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Mediators leading to fibrosis - how to measure and control them in tissue engineering

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Abstract

Fibrosis is the result of an excessive amount of fibrous connective tissue deposited into the extracellular matrix (ECM) space of damaged tissues from injury or disease. Collagens, particularly types I and III are the main constituents of the fibrotic scar tissue as well as a mixture of fibrotic cells. Severely fibrotic tissue will develop chronic healing problems resulting in tissue/organ dysfunction. More attention needs to be given to the fibrotic differentiation and related effects in bioengineered tissues. The current review provides an update on the mechanism behind fibrosis formation as well as technical measurements and preventions.

Keywords

Fibrosis; mediator; extracellular matrix; collagens; TGF- β 1

Introduction

The widespread interest in the application of tissue engineering for clinical settings has caused a dramatic increase in the development of engineered tissue constructs and biomaterials over the past ten years. While recognition in this scientific field has brought a moderate amount of success, challenges still remain. For instance, the implantation of cell-seeded biomaterial constructs for the purpose of improving tissue function has often resulted in the development of fibrous scar tissue for long term investigations [1,2]. Fibrosis is the final common pathway that transpires for nearly all forms of tissue injuries or diseases, which under extreme conditions will lead to end-stage organ failure [3]. While the goal of scientists and doctors is to restore the natural properties of dysfunctional tissues with bioengineered tissue constructs, the integrity of the therapeutic application is compromised at the onset of fibrosis development. Therefore, understanding the key mechanisms behind fibrotic scarring will not only help

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generate methods of prevention and treatment, but overall efficaciously improve the field of tissue engineering.

Mediators of fibrosis

Fibrosis formation is the result of the interactions between many cell types and various fibrogenic growth factors/cytokines in a pro-fibrotic environment. The mediators of fibrosis common in different tissues include pro-fibrotic cells and pro-fibrotic molecules (growth factors/cytokines), as well as other influences such as from the extracellular matrix (ECM), mechanical tension, or oxidative stress (Figure 1).

1. Cellular pro-fibrotic mediators

The main cell type contributing to the collagen synthesis and turnover of the ECM in tissue injuries and fibrogenic diseases are myofibroblasts or myofibroblast-like cells in different tissues [4-7]. Myofibroblasts have some of the phenotypic characteristics of smooth muscle cells [i.e. expression of α -smooth muscle actin (α -SMA), vimentin, myosin, and desmin], and are usually derived from the transdifferentiation of fibroblasts, which are ubiquitous mesenchymal cells distributed throughout the body [8,9]. Transdifferentiation is a process regarding the conversion of one differentiated cell type into another differentiated cell type outside of its original cell lineage. Myofibroblasts are capable of speeding wound repair by contracting the edges of the wound in favor of healing, despite non-functional scar tissue formation.

Fibroblasts are not the only cell type to differentiate to myofibroblasts in fibrosis. Other studies have shown that myofibroblasts are derived from myogenic cells during injury to the skeletal muscle, both *in vitro* and *in vivo* [10,11]. Evidence suggests that an important source of fibrosis-associated myofibroblasts in many organs is through transdifferentiation from epithelial cells via epithelial-mesenchymal transition (EMT) (i.e. kidney, lung, liver, eye, and serosal membranes), or from endothelial cells via endothelial-mesenchymal transition (EndMT) (i.e. heart and lung) [12-18]. Bone marrow-derived cells or fibrocytes (circulating fibroblast-like cells) are also able to differentiate into myofibroblasts during fibrosis formation of various solid organs, such as for pulmonary fibrosis, renal fibrosis, and the fibrosis surrounding tumors [19-21]. Additionally, hepatic satellite cells (HSC) were found to transdifferentiate into collagen-producing myofibroblast-like cells following fibrogenic stimuli such as alcohol, which in excessive amounts, can cause liver fibrosis [22-24].

