# New Strategies for Muscular Repair and Regeneration

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## Contents



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## 14.1 Introduction

Skeletal muscle injuries can stem from a variety of events, including direct, such as muscle lacerations and contusions, and indirect trauma, such as strains, and also degenerative diseases, such as muscular dystrophies (Huard et al. 2002).

Many classifications are used for muscle injuries, which sometimes causes limitations to the comprehensive study of muscle injuries and discrepancies in the uniformity for their categorization and description. In human sport clinics, the diagnosis of muscle injury is adequate in most cases, but imaging modalities such as ultrasonography, computed tomography (CT), or magnetic resonance imaging (MRI) are important to differentiate between structural lesions and functional disorders and to determine the extent of the injury.

A minor muscle injury can regenerate completely and spontaneously, whereas after severe injuries, muscle healing is incomplete, often resulting in the formation of fibrotic tissue that compromises muscle function. Despite the frequent occurrence and the presence of a body of data on the pathophysiology of muscle injuries, none of the treatment strategies adopted to date have been shown to be really effective in strictly controlled trials. Most current muscle injury treatments are based on limited experimental and clinical data and/or were only empirically tested.



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## 14.2 Physiopathology and Muscle Healing Process

The pathogenic process consists in consequent phases:

- Degeneration and necrosis
- Inflammation and cellular response
- Regeneration and repair
- Remodeling and fibrosis

### 14.2.1 Degeneration and Necrosis

The initial phase is characterized by rupture and necrosis of the myofibers. The gap created is filled with a hematoma. Late elimination of the hematoma is known to delay skeletal muscle regeneration (Beiner et al. 1999) and to promote fibrosis interfering with functional recovery.

### 14.2.2 Inflammation and Cellular Response

Neutrophils are the first inflammatory cells infiltrating the lesion. A large number of pro-inflammatory molecules such as cytokines (TNF-α, IL-6), chemokines (CCL17, CCL2), and growth factors (FGF, HGF, IGF-I, VEGF, TGF-β1) are secreted by neutrophils in order to create a chemo-attractive microenvironment for other inflammatory cells such as monocytes and macrophages (Tidball 1995; Toumi and Best 2003). Two types of macrophages are identified during muscle regeneration (McLennan 1996). Macrophages, infiltrating injured muscle, are key players of the healing process (Zhao et al. 2016), able to participate in the muscle regeneration process or to favor fibrosis (Munoz-Canoves and Serrano 2015).

Finally, the arrival in the damage tissue of the T lymphocytes plays an important role in the local vascularization through adhesion molecule secretion, product of growth factors (GFs), and cytokines. With the intervention of the T lymphocytes, the inflammatory response undergoes an acceleration.

### 14.2.3 Regeneration and Repair

Phagocytosis of damaged tissue is followed by myofibers regeneration, leading to satellite cell activation. Muscle regeneration usually starts during the first 4–5 days after injury, peaks at 2 weeks, and then gradually diminishes 3–4 weeks after injury. It is a multiple-step process including activation/proliferation of satellite cells (SCs), repair and maturation of damaged muscle fibers, and connective tissue formation. A fine balance between these mechanisms is essential for a full recovery of the contractile muscle function. Muscle fibers are postmitotic cells, which do not have the capacity to divide. Following an injury, damaged muscle fibers cannot be repaired without the presence of adult muscle stem cells (Relaix and Zammit 2012; Sambasivan et al. 2011). SCs are skeletal muscle stem cell located between the plasma membrane of myofibers and the basal lamina. Their regenerative capabilities are essential to repair skeletal muscle after injury (Hurme and Kalimo 1992; Lipton and Schultz 1979; Dumont et al. 2015). In adult muscles, SCs are found in a quiescent state and represent, depending on species, age, muscle location, and muscle type, around 5–10% of skeletal muscle cells (Rocheteau et al. 2015).

