summary of the included studies with their relevant populations and subgroups. Authors who used multiple laser dosimetric parameters are marked by '*[1]' where groups were split. Morais et al. and Beasi et al., marked by '*[2]', included two sub-groups per experimental group, one being exercised and the other not [24, 25]

Author	Population (total number)	Injury laser group	Injury control group	Control group	Laser only group	Other groups
Alves et al. [26]	Male Wistar rats (n = 105)	Yes (n = 28)	Yes (n = 28)	Yes (n = 7)	Yes (n = 7)	Sham (n = 7) Pre-injury laser (n = 28)
Assis et al. [27]	Male Wistar rats (n = 60)	Yes (n = 20)	Yes (n = 20)	Yes (n = 20)	No	No
Beasi et al. [24]	Wistar rats (n = 75)	Yes (n = 15 × 3)*[2]	Yes (n = 15)	Yes (n = 15)	No	Injury and swimming Injury, swimming and laser
de Freitas et al. [28]	Male Wistar rats (n = 30)	Yes (n = 10)	Yes (n = 10)	Yes (n = 10)	No	No
Morais et al. [25]	Male Wistar rats (n = 42)	Yes (n = 7 × 2)*[2]	Yes (n = 7 × 2)*[2]	Yes (n = 7 × 2)*[2]	No	No
Rodrigues et al. [29]	Male Wistar rats (n = 63)	Yes (n = 21 × 2)*[1]	Yes (n = 21)	No	No	No
Rodrigues et al. [30]	Male Wistar rats (n = 63)	Yes (n = 21 × 2)*[1]	Yes (n = 21)	No	No	No
Santos et al. [31]	Male Wistar rats (n = 21)	Yes (n = 7)	Yes (n = 7)	Yes (n = 7)	No	No
Trajano et al. [32]	Male Wistar rate (n = 15)	Yes (n = 10 [5 × 2])	Yes (n = 5)	No	No	No
Vatansever et al. [33]	Male Wistar rats (n = 56)	Yes (n = 7 × 2)	Yes (n = 7 × 2)	Yes (n = 7 × 2)	Yes (n = 7 × 2)	No

TABLE 3 Summary of dosimetric parameters of the included studies

Author	Injury mode	Laser type	Wavelength (nm)	Power output (mW)	Power density (W/cm^2)	Energy per point (J)	Energy density (J/cm^2)	Beam size (cm^2)	Pulsed/ continuous	Contact/ non-contact	Total time (s) (per point)	Points treated	Treatment frequency
Alves et al. [26]	TA C/I	LLLT AlGaAs	780	40	1	0.4	10	0.4	N/S	Contact	80 (10)	Eight points at lesion	2 h PI and daily for 1, 3, 7 and 14 d
Assis et al. [27]	TA C/I	LLLT AlGaAs	808	30	3.8	1.4	180	0.00785	Continuous	Contact	47	MoL	Immediately PI then daily for 4 d
Beasi et al. [24]	TA C/I	LLLT AlGaAs	830	100	3.086	5.8	180	0.0324	Continuous	Contact	58	Three points at lesion	Once daily for 7, 14 and 21 d
de Freitas et al. [28]	TA C/I	LLLT GaAs	904	50	1.438*	2.4	69	0.035	Pulsed	Contact	96 (48)	Two spots at lesion site	24 h PI then every 24 h for 5 d
Morais et al. [25]	TA C/I	LLLT InGaAiP	660	35	N/S	4.9	14.7	0.028	N/S	Contact	N/S	MoL and 2 mm to left and right of injury	Once daily for 14 d
Rodrig-ues et al. [29]	TA C/I	LLLT InGaAlP	660	20 and 40	0.5 and 1*	0.4 and 2	10 and 50	0.4	Continuous	Contact	20 and 50	One point above injury	48 h PI then daily for 5 d with a 48 h interval
Rodrig-ues et al. [30]	TA C/I	LLLT AlGaAs	780	20 and 40	0.5 and 1*	0.4 and 2	10 and 50	0.4	Continuous	Contact	20 and 50	One point above injury	48 h PI then daily for 5 d with a 48 h interval
Santos et al. [31]	TA C/I	LLLT GaAs	904	50	1.42	2.4	69	0.035	Pulsed	Contact	96 (48)	Two spots at injured area	24 h PI then daily for 5 d
Trajano et al. [32]	TA C/I	LLLT GaAs	904	25 and 75	0.36 and 1.10	0.5	13.68 and 14.3*	0.69	Pulsed	Contact	38 and 13	MoL	Immediately PI and daily for 3 d
Vatans-ever et al. [33]	TA C/I	LLLT GaAlAs	830	30	1.034*	0.87	30	0.06	N/S	Contact	29	MoL	Daily from 2 to 6 d

