



Regenerative Medicine (Biological) Therapies for Acute Muscle Injury

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17.1 Introduction

Muscle injuries are the most common injuries in professional athletes forced to high-intensity sprinting efforts [1, 2]. In international track and field competitions between 2007 and 2015, muscle injuries accounted for 41% of all injuries. The hamstrings were the most commonly affected muscle group [3–5]. Muscle injuries lead to absence from training and competition and to loss of performance, with financial and potentially lasting athletic consequences. Due to a high rate of recurrence of muscle injuries, it is one of the most challenging tasks for a sports medicine team to prepare a professional athlete for a return to competition and ultimately performance [4]. A recurrent injury leads to 30% longer absence, before athletes can return to competitive matches [6].

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In the literature, a variety of treatments for muscle injuries is described and yet the search for new treatments to improve and stimulate muscle healing is an ongoing process. In this chapter, we describe the basics of muscle healing and we discuss biological therapies and the scientific evidence on their efficacy.

17.2 Muscle Structure

Skeletal muscle is composed of two main components, muscle fibers, and the connective tissue. Muscle contraction is induced by the muscle fibers and the innervating nerves of these muscle fibers. The connective tissue is responsible for interconnecting all muscle cells and to shield the capillaries and nerves during a muscle contraction [7].

Muscle fibers originate from numerous myoblasts or (mononucleated) myogenic progenitor cells that are fused to build multinucleated myotubes. These myotubes will mature into the muscle fibers [8, 9]. For muscle contractions, contractile units (sarcomeres) contract by interaction (“sliding mechanism”) of the filamentary proteins (actin and myosin). These sarcomeres are the fundamentals of a myofibril, and myofibrils are the main elements of a muscle fiber [9, 10].

Now that the composition of muscle fibers is delineated, we can describe the organization of

the connective tissue. The connective tissue organizes the muscle fibers on three levels: the endomysium, the perimysium, and the epimysium. The endomysium (basement membrane) envelops an individual muscle fiber and includes arteries and veins. The perimysium is a sheath of connective tissue that surrounds a group of muscle fibers (fascicles), and the epimysium is the outer layer of connective tissue that envelops the entire muscle. The connective tissue is not only a supportive skeleton for the muscle fibers. It unites the contractions of all muscle fibers into a joint effort and thus converts all individual contractions into efficient locomotion [7, 11]. Musculotendinous junctions (MTJs) are responsible for the transmission of forces generated by contracting the muscle fibers to the tendon and eventually to the bone. The MTJs are located at both ends of the muscle fibers [12].

Motor neurons are responsible for initiation of muscle contraction. The motor point is the location where the motor neuron enters the muscle. Neuromuscular junctions connect muscle fibers with axon terminals. The muscle fibers innervated by a nerve axon and the axon itself are referred to as a “motor unit.” The amount of motor units per muscle and the amount of muscle fibers per motor unit differ between skeletal muscles [9, 12] (Fig. 17.1).

17.3 Muscle Healing

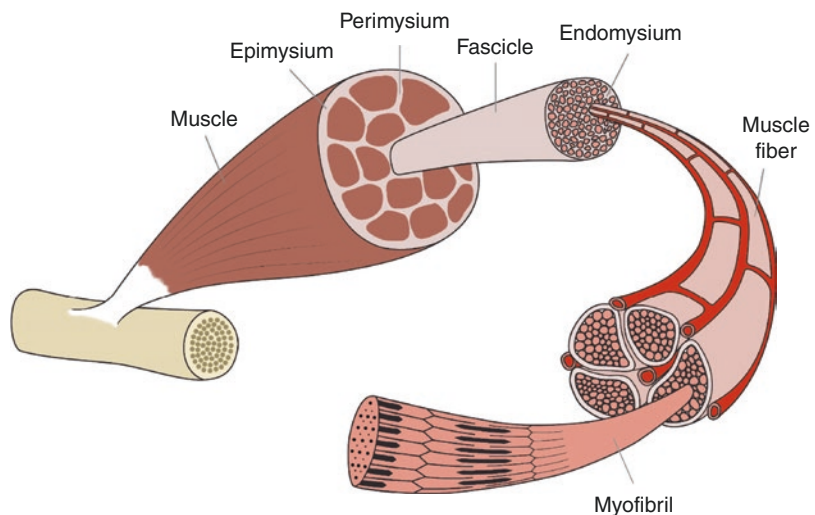
Skeletal muscle injury will heal with scar tissue, which is different from normal skeletal muscle tissue. Different causes of muscle injuries are described in the literature. For a contusion type of muscle injury, the rupture of muscle fibers occurs at or adjacent to the location of impact. In the muscle strain type of injury, the rupture of muscle fibers is located close to the MTJ [7]. The healing process is similar for muscle injuries resulting from different mechanisms of injury. The healing process is divided into the following phases: degeneration, inflammation, regeneration, and remodeling [7, 14].

