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Review

Photobiomodulation, Cells of Connective Tissue and Repair Processes: A Look at In Vivo and In Vitro Studies on Bone, Cartilage and Tendon Cells

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Abstract: The use of light in the red and near-infrared light spectrum influences cell viability and proliferation in both cell and animal experimental models. In wounded models, photobiomodulation (PBM) at various laser parameters may stimulate or inhibit the tissue repair process by affecting cells important to healing. Connective tissue cells include osteocytes and osteoblasts in bone, chondrocytes and chondroblasts in cartilage, and tenocytes and tenoblasts in tendons. PBM, at various wavelengths, energy densities and power output, has various effects on cell viability, proliferation, migration and gene expression. This narrative review will briefly encapsulate the effectiveness of PBM on connective tissue cells, and its possible role in tissue repair. Relevant journal articles were obtained through PubMed and Google Scholar.

Keywords: photobiomodulation; bone; cartilage; tendon; wound healing

Citation: Shaikh-Kader, A.; Houreld, N.N. Photobiomodulation, Cells of Connective Tissue and Repair Processes: A Look at In Vivo and In Vitro Studies on Bone, Cartilage and Tendon Cells. *Photonics* **2022**, *9*, 618. https://doi.org/10.3390/photonics9090618

Received: 20 July 2022
Accepted: 25 August 2022
Published: 30 August 2022

1. Introduction

Photobiomodulation (PBM) involves low-level light in the red or near-infrared (NIR) spectrum to stimulate or inhibit processes at the cellular and molecular level. Light in the red (600–700 nanometers (nm)) and NIR (780–1100 nm) spectrum penetrates skin at a much deeper level than light in the blue spectrum (400–490 nm) or ultraviolet radiation (200–400 nm), as human skin consists of chromophores with light scattering and absorptive properties [1–5]. Chromophores are endogenous compounds such as nucleic acids, aromatic amino acids, and melanins [6]. In PBM, both lasers and light-emitting diodes (LEDs) are employed, and the differences between the two application methods include spectral width and beam divergence [7]. Laser parameters are based on the characteristics of light and include wavelength spectrum measured in nm (determines light color), amplitude (the brightness of light), energy density or fluence measured in joules per centimeter squared (J/cm^2) (dose applied), spot size (the area to which light is applied or the treatment area measured in cm^2), and irradiance (power density or intensity) measured in milliwatts per centimeter squared (mW/cm^2) [4,8]. The parameters chosen to affect physiological processes are crucial in determining whether light has a stimulatory or inhibitory effect on tissue.

Light in the red and NIR spectral ranges has been associated with positive tissue effects due to substantial tissue penetration by longer wavelengths [9]. The mechanisms of PBM action on cells and tissues are still being researched, and some known mechanisms include chromophore absorption and the activation of signaling molecules, transcription factors and effector molecules [4]. PBM application leads to a "photochemical reaction" in the cell, and its effectiveness on the cell or tissue is determined by photon absorption by a chromophore within the tissue itself [10,11]. These chromophores include mitochondrial cytochrome C oxidase and ion channels receptive to light and heat [11].

Connective tissue, one of the basic tissue types of tissue (the other being epithelial, nervous and muscle tissue), consists of various components that function together to provide durability by binding and supporting other types of tissues. The three categories of connective tissue are loose and dense connective tissue (also known as connective tissue proper) and specialized connective tissue types. Connective tissue consists of the extracellular matrix (ECM) made up of fibers and ground substance, and either fixed or transient cells [12]. Fixed cells include fibroblasts, fixed macrophages, mast cells, adipocytes, mesenchymal stem cells and reticular cells. Transient cells such as eosinophils, neutrophils and dendritic cells migrate into connective tissue in response to specific stimuli [12]. Specialized connective tissue cells comprise bone cells (osteocytes and osteoblasts) and cartilage cells (chondrocytes and chondroblasts), while dense regular connective tissue include tenocytes, the specific fibroblasts found in tendons [12,13].

In general, tissue healing in bones and tendons follow a similar overlapping process that is initiated by damaged or necrotic cells around the injury site: (i) the inflammatory phase aims to protect tissue by stimulating the removal of tissue debris and promoting white blood cell migration, particularly phagocytes, to the injury site; (ii) this is followed by the migration and proliferation of cells crucial for the production of ECM components; and (iii) finally scar tissue formation and remodeling to re-establish the durability of the connective tissue. In cartilage, the repair process is not as structured and efficient due to the avascular nature of the tissue [14]. Wound repair in cartilage therefore lacks the intrinsic mechanisms central to tissue repair in skin, bone and tendons. The purpose of this review is to compare the effects of PBM on bone, cartilage and tendon cells found in connective tissue, as well as the wound healing capabilities of PBM in these tissues. The information will be useful in determining how light can be used to promote physiological processes, such as proliferation, apoptosis and tissue repair in bone, cartilage and tendons, as different tissues have contrasting responses to the laser parameters applied.