Note: All domains marked '*' have been calculated from other dosimetric parameters.

Abbreviations: C/I, cryoinjury; J, Joules; LLLT, low-level laser therapy; MoL, middle of lesion; mW, milli Watts; N/S, not stated by author; nm, nanometres; PI, post-Injury; s, seconds; TA, tibialis anterior.

TABLE 4 Summary of the assessment time points and results for each included study

Author	Factor of interest	Analysis time points	Outcome
Alves et al. [26]	MyoD	1, 3, 7 and 14 d	NS at 1 and 14 d ($P > .05$). SI at 3 d ($P < .05$) and 7 d ($P < .01$).
	Myogenin		NS at 1, 3 and 7 d ($P > .05$). SI at 14 d ($P < .01$).
Assis et al. [27]	MyoD	4 d	Twofold SI at 4 d ($P < .01$).
	Myogenin		SI at 4 d ($P < .01$).
Beasi et al. [24]	MyoD	7, 14 and 21 d	SDe between 7 and 21 d ($P = .0064$) for PBMG. SI between 7 and 21 d ($P < .0001$) and 14 and 21 d ($P = .0132$) for swimming + PBMG. SI in PBMG compared to ICG at 7 and 21 d ($P < .05$).
	Myogenin		SDe between 7 and 14 d ($P < .0001$) and 7 and 21 d ($P < .0001$) for PBMG. SI between 7 and 21 d ($P = .0001$) and 14 and 21 d ($P = .0211$) for swimming + PBMG. SI in PBMG compared to ICG at 7 d ($P < .05$). SDe in swimming + PBMG compared to ICG at 21 d ($P < .05$).
de Freitas et al. [28]	MyoD	5 d	NS compared to ICG ($P > .05$).
	Myogenin		SDe compared to ICG ($P < .05$).
Morais et al. [25]	MyoD	14 d	SI in all post-injury PBMGs, with strength training potentiating the effect (no P value given).
	Myogenin		SDe only in strength-trained PBMG (no P value given).
	Myf5		SI only in strength-trained PBMG (no P value given).
Rodrigues et al. [29]	MyoD	7, 14 and 21 d	50 J/cm^2 (2 J) SI compared to ICG and 10 J/cm^2 (0.4 J) at 7 and 14 d ($P < .05$). At 21 d, both energy doses SI compared to ICG ($P < .05$).
	Myogenin		SDe for both 10 J/cm^2 (0.4 J) and 50 J/cm^2 (2 J) at 7 d compared to ICG ($P < .05$). No change at 14 d. At 21 d, 10 J/cm^2 (0.4 J) SI compared to 50 J/cm^2 (2 J) and ICG at 21 d ($P < .05$).
Rodrigues et al. [30]	MyoD	7, 14 and 21 d	SI in 10 J/cm^2 (0.4 J) and 50 J/cm^2 (2 J) compared to ICG at all time points ($P < .05$); at 7 d, 10 J/cm^2 (0.4 J) had SI in expression compared to 50 J/cm^2 (2 J), with the inverse at 14 and 21 d ($P < .05$).
	Myogenin		NS at 7 d. SI at 14 d for 10 J/cm^2 (0.4 J) and 50 J/cm^2 (2 J) compared to ICG ($P < .05$). At 21 d, only 10 J/cm^2 (0.4 J) showed a SI out of all experimental groups ($P < .05$).
Santos et al. [31]	MyoD	5 d	NS from PBMG compared to ICG, but a SI in comparison to control group ($P < .05$).
Trajano et al. [32]	MyoD	5 d	SI at 25 mW (0.5 J) ($P < .01$) and 75 mW (0.5 J) ($P < .05$) compared to the ICG.
	Myogenin		SI at 25 mW (0.5 J) ($P < .01$) and 75 mW (0.5 J) ($P < .01$) compared to the ICG.
	Myf5		SI at 75 mW (0.5 J) ($P < .05$) compared to the ICG and 25 mW (0.5 J).
	MRF4		SDe at 75 mW (0.5 J) ($P < .05$) compared to the ICG; no significant change at 25 mW (0.5 J).
Vatansever et al. [33]	MyoD	7 d	SI for both age ranges in all experimental groups ($P < .05$).