17.3.1 Degeneration and Inflammation

Following injury, the resulting gap between the ruptured muscle fibers is filled with hematoma, due to hemorrhage from the torn blood vessels surrounding the muscle fibers [15].

Necrosis of the muscle fibers is initiated due to disruption of the plasma membrane. Cell permeability is increased and will result in a higher influx of calcium and an increase in activation of calcium-dependent proteases [16–18].

Fig. 17.1 Schematic overview of skeletal muscle structure. Reprinted from “Emerging Biological Approaches to Muscle Injuries” in *Bio-orthopaedics* (p 228), by van der Made A.D., Reurink G., Tol J.L., Marotta M., Rodas G., Kerkhoffs G.M., 2017, Berlin: Springer. Copyright ISAKOS 2017. Reprinted with permission [13]



The inflammatory cells in blood from the torn blood vessels have direct access to the injured site. This, in combination with the released substances of the necrotized parts of the muscle fibers that serve as chemoattractants, results in an extravasation of inflammatory cells [7, 15]. In the early acute phase after a muscle injury, polymorphonuclear leukocytes are the most abundant cells at the injury site. These leukocytes are replaced by monocytes within a day. The monocytes differentiate into macrophages that actively engage in the proteolysis and phagocytosis of the necrotic material by release of lysosomal enzymes [7, 19]. Because of the ability to adapt to the microenvironment and the multiple states of activation, macrophages have been associated with different (in vitro) phenotypes and functions [19, 20]. After several days in the healing process of muscle injuries, the macrophages switch to an anti-inflammatory profile and will contribute further in the cascade of muscle healing [17, 19–21].

17.3.2 Regeneration and Remodeling

After the destructive phases (degeneration and inflammation), the repair of the muscle injury starts with new processes: the healing process of the disrupted muscle fibers and the formation of the connective scar tissue [7].

Satellite cells are a divergent group of cells adjacent to the muscle fibers and consist of tissue-resident myogenic precursor cells. The satellite cells are located between the basal lamina and the plasma membrane (sarcolemma) and are essential cells in the cascade of the healing process of the muscles [7–10, 16, 22–24].

During the healing process of the muscles, satellite cells become activated through multiple stimuli and will migrate to the location of injury. Normally, satellite cells are in a quiescent state, which means that there is no cell cycling. At the site of injury, the satellite cells will re-enter the cell cycle to form myogenic

precursor cells (myoblasts) that will differentiate into multinucleated myotubes that will adhere to the existing damaged muscle fibers [7, 10, 25]. Revascularization of the injured site is also an essential process of muscle healing. The formation of new capillaries from surrounding blood vessels is one of the first signs of muscle healing [7].

Simultaneously with the regeneration phase, the remodeling phase will start. Due to the inflammatory process, the hematoma at the injured site will form a blood clot. The blood-derived fibrin and fibronectin will form early granulation tissue, which functions as an anchorage site for fibroblasts to invade [7, 24]. Fibroblasts are activated by the release of pro-fibrotic factors. One of these pro-fibrotic factors is transforming growth factor- β (TGF- β). These pro-fibrotic factors can be released by anti-inflammatory macrophages [19, 26]. Activated fibroblasts produce remodeling factors and extracellular matrix components (EMCs) such as collagen [26]. This gives the scar tissue its initial strength to cope with the forces that will be applied during the muscle healing [7, 24].

The new muscle fibers will form mini-MTJs between the regenerated muscle fibers and the scar tissue. Gradually, the scar tissue decreases in size and will bring the ends of the damaged muscle fibers at the injury site closer to each other [23, 24]. The muscle fibers will mature, and the rise of newly formed axons will stimulate the formation of new neuromuscular junctions (NMJs). The formation of new NMJs and thus re-innervation plays a key role in muscle healing and the recovery of muscle function [22, 24, 27].