After injury, SCs become activated, proliferate, and give rise to myogenic precursor cells, known as myoblasts. After entering the differentiation process, myoblasts form new myotubes or fuse with damaged myofibers and ultimately mature in functional myofibers. Following activation, SCs proliferate and generate a population of myoblasts that can either differentiate to repair damaged fibers or, for a small proportion, self-renew to maintain the SC pool for possible future demands of muscle regeneration (Collins 2006; Dhawan and Rando 2005).

### 14.2.4 Remodeling and Fibrosis

The last phase is characterized by maturation of regenerated myofibers with recovery of muscle functional capacity and also fibrosis and scar tissue formation. The presence of fibrin and fibronec-

tin into the injury site initiates the formation of an extracellular matrix that is rapidly invaded by fibroblasts (Darby et al. 2016; Desmouliere and Gabbiani 1995). Fibrogenic cytokines such as transforming growth factor-β1 (TGF-β1) participate to excessive fibroblast/myofibroblast proliferation and to an increase in type I/III collagens and laminin and fibronectin production (Lehto et al. 1985). In its initial phase, the fibrotic response is beneficial, stabilizing the tissue and acting as a scaffold for myofiber regeneration. Many growth factors are involved in the development of fibrosis, such as connective tissue growth factor (CTGF), platelet-derived growth factor (PDGF), or myostatin. TGF-β1, by stimulating fibroblasts/myofibroblasts to produce extracellular proteins such as fibronectin and type I/III collagen, has been identified as the key element in this process (Mann et al. 2011). Although fibroblasts are the major collagen-producing cells in skeletal muscle, TGF-β1 has also an effect directly on myoblasts causing their conversion to myofibroblasts.

The phases of muscle healing are almost the same in all muscle injuries (Fig. 14.1) but the functional recovery changes. Usually the healing process leads to muscle regeneration with a different scar tissue area. In the best of case the healing provides to complete resorption of the hematoma, in the almost complete regeneration of muscle damage tissue and so in a complete functional recovery that means the athlete is able to produce the same pre-injury muscle work (Huard et al. 2002). Late elimination of the hematoma is known to delay skeletal muscle regeneration, to improve fibrosis, and to reduce biomechanical properties of the healing muscle that have a negative influence on the functional recovery of the athlete. Furthermore, in rare case of major muscle injuries, some complications like myositis ossificans, cystic degeneration, heterotopic ossifications, and liquid flapper may occur.

## 14.3 Treatment

Most muscle injuries respond well to conservative treatment. The main indication to surgery, depending on the sport activity and the muscle group involved, is a subtotal or complete lesion of the muscle belly or an avulsion of the tendon.

The treatment strategy should be rapid and based on a correct clinical examination and instrumental diagnosis. The main objectives of the treatment are to reduce recurrence rates particularly in elite athletes, where decisions regarding return to play and player availability have significant financial or strategic consequences for the player and the team, and to minimize the absence from sport.

The pain is the first symptom and should be treated because it generates a state of muscular contraction or analgesic attitude that often affects the healing process.

**PRICE** (protection, rest, ice, compression, and elevation) has been central to acute soft tissue injury management for many years.

**POLICE**, a new acronym, which represents protection, optimal loading, ice compression, and elevation, is not simply a formula but a reminder to clinicians to think differently and seek out new and innovative strategies for safe and effective loading in acute soft tissue injury management. Optimal loading is an umbrella term for any mechanotherapy intervention and includes a wide range of manual techniques currently available; indeed, the term may include manual techniques such as massage refined to maximize the mechano-effect (Bleakley et al. 2012).

After 24–48 h from the injury, it is possible to know the lesion severity and to make decisions for recovery program. The treatment should be based on natural evolution: in the first 24–48 h, the edema and hematoma promote the fibroblast organization, and they realize the connective neoformation between 7th and 15th days.

Therefore, it is important to consider this process, because the treatment affects the scar, depending on the supplied stimuli.

The new tissue is composed of collagen maturation and it is breakable and sensible to mechanical stress. Tensile stresses allow an increase of the elasticity up to a maximum of 20%, while a load of 10–12 kg per $mm^2$ leads to the breakdown of the collagen fibers (Sallay et al. 1996).

