REVIEW

Journal of Cachexia, Sarcopenia and Muscle (2019)
Published online in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/jcsm.12416

Cell therapy to improve regeneration of skeletal muscle injuries

Taimoor H. Qazi[1,2], Georg N. Duda[1,2,3], Melanie J. Ort[1,3], Carsten Perka[4], Sven Geissler[1,2†] & Tobias Winkler[1,2,4*†]

[1]*Julius Wolff Institute, Charité—Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany,* [2]*Berlin-Brandenburg Center for Regenerative Therapies, Charité—Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany,* [3]*Berlin-Brandenburg School for Regenerative Therapies, Charité—Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany,* [4]*Center for Musculoskeletal Surgery, Charité—Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany*

Abstract

Diseases that jeopardize the musculoskeletal system and cause chronic impairment are prevalent throughout the Western world. In Germany alone, ~1.8 million patients suffer from these diseases annually, and medical expenses have been reported to reach 34.2bn Euros. Although musculoskeletal disorders are seldom fatal, they compromise quality of life and diminish functional capacity. For example, musculoskeletal disorders incur an annual loss of over 0.8 million workforce years to the German economy. Among these diseases, traumatic skeletal muscle injuries are especially problematic because they can occur owing to a variety of causes and are very challenging to treat. In contrast to chronic muscle diseases such as dystrophy, sarcopenia, or cachexia, traumatic muscle injuries inflict damage to localized muscle groups. Although minor muscle trauma heals without severe consequences, no reliable clinical strategy exists to prevent excessive fibrosis or fatty degeneration, both of which occur after severe traumatic injury and contribute to muscle degeneration and dysfunction. Of the many proposed strategies, cell-based approaches have shown the most promising results in numerous pre-clinical studies and have demonstrated success in the handful of clinical trials performed so far. A number of myogenic and non-myogenic cell types benefit muscle healing, either by directly participating in new tissue formation or by stimulating the endogenous processes of muscle repair. These cell types operate via distinct modes of action, and they demonstrate varying levels of feasibility for muscle regeneration depending, to an extent, on the muscle injury model used. While in some models the injury naturally resolves over time, other models have been developed to recapitulate the peculiarities of real-life injuries and therefore mimic the structural and functional impairment observed in humans. Existing limitations of cell therapy approaches include issues related to autologous harvesting, expansion and sorting protocols, optimal dosage, and viability after transplantation. Several clinical trials have been performed to treat skeletal muscle injuries using myogenic progenitor cells or multipotent stromal cells, with promising outcomes. Recent improvements in our understanding of cell behaviour and the mechanistic basis for their modes of action have led to a new paradigm in cell therapies where physical, chemical, and signalling cues presented through biomaterials can instruct cells and enhance their regenerative capacity. Altogether, these studies and experiences provide a positive outlook on future opportunities towards innovative cell-based solutions for treating traumatic muscle injuries—a so far unmet clinical need.