2. Methodology

Google Scholar and PubMed were searched for relevant research articles regarding the influence of PBM on bone, cartilage and tendon cells published between 2012 and 2022. The following keyword search strategy was used: 'Photobiomodulation AND osteoblast', 'Photobiomodulation AND osteocyte', 'Photobiomodulation AND osteoclast', 'Photobiomodulation AND chondrocyte', 'Photobiomodulation AND chondroblast', 'Photobiomodulation AND tenocytes', 'Photobiomodulation AND tenoblasts', Photobiomodulation AND tendon stem progenitor cells', 'Photobiomodulation AND tissue repair', 'Bone fracture AND healing OR repair', 'Cartilage AND healing OR repair', 'Tendon AND healing OR repair'. The articles chosen were published in the English language as full-length articles that focused on some aspect of the current subject conducted in either in vitro or in vivo experimental models. This article is not intended to be a systematic review or meta-analysis, but a 'narrative overview' of the influence of PBM on the specialized cells found in bone, cartilage and tendons.

3. Bone Cells

Osteoblasts are specialized fibroblast-like cells crucial for synthesizing bony matrix components such as type I collagen, proteoglycans and proteins important for cell attachment. Osteoblasts also play a role in stimulating bone mineralization. When osteoblasts become entrapped within the bony matrix, they become osteocytes—the most abundant cell type localized in bone. The main role of osteocytes is to secrete paracrine factors in response to mechanical stress. When the need arises, osteocytes may revert to becoming osteoblasts. The larger multinucleated osteoclasts present in bone serve to resorb the bony matrix for processes such as bone remodeling or calcium ion homeostasis by releasing proteolytic enzymes and acids [15,16].

The process of bone tissue repair starts with hematoma formation, which is the formation of a blood-filled swelling, followed by the inflammatory response. In response to

necrotic tissue and damaged ECM, inflammatory cells migrate to the injured site. Similar to wound healing in skin, the initial acute inflammatory phase is critical in establishing the area for successful regeneration. Inflammatory cells secrete proinflammatory mediators, including tumor necrosis factor alpha (TNF-α) and various interleukins. Crucial to the bone repair process is the proliferation and differentiation of mesenchymal stem cells into osteogenic cells. It has been suggested that the stem cells arise from the surrounding soft tissues, bone marrow and through circulatory recruitment [17]. Osteogenic differentiation of these stem cells is mediated by TNF-α in a 3D cell culture model [18]. Osteoblasts and osteoclasts contain cell-surface receptors for TNF-α, namely, TNFR1 and TNFR2, and the proinflammatory effects of TNF-α occur when it binds to these receptors, particularly TNFR2, which is expressed following bone injury [19]. Two important interleukins (IL) released during the inflammatory phase include IL-1 and IL-6. Both ILs are important in promoting angiogenesis, but IL-1 is important in establishing the cartilaginous callus for the next stage of bone fracture repair, while IL-6 stimulates the production of vascular endothelial growth factor (VEGF) and osteoblast and osteoclast differentiation. Following inflammation and hematoma formation, a cartilaginous (or soft) callus forms from the granulation tissue. Through the ossification processes, the cartilage is eventually replaced with a hard callus which undergoes remodeling to restore the bony tissue [17,20]. Figure 1 represents a summary of the bone repair process in response to injury.