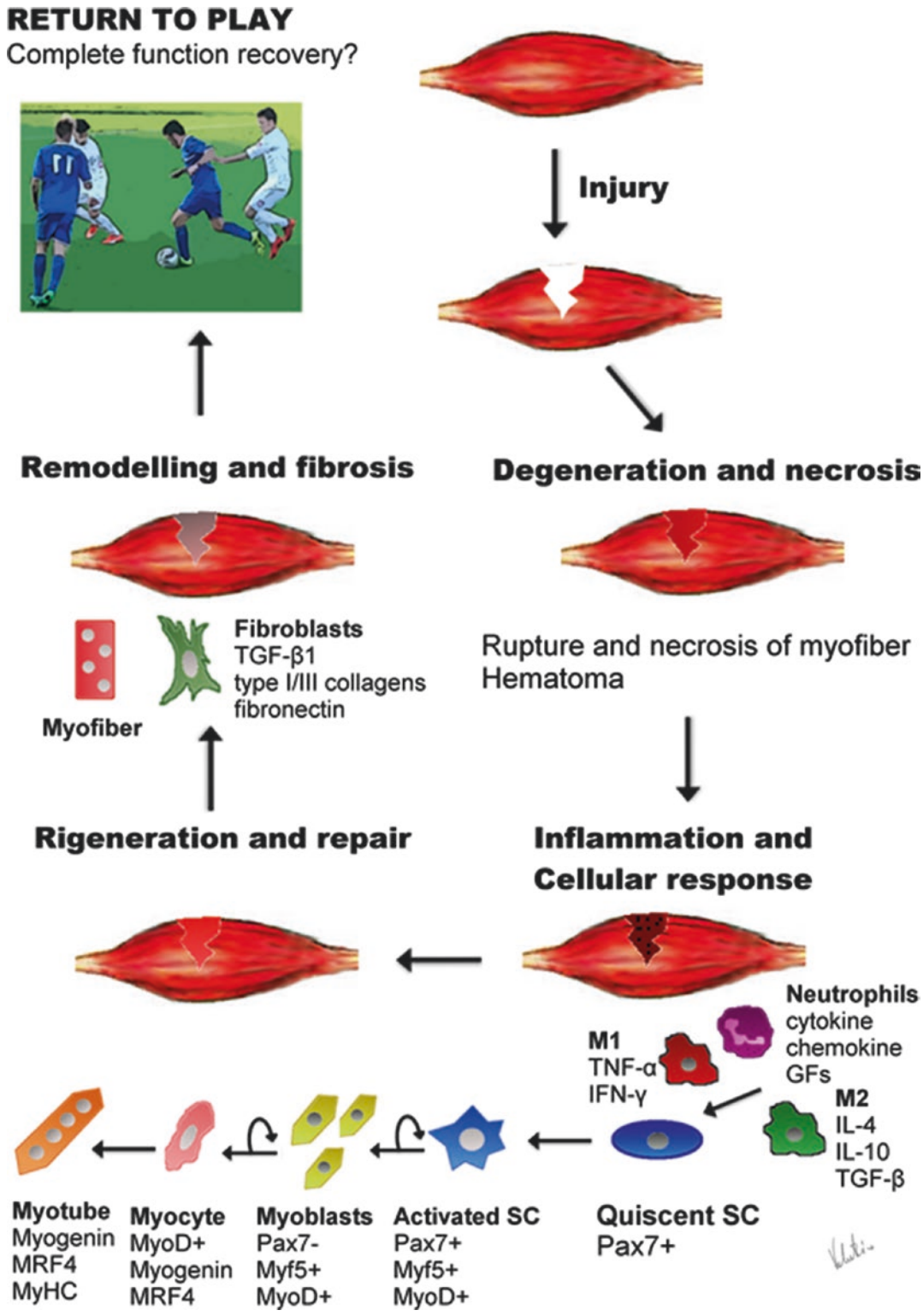


**Fig. 14.1** The phases of muscle healing are almost the same in all muscle injuries after indirect trauma, but the functional recovery changes, and it is almost always incomplete for the presence of fibrotic tissue

**Fisiochinesiterapic treatment** in this phase should respect the healing process. The prevention of adhesions is by massage therapy initially distant from the outbreak and then, depending on the evolution, even in the scar but not earlier than 10–15 days (Järvinen et al. 2007). We should try to obtain an elastic scar in the muscle and a solid scar in the transmission structures (tendons and apparatus myoentesico) or in stabilization structures (ligaments, capsule, bands).

