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REVIEW

Targeted Therapy for Skeletal Muscle Fibrosis: Regulation of Myostatin, TGF-β, MMP, and TIMP to Maintain Extracellular Matrix Homeostasis

Nurrani Mustika Dewi[1,2], Anna Meiliana[2,3], Irma Ruslina Defi[4], Riezki Amalia[3,5], Cynthia Retna Sartika[6], Andi Wijaya[3,6], Melisa Intan Barliana[1,5]

[1]Department of Biological Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Jatinangor, Indonesia; [2]PT Prodia Widyahusada, Jakarta, Indonesia; [3]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Jatinangor, Indonesia; [4]Department of Physical Medicine and Rehabilitation, Hasan Sadikin General Hospital/Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia; [5]Center of Excellence for Pharmaceutical Care Innovation, Universitas Padjadjaran, Jatinangor, Indonesia; [6]PT Prodia StemCell Indonesia, Jakarta, Indonesia

Correspondence: Melisa Intan Barliana, Department of Biological Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Jl. Ir. Soekarno KM 21, Jatinangor, 45363, Indonesia, Tel +6281214451177, Email melisa.barliana@unpad.ac.id

Abstract: Muscle fibrosis, defined by the excessive deposition of extracellular matrix (ECM) components, is a key pathological process that hinders muscle regeneration following injury. Despite muscle's inherent regenerative potential, severe or chronic injuries often result in fibrosis, which compromises muscle function and impedes healing. This review explores a range of therapeutic strategies aimed at modulating the molecular pathways involved in muscle fibrosis, with a focus on the inhibition of myostatin and transforming growth factor-β (TGF-β), as well as the regulation of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs). Some therapy modalities, including physiotherapy and exercise therapy, which are commonly used, have demonstrated the ability to regulate extracellular matrix (ECM) components and promote muscle repair. In addition, the use of TGF-β inhibitors, herbal plants, and other biochemically relevant compounds, holds promise in controlling fibrosis by targeting key signaling pathways that drive ECM accumulation as well as having anti-fibrotic and anti-inflammatory properties. Regenerative medicine, including therapies using stem cell, secretome, and platelet-rich plasma (PRP), have also been used as single or adjuvant treatment for muscle fibrosis, and represents a novel and minimally invasive approach. Although these therapeutic strategies show considerable promise, translating preclinical findings to clinical practice remains challenging owing to variability in patient responses and the complexity of human muscle injuries. In conclusion, a multifaceted approach targeting ECM regulation, either as single treatment or combined treatment, offers a promising avenue for the treatment of muscle fibrosis.

Keywords: muscle fibrosis, extracellular matrix, myostatin, TGF-β, MMP, TIMP

Introduction

Skeletal muscle is one of the most important components in the human body as it plays vital roles in maintaining an upright posture, as well as producing movement and mobility.[1] Any decline in skeletal muscle function will significantly affect a person's health and quality of life.[2] Skeletal muscle injuries are common occurrences that may result from acute trauma or chronic overuse. Acute muscle injuries mostly occur as a result of trauma during sports, exercise, or other physical activities, whereas chronic muscle injuries may develop gradually over time owing to repetitive strain or overuse, as well as a continuous sedentary lifestyle.[3] In sports alone, the incidence of skeletal muscle injuries represents around 10–55% of all sustained injuries.[4]

Even though skeletal muscles have an impressive ability to regenerate and heal, severe injuries can sometimes result in complications such as muscle fibrosis.[5] Muscle fibrosis, which is marked by the excessive accumulation of scar tissue, can impair muscle function and hinder recovery, resulting in a decline in skeletal muscle function.[5,6] In response to muscle injury, the formation of normal extracellular matrix (ECM) occurs during the injury recovery process. However,

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when fibrosis occurs, there is excessive and uncontrolled production of ECM components around the injured tissue that often results in abnormal ECM deposition.[7,8]

The development and progression of muscle fibrosis are mainly related to ECM. In normal skeletal muscle, ECM provides structural integrity, facilitates cell-to-cell communication, and regulates various cellular processes, such as proliferation, differentiation, and tissue remodeling. However, some dysregulation of ECM homeostasis may occur and result in the development of fibrosis.[9] The accumulation of ECM deposition can be reduced by inhibiting ECM synthesis or enhancing ECM degradation through regulation of myostatin, transforming growth factor-β (TGF-β), matrix metalloproteinase (MMP), and tissue inhibitors of metalloproteinases (TIMPs).[6,7] Therefore, targeting ECM-related pathways and regulating the fibrotic response may be promising therapeutic strategies for managing the development of muscle fibrosis.[10] Approaches that promote tissue regeneration and restore ECM homeostasis, such as stem cell therapy or growth factor delivery, also have great potential for attenuating fibrosis and improving skeletal muscle function.[11] In this review article, several modalities for the targeted therapy of skeletal muscle fibrosis through the regulation of ECM homeostasis will be discussed.

Accumulation of ECM Components in Muscle Fibrosis

ECM is a complex network of organized multidomain macromolecules including proteins, glycoproteins, and polysaccharides which surrounds and supports the cells within tissues.[12] ECM is present in the muscle niche, and plays a vital role in the maintenance of homeostasis and the regulation of skeletal muscle development and regeneration.[13] There are two major compartments of muscle ECM, namely the basal lamina and the interstitial matrix. The basal lamina is mainly composed of type 4 collagen, laminin, and heparan sulfate proteoglycans; meanwhile, the interstitial matrix is mainly composed of collagen types 1, 3, and 5, fibronectin, and perlecan.[14] The destruction or removal of the basal lamina as well as other structural muscle components also hinders the regeneration of muscle loss,[15] thus prolonging the healing process of muscle injuries.

The pathogenesis of muscle fibrosis involves the process of differentiation of dormant satellite cells, myoblasts, and fibroblasts into myofibroblasts. All of these cells migrate to the injured muscle area, form connective tissue, and repair the wound in the injured muscle. In the normal injury recovery process, remaining myofibroblasts are cleared through the process of apoptosis. Acute injury to healthy muscle causes rapid and controlled inflammation, which promotes the activation of satellite cells that speed up the turnover process of the injured muscle.[14,16] During the recovery process, neutrophils are recruited to the injured location to eat the damaged cells and induce regeneration. The recruited neutrophils secrete chemoattractant cytokines, facilitating the infiltration of monocytes and macrophages. There are two heterogeneous phenotypes of macrophages that have roles in muscle fibrosis, namely proinflammatory macrophages (M1) and anti-inflammatory macrophages (M2). Any disturbance of the balance between the activation of these two phenotypes of macrophages will increase the expression of TGF-β1, which is known to regulate ECM deposition.[5] The anti-inflammatory macrophages, which are promoted by T-helper 2 cells, are important in the healing process and in controlling inflammation due to the proinflammatory macrophages. Therefore, unregulated anti-inflammatory macrophages may induce fibrosis.[17] If the injury recovery process takes a long time and is followed by a prolonged inflammatory process, this will result in excessive and uncontrolled accumulation of ECM, which can inhibit repair by myogenesis. The process of myogenesis regeneration will lead to the formation of scar tissue and then reduce muscle function, so that the muscles become weak.[14,16]

