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Stem Cells for the Treatment of Skeletal Muscle Injury

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Abstract

Skeletal muscle injuries are extremely common, accounting for up to 35-55% of all sports injuries and quite possibly impacting all musculoskeletal traumas. These injuries result in the formation of fibrosis that may lead to development of painful contractures, increases their risk for repeat injuries, and limits their ability to return to a baseline or pre-injury level of function. The development of successful therapies for these injuries must consider the pathophysiology of these musculoskeletal conditions. We discuss the direct use of muscle-derived stem cells and some key cell population dynamics, as well as the use of clinically applicable modalities which may enhance the local supply of stem cells to the zone of injury by promoting angiogenesis.

Keywords or phrases

Sports injury; stem cells; tissue engineering; fibrosis; regeneration; skeletal muscle

Introduction

Skeletal muscle injury can result from a variety of mechanisms, including contusion, strain, laceration, or a combination of these mechanisms.¹⁻⁵ It is also possible for skeletal muscle injury to result from indirect sequelae of over-exertion or direct injury, such as via ischemia and neurologic impairment secondary to exercise-induced or traumatic compartment syndromes.⁶⁻¹⁵ These injuries are extremely common, accounting for up to 35-55% of all sports injuries and quite possibly impacting all musculoskeletal traumas.¹⁶⁻¹⁸ The associated morbidity is considerable, as these injuries portend professional and recreational athletes to develop painful contractures and muscle atrophy, require prolonged recovery periods, increase the risk for recurrent injury, and in some cases limit patients' abilities to return to baseline or

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pre-injury levels of activity.^{1, 6, 19} Accordingly, significant efforts are being made to improve the current treatment of skeletal muscle trauma.

Currently, the treatment of these injuries by and large consists of rest, ice, compression, and elevation (RICE), although other advocated treatments include the local application of heat, immobilization, and passive range of motion exercises, as well as non-steroidal anti-inflammatory drugs (NSAIDs), intramuscular corticosteroids, and, in some cases, surgery.¹⁻⁴ In many instances, however, these therapies remain sub-optimal. During the past decade, there have been sophisticated advances in rehabilitation, biomechanics, cell therapies, and tissue engineering with the goal of enhancing current therapies. As research in cell therapy and tissue engineering has progressed, it is clear that successful therapies must be based on an understanding of the basic pathophysiology of skeletal muscle injury.

Pathophysiology of Skeletal Muscle Injury

The pathophysiology of skeletal muscle injury is characterized by a sequence of events consisting of degeneration, inflammation, myofiber regeneration, and the formation of fibrotic scar tissue, as described below in detail and illustrated in Figure 1.

Degeneration and Inflammation

Immediately following injury, there is a phase of myofiber degeneration that is initiated by the release of proteases into the tissue stroma; these proteases autodigest myofibers and thereby release tissue debris along the zone of injury.²⁰ Within the time frame that this occurs, there is a chemotaxis of neutrophils and macrophages to this area at which point the local debris is phagocytosed and processed by macrophages to induce a local inflammatory response.²¹⁻²⁴ Although it appears that macrophages may in part be a culprit by initiating an inflammatory response, some studies indicate that these cells also secrete various growth factors that directly contribute to tissue regeneration. Additionally, macrophages stimulate the paracrine release of cytokines and other chemotactic factors by T-cells that may locally recruit progenitor and satellite cells with the capacity for muscle regeneration.²⁵⁻³¹ Some of the critical cytokines that orchestrate this local response include interleukin [IL]-1, -6, and -8, as well as insulin growth factor [IGF]-1.

It is clear from this initial sequence of events, then, that the inflammatory response may be conducive to the repair of skeletal muscle after injury. In the event that this event is blunted, such as through the use of NSAIDs or intramuscular corticosteroid injections, the tangible clinical benefits of also blunting the classic inflammatory symptoms of pain (*dolor*), heat (*calor*), erythema (*rubor*), and swelling (*tumor*) must be weighed against the cost of potentially delaying and reducing the extent of tissue healing that may be mediated by infiltrating progenitor cells. Some evidence from animal studies suggests that the blocking the cyclooxygenase-2 pathway with prostaglandin inhibitors such as NSAIDs does indeed compromise the histological quality of muscle repair and may even result in a functional compromise.^{32, 33} This may result in large part from the upregulation of transforming growth factor [TGF]- β 1, which inhibits myogenic precursor cells and augments fibrosis.³⁴