About **NSAIDs** some disputes exist on administration time. Some authors recommend using these drugs from the start and to suspend them after 3–5 days. Other authors indicate that the use of anti-inflammatory interferes with chemotaxis cell; with an inhibition of regenerative response, necessary for the formation of new muscle cells; and with pain that is an important parameter to the management of the first phases. These studies suggest to postpone the administration of anti-inflammatory on the 2nd or 4th day following the accident. It is considered more appropriate to use anti-inflammatory drugs from 3rd to 6th day and suspend them after the injury.

**Mechanical stimulation** may offer a simple and effective approach to enhance skeletal muscle regeneration. Muscle stretching can be passive or active assisted. There is no evidence that passive stretching is superior to an active protocol in terms of stretching and muscle elasticity. Mechanical forces are as important biological regulators as chemicals and genes and underline the immense potential of developing mechanotherapies to treat muscle damage (Cezar et al. 2016). Concentric and eccentric muscle contraction exercises can be started when the isometric contraction can be performed without pain. A recent study also demonstrated that a treatment based on ultrasound-guided intra-tissue percutaneous electrolysis (EPI technique) enhances the treatment of muscle injuries (Abat et al. 2015). Altogether, these results suggest that mechanical stimulation should be considered as a possible therapy to improve muscle regeneration and repair.

**Kinesiotaping** (neuromuscular bandage) has been introduced in recent decades. The rationale is to reduce tension on the lesion site by lifting the skin from the subcutaneous and deep tissue: the probable analgesic effect on the drainage process of these materials should get better the edema and swelling.

After scar formation and joint stability gained, we will begin on specific recovery that aims to rebuild muscle tropism, the motor pattern, and muscular strength. This stage use for the muscular tropism isometric exercises without loads and isotonic exercises (concentric and eccentric) with variable loads (from 2 to 5 kg). The sessions are to be divided in the day to avoid muscle overstress.

The different muscle contraction used in therapy must comply with the real operating conditions in athletic performance, so that the recovery of sports is as fast as possible. These treatments allow the athlete recovering quickly, while the athlete field observation in the post-treatment phases can confirm the healing and allow return to play (Gigante et al. 2014).

## 14.4 New Strategies for Muscular Repair and Regeneration

### 14.4.1 Growth Factors

Growth factors (GFs) are biologically active molecules, synthetized by the injured tissue or by other cell types into the inflammatory site, which are released in the extracellular space and modulate the regenerative response. They play a variety of roles in the different stages of muscle regeneration (Table 14.1).

Hepatocyte growth factor (HGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF) are of interest because of their capacity to stimulate satellite cells (Sheehan et al. 2000).

Insulin-like growth factor-1 (IGF-I) appears to be of particular importance for the muscle regeneration process. IGF-I stimulates myoblast proliferation and differentiation (Engert et al. 1996) and is implicated in the regulation of muscle growth (Schiaffino and Mammucari 2011). IGF-I improved muscle healing, and histology of the injected muscle revealed fibrosis within the