The major constituents of these fibrotic lesions are usually the interstitial collagens, such as type 1 and 3 collagens.[14] Interstitial collagen within the skeletal muscle ECM represents the primary load-bearing structural protein.[18] Collagens constitute 30% of the total proteins of an organism, and are the main structural components of ECM.[19] In the condition of fibrosis, increased collagen expression and deposition take place in ECM, especially of collagen 1, which can reach levels of more than 100-fold higher than under normal conditions.[20] The formation of a collagen-rich scar, which usually occurs in fibrosis, is regulated by myofibroblasts and their response as the agents of wound closure. Myofibroblasts are also responsible for the synthesis and secretion of specific ECM components, such as fibronectin.[21]

ECM is not only a pathological feature of fibrosis, but also an aggravating factor of fibrosis by the facilitation of ECM accumulation. When various components of ECM accumulate, they can exacerbate the initial fibrotic state. The

deposition of ECM generates a positive feedback loop in fibrotic conditions because the mechanisms driving fibrosis involve increased stiffness of the matrix due to ECM accumulation, resulting in an overabundance of ECM in the tissue microenvironment.[8] A fibrotic environment induces fibroadipogenic progenitors (FAPs), which comprise a mesenchymal-like cell population within skeletal muscle, to differentiate into myofibroblasts. These myofibroblasts increase ECM deposition, which leads to muscle tissue stiffness and the fibrotic condition.[22] The increased activation of FAPs into myofibroblasts is negatively associated with muscle satellite cell differentiation, which delays the recovery process. Since FAPs reside in the interstitial space, which directly interacts with the interstitial collagens, any changes in collagen cross-linking or accumulation will affect the activation of FAPs.[22,23]

As fibrosis develops, lysyl oxidase (LOX) facilitates the conversion of lysine residues in collagen and elastin into aldehydes, which later react to form cross-links between collagen fibers. This process increases matrix stiffness and hinders ECM degradation. In addition, the heightened mechanical stress can stimulate further ECM production by activating downstream pathways, including TGF-β1 and Wnt-β-catenin, as well as promoting epithelial–mesenchymal transition.[8] ECM can preserve the activation of ECM-secreting cells, including myofibroblasts, which will eventually accelerate the process of fibrosis.[24]

Myostatin, TGF-β, MMP, and TIMP Regulate ECM Homeostasis

ECM homeostasis is the dynamic and regulated process of maintaining the balance between the synthesis and degradation of ECM components to preserve tissue structure and function. Any alteration in the balance among different ECM components will lead to altered tissue architecture. The deposition of ECM components is mainly affected by factors such as cytokines, proteases, and protease inhibitors.

ECM proteins such as fibromodulin, decorin, fibronectin, and laminins, bind and modulate the function of myostatin; while other ECM proteins, including fibromodulin, matrix gla protein, and dermatopontin, play important roles in the regulation of myogenesis.[25,26] Myostatin inhibits myogenesis and, thereby, regulates the proliferation and differentiation of muscle satellite cells.[27] Myostatin, a member of the TGF-β superfamily, regulates skeletal muscle growth and development in an opposing manner through autocrine and paracrine signaling pathways.[28] The characteristics of myostatin protein are similar to those of other TGF-β family members, giving it the same importance as TGF-β in the accumulation of ECM components.

TGF-β1 has a role as a multifunctional cytokine, which assists the regulation of muscle repair by activating muscle satellite cells.[29] TGF-β1, which is released by anti-inflammatory macrophages, is recognized as an important factor in the development of fibrosis.[17] Elevated TGF-β1 has been found after acute muscle injury, which resulted in increased scar tissue formation in the injured muscle. The overexpression of TGF-β1 stimulates fibrous scar tissue formation via receptor-mediated Smad signaling pathways, which results in a decline in muscle function.[6] Upon activation of the Smad signaling pathways, TGF-β1 binds to its receptors, leading to the phosphorylation of Smad2 and Smad3. These phosphorylated Smads form a complex with Smad4, which translocates to the nucleus to regulate the transcription of profibrotic genes.[30] Targeting the TGF-β1/Smad signaling pathway offers potential therapeutic benefits for treating fibrosis. Inhibitors of TGF-β1 or its downstream signaling components, such as Smad2 and Smad3, have shown promise in reducing fibrosis in various models, including muscle fibrosis due to injury.

The formation and degradation of ECM are also regulated by several enzymes, such as specific proteases and inhibitors that are expressed during tissue repair.[31,32] Of these important enzymes, the MMP and TIMP families have been extensively studied with regard to their relationship with ECM degradation. MMP family members, such as MMP-9, have been reported to help the ECM degradation process, specifically the collagen 4 component. Meanwhile, TIMP-1 has the opposite role, which is to inhibit the function of MMP-9 in degrading ECM components.[6,7,32] When a muscle injury occurs, an increase in the levels of MMP-9 and TIMP-1 will occur.[33] Since MMP-9 promotes tissue degradation and TIMP-1 inhibits the substrate-degrading activity of MMP-9, maintaining a proper balance between these enzymes is essential for facilitating muscle regeneration and managing fibrous development. However, any imbalance or excessive activity of these enzymes can result in fibrosis.[6] The excessive activation of MMP will also increase tissue degradation, hindering myogenesis. Therefore, the balance between MMPs and TIMPs should be well managed to optimize the post-damage ECM remodeling.[32]