Abbreviations: Myf5, myogenic factor 5.

TABLE 5 Risk of bias assessments for each domain using the SYRCLE risk of bias tool for all of the included studies (Hooijmans et al. [34])

Authors	Selections bias			Performance bias		Detection bias		Attrition bias	Reporting bias	Other
	Sequence generation	Baseline characteristics	Allocation concealment	Random housing	Blinding	Random outcome assessment	Blinding	Incomplete data outcome	Selective outcome reporting	Other sources of bias
Alves et al. [26]	U	L	H	H	H	L	H	L	L	L
Assis et al. [27]	U	L	H	H	H	L	H	L	L	L
Beasi et al. [24]	U	L	H	H	H	H	H	L	L	L
de Freitas et al. [28]	U	L	H	H	H	H	H	L	L	H
Morais et al. [25]	U	L	H	H	H	H	H	L	H	L
Rodrigues et al. [29]	U	L	H	H	H	H	H	L	L	L
Rodrigues et al. [30]	U	L	H	H	H	H	H	L	L	L
Santos et al. [31]	U	L	H	H	H	H	H	L	L	L
Trajano et al. [32]	U	L	H	H	H	H	H	L	L	L
Vatansever et al. [33]	U	L	H	H	H	H	H	L	L	L

Note: U – unclear risk of bias; L – low risk of bias; H – high risk of bias.
Abbreviation: SYRCLE, SYstematic Review Centre for Laboratory animal Experimentation tool.

group receiving concurrent strength training exhibited an increase in Myf5, while the PBMT-only treatment group demonstrated no significant change. Trajano et al. also noted a significant increase in Myf5 mRNA expression ($P < .05$), yet only in the 75 mW (0.5 J) group when compared to the ICG and the 25 mW (0.5 J) group [32].

3.3 | Myogenin

Myogenin expression was measured by 8 of the 10 included studies [24–30, 32]. Myogenin demonstrated heterogenous results from the included studies; overall, there was no definitive trend in the results, and based on the current scientific literature, it was not possible to conclude that PBMT either promoted increased or decreased myogenin expression over the course of muscle regeneration (Figure 3B).

3.4 | Myogenic regulatory factor 4

Only one study examined MRF4, where a significant decrease ($P < .05$) in mRNA expression was observed when compared to the ICG, at a power output of 75Mw (0.5 J) and wavelength of 904 nm [32]. This is in contrast with the 25 mW (0.5 J) treatment group, which showed no significant change in MRF4 expression ($P > .05$). These data may suggest a power output-specificity in the response of MRF4.

3.5 | Risk of bias

The overall risk of bias was high throughout the studies, as summarized in Table 5. Most studies employed assessor blinding for histopathological assessment but did not blind the investigator for the measurement of MRF expression. This results in a high risk of bias which may potentially affect the overall results.