Aside from producing and modifying the ECM, myofibroblasts can also promote fibrosis by secreting angiogenic, pro-inflammatory, and pro-fibrotic/fibrogenic factors [7,9,25]. In fact, a large number of the pro-fibrotic factors are secreted by many other cell types, such as inflammation-related lymphocytes, macrophages, and activated mast cells located in various tissues, such as skeletal muscle, liver, lung, skin, heart and kidney [26-30]. Meanwhile, paracrine signals derived from lymphocytes and macrophages in a fibrotic site can further promote the transdifferentiation and activation of myofibroblasts [25].

2. Molecular pro-fibrotic mediators

Previous literature has demonstrated the role of molecular pro-fibrotic mediators of fibrosis. They were indicated in myofibroblast transdifferentiation and proliferation, both paracrine and autocrine signaling for upregulation of pro-fibrotic cells and molecular factors, ECM remodeling by increased collagen deposition and inhibition of enzymes related to the breakdown of collagen fibers [25,31,32]. Pro-fibrotic factors increase the production and density of collagen during the fibrotic formation stage of muscle healing (Figure 2). It is typically an irreversible process, regardless of when these molecular factors have been cleared.

Studies have determined that the multifunctional cytokine TGF- β 1, known as an important immune regulator during an inflammatory response, is a key factor in the activation of the pro-fibrotic cascade that occurs following the injuries and diseases [25,31,33-36]. Excessive signaling of TGF- β 1 will have negative consequences on muscle healing by activating intracellular signaling pathways involving the TGF- β 1 receptor (Activin receptor-like kinase 5, ALK5), signaling molecule Smad3 and a downstream mediator, connective tissue growth factor (CTGF) by promoting a fibrotic environment [25,31,35,37,38].

Additional molecular factors have also been identified as important mediators in fibrosis, including: Th2-type cytokines (IL-4, IL-5, IL-13, IL-21), chemokines (MCP-1, MIP-1 β), angiogenic factors (VEGF), growth factors (CTGF, PDGF, TNF- α), peroxisome proliferator-activated receptors (PPARs), acute phase proteins (SAP), caspases, and components of the renin-angiotensin-aldosterone system (Angiotensin II/ANG II) [25]. Also, TGF- β 2 and endothelin (ET) were found to mediate fibrosis in situations like subepithelial layer of the airways when brought on by chronic asthma [39]. Moreover, the severity of fibrosis is dependent on the degree of ECM remodeling from collagen degrading matrix metalloproteinases (MMPs) and the tissue inhibitors of metalloproteinases (TIMPs). An imbalance in the MMP/TIMP ratio due to increased activity of TIMPs will promote renal, liver, and pulmonary fibrosis [40-42]. Actually, many of these mediators are often correlated with or directly cooperate with TGF- β 1 signaling in regulating fibrosis. ANG II is produced locally at the site of injury by activated macrophages and myofibroblasts, and exerts its pro-fibrotic effects in part by promoting TGF- β 1 secretion and activation. It shares many intracellular signals with TGF- β 1 that are implicated in fibrosis, such as the TGF- β /Smad3 signaling pathway [25,32,43-45]. MMPs are one source of proteins that help degrade cytokines like TGF- β 1, while TGF- β 1 production in myocardial or renal fibrosis was found to induce the activation of TIMPs, inhibitors of MMPs, which prevent the degradation of collagen [46-48]. In some instances, pro-fibrotic mediators such as IL-13 and IL-21 can act independently of the TGF- β /Smad3-signalling pathway to stimulate collagen deposition in the lung and other tissues [49,50].

3. Other pro-fibrotic mediators

The microenvironment of injured or diseased tissues can also be critical to inducing fibrosis. Excluding molecular pro-fibrotic factors, the environmental factors involved with the up-regulation of fibrosis may include: vascular damage, inflammation, oxidative stress, ECM, and mechanical tension. For tissue engineering materials, certain characteristics of exogenous biomaterials are often related with fibrosis formation.