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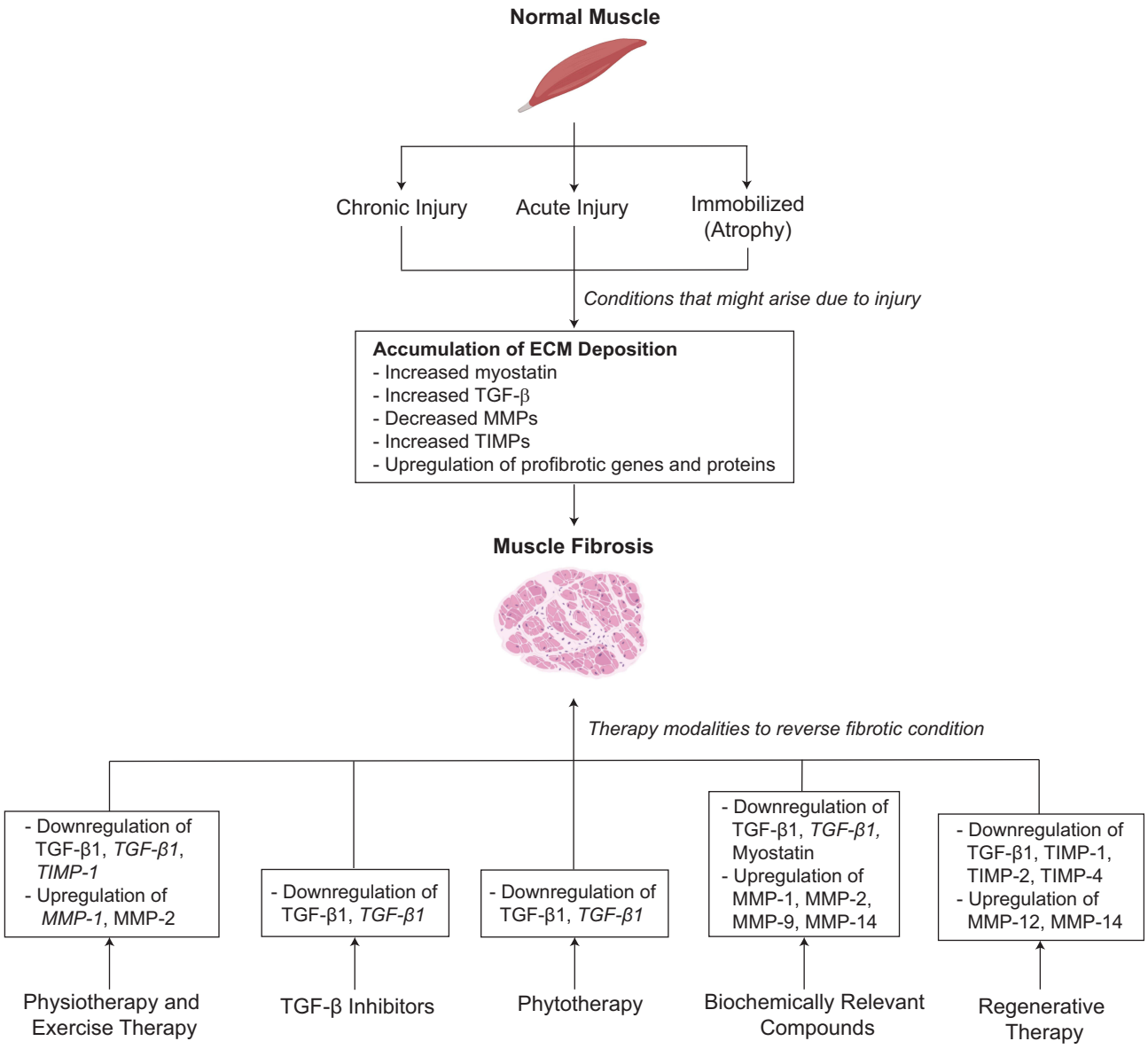

Figure 1 Factors that may cause muscle fibrosis and various modalities of therapy to reverse the condition.

Therapy Targeting the Reversal of Muscle Fibrosis

Knowing the importance of myostatin, TGF-β, MMPs, and TIMPs in fibrosis formation, especially in muscle fibrosis, in this review, various modalities of therapy targeting these four factors or directly related to these factors will be discussed. To stay focused, only primary studies with muscle fibrosis models due to injury (both acute and chronic injury) and immobilization are included in this review. Figure 1 summarizes the mechanism of each modality in modulating myostatin, TGF-β, MMPs, and TIMPs in muscle fibrosis formation.

Physiotherapy and Exercise

Physiotherapy is known to have a role in minimizing fibrotic changes due to skeletal muscle injury. Some of the physiotherapy modalities that have been reported to have an effect on ECM homeostasis by regulating TGF-β, MMPs, and TIMPs are electroacupuncture,[34–38] electrical stimulation,[39] twitch contraction,[40,41] low-level laser therapy (LLLT),[42–44] and shock-wave and ultrashort-wave diathermy.[45]

In acute rat models of tibial anterior muscle injury, electroacupuncture treatment was shown to alleviate skeletal muscle inflammation and fibrosis by reducing interleukin (IL)-6 and tumor necrosis factor (TNF)-α, as well as reducing collagen 2 deposition, which became increasingly apparent with prolonged intervention time. The electroacupuncture-treated rats were also shown to have decreased expression of TGF-β1, and the activation of Smad3 and p38 was inhibited compared to the injury model rats. In addition, this treatment also elevated extracellular signal-regulated kinase (ERK) 1/2 in rat models, suggesting that the involved mechanism was correlated with TGF-β1/Smad3/p38/ERK1/2 signaling.[34] In another acute injury model caused by contusion, in rats, electroacupuncture was able to reduce the cytokine level of interferon (IFN)-γ, but increased IL-4, IL-3, and IFN-α. These cytokines are assumed to induce macrophage polarization during the fibrosis recovery process in this contused skeletal muscle injury model. Electroacupuncture was also able to increase the expression of MyoD in muscle tissues.[35] Similarly to the previous study, electroacupuncture therapy in acute models of lumbar multifidus muscle injury in rats showed notable downregulation of the expression levels of collagen, especially collagen 1, while the expression of MMP-2, MyoD, and Pax7 proteins was obviously upregulated in the electroacupuncture-treated rats compared with the acute injury rat model.[36] Electroacupuncture was able to upregulate not only MMPs on the protein level, but also the mRNA expression of *MMP-1*. Knowing the opposite role of MMPs and TIMPs, in this acute rat model of blunt trauma, the mRNA expression of *TIMP-1* was downregulated. This study also showed that electroacupuncture lowers mRNA levels of *TGF-β1* and connective tissue growth factor (*CTGF*).[37] As a downstream mediator of TGF-β1, CTGF also plays a significant role in the development of fibrosis by stimulating the production of ECM proteins. Therefore, it was suggested that electroacupuncture is potentially effective in reducing fibrosis after acute injury by promoting the transformation of proinflammatory macrophages into anti-inflammatory macrophages, therefore reducing some proinflammatory cytokines, such as IL-6, TGF-β1, and IFN-γ, as well as increasing the anti-inflammatory cytokines, such as IL-4 and IL-13.[34,35] Meanwhile, its role in ECM homeostasis is to upregulate MMP-2, which degrades the ECM components, upregulating *MMP-1* mRNA expression, downregulating *TIMP-1* mRNA expression, as well as reducing the TGF-β1 and collagen levels which are usually associated with ECM accumulation.[34,36,37] Electroacupuncture also promotes satellite cell activation and muscle regeneration by increasing MyoD expression.[35,36]

Aiming to enhance the results of electroacupuncture treatment, a study of acute rat models also suggests that the combination with massage could produce more effective results in reducing skeletal muscle fibrosis caused by blunt trauma in rats compared to either treatment used alone. This combination not only reduces fibrosis development by downregulating the mRNA expression and protein expression of *TGF-β1* and *CTGF*, but also regulates the MMP-1/TIMP-1 balance (by downregulating *TIMP-1* and upregulating *MMP-1*) that is associated with ECM production.[37] Furthermore, in a rat chronic injury model of skeletal muscle fibrosis due to 3 weeks of eccentric exercise, following electroacupuncture treatment, expression levels of collagen 1, TGF-β1, and CTGF were reversed after having been initially elevated after the eccentric exercise. Meanwhile, the ratio of phosphorylated-ERK/ERK continued to decrease beyond the initial value. Since phosphorylated-ERK is also involved in ECM formation, and especially in cell migration, this suggests that acupuncture can inhibit the formation of skeletal muscle fibrosis by downregulating the TGF-β1/ERK/CTGF signaling pathway.[38] From these findings, it was suggested that electroacupuncture is effective not only in reducing muscle fibrosis due to acute muscle injury, but also in chronic muscle injury, by lowering the expression of TGF-β1 and its downstream cytokines, as well as lowering the level of collagen 1, which is one of the main components of ECM formation.