4 | DISCUSSION

4.1 | Summary of results

Overall, the results of this systematic review exhibit a broad array of outcomes from a mixture of treatment parameters. This concurs with previous findings in this field by Alves et al. and Teles et al., who stated that variety in dosage parameters leads to a notable variation in outcomes [3, 21]. MyoD is the primary factor responsible for promoting SC proliferation and myogenic

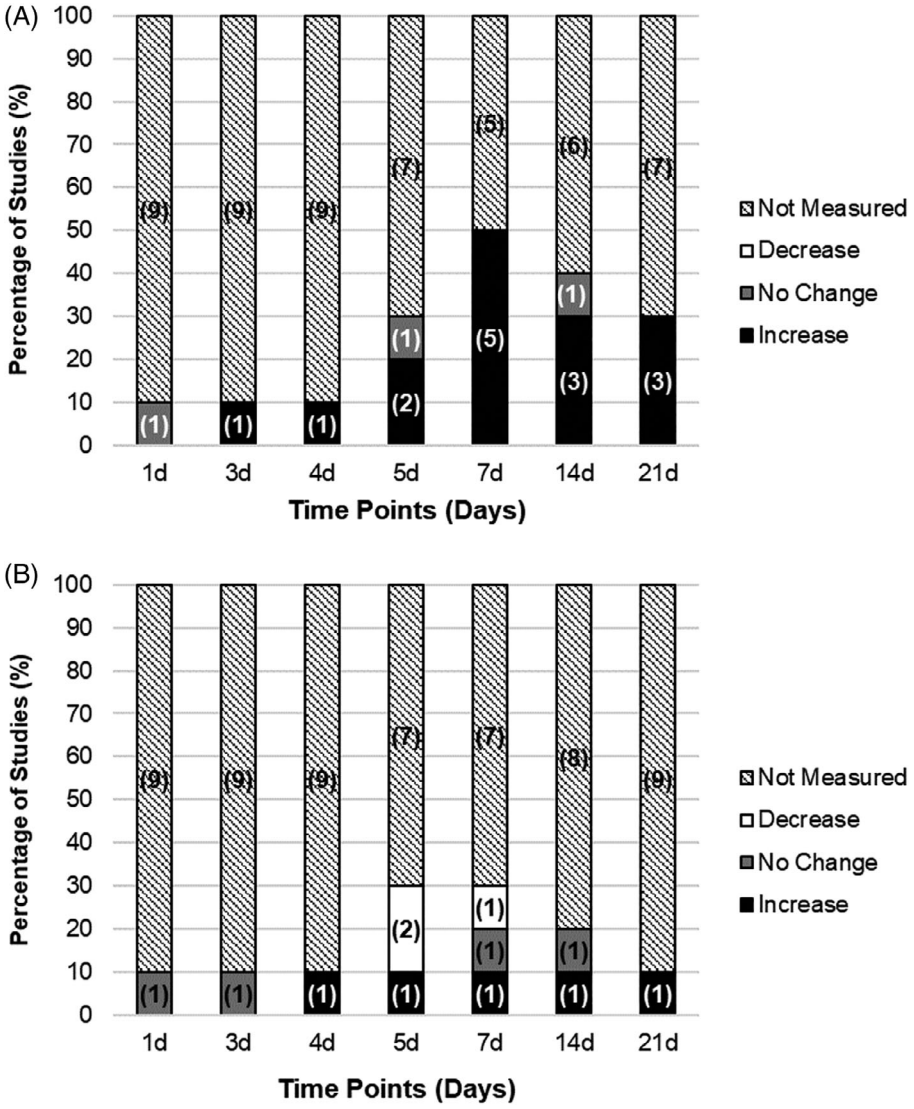


FIGURE 3 (A) Summary of changes in myogenic determination factor (MyoD) expression measured over the period of 1 to 21 days across all 10 studies. The bracketed number denotes the number of studies for each distribution. (B) Summary of changes in myogenin expression measured over the period of 1 to 21 days across all the 10 studies. The bracketed number denotes the number of studies for each distribution

commitment and this factor was analysed by all the studies. MyoD was the MRF that was consistently expressed in response to PBMT. Myogenin exhibited both increased and decreased expression, in addition to no change in expression at multiple time points. Myf5 and MRF4 received little attention within the literature, making it difficult to draw conclusions on the effect of PBMT on the expression of these factors.