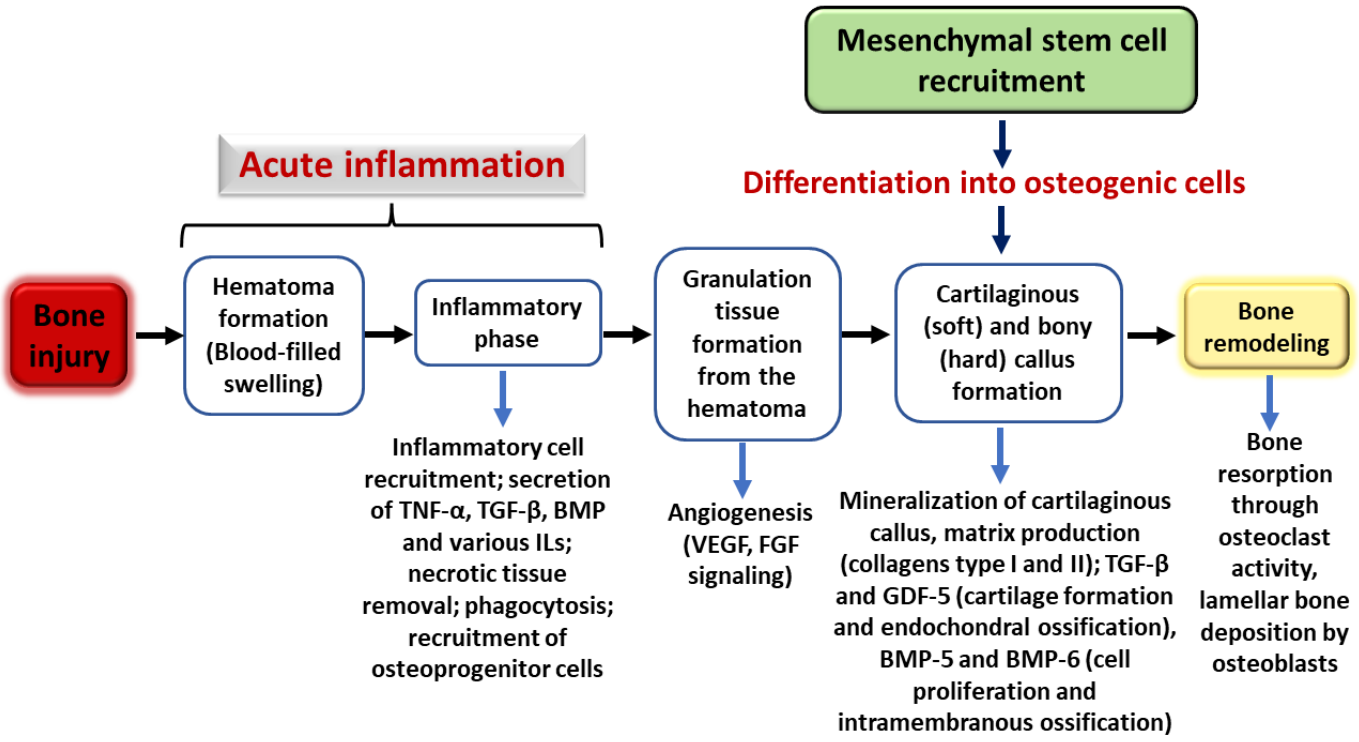

Figure 1. A brief summary of the overlapping phases during the bone healing process (indirect/secondary healing). TNF-α—tumor necrosis factor alpha; TGF-β—transforming growth factor beta; BMP—bone morphogenetic proteins; IL—interleukin; VEGF—vascular endothelial growth factor; FGF—fibroblast growth factor; GDF—growth differentiation factor.

The idea that PBM at various wavelengths and energy densities can improve cell viability and proliferation has been supported in numerous findings. Bölükbaşı Ateş et al. (2017) compared PBM at two different wavelengths (635 and 809 nm) and various energy densities (0.5, 1 and 2 J/cm^2) on osteoblast viability and proliferation. Osteoblast viability and proliferation did not differ after a single laser application at both wavelengths in the experimental and control groups, and no changes in cell morphology were noted [21]. In order to reduce the risk of contamination, the culture lids remained on during irradiation which may have led to a loss in the energy delivered, and hence the reason for not observing any changes. Furthermore, Bölükbaşı Ateş et al. (2017b) investigated the effect of a 635 nm laser with a power density of 50 mW/cm^2 at three different energy densities (0.5, 1 and 2 J/cm^2) on human osteoblasts in vitro. The osteoblasts were incubated with methylene blue, a photosensitizer, for an hour prior to receiving irradiation. Methylene blue-mediated PBM resulted in a decrease in cell number, particularly at 72 h after irradiation, and the main findings of the study showed that PBM (with methylene blue) did not significantly affect osteoblast viability or proliferation [22]. This, however, would be considered more as photodynamic therapy (PDT) than PBM, due to the use of a photosensitizer in the experiments, which is also the likely cause of the decrease in cell number observed at 72 h.

Cardoso et al. (2021) studied the effect of PBM on rat calvaria osteoblasts using red (660 nm) and infrared (808 nm) lasers and LED (637 $\pm$ 15 nm) at various energy densities. Their results showed that the red laser (8.3 J/cm^2) and LED (0.02 J/cm^2) stimulated osteoblast proliferation and viability, increased wound closure made by a central scratch, and increased alkaline phosphatase (ALP) levels and mineralization in rat calvaria osteoblasts [23]. ALP is an enzyme generated during osteoblast metabolism and is used as an osteogenic marker for osteoblast differentiation and maturation [24]. Furthermore, osteoblasts exposed to an osteogenic medium and PBM (red laser and LED) showed elevated proliferation and migration in a dose-dependent manner [23]. It should be noted that these stimulatory effects of PBM occurred in osteoblasts that were in a quiescent state for 24 h. In animal models, the use of PBM to stimulate bone healing after dental extraction has been reported. Wistar rats exposed to LED (850 nm) treatment after molar extraction showed increased bone remodeling activity as compared to the control group (no LED treatment), as well as increased osteoblast and osteocyte numbers at days 15 and 30 post-treatment, respectively [25]. Furthermore, LED treatment in conjunction with a biomaterial scaffolding resulted in better bone tissue organization at 15 days post-irradiation. Bone resorption was also less in the groups treated with LED and in the groups with the scaffold, as indicated by acid phosphatase (AcP) activity, the enzyme released by active osteoclasts [25,26].

In addition to influencing the repair process in bone, PBM also affects bone remodeling. Rats fitted with an orthodontic appliance received PBM with a gallium–aluminium–arsenide semiconductor (GaAIAs) laser (780 nm; 10 J/cm^2 and 40 mW) for 7 or 14 days [27]. The authors noted an increase in newly formed bone and osteoblast numbers around the teeth that received the orthodontic force and PBM as compared to the control group that did not receive irradiation. Similarly, in diabetic rats inserted with an orthodontic appliance and treated with a 780 nm diode laser (power output 20 mW; energy density 640 J/cm^2), the number of osteoprotegerin-positive osteoblasts and osteopontin-positive osteocytes were greater in normal and diabetic rats with PBM treatment as compared to the groups without PBM at days 7 and 14 post-treatment [28].