Vascular damage—Tissue damage is often accompanied with vascular damage, which may occur at both the microvascular (single endothelial cell) and macrovascular levels (tissue vasculature) [51,52]. Researchers have indicated that vascular damage of the tissues is a critical step for the onset of fibrosis [52]. The process usually induces platelet aggregation, blood clot formation, and accumulation of a provisional ECM. It can further result in immediate damage to the epithelial/endothelial barrier, improved lymphocyte migration through endothelial barrier, cellular apoptosis, tissue hypoxia, rapid recruitment of inflammatory cells and fibroblasts to the site of injury, production of inflammatory cytokines and TGF- β 1, and transdifferentiation of myofibroblasts via mechanisms including EMT and EndoMT [18,32, 52,53]

Inflammation—Inflammation is the first stage of the wound healing process. It is necessary for the removal of cell debris and apoptotic bodies, and can promote proliferation of progenitor cells in various tissues to achieve efficient wound healing [26,53]. However, this reaction is closely related to fibrosis, especially in the pathological condition of chronic inflammation,

which is characterized by a prolonged inflammatory response and tissue destruction. Many inflammatory cytokines secreted by the inflammatory cells, such as TGF- β 1 and IL-13, are fibrogenic [25]. Studies have shown that inflammation-induced progressive fibrosis is dependent on the TGF- β 1/Smad3 pathway [31]. Macrophages are a major cell type that secretes TGF- β 1 as a promoter for organ fibrosis; TGF- β 1 itself is a potent chemoattractant for recruitment of inflammatory cells as well [52,54]. Additionally, inflammation is known to be directly linked to inducing oxidative stress and reducing cellular antioxidant capacity [52,55].

Oxidative stress—In damaged tissues, oxidative stress caused by increased reactive oxygen species (ROS), is also closely associated with fibrosis [54,56,57]. ROS is released primarily by cells located in proximity to the damaged tissue or inflammatory cells [22,54,55]. Oxidative stress is represented as an upregulation of NADPH oxidase and suppression of superoxide dismutase (SOD) expression [57]. This oxidant/antioxidant imbalance may further contribute to tissue damage [52,54]. An upregulation of ROS has also been shown to induce a MMP/TIMP imbalance, which is also a major contributor to fibrosis formation [56,58]. A study in the kidneys of hypertensive rats showed that renal oxidative stress was able to promote expression of TGF- β 1 and therefore increase the population of myofibroblasts. Furthermore, prolonged ANG II administration can induce oxidative stress by increasing NADPH oxidase and reducing SOD [57].

Fibrogenic ECM—The ECM provides cells with positional information and a mechanical scaffold for adhesion, migration, and proliferation. It also transfers specific signals to cells, and also influences their characteristics and fate, including fibrogenic activation or deactivation [59]. It consists mainly of collagens, glycoproteins and proteoglycans, and specifically binds with certain growth factors/cytokines and MMPs. Dynamic interactions between ECM and growth factors are suggested to be integral to wound healing [60]. The ECM binds to and releases growth factors in response to various stimuli, which regulate the degradation and activation of expressed growth factors. The biochemical composition of the ECM was found to exert a powerful control over the phenotypes of fibroblasts [61]. In fibrotic tissue, TGF- β 1 activation and an imbalanced ratio of MMP/TIMP expression results in excessive deposition of collagen in the ECM and gives rise to an environment for chronic fibrogenesis, leading to further collagen deposition in a non-degradative manner. Increasing deposition of abnormal ECM in fibrotic tissue was also suggested to accelerate the activation of collagen producing cells [52,62]. With stem cell-based tissue engineering, one study illustrated that the local fibrotic environment blocked the migration of transplanted myogenic cells and stem cells; and instead triggered these cells to transdifferentiate into myofibroblasts [10]. These cells in turn cause an increase in the collagen deposition, which gradually reduces the area for myofiber regeneration over time (Figure 3). As a result, the authors suggested the importance of maintaining a balanced local environment within the ECM to avoid fibrosis when regenerating damaged tissue. Moreover, the physical nature of the ECM, such as mechanical properties, rigidity, and stiffness are proposed to influence cell behavior and biological activity [61,63].