Different types of electrical stimulation have been performed in immobilized rats. Belt electrode–skeletal muscle electrical stimulation (B-SES) prevented muscle fibrosis due to immobilization, with lower mRNA expression of *IL-1β*, *TGF-β1*, alpha-smooth muscle actin (*α-SMA*), and monocyte chemoattractant protein 1 (*MCP-1*) in the B-SES group than in the immobilization group. The number of macrophages was elevated after the rats were immobilized, indicating that this macrophage accumulation occurred as a result of the increase in proinflammatory macrophages. However, this increase in macrophage number was able to be reversed after B-SES intervention.[39] This showed that B-SES treatment prevented muscle fibrosis via the inhibition of inflammation. Still in the immobilized rats model, electrical-based twitch stimulation contributed toward the inhibition of skeletal muscle fibrosis by enervating the upregulation of TGF-β1 and by circumvention of the hypoxic condition. Collagens 1 and 2, as well as mRNA expression of *collagen 1* and *collagen 3*,

were also significantly decreased compared to the immobilized rats.[40] Magnetic-based twitch stimulation was able to decrease the elevated hydroxyproline content due to immobilization. It was also able to reduce fibrosis-related genes, including hypoxia-inducible factor-1α (*HIF-1α)*, *TGF-β1*, *α-SMA*, *collagen 1*, and *collagen 3*.[41] Since hydroxyproline also plays a crucial role in the formation of ECM by stabilizing the collagen triple-helix structure, it was assumed that twitch stimulation could also prevent ECM accumulation by downregulating or degrading the component that induced ECM accumulation, therefore preventing muscle fibrosis.

LLLT has demonstrated positive effects in modulating the inflammatory response, especially in relation to muscle regeneration following an injury. In rat models of acute injury, LLLT was shown to reduce collagen 1 expression and mRNA expression of *TGF-β1*, as well as to increase MMP-2 expression,[42,43] suggesting that LLLT has a role in maintaining ECM homeostasis. However, another study showed the opposite results regarding the collagen level, with the collagen intensity instead increasing after treatment with LLLT following acute injury.[44] Therefore, it is necessary to study further whether LLLT can reduce the collagen level or, instead, raise it. LLLT also reduced the inflammation after acute injury, which was shown by the decreased number of inflammatory cells.[43] With regard to muscle regeneration, LLLT was shown to be able to increase the mRNA expression of *MyoD* and *myogenin*, as well as improving the blood vessels, as examined through its vascular endothelial growth factor (VEGF) levels.[42,43]

One type of physiotherapy that is commonly performed for patients with muscle injury is the combination of radial extracorporeal shock-wave therapy (ESWT) and ultrashort-wave diathermy (USWD). A study reported that this combined treatment was more effective than radial ESWT or USWD alone against muscle fibrosis since it downregulates overexpression of TGF-β1 and HIF-1α.[45] In addition to fibrosis treatment, ESWT has been demonstrated to accelerate the regeneration of skeletal muscle tissue following acute injuries. It enhances the proliferation and differentiation of satellite cells, by enhancing Pax7-positive satellite cells, as well as increasing the expression of MyoD and myogenin, which are crucial for muscle repair, thereby improving muscle healing processes. This therapy is particularly relevant for acute muscle injury, especially sports-related muscle injuries, as it significantly increases the size and myonuclear content of regenerating muscle fibers.[46] USWD has a role in reducing inflammation, which is shown by the decreased levels of IL-60, TNF-α, and heat shock protein 70 (HSP70). The USWD in this study was performed before and after exercise, showing its ability to prevent the prolonged inflammation that might lead to muscle fibrosis.[47] Furthermore, the combination of USWD and stretching exercises was shown to be able to reduce fibrosis by decreasing collagen deposition and the mRNA expression of *TGF-β1* compared to the group that received USWD or stretching alone. This study showed that after the combination therapy, the group that received stretching exercises achieved better results in terms of reduced collagen levels and decreased *TGF-β1* mRNA expression compared to the group that received USWD treatment only.[48] This shows that stretching exercises alone were also effective in reducing possible ECM accumulation. Stretching exercises were reported to be able to reduce fibrosis not only by reducing the component of ECM formation, but also by reducing fibrosis through the tissue characteristic evaluation according to the Nakagami parametric index (NPI). They could also reduce the cytokines that are associated with muscle regeneration, MyoD, and myogenin.[49] To summarize, ESWT and USWD can be used to improve the fibrosis condition by reducing prolonged inflammation processes and enhancing muscle regeneration, but better results might be obtained when these treatments are combined with stretching.

Usually, along with physiotherapy procedures, the physiatrist will ask their patients to carry out some exercises at home. These exercises usually differ based on the condition of each patient. However, not many studies have directly assessed the effectiveness of exercise in improving skeletal muscle fibrosis in injury or immobilized models. Treadmill exercises have been performed in rat models of acute muscle injury, and the results show that such exercises can prevent muscle fibrosis by reducing the accumulation of FAPs in muscle injury. The reduced FAP accumulation occurs because exercise induces senescence in FAPs, leading to their clearance by macrophages.[50,51] Regarding the cytokines that are often associated with the regulation of FAP, TGF-β1 and TNF-α were found to be slightly decreased.[51] Similarly to the effects of electropuncture, treadmill exercises also increased the proportions of $MyoD^{+}Pax7^{+}$ muscle stem cells (MuSCs) and the relative number of MuSCs compared to the group that did not perform any exercises,[51] suggesting that treadmill exercise not only exacerbates muscle fibrosis by reducing the accumulation of FAPs, but also facilitates muscle regeneration.

TGF-β Inhibitors

Various TGF-β inhibitors, such as P144,[52] SB-505124,[53] or SB431542,[54] have also been used to ameliorate muscle fibrosis resulting from different muscle injuries. The inhibition of TGF-β using intravenous injection of synthetic peptide P144 (Disetertide) in rabbit models of acute injury due to radiation resulted in reduced ECM fibrosis, with a lower area of collagen deposition. P144 treatment also led to a significant depletion of Smad2/3 phosphorylation, which is the intracellular activation pathway of cells in response to TGF-β biological activity, compared with the placebo group.[52] The reduced level of Smad2/3 phosphorylation showed the downstream effect of TGF-β1 inactivation, which will help to prevent the overdeposition of ECM. In other types of fibrosis, P144 was able to prevent fibrosis through the inhibition of TGF-β1 and Smad2/3 signaling.[55,56]

In a similar study, applying therapy using another TGF-β inhibitor, SB-505124, in irradiated mice, lower fibrosis per slide was observed after Masson's trichrome staining compared to the radiation-only group.[53] Moreover, the inhibition of TGF-β using SB431542 in another acute muscle injury, due to rotator cuff tears, reduced the fibrotic tissue, as assessed by Masson trichrome staining. SB431542 also downregulated the adipogenic transcription factors sterol regulatory element-binding protein (SREBP)-1 and peroxisome proliferator-activated receptor (PPAR)-γ. Therapy with SB431542 depleted the number of FAP cells, an initiator of rotator cuff muscle fibrosis, by promoting the apoptosis of FAPs. The expression of the TGF-β1 target gene Plasminogen Activator Inhibitor-1 (*PAI-1)*, which is strongly linked to muscle fibrosis, as well as that of the profibrogenic gene *α-SMA*, were also decreased in the group receiving SB431542.[54] This shows that TGF-β inhibitors work by inhibiting TGF-β1 expression to prevent further accumulation on ECM formation.