PBM at 808 nm and 3.75 J/cm^2 reduced the expression of microRNA-503 (miR-503), but increased the levels of Wnt3a signaling in mouse pre-osteoblasts [29]. Studies have shown that miR-503-5p suppresses the differentiation of bone mesenchymal stem cells and Wnt3a is an important stimulator of pre-cursor osteoblast cells and osteoblast proliferation in mouse models [30–32]. Wnt3a is a member of the Wnt family that consists of 19 glycoproteins, and Wnt signaling may be divided into two pathways: canonical and non-canonical [33]. Wnt3a is a canonical Wnt ligand that has been shown to influence bone metabolism and

repair [34–36]. Interestingly, Sun and colleagues (2017) [37] showed that in a model of distraction osteogenesis, miR-503 may stimulate bone formation.

Not all studies have shown positive results in response to PBM, although there are more positive studies than there are negative. The complexity of selecting the correct illumination parameters and treatment intervals has led to some studies that show negative results. No changes in cell proliferation were observed in human osteoblasts extracted from a healthy donor and exposed to laser irradiation at 915 nm at various energy densities (0, 5, 15 and 45 J/cm^2) and power densities (0.12 or 1.25 W/cm^2) [38]. While the study produced similar results to other studies using a 915 nm laser [39,40], the authors acknowledge that direct comparison of the results is not ideal due to different experimental conditions. As the results are from a single donor, the results are not conclusive and further investigation is required. Using primary cells instead of immortalized cells has been encouraged as cell lines behave differently to primary cells [41]. However, Mergoni et al. (2018) also reported an increase in bone nodule formation in the primary cells treated with PBM at 915 nm and 5 J/cm^2 [38].

In a wounded in vitro model, Saos-2 human osteoblast-like cells exposed to a GaAlAs diode laser (915 nm and fluencies of either 5, 10 or 15 J/cm^2) exhibited an enhanced healing ability as compared to the control group; however, the groups exposed to 15 J/cm^2 showed decreased healing ability as compared to the other fluencies used [39]. While wound healing was stimulated through cell migration and collagen production, the authors noted that the wound environment did not mimic typical wounds due to a lack of an inflammatory prolife (lack of IL-1β expression, matrix metalloproteinases (MMP) 1 expression and prostaglandin E_2 (PGE_2) synthesis). This is an important noting as inflammation affects wound healing and if inflammation does not resolve, wounds do not heal effectively. Na and colleagues (2018) assessed cell proliferation, differentiation, viability and bone resorption activity in osteoblasts, osteocytes and osteoclasts exposed in vitro to LED emitting at a wavelength of 940 nm and four fluencies (0, 1, 5 and 7.5 J/cm^2). Results showed that osteoblast proliferation and osteoclast activity increased at 1 J/cm^2, while osteocytes exposed to 5 J/cm^2 showed decreased viability at 12 h after light exposure, and more importantly, cell death at 24 h after LED application. Furthermore, cell death occurred in osteoclasts (5 J/cm^2) 24 h post-application, and decreased cell viability was observed in osteoblasts (7.5 J/cm^2) after 24 h [42]. The importance of these findings suggests that PBM at higher doses may negatively impact bone remodeling and possibly bone repair through its effects on cell viability and enhanced apoptosis. The difference in results between the irradiated groups in the two studies above may be explained by the biphasic dose response, which is based on the Arndt–Schulz curve. Essentially, low levels of light have a better effect than higher levels; however, too low levels have no effect. If too much energy is applied, the stimulatory effects are no longer evident and instead bioinhibition is observed.

Ovariectomised rats with bone defects were treated using a 780 nm low-intensity GaAlAs laser (and energy densities of 0, 20 or 30 J/cm^2) for 3, 6 or 12 sessions [43]. The main finding from this study showed that PBM, especially at an energy density of 30 J/cm^2, was capable of prompting bone formation at the area of bone defect and stimulating the production of osteocytes. The results obtained using high-energy densities in vivo are different to in vitro studies. In in vitro models, light does not have to penetrate through several layers where the light is scattered, reflected and absorbed by various cellular components and cells types, as there is only a single layer of cells present. However, in animal models, light needs to penetrate through several layers to reach the cells or tissue of interest and this accounts for the differences in results obtained between in vitro and in vivo studies. Ansari et al. (2021) have recently reported on the need to source in vitro bone models from progenitor cells obtained from an individual's peripheral blood to "account for donor-specific differences and disease-related cell reactions" [44]. The idea could be applicable to PBM therapy and warrants studies that determine the effect of PBM on patient-derived in vitro bone models to factor in the differences with regard to patient-specific differences and disease.

4. Cartilage Cells

The three major types of cartilage are hyalin cartilage (found in articulating surfaces), fibrocartilage (located in the pubic symphysis, menisci and temporomandibular joint disc) and elastic cartilage (located in the epiglottis and pinna). Cartilage comprises chondroblasts, immature cells responsible for cartilage production, and chondrocytes, which are mature cells in charge of producing and maintaining the ECM [45]. Cartilage is hypocellular and lacks nervous tissue, lymphatics and a blood supply [45]. As the repair process in cartilage is adversely affected due to the lack of vascularity, cartilage heals very slowly. Additionally, the cells of the perichondrium divide slowly, and this may further delay the healing process in cartilage. Bos et al. (2001) showed that auricular cartilage exhibits an intrinsic healing capacity through the production of growth factors, particularly transforming growth factor beta (TGF-β) [46]. Medvedeva et al. (2018) have reviewed the current approaches and future direction in the repair of articular cartilage subject to traumatic stress and degenerative pathologies. Tissue engineering approaches include the use of a scaffold (synthetic or natural polymers) coupled with a variety of stem cells and a favorable natural environment for cartilage regeneration. The ultimate goal is to stimulate stem cell differentiation and expansion into chondrocytes.