Regeneration

While the degeneration phase is transient, the subsequent phase of myofiber regeneration is the first step in the schematic for skeletal muscle injury that has a long-term effect. This phase may begin as early as 24 hrs following injury, as evidenced by the cytokine-mediated induction of local satellite cells that previously lie dormant between the basal lamina and sarcolemma; it is not until at least 3-5 days after injury, however, that the complete formation of new, centronucleated myofibers can be detected histologically.^{7, 27, 35}

It is likely that a crucial event in the regeneration phase is the differentiation of satellite cells into myotubules and myofibers. To date, these progenitor cells are perhaps the best characterized, and are often referred to as “muscle stem cells” given their predilection to the myogenic lineage. There are, however, other populations isolated from skeletal muscle, including muscle side-population cells, mesoangioblasts, pericytes, and post-natal muscle-derived stem cells (MDSCs) that appear to be multipotent.³⁶⁻⁴²

While the origin and relationship of these additional progenitor cells to muscle stem cells remains to be fully elucidated, emerging evidence suggests that MDSCs represent a highly purified and unique population of stem cells that have several advantages for regenerative medicine over other populations. These advantages by and large consists of their longer-term survival after implantation into skeletal muscle as compared to myoblasts; their remarkable multi-potency (Figure 2), and their potential for long term regeneration, with up to 300 population doublings (PDs) prior to becoming senescent as compared to PDs of 130-250 for embryonic stem cells.^{37, 40, 43-46} Additionally, MDSCs can be efficiently transduced with antifibrotic and regenerative factors that may enhance skeletal muscle healing.^{44, 47, 48}

Since the discovery of MDSCs, a topic of interest has been their origin. Recently, there is convincing evidence that these cells are likely derived from the vascular endothelium.^{49, 50} Accordingly, a growing focus in research on skeletal muscle repair has not only involved finding ways to use MDSCs for repairing the zone of injury, but also to augment the local vascular supply to this site as a way to provide a steady source of these cells.^{42, 44, 50-52}

Fibrosis

Perhaps the greatest limitation for patients that results from the pathophysiology of skeletal muscle injury is the formation of dense fibrotic scar tissue. It is clear that fibrosis is induced by a deleterious rise in the cytokine Transforming Growth Factor (TGF)-B1 after injury.⁴⁶ In the presence of this cytokine, MDSCs and other myogenic cells differentiate into myofibroblasts that produce collagen type I, the major component of fibrotic tissue.^{8, 34, 45, 53} Ultimately, fibrosis can prevent patients from returning to their baseline function, in part by preventing the formation of new axons toward myofibers, and contributes to a decline in muscle contractility and range of motion.^{54, 55} The pain that results from fibrosis also is a limiting factor in the recovery of patients, both during rehabilitation and in the long-term.

While not currently used clinically in this capacity or at all, several agents that block TGF-B1 have proven to be remarkably antifibrotic, including gamma-interferon, suramin and decorin.⁵⁶⁻⁵⁸ Fortunately, the commercially-available diuretic, losartan, has also been shown to have a significant antifibrotic effect along the zone of skeletal muscle injury in Sprague Dewy rats.⁵⁹ While clinical trials with this medication are feasible, their use must be cautioned in settings of musculoskeletal traumas and athletic injuries, where patients may oftentimes be dehydrated and thereby be at increased risk for developing acute renal insufficiency.

Skeletal Muscle Engineering with Muscle Derived Stem Cells

The transplantation of stem cells into aberrant or injured tissue has long been a central goal of regenerative medicine and tissue engineering. The translation of basic science research on muscle repair with autologous MDSCs to the bedside has been spearheaded by preliminary trials to treat stress urinary incontinence. One trial has resulted in successful cases over a one-year period following the implantation of these cells to restore detrusor muscle function, with 5 out of 8 females reporting improvements, one achieving complete continence, and none sustaining any adverse outcomes.⁶⁰ Larger clinical trials using MDSCs to treat this disease entity are planned for the near future.