Losartan, an angiotensin II type 1 receptor blocker, has been studied for its potential role in inhibiting TGF-β signaling, which is crucial in muscle fibrosis and various muscle disorders. The results showed that the use of losartan (as well as its combination with exercise) could improve muscle regeneration and decrease fibrosis.[57] In another model of acute muscle injury, losartan (and its combination with adipose tissue-derived stem cells [ASCs]) was effective in ameliorating muscle fibrosis after the evaluation of Masson's trichrome staining. In terms of muscle regeneration, the administration of losartan elevated the mRNA expression levels of *MyoD* and *myogenin*, MyoD protein, and Pax7 (although the elevation of Pax7 following treatment with losartan only was no better than with a combination of losartan and ASCs).[58] Myogenic satellite cell differentiation and muscle fibrosis are interconnected processes that play crucial roles in muscle regeneration. Injured muscle often exhibits myogenic cells differentiating into myofibroblasts, which later contribute toward fibrosis.[59] Hence, treatment with losartan and any other combination therapy can regulate the differentiation of myogenic satellite cells and inhibit the TGF-β signaling pathway, thus reducing muscle fibrosis.

Phytotherapy

Phytotherapy, the use of plant-based treatments, is being explored as a potential therapy for muscle fibrosis owing to the limitations and side effects that may arise with the use of conventional pharmacological treatments. In an acute injury model due to irradiation, sulforaphane, a compound found in cruciferous vegetables, has been reported to reduce collagen fiber production and the proportion of collagen fibers, which were initially elevated as the result of irradiation. The expression of collagen 1 and CTGF was decreased, suggesting that sulforaphane can inhibit the overexpression of ECM components.[60] The transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) is known for its role in regulating antioxidant responses and has been shown to exert antifibrotic effects by modulating the TGF-β/Smad signaling pathway. Activation of Nrf2 can inhibit the TGF-β/Smad pathway, thereby reducing fibrosis.[61] Sulforaphane is reported to have a significant role as an antioxidant, by activating Nrf2 and its downstream antioxidant genes. Sulforaphane intervention also downregulates the expression of TGF-β1 and phosphorylated Smad2/3 expression, confirming its role in the inhibition of TGF-β1/Smad pathway activation.[60] Therefore, a sulforaphane-rich diet, including food such as broccoli and cabbage,[62] may be a potential treatment modality for muscle fibrosis, through the activation of Nrf2 and the inhibition of TGF-β/Smad signaling, thus inhibiting muscle fibrosis.

Geniposide, a bioactive constituent of gardenia fruit pomace, was also reported to be beneficial for skeletal muscle, thus suggesting a potential therapy to ameliorate skeletal muscle fibrosis. Decreased protein expression of α-SMA, vimentin, and collagen 1, as well as reduced mRNA expression of *α-SMA*, *Vim*, and *collagen 1*, in response to geniposide treatment were found. The decrease in mRNA expression was shown to be dose dependent. Geniposide also significantly

decreased the expression of profibrotic genes in vitro, and reversed profibrotic gene expression induced by TGF-β and Smad4. In addition, in vivo, treatment with geniposide was shown to reduce the profibrotic gene expression and enhance recovery from skeletal muscle injury in a mouse contusion model. Therefore, it can be inferred that geniposide treatment inhibits skeletal muscle fibrosis in acute injury through modulation of the TGF-β/Smad4 signaling pathway.[63]

Highland Barley tea, which is a nutritious drink that is rich with polyphenols, has been shown to have some beneficial effects in alleviating muscle fibrosis and muscle damage.[64,65] An acute injury model of skeletal muscle senescence and fibrosis was used as an intervention to reduce oxidative stress, inflammation, and fibrosis in muscle. A study found that Highland Barley tea increased levels of Sirtuin 3 (SIRT3) protein, which further inhibited the expression of fibrotic markers, collagen 1, and α-SMA.[64] The effect of Highland Barley tea in reducing muscle fibrosis may be due to the ability of SIRT3 to modulate oxidative stress and mitochondrial function. It enhances mitochondrial respiration and reduces oxidative stress, which is critical in preventing fibrotic changes.

In another study, an antioxidant supplement, astaxanthin, which is a pinkish-orange pigment (carotenoid) naturally found in many marine organisms, such as crustaceans and fish, was shown to be able to attenuate the increased collagen fiber area, TGF-β1 expression, and α-SMA in immobilized rats.[66] Reactive oxidative species (ROS) production was measured by dihydroethidium (DHE) staining, and the results showed that immobilized rats that were given astaxanthin had equivalent results to the control rats that were given astaxanthin,[66] suggesting that astaxanthin was able to restore the ROS level before injury occurred. Similarly to other fibrotic conditions, astaxanthin exerts its antifibrotic effects primarily through its antioxidant properties. By decreasing ROS, astaxanthin helps to prevent the activation of profibrotic pathways, such as those mediated by TGF-β1 or nuclear factor-kappa B (NF-κB).[67,68] Summarizing this section, it can be assumed that the use of phytotherapy to alleviate muscle fibrosis was mainly effective through the inhibition of TGF-β1/Smad pathway activation or through its antioxidant properties.

Biochemically Relevant Compounds

Lysyl oxidase-like 2 (LOXL2) is an enzyme involved in the cross-linking of ECM proteins, such as collagen and elastin, which contribute to the development of fibrosis in various tissues. LOXL2 plays a critical role in fibrosis by facilitating collagen cross-linking, which leads to tissue stiffening and impaired function. Therapy targeting the inhibition of LOXL2 has been explored as a strategy to manage fibrosis in various tissues, including muscle.[69,70] A chemical compound, [2-chloropyridin-4-yl]methanamine hydrochloride, which is known to be a LOXL2 inhibitor, was also investigated regarding its involvement in the development of skeletal muscle fibrosis. In an in vitro experiment on fibroblasts induced by D-galactose, treatment with LOXL2 inhibitor was able to alleviate fibrosis through the inhibition of the TGF-β1/p38 mitogen-activated protein kinase (MAPK) pathway. The intensity of the ECM formation component collagen 1 was also decreased with the increased concentration of LOXL2 inhibitors. Intervention with [2-chloropyridin-4-yl]methanamine hydrochloride decreased senescence-associated β-galactosidase (SA-β-gal)-positive cells and the expression of senescence-related proteins, p53 and P16INK4a, which are known cellular senescence markers.[71] LOLX2 inhibitor has the ability to reduce skeletal muscle fibrosis not only through the inhibition of TGF-β1 signaling and the promotion of cell senescence, but also through its antioxidant properties by reducing the activities of xanthine oxidase (XOD) and nitric oxide synthase (NOS) and suppressing mitochondrial ROS production. Meanwhile in vivo, mice with fibrosis induced by D-galactose showed reduced skeletal muscle fibrosis through reduced collagen deposition after treatment with LOXL2 inhibitor. Although not statistically significant, decreases in TGF-β1, α-SMA, collagen 1, and collagen 3 were also observed after treatment with LOXL2 inhibitor.[71] These results suggest that LOXL2 inhibitor also inhibits fibrosis in muscle in acute injury mouse models via the inhibition of ECM formation components.