17.4 Biological Treatments

In this paragraph, we will discuss the most important biological treatments used for acute muscle injuries. We will provide a summary of the composition, the working mechanism, and the results based on the evidence available for each biological treatment.

17.4.1 Platelet-Rich Plasma (PRP)

In the media, products with autologous blood concentrates have received increasing attention over the years. Platelet-derived products like platelet-rich plasma (PRP) have gained popularity among professional and recreational athletes [28, 29]. PRP is defined as a suspension of platelets in plasma with a higher concentration in comparison with the physiological concentration in blood. When platelets are activated, they release growth factors (GFs) that play a role in regenerative processes [30].

PRP is obtained from autologous peripheral blood out of patients. A centrifuge is used to separate the platelet-rich plasma from other blood components, which result in a higher concentration of platelets in a smaller volume of plasma [29]. The platelet levels in autologous concentrated plasma could increase up to eight-fold [31]. Multiple PRP products are used in different studies. Various autologous platelet-rich products are available. These products differ in preparation methods, biomolecular characteristics, and composition of cellular components, such as platelets, growth factors, cytokines, red blood cells, and leukocytes. Due to the sample variability, the interpretation of the effect of PRP is difficult [30, 32].

The rationale for the use of PRP for muscle injuries is that growth factors such as transforming growth factor- β (TGF- β), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-I, IGF-II), fibroblast growth factor (FGF), epidermal growth factor, vascular endothelial growth factor (VEGF), and endothelial cell growth factor may improve tissue recovery. These growth factors may enhance the healing of tissue and improve angiogenesis, which could stimulate the healing process [29].

Multiple randomized controlled trials (RCTs) have been conducted to examine the effect of PRP on muscle injuries. The hamstrings are the most frequently studied muscle group for the effect of PRP. One RCT studied the effect of PRP for gastrocnemius and rectus femoris injuries [33]. Most studies showed no superiority of PRP in treating muscle injuries on the time to

return to pre-injury activities [34]. One RCT found a shortened time (4 days) to return to play for patients treated with PRP in hamstring muscle injuries in comparison with the control group with patients that did not receive an injection [35]. This study is at risk of bias due to the lack of presence of a placebo group, and no effect was found on the re-injury rate. In the placebo-controlled studies, no significant effect was found. A meta-analysis showed no superiority of PRP over placebo injections in hamstring injuries [34]. In one study with rats, the muscle force and the size of regenerating muscle fibers were adversely affected by the use of PRP injections as an addition to active rehabilitation [36].

In conclusion, given the lack of high-level evidence to support the efficacy of the use of PRP injections and the potential negative effect in an animal study, we do not recommend the use of PRP injections as a treatment for acute muscle injuries.

17.4.2 Actovegin

Actovegin is a drug that is used as an injection therapy for muscle injuries. Actovegin is a deproteinized hemodialysate of ultrafiltered calf serum from animals under 8 months of age. A recent in vitro study suggested that Actovegin could improve the intrinsic mitochondrial respiratory capacity in injured human skeletal muscle fibers [37]. Still, the exact working mechanism of Actovegin is unknown.

One pilot study with 11 football players diagnosed with hamstring injuries described a reduction of 8 days in return to playtime after intramuscular injections with Actovegin. These injections were an addition to a specific rehabilitation protocol for hamstring injuries. The control group consisted of patients following the specific rehabilitation protocol [38]. However, there is a high risk of bias, as there was no randomization, no blinding, and no placebo control group. Currently, there is insufficient evidence regarding its efficacy and safety profile to support the use of Actovegin for (acute) muscle injuries.

17.4.3 Traumeel

Traumeel is a fixed combination of diluted plant and mineral extracts that are currently used to treat acute muscle injuries. Traumeel has an anti-inflammatory effect because of the activity of various components that seize on different phases of the inflammatory response [39]. In vitro studies found that the systemic interleukin-6 production decreases and edema reduces, off-setting an unregulated inflammatory response. Furthermore, Traumeel inhibited the secretion of the pro-inflammatory mediators interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α), and interleukin-8 (IL-8). This suggests that Traumeel may have the potential to stabilize immune cells [39, 40].