4.1.1 | Myogenic determination factor

Evidence conflicts regarding the time points of when MyoD is expressed post-injury, although this may be relative to how the skeletal muscle is injured. Contusion injury in rat models often results in change to MyoD expression within 4 to 8 hours post-injury, which declines by 8 days [5]. In contrast, prior research with cryoinjury models has shown that MyoD and Myf5 initially downregulate for 7 days, after which PAX7 downregulation occurs, enabling SC proliferation through expression of the early MRFs [35]. This may explain why MyoD expression changes were analysed more often from 7 days onwards, and why a significant increase was noted by most of the studies analysing expression at this time. Cryoinjury generally exhibits distinct and well-characterized layers of damage, which are known to result in different MRF expression and regulation depending on the extent of tissue damage and should be considered a factor when analysing MRF expression [35]. Given the early expression of MyoD in some cases of skeletal muscle injury, it is unexpected that only one study analysed the effect at day 1 [26].

While not directly comparable to in vivo studies, in vitro research has indicated some concurrence with the in vivo studies included within this review in terms of MyoD expression and PBMT dosages. These studies

used the C2C12 cell lines demonstrated mixed results; Zhang et al. found PBMT decreased MyoD expression within 24 hours (632.8 nm and 6 mw/cm^2 for 3 minutes; no energy-per-point provided) [36]. Mesquita-Ferrari et al. had similar results, with no MyoD expression being promoted within 24, 48 and 72 hours from treatment sessions (780 nm and 10 mW, for 2 minutes at 0.2 J) [37]. Conversely, Monici et al. noted an increase in MyoD expression from PBMT in C2C12 myoblasts, with the increased formation of myotubes (905 nm and 550 mW and 808 nm and 25 mW, at ~68 J and 20 seconds for eight wells) [38]. While an in vitro cell line will prove to behave differently to an in vivo environment in response to PBMT, the above studies indicate that a differing dosimetric parameters still lead to an array of outcomes to varying degrees.

4.1.2 | Myogenic factor 5

Only two studies assessed the expression of Myf5, which is surprising given the factor's essential role in the early promotion of SC differentiation through the myogenic lineage [6]. While these two studies exhibited positive upregulation, Morais et al. only reported an increase in expression when strength training was used within the pre-treatment regime, with PBMT potentially enhancing the physiological benefits from exercise [25]. Conversely, Beasi et al. indicated that swimming exercise augments the effect of PBMT in promoting MRF expression, yet both studies show that PBMT mixed with exercise in some form may maximize PBMT in MRF expression enhancement [24]. As the two studies only observed a 14-day time point, early changes in Myf5 levels may have been missed when PBMT alone may have promoted a change without strength training. Trajano et al. only noticed a change in their 75 mW (0.5 J) treatment group at 5 days, suggesting that increased optical power may be more effective in promoting Myf5 expression [32]. Given no other skeletal muscle injury research has assessed Myf5, further research is needed to characterize its expression.

4.1.3 | Myogenin

Myogenin expression varied considerably from PBMT application, with some studies detecting a range of upregulation, downregulation, or no change, with no clear trend. Studies of myogenin expression, have shown that it follows a similar pattern as MyoD during contusion injuries, upregulating within 4 to 8 hours and eventually declining at 8 days [5]. Cryoinjuries can differ, exhibiting myogenin upregulation within the severely damaged areas of tissue but only a weak expression at 7 days [35]. Myogenin expression has only been observed in two PBMT in vitro studies by Zhang et al. and Mesquita-Ferrari et al., who observed myogenin expression at 24 hours and 24 and 48 hours, respectively [37, 39]. Zhang et al. discovered a significant decrease at 24 hours in myogenin expression, while Mesquita-Ferrari et al. showed no significant changes at both time points [37, 39]. This concurs with this current review's results, showing within the first few days post-injury, myogenin is not notably expressed. As myogenin is responsible for the terminal differentiation and fusion of myoblasts in mature muscle fibres, this may explain why it is not expressed during the early stages of injury [35]. Due to the limited numbers of studies MRF expression in the latter stages of injury at 7, 14 and 21 days, it is difficult to determine whether a trend of upregulation is present towards myotube maturation. Further quantitative studies are necessary to define whether this is the case.