**Table 14.1** Growth factor roles during the phases of muscle healing after a trauma

| Growth factors | Physiological effects | Potential benefit | Main role |
|---|---|---|---|
| IGF-1 | – Promotes myoblast proliferation and differentiation in vitro<br>– Hypertrophic effect essential for muscle growth during development and regeneration<br>– Existence of a muscle specific isoform (m IGF-1) | – Serial injections of IGF-1 improve muscle healing in vivo (Menetrey et al. 2000)<br>– Chemotactic for fibroblasts, increase collagen production, and fibrosis development | – Central role in muscle regeneration and hypertrophy |
| VEGF | – Promotes angiogenesis in the healing process<br>– Promotes myoblast migration, proliferation, and survival | – VEGF administration improves muscle regeneration (Deasy et al. 2009) | – Creates a neo-capillary network that improves the migration of satellite cell |
| HGF | – Promotes myoblast proliferation and inhibits myoblast differentiation (Anderson 2016)<br>– A second set of HGF production is crucial for inflammation resolution after injury (Proto et al. 2015) | – Injection of HGF into injured muscle increased myoblast numbers but blocked the regeneration process (Miller et al. 2000) | – Activates satellite cells in the early phase of regeneration |
| TGF-β1 | – Key regulator of the balance between muscle fibrosis and muscle regeneration<br>– Inhibits satellite cell proliferation and differentiation in vitro | – Anti fibrotic therapy by blocking overexpression of TGF-β1 improves muscle regeneration | – Pro-fibrotic factor |
| FGF | – FGF-6 and FGF-2 promote satellite cell proliferation but inhibit myogenic differentiation | – Anti fibrotic therapy by blocking overexpression of TGF-β1 improves muscle regeneration (Hwang et al. 2016) | – Stimulates fibroblast proliferation |

lacerated site, despite high level of IGF-I production (Lee et al. 2000). However, the efficacy of direct injection of recombinant proteins is limited by the high concentration of the factor typically required to elicit a measurable effect. This is mainly due to the bloodstream rapid clearance of these molecules and their relatively short biological half-lives.

Vascular endothelial growth factor (VEGF) has a potent angiogenic effect and is expressed in high concentration in healing flexor tendons 7–10 days following repair in animal models (Würgler-Hauri et al. 2007). Increased vascularity may improve tendon healing and contribute positively to the repair process. By targeting simultaneously angiogenesis and myogenesis, it was shown that combined delivery of VEGF and IGF-I enhances muscle regenerative process (Borselli et al. 2010).

In this direction, the use of platelet-rich plasma (PRP) is considered as a possible alternative approach based on the ability of autologous growth factors to improve skeletal muscle regeneration (Hamid et al. 2014; Hammond et al. 2009). Approximately 70% of the stored factors are secreted within the first 10 min following activation, and within the first hour almost 100% have been secreted. The degranulation of the α-granules results in the release of PDGF, TGF-β, insulin-like growth factor (IGF), and vascular endothelial growth factor (VEGF), with a host of other growth factors as well. These are native growth factors in their biologically determined ratios. These platelet growth factors enhance DNA synthesis, chemotaxis, and angiogenesis, increase collagen deposition, and stimulate synthesis of extracellular matrix.

Experimental studies on animal models showed that IGF-1, bFGF, and nerve growth factor (NGF) are potent stimulators of myoblast proliferation and fusion. Hammond et al. showed the capability of PRP to promote and accelerate myogenesis in an experimental study investigating the biomechanical and biochemical effects in rat muscle injuries (2009).

In a previous experimental study in a rat model of muscle injury, the effects of a platelet-rich fibrin matrix (PRFM) in the regeneration of damaged muscle tissue were histologically and immunohistochemically evaluated. This morphological study showed that the use of PRFM could improve muscle regeneration and long-term vascularization, suggesting that autologous PRFM may be a suitable and useful tool in the clinical treatment of muscle injuries (Gigante et al. 2012).

A side effect of the use of PRP and/or related products (e.g., PRFM) may be the occurrence of fibrosis. Visser et al. demonstrated that, in vitro, PRFM contains a significantly higher concentration of TGF-β1 compared with whole blood concentrate of similar volume. TGF-β1 has the ability to significantly increase connective cell proliferation over time, thus generating fibrotic tissue. Indeed, in an in vivo study, no increase in fibrotic tissue formation was observed during PRFM treatment in comparison with controls, suggesting that in vivo the amount of TGF-β released by PRFM might be not sufficient for this occurrence (Visser et al. 2010; Gigante et al. 2012).