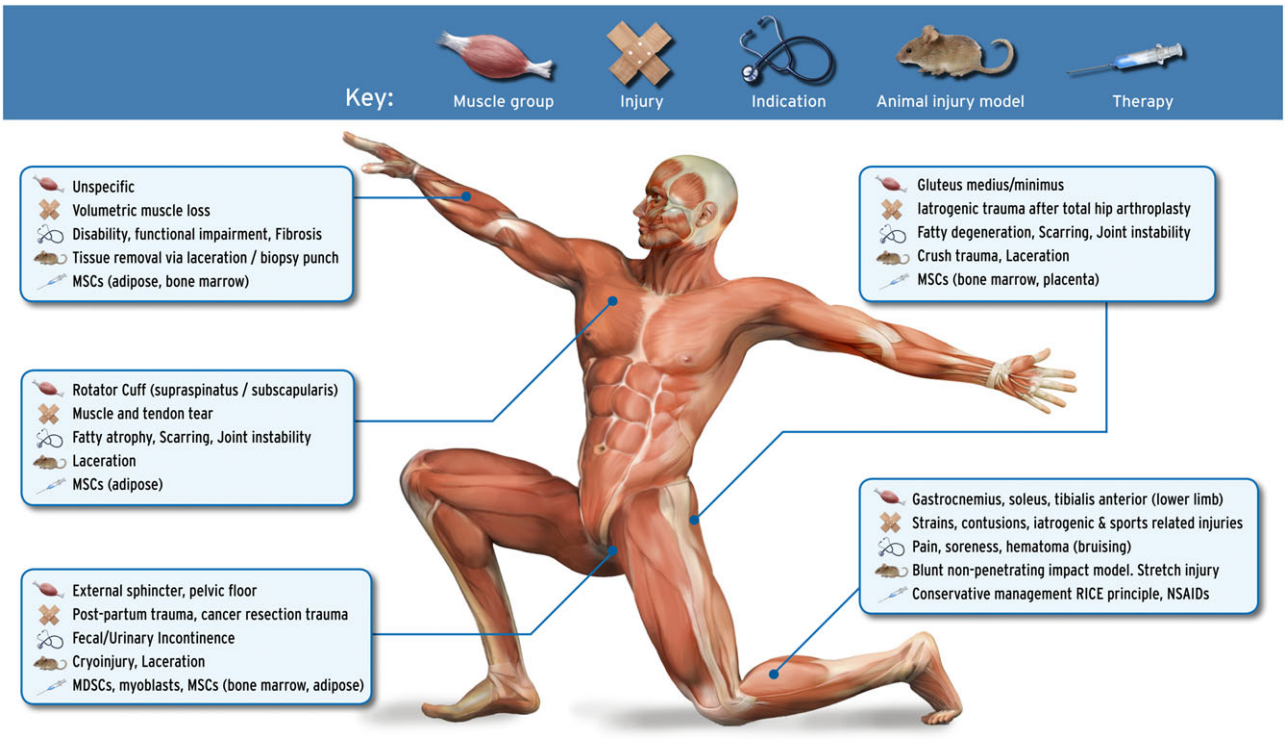
Keywords Muscle trauma; Stem cell therapy; Clinical translation; Injury models; Tissue engineering

Received: 3 August 2018; Accepted: 27 January 2019
**Correspondence to: Dr Tobias Winkler, Julius Wolff Institute, Charité—Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Augustenburger Platz 1, Charité Universitätsmedizin Berlin, 13353 Berlin, Germany. Tel: +49 (0)30 450-559084, Fax: +49 (0)30 450-559969, Email: tobias.winkler@charite.de*
†S. G. and T. W. contributed equally as senior authors of this work.

Introduction

Orthopaedics and traumatology departments in hospitals around the world are increasingly encountering patients suffering from traumatic skeletal muscle injuries. Skeletal muscles make up roughly 40–45% of the total human body mass and are essential to sustaining life. Their proper function permits mobility, joint stability, and postural maintenance, as well as breathing, metabolic control, thermoregulation, and energy storage.[1] Skeletal muscle injuries span a broad spectrum of causes, severities, and various treatment modalities (*Figure* 1). Sports-related injuries, for instance, in soccer players, typically occur in the lower limbs affecting the hamstring (37%), adductor (23%), quadriceps (19%), and calf (13%) muscles and range from minor strains and bruises to partial or complete muscle tears.[2] Strains can potentially be complicated to treat if a tear occurs at the myotendinous junction, as is often the case in injuries of the rotator cuff or lower limb muscles.[3] Muscle injuries comprise nearly a third (20–37%) of all injuries sustained by soccer players and as such represent a significant economic health care burden.[4] Military personnel having to keep up with a physically demanding and rigorous workload are especially prone to musculoskeletal injuries that require hospitalization. Furthermore, soldiers who are injured in combat often suffer from volumetric muscle loss and high-impact trauma that may lead to long-term immobilization, disability, or infection.[5] Post-partum damage and subsequent weakening of pelvic floor and external sphincter skeletal muscles can contribute to urinary and faecal incontinence, causing social anxiety and isolation. Incontinence affects $>$200 million people globally,[6,7] and places a significant economic burden ($16bn in the USA alone) on patients and national health care systems.[8,9] Patients undergoing surgical interventions such as tumour ablation, soft tissue reconstruction, or joint arthroplasty almost inevitably sustain iatrogenic muscle injuries.[10] Many of these debilitating conditions drastically impair muscle function, leading to intense physical pain, negatively affecting mental health, and compromising the quality of life of patients. Despite the high incidence rate, treatment of severely injured muscles and the restoration of their original structure and function remain an unmet clinical challenge.