In an animal model of osteoarthritis, PBM therapy using LED (850 nm, 200 mW, 6 J) enhanced the immunoreactivity of TGF-β and collagen type II, and resulted in better tissue organization in osteoarthritic rats [47]. Balbinot et al. (2021) used a GaAIA laser emitting at a wavelength of 850 nm and 57.14 J/cm^2 to treat monoiodoacetate-induced osteoarthritic pain in adult male Wistar rats. PBM was effective at preventing cartilage degradation and controlling central sensitization associated with chronic osteoarthritic pain [48]. Mesenchymal stem cell differentiation into chondrogenic cells using PBM has been reported. In response to PBM at 632.8 nm and 1.7 mW, SRY-Box transcription factor 9 (SOX9) and TGFβ3 gene expression increased, peaking at 7 days post-irradiation, and decreasing thereafter. The gene expression of collagen type XII alpha 1 chain (COL2A1) also increased in response to irradiation over the 21-day experimental period [49]. The results obtained show a promising link between PBM and cartilage injury since stem cell differentiation into chondrogenic cells is pertinent to cartilage healing. SOX9 is an important transcription factor in cartilage generation in both developing and adult cartilage. TGFβ3 has been shown to promote chondrogenesis in adipose-derived stem cells, mesenchymal stem cells and articular chondrocytes, and COL2A1 is responsible for type II collagen formation [50–54]. However, it is worth noting that in a co-culture of mesenchymal stem cells and chondrocytes, a reduced TGFβ3 concentration and exposure duration is required to achieve the same chondrogenic effect when compared to the monocultures [50]. The effect of PBM on co-cultures in cartilage degeneration is thus recommended.

Cartilage regeneration therapy includes an exercise regime to improve cartilage repair [55], and the effect of PBM with exercise on cartilage damage has been reported. In osteoarthritic rats, the combination of PBM using a GaAlAs laser (808 nm and 50 J/cm^2) and aquatic or aerobic exercises prevented cartilage damage and degeneration with anti-inflammatory effects [56–58]. Furthermore, PBM at 808 nm (energy density 28 J/cm^2 or 50 J/cm^2) has also been shown to have promising effects on cartilage regeneration in rat chondrocytes. PBM at the two energy densities increased cell proliferation and factors important in ECM generation such as IL-4, IL-10, COL-2, TGF-β and aggrecan, while decreasing the inflammatory cytokine IL-1β [59]. Human primary chondrocytes exposed to NIR irradiation (910 nm and 8 J/cm^2) showed anti-inflammatory properties due to reduced cytokine expression (IL-1β-induced IL-1β and IL-6) as well as the inhibition of nuclear factor-kappa B (NF-κB) activity in chondrocytes treated with Ilβ1 [60]. NF-κB is a transcription factor, and the phosphorylation of NF-κB leads to its translocation into the cell's nucleus. The activation of genes involved in cytokine production and NF-κB signaling has been linked to cartilage degeneration [61,62].

5. Tendon Cells

Tenocytes and tenoblasts are cells found in tendons, the dense connective tissue that connects skeletal muscle to bone. Tenoblasts are immature tenocytes consisting of large ovoid nuclei. These round cells are the main cell type in young tendons that mature into spindle-shaped tendon-specific fibroblasts, known as tenocytes, with ageing [63]. Tenocytes are, therefore, important in the production of the ECM (collagen, protein mediators important in repair, and proteoglycans) and are responsible for the maintenance of tendon tissue. The repair process in tendons initiates with inflammation, followed by a proliferative/repair/regenerative phase, and ends with remodeling [64]. In brief, during the inflammatory phase (which typically lasts 3 to 7 days), a blood clot forms and chemoattractant factors are released. In response to these factors, inflammatory cells arrive at the injury site to remove debris and prevent infection. Importantly, the recruitment of tenocytes and fibroblasts occur during the inflammatory response, and ECM components, including collagen (type III), are synthesized. During the second phase, tenocytes and fibroblasts proliferate to accelerate the production of ECM components crucial to the repair process, and the injured site now contains an abundance of type III collagen. During the final stage, the tenocytes and collagen fibers are aligned in the direction of the stress to re-establish the load-bearing capabilities of the tendon, and type III collagen is replaced with type I collagen. However, tendons display hypocellularity and hypovascularity and tendon repair is a challenging and difficult process to achieve, with tendons being more susceptible to injury after a successful repair process [64,65].

Tsai et al. (2012) determined the effect of PBM at 660 nm (energy densities at 1, 1.5, and 2 J/cm^2) on tenocyte migration during the regenerative phase of wound healing in an in vitro model. PBM at 660 nm was successful at stimulating tenocyte migration in the wounded models and the migration was enhanced in a dose-dependent manner, particularly at 2 J/cm^2, as observed by a Transwell filter migration assay. Additionally, PBM resulted in the up-regulation of mRNA and the protein expression of dynamin-2 in a dose-dependent manner [66]. Dynamins are GTPases (enzymes that hydrolyze guanosine triphosphate), which are important in cell migration [67]. Furthermore, dynasore, a dynamin inhibitor, negatively impacted cell migration in the 2 J/cm^2 group.