Until now, no clinical trials are performed to examine the efficacy of the use of Traumeel in treating acute muscle injuries. Therefore, the level of scientific evidence is considered as low [39]. In conclusion, there is no scientific evidence that supports the use of Traumeel as treatment for acute muscle injuries.

17.4.4 Stem Cell Therapy

Stem cells are undifferentiated cells that can divide, under activation of specific stimuli, into an identical stem cell and a cell that can contribute to growth or regeneration. This ability of stem cells is an interesting characteristic regarding the use of stem cells as treatment for muscle injuries [41].

Research has shown the presence of several stem cell populations in skeletal muscles. Muscle-derived stem cells (MDSCs), which possibly represent satellite cell predecessors, have the ability to differentiate into cells of the myogenic lineage. The MDSCs are relatively easy to harvest and can express growth factors or anti-fibrotic molecules, like decorin, by genetic modification [42–44]. As mentioned before, these cells can theoretically contribute in the regeneration phase in muscle healing.

The therapeutic use of stem cells for muscle injury could be an interesting approach, but for now the literature to support use of stem cells is

mainly focused on degenerative muscle disorders. The effect of MDSC transplantation on acute muscle injuries is studied in two studies utilizing murine contusion injury models [45, 46]. The use of intramuscular transplantation of MDSCs in mice yielded better angiogenesis and a significantly higher number of regenerative muscle fibers with a larger diameter at the fourth day post-injury in comparison with the control group or transplantations at other points in time. The MDSCs also significantly decreased fibrosis compared to the control group. When the MDSCs were transplanted during the inflammatory phase in muscle healing, a stimulation of fibrosis development occurs due to the differentiation of MDSCs in fibroblasts by the high expression of TGF- β 1 [45]. These results from animal studies cannot directly be translated to humans. Thus, research in humans should be conducted.

Due to the potential tumorigenicity, there are concerns on the application of stem cell transplantation. Therefore, it is necessary to evaluate the safety of the use of stem cell transplantation as treatment for acute muscle injuries in humans.

Tissue engineering is a concept with potential for treating muscle injuries in the future. The goal of tissue engineering is to design a matrix where stem cells, such as MDSCs, will differentiate into the required tissue through the activation of signaling molecules [47].

In conclusion, in murine studies the use of stem cells provided interesting findings, but the evidence advocating the use of stem cells as treatment for muscle injuries in humans is not available. Further development and evaluation of the potential concepts are needed to provide a deliberate advice on the (intramuscular) use of stem cells in humans. Accordingly, we do not advocate the use of stem cells in muscle injuries, because of the unidentified (long-term) efficacy and safety of its use in humans.

17.4.5 Anti-Fibrotic Therapy

As mentioned before, the formation of scar tissue in muscle injuries leads to fibrosis in the affected muscle and is part of healing process in muscles.

An overstimulation of scar tissue development may lead to disproportionate accumulation of fibrosis. Fibrosis can restrict the formation and re-innervation of new muscle fibers at the injured site because it may function as a mechanical barrier [7]. This could inhibit the recovery of the injured muscle tissue and muscle function [26, 48, 49].

TGF- β 1 plays a key role in formation of scar tissue by the activation of the fibrotic cascades [26, 49, 50]. With this in mind, anti-fibrotic therapies are mainly focused on the pathway of TGF- β 1 to enhance muscle healing [49].

The most pro-fibrotic growth factor identified in the literature is TGF- β 1. In the pathway of TGF- β 1, ligand binding activates the phosphorylation of receptor-regulated SMADs (R-SMADs), such as SMAD2 and SMAD3. Subsequently, the R-SMADs bind to the common mediator SMAD (SMAD 4). This activates the transcription of collagen by the translocation of the nucleus. SMAD7 suppresses the collagen transcription [51]. To inhibit to working mechanism of TGF- β 1, the anti-fibrotic therapies will aim on one of the upper mentioned steps in its pathway.

The various anti-fibrotic therapies described in the literature will be discussed.

17.4.5.1 Decorin

Decorin is a human proteoglycan serving as an anti-fibrotic agent and prevents TGF- β 1 action by binding on its receptor [48, 52]. In one murine study, which used direct injections of decorin into skeletal muscle, a significant decrease in fibrosis and a significant increase in the amount of regenerating muscle fibers were described. The comparison was made with skeletal muscle of mice treated with a direct injection with saline [48]. Although a significant improvement in muscle healing was observed, a large amount of decorin was required to enhance healing process in a very small mouse muscle. This, in combination with the unknown safety of the use of decorin agents on human beings, may limit the use of direct injections with decorin as treatment for muscle injuries in the future.