Presently, the majority of reports on research using stem cell therapies for muscle regeneration are limited to animal models. Successful results have been reported for Duchenne Muscular Dystrophy models, in which dystrophin can be restored following the systemic delivery of various stem cells.^{40, 61, 62} Perhaps more relevant to sports injuries, Kinnaird *et al* developed an ischemic model in which Balb/C mice underwent ligation of the femoral artery; compared to controls, mice that later received distal injections of marrow-derived stromal cells on the affected limb displayed significantly better perfusion and appearance, had a lower incidence of autoamputation, and developed less fibrosis and atrophy.⁶³

Although perhaps a more severe form of ischemia, this ischemic model may translate in part to the ischemia that may occur from an exercise-induced compartment syndrome, raising the possibility that the local injection of autologous stem cells early during the development of limb ischemia from such a mechanism is worthy of further investigation. Presently, although our laboratory is investigating the role of directly implanted MDSCs into contused skeletal muscle following contusion, we are unable to determine as of yet what the benefits to doing so may be. We hope that reports on this will follow in the near future.

Although the literature may often refer to MDSCs as a homogeneous population, these stem cells are quite heterogeneous, differing in how efficiently different populations regenerate skeletal muscle *in vivo*. Research on sex-related differences in the regeneration of skeletal muscle for Duchenne muscular dystrophy models shows that, regardless of the host's sex, female MDSCs are significantly superior to male MDSCs to regenerate and repair skeletal muscle.⁵² The use of MDSCs to repair bone and cartilage is also influenced by the gender of the cells and the host animals, with the male MDSCs displaying a superior regeneration index than their female counterpart.^{64, 65} This may in large part be due to different embryonic developmental patterns that occur in female and male embryogenesis. Deasy *et al* also found that in skeletal muscle experiments where donor MDSCs are sex-matched, female hosts are also superior, a finding that is supported by other studies suggesting that the hormonal milieu of the female host skeletal muscle is better-suited for donor cell transplantation.^{66, 67} The role of immune rejection does not appear to explain the lower regeneration index of male donor cells transplanted into female hosts, as these sex differences were also identified when using severe combined immunodeficient host mice. This confirms that these sex differences may indeed occur because females are superior donors and hosts for MDSC-mediated skeletal muscle regeneration. While further studies are necessary to show a similar phenomenon with human cells, future therapeutic advances on skeletal muscle healing with MDSCs can greatly benefit from these important gender differences observed with MDSC.

Skeletal Muscle Engineering with Angiogenic Modalities: Exercise and Neuromuscular Electrical Stimulation

Aside from directly implanting stem cells into skeletal muscle, significant attention now focuses on promoting angiogenesis to activate resident satellite cells and provide a long-lasting portal through which MDSCs can derive, ultimately to aid in skeletal muscle healing. With clear evidence that exercise promotes cardiac and skeletal muscle perfusion, several studies now show this is because muscle contraction, such as through voluntary exercise or neuromuscular electrical stimulation, induces the formation of new vessels and the expansion of existing vascular trees.⁶⁸⁻⁷⁰

Aside from promoting angiogenesis, several other mechanisms exist through which exercise can enhance healing. For instance, exercise increases the serum concentrations of matrix metalloproteinases (MMPs), which directly digest fibrotic scar tissue, regulate the secretion of pro-regenerative growth factors such as insulin-like growth factor, and may also mobilize stem cells.^{71, 72, 73, 74} Moreover, several studies show that exercise-induced hypoxia promoted skeletal muscle healing by elevating the circulating concentrations of hypoxia-induced factor,

stromal-cell derived factor, and erythropoietin, each of which mobilizes endothelial progenitor stem cells from the bone marrow to coordinate the neovascularization of hypoxic tissues.⁷⁵⁻⁸² In light of this information, there is certainly the potential for combining MMPs and stem cells for direct implantation, as well as MMPs with conservative means of promoting angiogenesis, such as voluntary exercise and, as will be discussed below, neuromuscular electrical stimulation.

Based on this information, it is possible that, at least for some instances of skeletal muscle injury, the more traditional therapy of rest may jeopardize an opportunity to locally recruit stem cells to the zone of injury. It is also possible that through controlled and monitored exercise regimens in appropriately selected patients, perhaps initiated prior to the completion or the regeneration phase of skeletal muscle injury, the activation and infiltration of stem cells to the zone of injury may increase and enhance regeneration. Further studies may be necessary to determine whether rest is deleterious to healing after injury, whether certain exercises are safe and clinically beneficial, and, if so, whether the timing of exercise rehabilitation relative to the onset of injury influences outcomes.