In rats, supplementation with one of the essential branched-chain amino acids, leucine, decreased the amount of collagen and the activation of phosphorylated TGF-β receptor type I (TβR-I) and Smad2/3 during the regeneration of muscles. These results indicate that leucine supplementation expedites the connective tissue repair and muscle regeneration function through the attenuation of TβR-I and Smad2/3 activation. In terms of alleviating muscle fibrosis, leucine was shown to be able to reduce the hydroxyproline content after acute injury, reaching values similar to those in the control group, thus inhibiting the formation of ECM. With regard to muscle regeneration, animals treated with leucine also had fewer neonatal myosin heavy chain (MyHC-*n*)-positive regenerating myofibers than those in the untreated

group.[72] Increased MyHC expression is often correlated with reduced myostatin levels during muscle repair and hypertrophy, therefore worsening the regeneration process.[73]

Although considered a dangerous gas, therapy using hydrogen sulfide (H_2S) has been reported to be beneficial in reducing skeletal muscle injury in mice. H_2S treatment has been shown to attenuate skeletal muscle fibrosis and partly improve skeletal muscle injury. The attenuation of muscle fibrosis was mainly explained by the inhibition of inflammation, since H_2S was reported to reduce proinflammatory macrophages, such as *CD68*; reduce the mRNA expression of *TGF-β*, *TNF-α*, *IFN-γ*, *IL-1β*, and *IL-6* profibrotic and proinflammatory cytokines; reduce inflammatory chemokines, such as C-C motif ligand 2 (*CCL2*), C-C motif chemokine receptor 2 (*CCR2*), *CCL3*, *CCL5*, C-X-C motif chemokine (*CXCL12*), and C-X-C chemokine receptor type 4 (*CXCR4*); as well as increase anti-inflammatory macrophages, such as *CD206*. H_2S intervention also decreased the previously increased levels of of *MMP-1*, *MMP-2*, *MMP-9*, and *MMP-14* after muscle contusion.[74] This shows that H_2S also has the ability to enhance the degradation of ECM by decreasing MMP expression. H_2S also lowered the expression level of the oxidative stress factor *gp91phox*,[74] which confirmed the capacity of H_2S to reduce inflammation and oxidative stress, thus inhibiting fibrosis in various organs,[75] including skeletal muscle.

Regenerative Therapy

Regenerative medicine, encompassing stem cell therapy, secretome-based approaches, and platelet-rich plasma (PRP), has emerged as a promising strategy for treating muscle fibrosis following injury.[3] Most conventional treatments focus on symptom management, rather than addressing the underlying pathological changes. However, regenerative therapies aim to modulate the fibrotic environment, promote myogenic regeneration, and restore muscle function by leveraging the regenerative potential of stem cells, bioactive factors within the secretome, and the growth factors present in PRP.

The transplantation of umbilical cord-derived mesenchymal stem cells (UC-MSCs) has been reported to reduce TIMP-1, TIMP-2, and TIMP-4 after acute muscle ischemic injury. The combination of UC-MSCs and *miR-29a*, which is known as a significant regulator in the context of fibrosis in muscle tissues, was able to reduce only TIMP-1 and TIMP-2, but not TIMP-4. Transplantation of UC-MSCs could increase MMP-12 and MMP-14 after injury, but did not increase the expression of MMP-2, MMP-3, MMP-9, and MMP-15,[76] although their overexpression is important in the degradation of ECM. In terms of muscle regeneration, UC-MSCs were able to enhance the expression of VEGF-A and VEGF-C,[76] which play a role in muscle regeneration. VEGF-A is the key player in muscle regeneration, by promoting angiogenesis, while VEGF-C is necessary for proper healing, by supporting vessel formation.[77] UC-MSCs demonstrated anti-fibrotic properties by suppressing the levels of various ECM components, including fibronectin 1, and collagens 1A1, 3A1, 4A1, 4A6, 5A1, 6A1, and 9A1.[76] This shows that UC-MSCs have the ability to improve angiogenesis, inhibit ECM accumulation, and protect against fibrosis post-injury.

Stromal vascular fraction stem cells (SVFCs) are a type of stem cell derived from the stromal vascular fraction, which is a component of adipose tissue that contains a population of stem cells with the potential to differentiate into various cell types, often used in regenerative medicine therapies.[78,79] An intervention using SVFCs at two different concentrations showed that SVFCs decreased the hydroxyproline content and ECM accumulation in a dose-dependent manner, with the higher concentration achieving better results.[78] Although there has not been much research on the use of SVFCs for muscle fibrosis, SVFCs have been reported to reduce ECM accumulation by reducing collagen 3 expression in fibrotic condition of other organ.[80] Since there are several types of stem cell sources, the selection of an appropriate stem cell source is critical for successful therapy. Different sources may give different results in alleviating muscle fibrosis and enhancing muscle regeneration.

The MSC secretome is a substance produced by stem cells, and refers to the conditioned culture medium (CM) collected after incubation. The use of secretome as a therapeutic approach has gained significant attention owing to its allogeneic application potential. The secretome is also rich in proteins that promote cell proliferation, differentiation, migration, and tissue repair, including muscle regeneration.[81] Adipose-derived mesenchymal stem cell CM (ADSC-CM) treatment has been reported to suppress α-SMA expression. ADSC-CM treatment was also able to downregulate *Acta2* gene expression and increase decorin.[82] Overexpression of *Acta2* is associated with the increase of TGF-β1, while decorin is an inhibitor of TGF-β1. This demonstrates the ability of ADSC-CM treatment to block TGF-β1. However,

pretreatment with ADSC-CM did not induce decorin expression.[82] This is the challenge in using stem cells, or any derivations of stem cells, since pretreatment or treatment given after injury may produce different results.

Exosomes, a specialized category of extracellular vesicles within the secretome, are enclosed within a single outer membrane and are secreted by all cell types. The exosomes produced by the skeletal muscle cells (MuSC-Exo) can be used as a therapy modality, other than stem cells, and have been reported to improve the muscle fibrosis condition by inhibiting the TGF-β1/Smad3 pathway. Intramuscular injection of MuSC-Exo has an inhibitory effect on the TGF-β1/Smad3 pathway, thus increasing the expression of Pax7, which is related to muscle regeneration.[83] Although their use in fibrosis has not been reported many times, stem cell secretomes, and especially exosomes, are known to have antifibrotic effects and are not associated with a higher risk regarding patient safety compared with the stem cell itself in the application of regenerative medicine.[84] Administration of secretomes or exosomes, especially through intramuscular injection, presents a promising modality for accelerating recovery from muscle injury, including muscle fibrosis. Secretome contains growth factors and exhibits anti-inflammatory properties that enhance satellite cell proliferation, thereby promoting muscle regeneration and restoring both function and strength. In addition, its angiogenic and anti-apoptotic effects contribute toward reducing cell death.[85] Furthermore, it may offer the potential to reduce the area of fibrosis.