Figure 1 Traumatic muscle injuries represent a heterogeneous spectrum of causes, severity, and intervention options. Whereas muscle groups in the lower extremities are more prone to sports-related strains, tears, and bruises, other injuries like iatrogenic trauma, contusions, and volumetric muscle loss can occur in any part of the body. A common feature of severe muscle injuries is the pathological observation of haematoma, muscle atrophy, fibrotic scar tissue, and fatty infiltration, with associated physical disabilities like functional impairment, limping, soreness, and pain. These pathological features have been replicated in small animal injury models using various approaches and techniques. Cell therapies using MSCs, myoblasts, or MDSCs have shown apparent benefit in pre-clinical settings, and some have been tested in human clinical trials with promising outcomes. Although few in number, these clinical studies demonstrate the potential to accelerate and significantly improve the healing of traumatic muscle injuries through autologous or allogeneic cell-based solutions.



DOI: 10.1002/jcsm.12416

In contrast to other tissues, injured skeletal muscles have an intrinsic capacity to adapt and initiate a synchronized biological response to prevent further muscle loss and eventually lead to regeneration. Minor injuries (e.g. muscle strains and contusions) heal spontaneously in healthy adults. External physical stimulation can support the intrinsic endogenous healing potential such as in sports medicine, where physicians employ training regimes and rehabilitation strategies to enable muscle repair and permit recovery in relatively short time periods (weeks to months). Although regimes such as cryotherapy, physical massage, and dedicated resting periods allow athletes to resume professional sport with restored muscle functionality, their efficacy is based largely on empirical evidence.[11] Similarly, exercise protocols targeting specific muscle groups in the pelvic region exist that help patients to partially overcome urinary and faecal incontinence.

Beyond a certain injury severity threshold, the endogenous process of muscle repair proves insufficient, leading to loss of contractile tissue, fatty degeneration, and fibrotic scar tissue formation, which can cause long-term deficits in muscle structure and strength. Severe muscle injuries do not just include complete muscle tears, volumetric muscle loss, or high-impact trauma. Iatrogenic muscle damage can also occur during various surgical interventions. Consequences for the affected patients are often detrimental, causing severe physical impairment that leaves the patient in extreme pain and discomfort.[12,13]

Thus, regeneration of severe muscle injuries is an unsolved medical need and is a topic of considerable scientific interest. Biomedical scientists in different areas of research have proposed various cellular and molecular approaches as potential therapeutic strategies. Many of these have shown beneficial effects in pre-clinical muscle injury models. However, translation to the clinic and successful demonstration of efficacy in humans has been lacking either owing to regulatory issues, appropriateness of the pre-clinical model, the selected patient cohorts, or low confidence in the therapeutic benefit. Consequently, this has limited the transfer of novel treatment options to the clinical and medical communities. More recent pre-clinical and some first clinical data give the impression that cell therapy might provide a treatment option with a relevant potential for reaching the clinical routine in treating skeletal muscle injuries. The promising outlook of this rapidly developing area of translational research has motivated the preparation of the current review.

Biological characteristics of muscle homeostasis, injury, adaptation, and regeneration

Skeletal muscle is a hierarchically organized tissue that consists of muscle fibres, a laminin-rich and collagen-rich extracellular matrix (ECM), and distinct cellular populations.[14,15] Satellite cells (SCs) are a rare population of muscle-specific progenitor cells (2–7% of all muscle cells) but play a central role in muscle maintenance and regeneration.[16,17] Under homeostatic conditions, SCs reside in a quiescent state between the sarcolemma and the basal lamina of myofibers and are characterized by the expression of the transcription factor paired box protein 7 (Pax7).[18,19] Injury causes activation of these cells, which then re-enter the cell cycle, proliferate, and subsequently differentiate into myoblasts. The myoblasts further extensively proliferate and migrate towards the site of injury where they undergo myogenic differentiation to fuse with new or existing myofibers. In parallel to producing myoblasts, SCs also self-renew via asymmetric cell division and maintain the stem cell pool in the skeletal muscle.[20] Recent reports have provided conclusive evidence that SCs are essential to the regeneration process,[21,22] but their optimal function is dependent on the properties of the microenvironmental niche. Muscle resident fibroblasts and fibro/adipogenic progenitor (FAPs) cells deposit the ECM that also acts as a conducive niche, allowing SCs and their progeny to participate in regeneration.