Mechanical tension/stress—The mechanical environment of fibroblasts influences their fibronectin fibril assembly and modifies their response to growth factors [63,64]. Studies have shown that mechanical tension is an important mechanism underlying tissue repair and fibrosis. Fibroblasts cultured in three-dimensional (3D) collagen matrices responded differently to growth factor stimulation under tension or relaxed condition [65]. Mechanical tension prevents fibroblast apoptosis, stimulates the transdifferentiation of fibroblasts into myofibroblasts in human burn scar, and is crucial for the maintenance of contractile activity of myofibroblasts in a rat model [66]. Mechanical tension also induces fibroblasts to synthesize abundant ECM and an assorted array of fibrotic cytokines/chemokines [61]. Moreover, cells are not only exposed to mechanical tension through the ECM, but some cells can also generate mechanical

tension via contraction [68]. It was shown that with increased expression of α -SMA, myofibroblasts have up-regulated contractile activity [69]. Myofibroblast contraction activates latent TGF- β 1 from the ECM, and can be improved with intercellular mechanical coupling of stress fibers via adherens junctions [70,71].

Fibrogenic biomaterials in tissue engineering—Tissue engineering-based regenerative therapies often apply biomaterial scaffolds to create bioartificial replacement organs, in which genetically modified cells or multipotent/pluripotent stem cells are seeded [2,72]. Biomaterials are used to create transplant substitutes for the ECM, while drug delivery system (DDS) signal molecules to enhance their biological repair [73]. The application of various synthetic and natural materials, such as polymers, ceramics and metals or their composites, as well as reconstructed ECM derived from various tissues, have been studied in different manners [73,74]. Appropriate selection of biomaterials and cell types are important for their integration into reconstructed tissue because their implantation different materials are known to influence inflammatory and immune responses as well as have negative consequences on wound healing (fibrosis formation) [1,2,72,75]. For example, the implantation of alginate-poly-L-lysine microcapsules causes a significant inflammatory response and instigates the fibrogenic process [76]. Another biomaterial, silk fibroin (SF) protein, when used in its pure form can potently stimulate myofibroblast transdifferentiation of adhering host fibroblasts; however, the modified lactoseconjugated SF biomaterials can greatly suppress the fibrogenic development [77]. Moreover, inflammation caused by biomaterial implantation can result in fibrosis, but is not a necessary factor for fibrosis [2]. Therefore, the biomaterial cues determining fibrosis in tissue engineering are important for evaluation. Like the natural influence of the ECM (tension, rigidity and stiffness), the physical properties of biomaterials will regulate cell adhesion, proliferation and migration to impact fibrosis development [52,61,68,78]. In addition, such biochemical characteristics as oxidant-generating potential, biodegradability, chemoattractivity, and biocompatibility with the local cells and surrounding growth factors/cytokines must be considered in order to minimize fibrosis.

How to measure fibrosis

Currently, the most promising method of determining the extent of a patient's tissue fibrosis is by obtaining a biopsy. Once the biopsy is harvested from the individual, a number of different strategies are suitable to evaluate his/her fibrotic state. Using the histological techniques of hematoxylin & eosin (HE) and trichrome staining can help verify the severity of the existing fibrosis based on the collagens deposition. Both methods specifically target collagen which appears as a pale pink color or blue for either HE or trichrome stain, respectively. Based on the collection of numerous images, the degree of fibrosis can be assessed by determining the percentage of collagen present versus the total tissue area.

Additionally, examining the severity of a patient's fibrotic tissue can be evaluated by fluorescent immunohistochemistry where primary and secondary antibodies are used to identify/quantify proteins associated with healthy skeletal muscle tissue (myofiber formation) or indicators of scar tissue. The presence of healthy muscle tissue markers including myosin, dystrophin, myogenin, MyoD, and myogenic regulatory factors (MRFs) can be examined in comparison to markers of fibrotic scar tissue, laminin, fibronectin and collagens I, III and IV. Furthermore, markers for myofibroblasts, who are leading culprits of fibrosis formation, can be assessed by staining for vimentin, α -smooth muscle actin and desmin [8,9].