17.4.5.2 Suramin

Suramin was originally designed as an anti-parasitic drug, but suramin also has an anti-fibrotic function by competitively binding the receptor of TGF- β 1. Therefore, it inhibits the TGF- β 1 pathway [50]. The anti-proliferative effect on fibroblasts is described in *in vitro* studies, and in murine models, it is shown that suramin enhances muscle healing and reduces the formation of connective scar tissue [50, 53]. Comparable to the use of decorin, the effects and the safety of the use of suramin in human beings are unknown. Therefore, more research should be done to provide a clear recommendation for the use of suramin.

17.4.5.3 Losartan

Losartan is an antihypertensive medication and has a well-tolerated profile of side effects. It works as an angiotensin-II receptor blocker. Angiotensin-II induces the formation of collagen type I via the TGF- β pathway that is mediated by the angiotensin-II type 1 (AT1) receptor. Losartan reduces fibrosis through upregulation of SMAD7, which inhibits the activation of the earlier mentioned R-SMADs [46]. Another effect of the use of losartan is the increase in follistatin at the site of injury. Follistatin is a secreted protein and is able to neutralize the actions of the TGF- β superfamily proteins and stimulates the satellite cell proliferation [46]. These effects of losartan are shown in murine models, where oral use of losartan reduced the amount of fibrosis and enhanced muscle healing [54, 55]. The dosage of losartan used in mice was an equivalent of the dosage used for hypertension in human beings and was proven to be effective [55]. These results were also found in studies in which losartan was used as an additional therapy to PRP [56] and the use of stem cells [46, 57].

Losartan tablets are generally used as antihypertensive therapy in human beings. With the positive effects on muscle healing in mice, the use of losartan could be a promising therapy in muscle healing in human beings. However, the use of losartan should be examined in human skeletal muscle before incorporating losartan as a treatment for muscle injury.

17.4.5.4 Interferon- γ

The working mechanism of interferon- γ on muscle healing is supposedly through inducing the expression of SMAD7. This inhibits the TGF- β 1 pathway and thus the formation of fibrosis. A murine study found a decrease in the amount of fibrosis, an increase in muscle fibers, and an improved muscle strength [58].

Despite the proven effect of the use of interferon- γ as treatment for acute muscle healing by blocking the TGF- β 1 pathway in murine models, the effects on human beings are unknown. Therefore, the efficacy and safety of interferon- γ should be evaluated in human beings before it can be integrated as treatment for acute muscle injury.

17.4.6 Safety of Intramuscular Injections

Intramuscular injection may have side effects that should be considered before it is applied in clinical practice. The myotoxic effects are evaluated in a systematic review that was performed in 2014 [59]. Evidence was found for myotoxicity of corticosteroids, local anesthetics, and nonsteroidal anti-inflammatory drugs (NSAIDs). For PRP, the evidence found for myotoxicity was ambiguous. One study found necrosis, edema, increase in inflammatory cells, and fibrosis after intramuscular injections of PRP, which were not reported in the control group. Other studies reported increased formation of muscle fibers, decrease in necrosis, and granulomatous tissue in muscle injected with PRP when compared to the control group.

For the intramuscular injections of Actovegin or Traumeel as treatment for acute muscle injuries, there is no evidence available on the myotoxicity. Due to the lack of high-level evidence on the efficacy of the use of these potential treatments in muscle injuries, more evidence is required to consider these therapies as a useful therapy in human beings.

17.4.7 Conclusion

In conclusion, multiple biological treatments for acute muscle injury are discussed. The knowledge on mechanisms of accelerating muscle tissue healing is described in the present chapter. To improve the standard of treating athletes with muscle injuries to achieve their full potential, high-quality evidence on the efficacy and the safety of these treatments should be assembled before incorporating these options into the standard of care for acute muscle injury.

As various treatments are promising, additional studies should be performed to provide this evidence. For now, the use of PRP, Actovegin, Traumeel, stem cell therapy, or anti-fibrotic agents are not advised as treatment for acute muscle injury.

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