As with exercise, another modality that appears to promote angiogenesis and skeletal muscle healing after injury is neuromuscular electrical stimulation (NMES). While the data linking stem cell activation and recruitment to NMES is lacking, there is evidence that this modality promotes angiogenesis.^{70, 83} As with exercise, it appears that tissue hypoxia induced during NMES may play a role in promoting angiogenesis, although the exact mechanism requires further elucidation.⁸⁴ Our laboratory has demonstrated that amongst 9-week old male C57BL/10J mice, prophylactic and post-injury NMES significantly enhances the percent capillary area of the tibialis anterior (unpublished data.) Additionally, at 5 and 10 days after injury, the percentage regeneration significantly increases and the percentage fibrosis significantly decreases along the zone of injury in mice undergoing prophylactic electrical stimulation 3 times weekly for 2 weeks (unpublished data.) Among mice undergoing post-injury NMES, we had the same findings for fibrosis, but were only able to detect a significant increase in the percentage regeneration at 10 days after injury (unpublished data).

One reason for why prophylactic NMES may be superior to post-injury stimulation is that by promoting angiogenesis early on, the regenerative phase of skeletal muscle injury will begin to occur in the presence of more MDSCs derived from the vascular endothelium, as well as more infiltrating growth factors that activate dormant satellite cells. With post-injury stimulation, these cells and factors may come into the zone of injury beyond the optimal time window for tissue repair. While speculative on our part at the present time, this would be consistent with our proposition above that early exercise rehabilitation programs may be beneficial in some cases of skeletal muscle injury, although clinical studies are necessary to support this. This may also be similar to the process of fracture repair, in which relatively early fracture stabilization is oftentimes necessary to prevent the progression to fracture non-union.⁸⁵ Accordingly, more studies are required to better delineate the temporal relationship of skeletal muscle injury, NMES, and other therapeutic interventions such as the implantation of stem cells directly into the zone of injury to optimize skeletal muscle healing responses.

Summary

Amongst the most commonly prescribed treatments for skeletal muscle injuries are rest, ice application, compression, and elevation (RICE), as well as heat application and either immobilization or passive range of motion exercises. In many instances, however, these therapies remain sub-optimal.

Based on our current knowledge, the inflammatory response that follows injury promotes skeletal muscle regeneration, perhaps in part by locally recruiting stem cells via chemotaxis to the zone of injury. While the therapeutic administration of steroids and NSAIDs may prove symptomatic relief by combating inflammation, there is evidence that this blunts the regenerative response and may actually promote fibrosis.

Current research has linked angiogenesis to skeletal muscle healing, and indicates that blood vessels are likely the origin of MDSCs. As voluntary exercise and neuromuscular electrical stimulation both promote angiogenesis, it is possible that in appropriately selected patients, a feasible therapeutic alternative to rest and immobilization for skeletal muscle injury may consist of controlled and monitored exercise programs as well as NMES; future studies may need to determine if rest is deleterious to skeletal muscle healing, as well as which exercises are safe and clinically efficacious for various patterns and locations of injury. Similarly, a feasible alternative to current pharmacologic therapies may include losartan, although the use of this medication for treating skeletal muscle injury is not currently approved by the United States Food and Drug Administration.

In light of recent successful clinical trials on the direct implantation of MDSCs to treat urinary incontinence secondary to detrusor muscle dysfunction, the future of stem cell therapy for skeletal muscle injury may be closer than ever to translation into clinical studies. Such studies must continue to characterize and make use of the optimal MDSC populations, as well as examine MDSC transplantation in combination with pro-regenerative and antifibrotic agents such as MMPs and losartan.

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Figure 1. Skeletal muscle injury pathology

After injury, there is a degeneration phase followed by inflammation. This inflammatory response locally recruits progenitor cells to the zone of injury for muscle repair. The reparative phase can last up to 3 weeks, and is followed by a deleterious rise of TGF- β that induces fibrosis, which places patients at increased risk for recurrent injury, developing painful contractures, and requiring lengthy recovery periods from which there is often an incomplete return to baseline function.

Figure 2. Multipotency of Muscle-Derived Stem Cells

Our laboratory has previously isolated muscle derived stem cells from mice. These cells are remarkably pluripotent, and can undergo long term expansion. Accordingly, MDSCs are ideal for regenerative medicine.