Indirect measurements of the severity of fibrosis can be obtained non-invasively by performing functional tests on patients. These include subjecting individuals to a variety of physical measurements including muscle strength, balance/coordination, and gait as well as tests to

measure response to neurological stimuli. Also, if the person under examination is being diagnosed for pulmonary fibrosis, measurements testing the lungs using a spirometer can be used to determine such parameters like vital capacity or the functional residual capacity.

How to measure fibrosis mediators in tissue engineering

The successful regeneration of injured/diseased tissues using tissue engineered constructs greatly depends on the structured scaffold microenvironment and the cells they are seeded with, which will influence cellular phenotypes and ECM production [79]. To reduce the activation of fibrosis mediators, it would be beneficial to immediately detect fibrotic mediators before scar tissue develops, thus allowing for possible therapeutic intervention. The measurement of fibrosis mediators should be quantified at the cellular, molecular, or environmental levels.

Because of the direct relevance of both inflammatory cells and activated myofibroblasts in mediating fibrosis, the amount of these cells present at the site of the biomaterial implantation are important parameters for diagnosing potential fibrosis development. A study with microcapsule injection suggested that the accumulation of various inflammatory cells is differentially activated by biomaterial implantation: neutrophil number can be quickly increased by surgical wounding and vascular damage, while macrophages are more attracted by an implanted biomaterial [76]. A significant relationship was found where macrophages involvement with chronic inflammation resulted in a higher degree of interstitial fibrosis [76, 80]. The reason for this phenomenon is macrophages are the major cell source for secreting fibrogenic TGF- β 1, therefore measuring the number of macrophages is a critical parameter for evaluating fibrosis from implanted tissue engineered constructs [52]. Immunoassays of either tissue biopsies or whole cell isolated populations allow for the detection of macrophages by specific cell markers such as CD68, CD1a, CD11b, and CD163 [81-83]. Myofibroblast transdifferentiation is a central event of the initiation and development of fibrosis, and is inducible by the implantation of biomaterials [79]. As the main contributor to collagen deposition in ECM, a greater percentage of activated myofibroblasts is measurable by detecting the neo-expression of myofibroblast markers such as α -SMA, tropomyosin-1, β -actin, and desmin [8,84,85].

As mentioned before, TGF- β 1 plays a critical role in mediating fibrosis by activating fibrogenic cells directly and cross-talking with almost all other molecular mediators [43,45,46,48]. Therefore, TGF- β 1 has been widely examined as the primary molecular marker of fibrosis in both experimental and clinical studies [86,87]. TGF- β 1 is secreted in an inactive, latent form, but upon activation, it is fully functional and fibrogenic [88]. Given the importance of functional TGF- β 1 in the pathogenesis of fibrotic development, detecting measurable amounts are highly significant for fibrosis prevention. Various methods, including enzyme-linked immunosorbent assays (ELISA) or sandwich immunoassays, are useful to quantify the levels of functional TGF- β 1 protein in blood or tissue samples [86,87,89,90]. TGF- β 1 concentration is measured from collected blood samples; however, elevated levels in TGF- β 1 concentration are attributed to platelet degranulation, where intracellular TGF- β 1 accumulates in the cytoplasm [86,89]. This excessive quantity of TGF- β 1 from platelets plays an important role in enhancing platelet aggregation to mediate subsequent vessel repair and does not contribute a considered amount to scar tissue [91]. Therefore, caution is used to minimize the release of TGF- β 1 from platelets and only measure the concentration of TGF- β 1 in the blood plasma [89]. In addition to protein assays, the mRNA level of TGF- β 1 can also be measured with quantitative-PCR assay on total RNA isolated from blood or bioengineered tissues [92].