In skeletal muscles, a highly orchestrated series of biological processes make up the endogenous response to injury or trauma. These processes occur regardless of the type or severity of injury.

(1) The degenerative and inflammatory phase: This initial phase is characterized by rupture and necrosis of myofibers and surrounding blood vessels, leading to haematoma formation and triggering a pro-inflammatory injury response. Neutrophils are the first cells to invade the site of injury (typically within 2 h) where they enzymatically degrade muscle membranes and produce free radicals that target tissue debris for macrophage-mediated phagocytosis.[23]
(2) The repair and regeneration phase: Both pro-inflammatory M1 (CD68+/CD163−) and anti-inflammatory M2 (CD68−/CD163+) macrophages play important roles during this phase.[24,25] M1 macrophages remove debris, execute structural degradation, and secrete cytokines that stimulate the proliferation and migration of SCs.[26] A timely switch in the expression of signalling molecules triggers the polarization of M1 into M2 macrophages, which persist in the muscle for a number of days during which they inhibit the deposition of excessive ECM by FAPs, while stimulating the fusion of myoblasts into multinucleated myotubes.[27]
(3) The remodelling and maturation phase: The final phase involves the maturation of myotubes into functional, contractile myofibers, remodelling of the connective tissue ECM, and establishment of neuromuscular junctions.[28]

While this endogenous process can achieve complete regeneration after minor injury, the natural healing cascade is

DOI: 10.1002/jcsm.12416

unable to meet the demands created by severe trauma. This can at least partially be attributed to an impaired SC function and dysregulated FAP cell activity, leading to excessive ECM deposition and near-irreversible scarring.[29,30] Scar tissue hinders the fusion of newly formed and existing myofibers and negatively affects the muscle's contractile function. It is important to acknowledge that not all cases of skeletal muscle injuries exclusively occur in the muscle belly, but many also cause damage to the myotendinous junction (muscle–tendon interface) and the associated tendon. Regeneration after such injuries can be especially problematic because the tendon and muscle each have distinct characteristics including endogenous cell types, repair mechanisms, ECM compositions, and mechanical properties.[31]

Clinical options for treatment

Clinical options for the treatment of skeletal muscle injuries can be categorized into conservative management practices or invasive surgical procedures.[32] On clinical presentation, a number of factors need to be carefully considered to determine which treatment strategy to pursue. These include type and severity of injury, muscle groups affected, and general health status of the patient.

Conservative treatment

The RICE principle

Athletes who sustain minor muscle tears with associated pain during mobility are typically administered first aid following the RICE principle that consists of rest, ice, compression, and elevation. Although lacking a thorough scientific basis, the RICE principle aims to reduce blood flow through the injured muscle tissue, thereby reducing the size of the haematoma and preventing the extension of the actual trauma into adjacent muscle regions. Questions revolving around the duration of the immobilization and the right time point for the mobilization of the patient are still controversial. While a short period of immobilization or resting time immediately after injury allows the scar tissue to develop enough strength to withstand local contraction forces,[32] prolonged inactivity is known to lead to excessive fibrosis, compromising biomechanical tissue properties.[33] On the other hand, early mobilization of the patient and exertion of local contraction can increase the chances of secondary rupture at the site of injury.[34] A recent randomized, controlled trial suggests that a brief initial immobilization period (~2 days) followed by a gradual increase in mobilization support repair and recovery.[35]