4.1.4 | Myogenic regulatory factor 4

Trajano et al. were the only authors assessing MRF4 response to levels, noting a significant decrease in response to PBMT [32]. This decrease was only observed by the 75 mW (0.5 J) treatment group, suggesting that increased optical power output may be required to alter its expression, but this requires further investigation. As MRF4 has been shown to be expressed at the terminal phase of differentiation, it might be expected that these factor levels are naturally decreased at 5 days [5]. The results for MRF4 are distinctly limited by the restrictive time analysis, justifying the need for future research to analyse the effect of PBMT towards the effect of myotube maturation.

4.2 | Result variability

The collective results were highly variable, in part be due to the breadth of dosimetric parameters utilized in the included studies. Considering that the depth of tissue and chromophore activation may be relative to the treatment wavelength, it is perhaps unsurprising that the array of wavelengths used only demonstrates a trend in expression changes for MyoD [16]. Several studies that utilized different dosimetric parameters garnered varied results for MyoD expression; Trajano et al. demonstrated a significant increase in MyoD expression at 25 and 75 mW dosages; however, these both utilized an energy-per-point of 0.5 J at 904 nm, and were therefore comparable [32]. Conversely, the two studies by Rodrigues et al. resulted

in varying outcomes for MyoD expression over the treatment, even though the authors employed the same treatment parameters across the two studies of 10 and 50 J/cm^2, being 0.4 and 2 J, respectively [29, 30].

Freitas and Hamblin discuss how excessive dosage may result in an inhibitory or negative effect on cellular or tissue response, however the studies formerly discussed may suggest that energy output is not the determinant of MRF promotion in vivo, indicating other factors are necessary, especially wavelength [19]. Given that specific wavelengths interact with different molecular components, the wavelengths employed by the Rodrigues et al. studies (660 and 780 nm), alongside the aforementioned in vitro studies, may indicate that wavelength is more detriment in MRF activation than energy dosage alone [29, 30]. Considering the distinct lack of homogenous treatment parameters and results, it is hard to discern what dosages are required to facilitate suitable regulation of MRFs throughout the regeneration period.

Animal numbers within each study may also have compounded result variability, especially as no study reported a power calculation method for animal numbers within their methods, thus risking underpowering, or using more animals than necessary. Furthermore, the studies included in this review analysed a wide variety of time points. Some studies examined MRF expression at critical time points of the healing process, enabling a timeline of MRF expression to be established in response to PBMT [26, 29, 30]. The remaining authors only assessed singular chronological points, specifically 4, 5, 7 and 14 days. This may result in studies missing critical time points for MRF regulation, as the factors are expressed in a highly controlled manner at different time points throughout the healing process.

4.3 | Limitations of included studies

Treatment blinding is difficult to accommodate in trauma-induced PBMT research, due to the apparent injury presented to the researcher and the need to apply PBMT to an obvious injury site. This may lead to a different approach to handling and treatment of the animals, due to the imposed injury [34]. Another major limitation for all the studies was the level of risk of bias domains; this brings into consideration the methodological quality of the included studies, primarily due to the lack of blinding for MRF measurement and PBMT application. Prior animal-based research has shown to vary in methodological quality, resulting in a mixture of experimental quality, which ultimately poses ramifications on the translation of results to human trials [22]. While the intrinsic nature of PBMT application to a lesion area is problematic around the use of blinding, the inclusion of an injury group with sham treatment (ie, non-therapeutic red light), with the treatment parameters being blinded to the researcher applying the treatment may be a viable option.