CTGF is a downstream mediator of Ang II and TGF- β -induced fibrosis, and currently there is no data revealing the positive effects of CTGF in the modulation of inflammatory and immune reactions [38,43,93,94]. As comparable to TGF- β 1, CTGF concentrations in circulation do not

adequately reflect the extent of fibrotic development, but rather ongoing fibrogenesis, which allows for potential therapeutic intervention treatments [95]. Hence, measuring the level of CTGF in plasma or serum has been indicated a useful method to predict the extent of fibrosis or verify its occurrence with fibrosis-related diseases [95-97]. Similarly, fibrogenesis resulting from implanted biomaterials can be evaluated by the present level of CTGF protein or mRNA in circulation. As mentioned previously, changes in the ratio of MMP/TIMP system during ECM remodeling and TGF- β 1 activity, is detectable by measuring both MMPs and TIMPs found in the serum or tissues, and is reflective of fibrotic activity [40-42,98,99].

Improved angiogenesis and vascularization of the regenerating tissues is essential for the success of tissue engineered constructs in developing functional tissues or multi-tissue organs [100,101]. Angiogenic factors such as VEGF and bFGF are beneficial when incorporated into tissue-engineering studies/trials by facilitating blood-vessel growth [100,102]. However, in many pathological conditions, angiogenesis and fibrosis are a codependent phenomena, sharing some common features [3]. For instance, increased angiogenic activity and elevated concentrations of circulating serum VEGF were found in idiopathic myelofibrosis [103]. Similarly, elevated VEGF concentration were also shown in pulmonary fibrosis and cystic fibrosis [104,105]. Unfortunately, some controversy has erupted, prompting arguments among critics about the reliability of measuring VEGF as a parameter of fibrosis [106]. Serum hyaluronic acid has also been a useful indicator of fibrosis in tissues such as liver and lung [107,108]. Based on the number known fibrotic indicators, further examination is required to determine if measuring a combination of fibrotic mediators is more reliable for predicting the extent of fibrosis formation.

How to prevent or control fibrosis mediators in tissue engineering

The main idea to reduce fibrosis from tissue engineered constructs is by creating instructive non-fibrogenic cellular microenvironments for selected cell types with the highest regenerative capacity and minimal fibrogenic potential. This is possible by regulating various fibrosis mediators during the construct development. Evidence has indicated that fibrosis occurs as a competing event to the normal regeneration of damaged or diseased tissues, and an improvement in tissue repair is accomplished by reducing fibrotic promoters [109,110].

Stem cell therapies have been intensely studied and successfully applied in tissue engineering for a range of tissues, such as epithelial surfaces (skin, cornea and mucosal membranes) to skeletal tissues [111,112]. These stem cells under investigation include both pluripotent stem cells, embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, as well as adult multipotent stem cells (i.e., mesenchymal stem cells (MSCs) or muscle derived stem cells (MDSCs)). Stem cells are described as self-renewable, non-immunogenic (if derived from the patient's own tissue), highly anti-oxidant, and capable of differentiating into various cell types needed for regenerating functional tissues [112-115]. Since all these qualities improved tissue regeneration, competing fibrosis formation is reduced with minimal myofibroblast transdifferentiation, when stem cells are seeded in implantable tissue engineered constructs. In fact, reduced fibrosis formation has been widely reported with stem cell-based therapies treating injuries or diseases in multiple tissue, such as skeletal muscle, heart, lung, nerves, and more [115-117]. In combination with stem cell therapies, the administration of various growth factors (i.e., bFGF) and controlled physical interactions with ECM have helped lower myofibroblast transdifferentiation [118-120].