Platelet-rich plasma (PRP), another modality of regenerative medicine, is a concentrated form of plasma derived from autologous blood, enriched with platelets and growth factors for muscle repair. PRP also contains high concentrations of growth factors that are deleterious for optimal muscle healing, such as TGF-β1.[86] A study assessing the effects of PRP and a combination of PRP and neutralizing antibody (PRP+Ab) showed that both could enhance angiogenesis and Pax7 positive satellite cells compared to the control group. However, the group that received PRP only did not show better results compared to the groups that received PRP+Ab, in terms of decreasing collagen deposition and increasing anti-inflammatory macrophages.[87] This shows that different molecules that are combined with PRP may affect the results during the muscle recovery process. Another study found no significant results of PRP treatment in an acute injury model due to radiotherapy.[88] This raises the question of whether muscle fibrosis treatment using PRP is worthwhile. Further study using various combinations of molecules at various concentrations, as well as studies with more subjects, should be implemented to confirm the role of PRP as a therapeutic modality for muscle fibrosis.

Future Directions for Clinical Translation

Based on the summary presented in Table 1, it was noted that the reported modalities of therapy can prevent or reduce the progression of muscle fibrosis through the inhibition of myostatin expression, inhibition or downregulation of TGF-β expression and *Tgfb1* mRNA expression, upregulation of MMP family expression, downregulation of TIMP family expression, as well as modulation of other fibrotic-related genes and proteins, in which all of these mechanisms of action are related to the regulation of ECM homeostasis. Currently, physiotherapy is the most common modality of therapy used to improve skeletal muscle fibrosis. Physiotherapy, including exercise programs and physical therapy, has been shown to be effective in improving muscle strength, range of movement, and quality of life in various muscle injury conditions, including muscle fibrosis.[89] As current treatments continue to improve, researchers and clinicians may question whether further enhancements can accelerate recovery while maintaining safety and efficacy. Some research has demonstrated that antifibrotic treatments, when combined with regenerative therapies, can enhance muscle regeneration and reduce fibrotic scarring, and therefore might have potential as an adjunctive modality in muscle recovery therapy.[90,91] Hence, further study to investigate the use of potential of therapy modalities with minimal possible side effects, such as stem cell exosomes,[14] in combination with currently used therapy, such as physiotherapy, exercise therapy, or FDA-approved medication, needs to be considered. Integrating regenerative medicine approaches with conventional treatments may offer improved outcomes for patients suffering from muscle injuries and fibrosis.

Unfortunately, not many studies relating to muscle fibrosis have been conducted in humans, since most studies were performed in vitro or in vivo with mice, rats, and rabbits. In vivo preclinical studies often show promising antifibrotic effects in controlled environments; however, these results may not be fully replicated in human clinical studies owing to variability in subjects' genetics, disease stage, and comorbidities. The limited number of studies focusing on therapy for muscle fibrosis conducted in humans demonstrates a necessity for further translational study. Clinically, muscle is considered a regenerative

Table 1 Summary of Reported Therapy Modalities for the Treatment of Muscle Fibrosis in Various Muscle Injuries

Modality of Therapy	Type of Injury	Mechanism of Action	Results	References
Physiotherapy and Exercise				
Electroacupuncture	Acute injury	Downregulation of IL-6, TNF-α, TGF-β1, IFN-γ, collagen 1, *MMP-1* mRNA, *TGF-β1* mRNA, *CTGF* mRNA Inhibition of Smad3, p38 Upregulation of IL-4, IL-13, MMP-2, IFN-α, MyoD, Pax7, *TIMP-1* mRNA	Promotes the transformation of proinflammatory macrophages into anti-inflammatory macrophages in response to acute injury, therefore improving the inflammatory process during recovery and avoiding ECM accumulation, thus alleviating prolonged muscle fibrosis	[34–37]
	Chronic injury	Downregulation of collagen 1, TGF-β1, CTGF, phosphorylated ERK/ERK	Inhibits the accumulation of ECM formation, therefore avoiding the formation of muscle fibrosis	[38]
Electroacupuncture + massage	Acute injury	Downregulation of *MMP-1* mRNA, *TGF-β1* mRNA, *CTGF* mRNA Upregulation of *TIMP-1* mRNA	Maintains ECM homeostasis, therefore avoiding the formation of muscle fibrosis	[37]
Belt electrode–skeletal muscle electrical stimulation	Immobilization	Downregulation of *IL-1β* mRNA, *TGF-β1* mRNA, *α-SMA* mRNA, *MCP-1* mRNA Reduction of macrophage numbers, hydroxyproline content	Prevents muscle fibrosis progression via the inhibition of inflammation and ECM accumulation; and restores muscle function	[39]
Twitch stimulation	Immobilization	Downregulation of TGF-β1, *HIF-1α*, *TGF-β1*, *α-SMA*, *collagen 1*, *collagen 3* Degradation of hydroxyproline content	Prevents muscle fibrosis by downregulating or degrading the component that induces ECM accumulation	[40,41]
Low-level laser therapy	Acute injury	Downregulation of *TGF-β1* mRNA, collagen 1, inflammatory cells Upregulation of *MyoD* mRNA, *myogenin* mRNA, MMP-2, VEGF	Maintains ECM homeostasis and decreases prolonged inflammation, therefore preventing muscle fibrosis; and improves the muscle regeneration process	[42,43]
ESWT and USWD	Acute injury	Downregulation of TGF-β1, HIF-1α, IL-60, TNF-α, HSP70 Upregulation of Pax7-positive satellite cells, myoD, myogenin	Prevents muscle fibrosis progression by inhibiting prolonged inflammation; and improves muscle regeneration	[45–47]
USWD + stretching	Immobilization	Downregulation of *TGF-β1* mRNA, collagen	Prevents muscle fibrosis by inhibiting the component that may induce ECM accumulation	[48]
Stretching	Acute injury	Upregulation of *MyoD*, *myogenin* Reduction of NPI	Reduces muscle fibrosis and increases myogenesis in injured muscles	[49]
Treadmill exercise	Acute injury	Downregulation of TGF-β1, TNF-α Induces FAP senescence Increases $MyoD^{+}Pax7^{+}$ MuSCs, MuSC number	Reduces the accumulation of FAPs that are often associated with ECM deposition, therefore preventing muscle fibrosis formation; and improves muscle regeneration	[50,51]
TGF-β Inhibitors				
P144	Acute injury	Downregulation of TGF-β1, Smad2/3 phosphorylation	Prevents muscle fibrosis by inhibiting the main component of ECM accumulation	[52]

(*Continued*)

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Table 1 (Continued).