Furthermore, it has been demonstrated a correlation between the concentration of growth factors and muscle regeneration. In order to closely simulate a clinical approach, Cianforlini et al. injected two different concentrations of PRP intramuscularly 24 h after the surgical trauma and evaluated, by means of histological and immunohistochemical analyses, the dose-dependent effects. Histological results confirmed the effectiveness of PRP in muscle healing and showed that the increase in PRP concentrations (i.e., GFs) in damaged muscle tissue accelerates the tissue regeneration process as well as neovascularization. Immunohistochemical data further strengthened this hypothesis detecting MyoD and myogenin-positive cells, located both inside the basal lamina of the fiber and in the interstitial spaces in the muscle sacrificed at three days and in a dose-dependent manner. It is well known that MyoD and myogenin play a key regulatory role in the processes of plasticity, adaptation, and regeneration in adult muscle. At last no significant side effects related to a higher dose of GFs were detected (Cianforlini et al. 2015).

About the efficacy of GH and IGF-1, Cianforlini et al. wanted to verify the role of GH by means of a single systemic administration in the treatment of acute muscle injury in an experimental model, verifying a possible correlation between the concentration of GH administered and tissue regeneration and fibrosis.

The action of GH is found to be ubiquitous, being increased the amount of muscle tissue and also of the endomisial and perimisiali connective tissue, with a consequent presence of exuberant scar tissue, directly proportional to the concentration of the administered GH. These are only preliminary data, but muscle regeneration and fibrosis could be both dependent from GH concentration, with an effect that would not be positive from a functional point of view, being present abundant scar tissue.

Considered as safe products, autologous PRP injections are increasingly used in patients with sports-related injuries (Engebretsen et al. 2010). Nevertheless, a recent randomized clinical trial shows no significant positive effects of PRP injections, as compared with placebo injections, in patients with muscle injuries, up to one year after injections (Reurink et al. 2014, 2015).

By recent findings, some scientific works combine PRP with Losartan (an angiotensin II type 1 receptor antagonist) (Terada et al. 2013) or PRP with the use of TGF-β1 neutralizing antibodies (Li et al. 2016). These strategies are a promising alternative to promote muscle regeneration while significantly reducing fibrosis.

### 14.4.2 Stem Cells

Transplantation of satellite cell-derived myoblasts has long been explored as a promising approach for treatment of skeletal muscle disorders. After

an initial demonstration that normal myoblasts can restore dystrophin expression in mdx mice (Partridge et al. 1989), clinical trials, in which allogeneic normal human myoblasts were injected intramuscularly several times in dystrophic young boys muscles, have not been successful (Law et al. 1990; Mendell et al. 1995). Even recently, despite clear improvement in methodologies that enhance the success of myoblast transplantation in Duchenne patients (Skuk et al. 2007), outcomes of clinical trials are still disappointing. These experiments have raised concerns about the limited migratory and proliferative capacities of human myoblasts, as well as their limited life span in vivo. It led to the investigations of other muscle stem cell sources that could overcome these limitations and outperform the success of muscle cell transplantation. Among all these non-satellite myogenic stem cells, human mesangioblasts, human myogenic-endothelial cells, and human muscle-derived CD133+ have shown myogenic potentials in vitro and in vivo (Sampaolesi et al. 2006; Meng et al. 2014). The use of myogenic progenitor cells for improving muscle healing may become an interesting therapeutic alternative (Tedesco and Cossu 2012).

The functional recovery of muscle in a young rat model of contusion injuries is significantly improved with the combined use of Losartan and muscle-derived stem cells (Kobayashi et al. 2016). New perspectives are provided by the combination of stem cells with anti-fibrotic therapies.

### 14.4.3 Anti-fibrotic Agents

Considering the important role of TGF-β1 in the fibrotic cascade, the neutralization of TGF-β1 expression in injured skeletal muscle should inhibit the formation of scar tissue.

Indeed, the use of anti-fibrotic agents (decorin, relaxin, antibody against TGF-β1, AII antagonist, interferon gamma) that inactivate TGF-β1 signaling pathways reduces muscle fibrosis and, consequently, improves muscle healing, leading to a near complete recovery of lacerated muscle (Fukushima et al. 2001; Li et al. 2007).