In some cases, stem cell therapy alone may not be adequate to reverse tissue damage from degenerative diseases [121]. The administration of biological or chemical molecules is often needed as “molecular adjuvants” or enhancers of stem cell therapy [121]. Since normal tissue regeneration and fibrosis are competing events, the administration of these molecules are

expected to promote stem cell mobilization, proliferation, and secretion of growth factors as well as inhibiting the activation of fibrosis mediators or even inducing cell death in myofibroblasts [24]. Among all fibrosis mediators, TGF- β 1 has been the main target in the medical interventions and antifibrotic therapies. The administration of several remedies such as basic-fibroblast growth factor (bFGF), insulin growth factor type 1 (IGF-1), losartan, suramin, decorin, relaxin, interferon- γ (INF γ) and ANG II receptor blockers successfully improve tissue healing by either promoting stem cell proliferation or blocking TGF- β 1 expression [11,109,110,122-127].

Blocking TGF- β 1 expression has been shown to prevent fibrosis formation in experimental models; however, TGF- β 1 is a multi-functional growth factor, necessary for many normal biological functions. Therefore, different antifibrotic targets are under examination to effectively prevent fibrosis development [43]. The early fibrogenic marker protein CTGF has been recognized as an alternative target for antifibrotic therapies because of its critical role in mediating the fibrogenic effects of TGF- β , particularly during the initiation phases of fibrosis [43,95]. Administering angiotensin blockers against ANG II activity have demonstrated beneficial effects in cardiovascular and kidney diseases [43,128,129]. Moreover, because of the important roles of MMPs and TIMPs in tissue remodeling by influencing cell proliferation, migration, differentiation, and apoptosis; a closer examination of the relationship between MMPs/TIMPs may provide a greater understanding scarless healing with tissue regeneration [130-132]. Systematically regulating the activities of MMPs and TIMPs in bioengineered tissues will be important in creating anti-fibrotic strategies [41]. Targeting mediators of fibrosis and appropriately selecting non-fibrogenic materials will also help limit fibrosis development that results from inflammation and vascular damage, mechanical tension or oxidant stress [133,134].

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Abbreviations

ALK5	Activin Receptor-Like Kinase 5
α -SMA	α -Smooth Muscle Actin
ANG II	Angiotensin II
bFGF	basic-fibroblast growth factor
CTGF	Connective Tissue Growth Factor
ECM	Extracellular Matrix
ET	Endothelin
IGF-1	insulin growth factor type
IL	Interleukin
INF γ	interferon- γ
MCP-1	Monocyte Chemoattractant Protein-1
MIP-1 β	Macrophage Inflammatory Protein-1 β
MMP	Matrix Metalloproteinases
MRF	Myogenic Regulatory Factors

NADPH	Nicotinamide Adenine Dinucleotide Phosphate-Oxidase
PDGF	Platelet Derived Growth Factor
PPAR	Peroxisome Proliferator-Activated Receptor
ROS	Reactive Oxygen Species
SAP	Serum Amyloid P
SOD	Superoxide Dimutase
TGF- β	Transforming Growth Factor- β
TIMP	Tissue Inhibitor of Metalloproteinases
TNF- α	Tumor Necrosis Factor- α
VEGF	Vascular Endothelial Growth Factors

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Figure 1.

A diagram representative of the different factors that mediate fibrosis formation. Mediators of fibrosis: Cellular (green), Molecular (blue) and Other (Red).

Normal Tissue

Fibrotic Tissue

Figure 2.

A comparison of the tissue architecture of normal and fibrotic tissue. Normal tissue is organized with a balanced distribution of cells (rounded objects) and the ECM (long fibrils). In fibrotic tissue, an excessive amount of collagen fibers are secreted in a disorganized manner, which detrimentally inhibits cell proliferation and migration.

Fibrosis Develops in Muscle Healing

Figure 3.

A timeline illustrating the development of fibrosis during muscle healing after traumatic injuries. During the initial days following injury, regenerative myofibers have the ability to migrate through the injury site of the ECM to promote muscle regeneration. By 10 days, the secreted collagen fibers begin to accumulate and severely limit myofiber migration throughout. Fibrotic scar tissue formation is established by two weeks and potentially prevents any further muscle regeneration.