Under stressful conditions, tenocytes release heat shock proteins (HSPs). In response to HSP release, certain cytokines and chemokines are released and natural killer cells are stimulated. The HSPs have various roles in wound repair, including the regulation of the inflammatory response, the stimulation of cell migration and proliferation, the promotion of collagen synthesis, and wound debris clearance [68]. HSP70, in particular, plays a pivotal role in tendinopathies by controlling the toxic effects of TNF-α and nitric oxide on cells [69]. The effect of PBM on HSP70 in an experimental acute Achilles tendinitis rat model was reported by Evangelista et al. (2021). The study reported that PBM therapy with an LED (parameters 630 ± 20 nm; 300 mW; 9 J/cm^2) was successful at increasing HSP70 expression, fibroblast numbers, and hence collagen production [70].

In an in vivo model of partial injury of the calcaneus tendon, Wistar rats received treatment including heterologous fibrin biopolymer application; PBM treatment; and a combination of heterologous fibrin biopolymer and PBM over a period of 7, 14 and 21 days [71]. The fibrin biopolymer is a biomaterial that has sealant, adhesive and haemostatic properties, to name a few [72]. The study by de Freitas Dutra Júnior et al. (2022) showed that tenocyte proliferation increased in the injured area in response to heterologous fibrin biopolymer application and the combination of heterologous fibrin biopolymer and PBM. PBM treatment only, however, resulted in greater tendon injury as observed in the histological findings, as compared to the control and other treatment groups. This interesting finding occurred after 7 days of PBM treatment, but the results show an improved repair process in the PBM group on days 14 and 21 day when compared to the control [71].

In a diabetic rat model, Wistar rats were injected with Streptozotocin (STZ) (dose of 40 mg/kg), and one week after STZ-induced diabetes, an injury was made to the Achilles tendon. An indium–gallium–aluminum–phosphide laser emitting at a wavelength of 660 nm and 4 J/cm^2 was applied to the injured site for 3 weeks. Fibroblast number and orientation were determined with immunohistochemistry showing that fibroblast numbers increased in the injured diabetic rats receiving PBM and the injured diabetic rats who received aerobic exercise as compared to the control group (injured non-diabetic only). Interestingly, in the injured diabetic rats with PBM and aerobic exercise, the number of fibroblasts decreased when compared to the injured diabetic and injured diabetic with exercise groups [73]. In tenocytes isolated from sheep, the tenocytes treated with a 5% platlet-rich plasma, either alone or in combination with PBM using an LED, displayed increased viability as compared to the control group after 48 h [74]. The parameters of the LED were an energy dose of 4 J/cm^2 and wavelengths of 630 nm/150 mW (standard small probe), and 625 nm and 850 nm/1200 mW (large probe). Additionally, tenocytes exposed to PBM only showed enhanced migratory abilities.

Tendon stem/progenitor cells (TSPCs) are resident cells discovered in mice and humans by Bi et al. (2007), and contribute to tendon regeneration through the release of trophic factors [65,75]. Zhang et al. (2016) showed that moderate exercise may stimulate tendon repair through the stimulation of TSPCs in a rat model with tendon injury [76]. To the best of our knowledge, there are currently no studies looking at the effects of PBM on TPSCs and this area of research is required due to the ability of TSPCs to accelerate repair processes in damaged tendons using light. Table 1 provides an overview of the studies reported in this review.

Table 1. An overview of the in vitro and in vivo studies included in this review. AcP—acid phosphatase; AIGaInP—aluminum gallium indium phosphide; ALP—alkaline phosphatase; COL—collagen; GaAIAs—gallium–aluminum–arsenide semiconductor; h—hours; HSP70—heat shock protein 70; IL—interleukin; J/cm^2—joules per centimeter squared; LED—light-emitting diode; MB—methylene blue; miR-503—miRNA-503; min—minutes; MMP—matrix metalloproteinases; mW—milliwatts; NF-κB—nuclear factor kappa B; nm—nanometers; PBM—photobiomodulation; s—seconds; TGFβ—transforming growth factor beta; W/cm^2—watts per centimeter squared.

Cells of Interest	Study Design	Laser Parameters				Main Outcome/s	Reference
		Wavelength	Fluency	Power Output or Power Density	Irradiation Time		
Bone							
Osteoblasts	in vitro	635 nm (diode laser) 809 nm (diode laser)	0 J/cm^2 0.5 J/cm^2 1 J/cm^2 2 J/cm^2	50 mW	10, 20, 40 s	No change in cell viability or cell proliferation between irradiated and control groups. PBM had no effect on ALP activity.	Bölükbaşı Ateş (2017) [21]
Osteoblasts	in vitro	635 nm (diode laser)	0 J/cm^2 0.5 J/cm^2 1 J/cm^2 2 J/cm^2	50 mW/cm^2	10, 20, 40 s	Decreased cell viability at 72 h when in irradiated osteoblasts previously incubated in MB. Increased ALP activity in groups with MB and PBM on day 7. Decreased mineralization reported in all treated groups.	Bölükbaşı Ateş (2017b) [22]
Osteoblasts	in vitro	660 nm (AlGaInP) 808 nm (GaAlAs) 637 ± 15 nm (LED)	5 J/cm^2 8.3 J/cm^2	40 mW	3 s 5 s	Increased cell viability and wound closure occurred in groups exposed to the 660 nm laser and LED. All groups exposed to 5 s irradiation showed increased viability, greater cell density, and faster closure of the wound gap. PBM increased ALP activity.	Cardoso et al. (2021) [23]
Osteoblast; osteocyte	in vivo (Wistar rats)	850 nm (LED)	2.14 J/cm^2	100 mW	60 s	Groups treated with LED displayed better bone remodeling and maturation, but not bone formation, with and without the biomaterial scaffold. Increased ALP and decreased AcP activity reported in the LED groups with biomaterial.	Dalapria et al. (2022) [25]
Osteoblast	in vivo (Wistar rats)	780 nm (GaAlAs)	10 J/cm^2	40 mW	10 s	Increased osteoblast numbers and enhanced bone formation in the area surrounding the central incisors in groups with a fitted orthodontic appliance (orthodontic force) and PBM exposure.	Gonçalves et al. (2016) [27]