4.4 | Review limitations

One limitation of this systematic review is the omission of research and results pertaining to inflammatory markers and infiltrate. Inflammatory cells are an important component in regulating muscular regeneration due to the infiltrate releasing growth factors and cytokines; however, this study was expressly focused at determining MRF expression within an in vivo environment due to their significance in regulating myogenesis.

Research implementing toxins as the lesion modality were also omitted from inclusion, as this research was aimed at the effect of laser in orthopaedic and musculoskeletal injuries through physical trauma. Additionally, some animals may show resistance to toxins, resulting in variable MRF expression [40]. Furthermore, given that these toxins can cause both local and systemic myotoxicity and significant disruption to the surrounding tissues, it may not be as reproducible as a contusion or cryoinjury, as featured throughout the included studies [41, 42]. Studies were also excluded if the animals had induced comorbidities, such as diabetes, in order to represent healthy population and keep the results comparable.

Finally, studies were omitted from this review if they did not utilize qRT-PCR to analyse MRF expression, meaning three studies were excluded as they relied upon immunohistochemistry analysis. As immunohistochemistry analysis is only semi-quantitative and mRNA and protein expression are known to correlate poorly it would not allow a comparable cross-study assessment.

This study was also limited by the lack of any meta-analysis. Given the limited and often heterogenous results of the included studies, formal meta-analysis was not possible. This may also be considered a limitation of the included studies in this review since inappropriate or insufficient data were reported and any future studies should be encouraged to supply more information regarding results to facilitate meta-analyses and validate their inferential results.

5 | CONCLUSIONS AND RECOMMENDATIONS

While the results from this systematic review are varied, the collated research has shown some MRF regulatory changes in response to PBMT, especially MyoD, given the increased attention it received within the included studies. The studies showed a mixture of methodological

quality, although the lack of blinding may cause concern on the presence of bias. The consensus amongst the included studies was that PBMT promotes lesion area reduction. However, this needs to be confirmed as a response to MRF upregulation and not just attenuation of the inflammatory infiltrate and natural healing occurring. Although these results may be limited clinically, the aim is that this study will encourage further, high-quality research in PBMT for skeletal muscle regeneration, focusing on the use of quantitative measures, being primarily qRT-PCR and protein expression analyses. It is critical that more studies attempt to assess MRF and related factor expression over the whole injury period, not a singular arbitrary time point in the healing process, as MRF expression seemingly changes throughout the period of the injury and important time points may be missed all together. This should be combined with histological tissue analysis alongside investigation into protein-level MRF expression to determine if MRFs are expressed for sufficient periods to result in functional myogenesis.

PBMT research is progressively improving and becoming more prevalent, yet more studies are required for the timely advancement of the field, especially research aiming to define the optimal parameters for stimulating MRF activation to promote muscle regeneration. It is apparent from the results of this study that several different sets of dosimetric parameters can produce comparable results suggesting a wide treatment window that should be relatively simple to define. Developing an understanding of the mechanisms regulating MRF expression would assist in defining the most efficacious dose for animal models, and eventually humans, to promote skeletal muscle regeneration. This may include using advanced in vitro cell and tissue models to explore the mechanisms through which Cytochrome c oxidase activation ultimately leads to a MRF expression.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

David W. Shepherd, **Darren J. Player** and **Hayley C. Whitaker**: Developed the concept for the research. **David W. Shepherd**, **Joseph M. Norris**, **Benjamin S. Simpson**, **Darren J. Player** and **Hayley C. Whitaker**: Developed and supported methods used in the research. **David W. Shepherd**, **Joseph M. Norris**, **Benjamin S. Simpson**, **Darren J. Player** and **Hayley C. Whitaker**: Generated data or involved in data analyses. **David W. Shepherd**, **Joseph M. Norris**, **Benjamin S. Simpson**, **Darren J. Player** and **Hayley C. Whitaker**: Wrote or edited the manuscript.

DATA AVAILABILITY STATEMENT

All data used in this article are available on request.

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