Modality of Therapy	Type of Injury	Mechanism of Action	Results	References
SB-505124	Acute injury	Downregulation of TGF-β1	Reduces muscle fibrosis due to overexpression of TGF-β1	[53]
SB431542	Acute injury	Downregulation of TGF-β1, SREBP-1, PPAR-γ, *TGF-β1* mRNA, α-SMA Induces FAP senescence	Reduces the accumulation of FAPs, so that accumulated ECM deposition can be prevented to avoid muscle fibrosis	[54]
Losartan	Acute injury	Upregulation of *MyoD* mRNA, *myogenin* mRNA, MyoD, Pax7 Downregulation of TGF-β1 Reduction of fibrotic tissue	Regulates the differentiation of myogenic satellites cells and inhibits the TGF-β signaling pathway, thus reducing the muscle fibrosis condition	[57,58]
Phytotherapy				
Sulforaphane	Acute injury	Promotion of Nrf2 activation Inhibition of TGF-β1/SMAD pathway activation	Prevents muscle fibrosis by the activation of Nrf2 and inhibition of the TGF-β/Smad signaling pathway that is associated with muscle fibrosis	[60]
Geniposide	Acute injury	Downregulation of *TGF-β*, *Smad4*, *α-SMA*, *Vim*, *collagen 1*, α-SMA, vimentin, collagen 1	Prevents muscle fibrosis by the inhibition of the TGF-β/Smad signaling pathway and its downregulated genes and proteins	[63]
Highland Barley tea	Acute injury	Upregulation of SIRT3 Downregulation of collagen 1, α-SMA	Reduces muscle fibrosis through the enhancement of mitochondrial respiration and reduction of oxidative stress	[64]
Astaxanthin	Immobilization	Decreases collagen fiber area, ROS production Downregulation of TGF-β1, α-SMA	Inhibits the TGF-β1/Smad pathway and with its antioxidant properties inhibits the activation of the profibrotic pathway, thus preventing muscle injury	[66]
Biochemically Relevant Compounds				
[2-Chloropyridin-4-yl]methanamine hydrochloride (LOLX2 inhibitor)	Acute injury	In vitro Reduction of SA-β-gal-positive cells, XOD, NOS, mitochondrial ROS production Downregulation of p53, P16INK4a, TGF-β1 In vivo - Downregulation of TGF-β1, α-SMA, collagen 1, collagen 3	Inhibits TGF-β1 signaling, promotes cell senescence, reduces oxidative stress with its antioxidant properties, thus reducing fibrosis (in vitro). Inhibits muscle fibrosis by degrading the component of ECM formation (in vivo)	[71]
Leucine	Acute injury	Downregulation of collagen, TβR-I, Smad2/3, MyHC-n Reduction of hydroxyproline content, myostatin	Prevents the accumulation of ECM deposition, thus inhibiting muscle fibrosis; and improves muscle regeneration and tissue repair	[72]
Hydrogen sulfide (H_2S)	Acute injury	Upregulation of *CD206* Downregulation of *CD68*, *TGF-β*, *TNF-α*, *IFN-γ*, *IL-1β*, *IL-6*, *CCL2*, *CCR2*, *CCL3*, *CCL5*, *CXCL12*, *CXCR4*, *MMP-1*, *MMP-2*, *MMP-9*, *MMP-14*, *gp91phox*	Reduces inflammation, enhances the degradation of ECM deposition, and reduces oxidative stress; thus alleviating muscle fibrosis	[74]

(*Continued*)

Table 1 (Continued).

Modality of Therapy	Type of Injury	Mechanism of Action	Results	References
Regenerative Therapy				
Umbilical cord-derived mesenchymal stem cells (UC-MSC) + miR-29a	Acute injury	- Downregulation of TIMP-1, TIMP-2, TIMP-4, VEGF-A, VEGF-C, fibronectin 1, collagen 1A1, collagen 3A1, collagen 4A1, collagen 4A6, collagen 5A1, collagen 6A1, collagen 9A1 - Upregulation of MMP-12 and MMP-14	Improves angiogenesis and inhibits ECM accumulation, thus protecting against fibrosis post-injury	[76]
Stromal vascular fraction stem cell (SVFC)	Chronic injury	Reduction of hydroxyproline content	Prevents the accumulation of ECM deposition, thus inhibiting muscle fibrosis	[78]
Adipose-derived mesenchymal stem cell CM (ADSC-CM / secretome)	Acute injury	In vitro Upregulation of decorin Downregulation of α-SMA, *Acta2*	Prevents the accumulation of ECM deposition by blocking TGF-β, thus inhibiting muscle fibrosis	[82]
Exosome derived from skeletal muscle satellite cells (MuSC-Exo)	Immobilization	Inhibition of TGF-β1/Smad3 pathway Upregulation of Pax7	Prevents muscle fibrosis by inhibiting the TGF-β/Smad signaling pathway	[83]
Platelet-rich plasma (PRP) + neutralizing antibody	Acute injury	Upregulation of Pax7-positive satellite cells, anti-inflammatory macrophages Downregulation of collagen Enhancement of angiogenesis	Reduces inflammation, therefore avoiding ECM accumulation, and thus alleviating prolonged muscle fibrosis	[87]

tissue, and patients with muscle injuries that may lead to muscle fibrosis often do not receive treatment beyond the rest, ice, compression, and elevation (RICE) protocol and anti-inflammatory medication. While in vivo studies on muscle fibrosis therapies show great potential in maintaining ECM homeostasis, concerns remain about the possible side effects and interference with normal adaptive mechanisms,[14] or whether the therapy is efficient for treatment in human subjects. Translating fibrosis-related muscle regeneration research from animal models to clinical applications for humans is indeed a critical step in developing effective therapies. Therefore, rigorous clinical trials are essential to evaluate the safety, efficacy, and optimal dosing of these therapies in human patients. In addition, regulatory considerations, ethical concerns, and large-scale production of standardized therapeutic products must be addressed. The integration of these approaches holds promise for bridging the translational gap and improving outcomes for patients with muscle fibrosis.

Conclusion

Skeletal muscle fibrosis remains a significant challenge in the management of muscle injuries, as it can affect the recovery and long-term function of the muscle. Reversing or improving muscle fibrosis conditions can be achieved through the management of ECM homeostasis. Several factors are involved in maintaining ECM homeostasis, including myostatin, TGF-β, MMPs, and TIMPs. Various therapeutic modalities have been studied and demonstrate substantial potential in mitigating fibrosis, with the most commonly performed method being physiotherapy. Other modalities, such as exercise therapy, phytotherapy, and therapy using TGF-β inhibitors, biochemically relevant compounds, stem cells, and secretomes, also demonstrate benefits in improving the fibrosis condition through various pathways to maintain ECM homeostasis. However, it is necessary to integrate regenerative

medicine approaches with minimal possible side effects, such as stem cell exosomes, with conventional treatments, such as physiotherapy, to offer better results for the treatment of muscle fibrosis. While preclinical findings are encouraging, more clinical studies are also needed to ensure the safety and efficacy of these therapies in humans.

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