Table 1. *Cont.*

Cells of Interest	Study Design	Laser Parameters				Main Outcome/s	Reference
		Wavelength	Fluency	Power Output or Power Density	Irradiation Time		
Osteoblast; osteocyte	in vivo (Wistar rats)	780 nm (GaAlAs)	640 J/cm^2	20 mW	40 s	PBM enhanced bone remodeling of the alveolar bone. At days 7 and 14, the number of osteopontin-positive osteocytes was higher in the groups receiving laser treatment (in normoglycemic and diabetic rats). PBM increased the number of osteoprotegerin-positive osteoblasts in the groups receiving laser treatment (in normoglycemic and diabetic rats).	Gomes et al. (2017) [28]
Osteoblast	in vitro	808 nm (GaAlAs)	3.75 J/cm^2	0.401 W, 0.042 W/cm^2	90 s	PBM down-regulated miR-503 expression and up-regulated Wnt3a expression. miR-503 stimulated apoptosis and caspase-3 expression, but repressed cell proliferation and decreased the expression of Wnt3a, β-catenin, Runx2 and Bcl-2.	Li et al. (2019) [29]
Osteoblast	in vitro	915 nm (GaAlAs)	5 J/cm^2 15 J/cm^2 45 J/cm^2	1.5 W 0.12 W/cm^2 1.25 W/cm^2	0.12 W/cm^2 (41.7, 125 and 375 s) 1.25 W/cm^2 (4, 12 and 36 s)	Osteoblast proliferation did not change in the groups receiving PBM (single treatment per day for 3 days) at 5, 15 and 45 J/cm^2 and the control group. PBM stimulated bone nodule formation in groups treated with 5 J/cm^2 and 0.12 W/cm^2 as compared to control groups.	Mergoni et al. (2018) [38]
Osteoblasts, osteocytes and osteoclasts	in vitro	940 nm (LED)	0 J/cm^2 1 J/cm^2 5 J/cm^2 7.5 J/cm^2	1.67 mW/cm^2 8.33 mW/cm^2	10 min	PBM increased osteoblast proliferation after 48 h post-irradiation (1 J/cm^2 promoted 100% increase, while 5 J/cm^2 promoted a 25% increase). PBM did not affect osteocyte proliferation. Osteoclast differentiation and resorption activity stimulated at 1 J/cm^2. Osteocyte and osteoclast viability decreased when irradiated with a dose of 5 J/cm^2, while PBM at 7.5 J/cm^2 decreased osteoblast viability.	Na et al. (2018) [42]

Table 1. *Cont.*

Cells of Interest	Study Design	Laser Parameters				Main Outcome/s	Reference
		Wavelength	Fluency	Power Output or Power Density	Irradiation Time		
Osteocytes	in vivo (Wistar rats)	780 nm (GaAlAs)	0 J/cm^2 20 J/cm^2 30 J/cm^2	70 mW	20 J/cm^2 (100 s) 30 J/cm^2 (150 s)	PBM at higher fluencies promoted bone formation (increased trabecular surface area) and increased osteocyte number.	Scalize et al. (2019) [43]
Saos-2 human osteoblast-like cells	in vitro	915 nm (GaAlAs)	5 J/cm^2 10 J/cm^2 15 J/cm^2	6 W ± 20%	48, 96, 144 s	Wound closure occurred faster (after 72 h) in groups treated with 5 J/cm^2 and 10 J/cm^2 and after 96 h in the 15 J/cm^2 as compared to the control. PBM did not influence cell viability for each experimental period. PBM increased COL1A1 gene expression and decreased TGF-β1 expression (5 and 15 J/cm^2).	Tschon et al. (2015) [39]
Cartilage							
Chondrocytes	in vivo (Wistar rats)	808 nm (GaAIAs)	50 J/cm^2	50 mW	28 s	Decreased caspase-3 expression in groups treated with irradiation coupled with exercise. Decreased IL-β and MMP-13 expression in groups receiving irradiation, exercise or both.	Assis et al. (2016) [56]
Chondrocytes	in vivo (Wistar rats)	808 nm (GaAIAs)	50 J/cm^2	50 mW	28 s	IL-10 and COL-2 expression increased in response to aerobic and aquatic exercise, with and without PBM intervention. Aerobic exercise with and without PBM stimulated TGF-β expression.	Assis et al. (2018) [58]
Chondrocytes	in vivo (Wistar rats)	850 nm (GaAIAs)	57.14 J/cm^2	100 mW/ 1.43 W/cm^2	40 s per site	PBM stimulated cartilage regeneration.	Balbinot et al. (2021) [48]
Chondrocytes	in vivo (Wistar rats)	808 nm (GaAIAs)	50 J/cm^2	50 mW	28 s	Aquatic exercise, with or without PBM, resulted in better tissue organization as well as improved chondrocyte organization along the articular surface. Aquatic exercise coupled with PBM decreased MMP-13 expression.	Milares et al. (2016) [57]