The expression of myogenesis factor increased in mice skeletal muscles of the CCl4 + losartan group compared to the corresponding levels in the control group. It could be hypothesize that systemically elevated TGF-β1 as a result of CCl4-induced liver injury causes skeletal muscle injury, while losartan promotes muscle repair from injury via blockade of TGF-β1 signaling (Hwang et al. 2016).

Losartan, an angiotensin II receptor antagonist, neutralizes the effect of TGF-β1 and reduces fibrosis, making it the treatment of choice, since it already has FDA approval to be used clinically (Park et al. 2012; Terada et al. 2013). Suramin, also approved by the FDA, blocks TGF-β1 pathway and reduces muscle fibrosis in experimental model (Chan et al. 2003; Taniguti et al. 2011).

Also studies on the rotator cuff repair suggest the benefit effect of Licofelone (inhibitor of 5-LOX, COX-1, COX-2) on tendon healing, muscle fibrosis, and lipid accumulation (Oak et al. 2014).

In a mouse laceration model, the area of fibrosis decreased when γINF was injected at either 1 or 2 weeks after injury. More importantly, it found to improve muscle function in terms of both fast-twitch and tetanic strength. Demonstrating that γINF is a potent anti-fibrosis agent that can improve muscle healing after laceration injury (Foster et al. 2003).

### 14.4.4 Scaffolds

Appropriately configured materials have the ability to modulate different stages of the healing response by inducing a shift from a process of inflammation and scar tissue formation to one of constructive remodeling and functional tissue restoration. The events that facilitate such a dramatic change during the biomaterial-host interaction are complex and necessarily involve both the immune system and mechanisms of stem cell recruitment, growth, and differentiation. The biological scaffolds derived from animal ECM after a decellularization process that consists in the removal of cells associated antigens, preserving the ultrastructure and composition of the ECM.

When properly manufactured, the scaffold material increases the migration and cell survival of myogenic precursor cells (Boldrin et al. 2007). Controlling the microenvironment of injected myogenic cells using biological scaffolds enhances muscle regeneration (Borselli et al. 2011). Ideally, using an appropriate extracellular matrix (ECM) composition and stiffness, scaffolds should best replicate the in vivo milieu and mechanical microenvironment (Gilbert et al. 2010; Engler et al. 2006).

With enzymatic and chemical decellularization process, we can isolate skeletal muscle ECM; this shows to contain growth factors, glycosaminoglycans, and basement membrane structural proteins. Myogenic cells survive and proliferate on muscle ECM scaffolds in vitro, and when implanted in a rat abdominal wall injury model in vivo shows to induce a constructive remodeling response associate with scaffold degradation and myogenesis in the implant area (Wolf et al. 2012).

The Food and Drug Administration has not established standards for tissue decellularization. As a result, commercially available ECM-derived scaffolds contain different amounts of cell-associated antigenic material.

A recent study demonstrated that an acellular scaffold composed of urinary bladder porcine ECM can promote formation of new muscle tissue in mice and in humans after a volumetric muscle loss. Further more encouraging histological results, three of five patients also show a functional improvement of muscle injuries (Sicari et al. 2014).

A combination of stem cells, biomaterial-based scaffolds, and growth factors may provide a therapeutic option to improve regeneration of injured skeletal muscles (Jeon and Elisseeff 2016).

### Conclusions

Skeletal muscle injuries are very frequently present in sports medicine and sport traumatology. Despite their clinical importance, the optimal rehabilitation strategies for the treatment of these injuries are not well defined. The healing process required the presence of different cell populations, up- and downregulation of various gene expressions, and participation of multiple growth factors. Scientific research so far has focused on individual elements; nowadays strategies based on the match and combination of stem cells, growth factors, and biological scaffolds have already shown promising results in animal models. A better understanding of the cellular and molecular pathways as well as a better definition of the interactions (cell-cell and cell-matrix) that are essential for effective muscle regeneration should contribute to the development of new therapies in athletes.

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