Table 1. *Cont.*

Cells of Interest	Study Design	Laser Parameters				Main Outcome/s	Reference
		Wavelength	Fluency	Power Output or Power Density	Irradiation Time		
Chondrocytes	in vitro	910 nm (GaAs)	8 J/cm^2	300 mW	256 s	PBM decreased inflammatory cytokine expression (IL1β and IL-6) and NF-κB in IL1β-treated chondrocytes.	Sakata et al. (2022) [60]
Chondrocytes	in vitro in vivo (Wistar rats)	808 nm	28 J/cm^2 (in vitro only) 50 J/cm^2	50 mW	16 s (in vitro only) 28 s	PBM at a higher energy dose stimulated chondrocyte proliferation (in vitro). Decreased IL-1β expression in PBM groups after 4 and 8 weeks. Greater IL-10, COL-2 and IL-4 expression in PBM group after 8 weeks of treatment. Increased gene expression in TGF-β, COL-2, aggrecan after 4 weeks of PBM treatment (in vivo).	Tim et al. (2022) [59]
Chondrocytes	in vivo (Wistar rats	850 nm (GaAIAs)	Not given	200 mW/ 0.4 mW/cm^2	30 s	Groups treated with PBM showed enhanced COL-2 and TGFβ expression as compared to control.	Trevisan et al. (2020) [47]
Tendon							
Tenocytes	in vitro	630 nm (small probe) 625 nm (large probe) 850 nm (large probe)	4 J/cm^2	4150 mW (small probe) 1200 mW (large probe)	18 min	PBM alone did not change cell viability; however, PBM increased viability of cells grown in a platelet-rich plasma culture medium. LED application increased the closure of the wound gap.	Alzyoud et al. (2019) [74]
Tenocytes	in vivo (Wistar rats)	660 nm	4 J/cm^2	10 mW/ 250 mW/cm^2	16 s	PBM and exercise increased COL-1 immunoreactivity and resulted provided better cellular alignment. MMP3 and MMP13 expression was reduced in the PBM groups.	de Oliveira et al. (2019) [73]
Tenocytes	in vivo (Wistar rats)	630 ± 20nm	9 J/cm^2	300 mW/ 0.3 W/cm^2	30 s	LED increased HSP70 expression and collagen production	Evangelista et al. (2021) [70]

Table 1. *Cont.*

Cells of Interest	Study Design	Laser Parameters				Main Outcome/s	Reference
		Wavelength	Fluency	Power Output or Power Density	Irradiation Time		
Tenocytes	in vivo (Wistar rats)	660 nm	6 J/cm^2	0.04 W/ 1 W/cm^2	5.70 s	Heterologous fibrin polymer and PBM, either alone or coupled together, were successful at decreasing edema. After 7 days, the PBM group showed greater tendon injury, which reduced after 14 and 21 days. No differences in collagen quantification were found in treated and control groups over the 3-week period.	de Freitas Dutra Júnior et al. (2022) [71]
Tenocytes	in vitro	660 nm	1 J/cm^2 1.5 J/cm^2 2 J/cm^2	50 mW	5.2 min 7.8 min 10.4 min	PBM stimulated cell migration and wound closure. Dynamin-2 expression up-regulated in groups exposed to PBM. Dynasore treatment reduced cell migration in the 2 J/cm^2 irradiated group	Tsai et al. (2012) [66]

6. Conclusions

PBM (lasers and LEDs) has an effect on the cells of connective tissue and these effects may enhance repair processes in injured bone, cartilage and tendon. In bone, PBM at specific wavelengths and energy densities stimulates osteoblast proliferation, viability and migration. PBM also enhances tissue organization in bone. Furthermore, PBM promotes bone remodeling, a crucial final step in the repair of injured bone. PBM stimulates the differentiation of stem cells into cells with chondrogenic capabilities and in cartilage, irradiation prevents cartilage degradation and leads to better tissue organization. Tenocyte proliferation and migration increases in response to irradiation. While it appears that PBM at various wavelengths and fluencies may positively influence repair, it is challenging to select the correct parameters that will ideally stimulate tissue repair without any negative consequences, and laser parameters applied to in vitro models are not necessarily the same parameters that should be used in vivo.

Author Contributions: Conceptualization, A.S.-K.; writing—original draft preparation, A.S.-K.; writing—review and editing, N.N.H.; All authors have read and agreed to the published version of the manuscript.

Funding: South African Research Chairs Initiative of the Department of Science and Technology and National Research Foundation of South Africa [Grant No 98337], as well as grants received from the University of Johannesburg (URC), the National Research Foundation (NRF) [129327], and the Council for Scientific and Industrial Research (CSIR)-National Laser Centre (NLC) Laser Rental Pool Programme.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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