Medication

Pharmaceutical approaches primarily address the initial pro-inflammatory response in the early phase of muscle healing. Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to treat patients with minor muscle damage and to alleviate post-traumatic pain.[36] However, experimental evidence suggests that an early and short-term use of such anti-inflammatory drugs only moderately reduces the extent of muscle damage,[37–39] while persistent use of NSAIDs negatively influences muscle regeneration and contractile function.[40] This could be due to the broad effects of these drugs on the immune system, potentially interfering with its central role in the progression of the healing process or final resolution of muscle injury.[41,42] Furthermore, a study by Rahusen *et al*. questioned the exclusivity of using NSAIDs because they did not demonstrate a superior effect than do low-cost painkillers and analgesics.[43] Despite lack of supportive evidence from human clinical trials and known negative side effects,[44] NSAIDs continue to be a popular medication among athletes, and further investigation is needed to elucidate the local effects of these drugs on the healing cascade of skeletal muscles.[45]

Surgical treatment

Surgical intervention is normally recommended for patients suffering from volumetric muscle loss that requires soft tissue reconstruction.[46–48] The current clinical standard for treating volumetric defects is the replacement of lost tissue by muscle flaps.[49] This involves the autologous transfer and engraftment of healthy, innervated, and vascularized muscle tissue either from the direct vicinity of the injured area or from a distant site of the body as a free flap. The biggest drawback of the muscle flap procedure is donor site morbidity that further prolongs patient recovery time.[50]

A ubiquitous cause of severe muscle trauma is iatrogenic injury. These injuries occur during almost any invasive surgical procedure including aggressive tumour ablations and joint revision arthroplasties. Among orthopaedic procedures, total hip arthroplasty (THA) is known to cause severe muscle damage mainly due to lacerations or crush trauma, procedures that are necessary to gain access to the hip joint.[51] The frequency of joint replacement surgeries has reached many hundreds of thousands per year in accordance with the desire of an ageing population to improve their quality of life.[52,53] An overwhelmingly large proportion of these patients shows signs of impaired muscle functionality, fatty degeneration, and scar tissue formation in their yearly follow-ups.[54,55] The gluteus minimus, gluteus medius, the abductor, and adductor muscle groups are affected the most by trauma due to THA.[51,56] Gradual muscle degeneration after the surgical procedure not only causes pain and irritation but also predisposes the patient to limping, hip dislocation, and re-injury.[57]

DOI: 10.1002/jcsm.12416

It is pertinent to note that these iatrogenic injuries and consequential pathologies even occur after what is commonly considered as a successful primary or revision THA procedure. In case of surgical complications such as an infected joint or prosthetic component failure, a last resort is sometimes muscle flap transfer, which despite its invasiveness is unable to restore the original function of the joint.[58]

Advances in treatment of local muscle injuries

Muscle injury models

Although a number of small animal injury models have been reported in literature to evaluate muscle regeneration, there is a large variability in the choice of the muscle group and the mode of injury.[59] The most commonly used routes of inducing injury are myotoxin injection (e.g. cardiotoxin),[60] freeze/cryoinjury (liquid nitrogen-cooled metal rod),[61] chemical injury (e.g. barium chloride),[62] and physical injury (e.g. crush, laceration, denervation, and ischaemia).[63] Hardy and colleagues published a study comparing four commonly used injury models in mice. This study revealed that despite similar initial necrosis and complete regeneration 1 month post-injury in all models, the cellular composition, revascularization, and immune profile varied significantly among the groups over the time course of regeneration.[64] The fact that the injury was resolved without any intervention also indicates that those may not be appropriate models to study severe clinically relevant injuries. Surprisingly, it seems that in most pre-clinical work, the injury model has been chosen on the basis of convenience, or experience with certain protocols, and not necessarily because the model mimics a human pathology. For example, cardiotoxins are usually derived from snake venom. Their use to induce tissue injury mimics nothing but a snakebite, which as such constitutes its own pathomechanism and is a rather rare event in the Western world. However, cardiotoxins are used in the vast majority of *in vivo* muscle regeneration studies. Moreover, the arbitrary use of injury models in different laboratories and research groups leads to different observations and outcomes, which makes it difficult to compare results and derive conclusions about the efficacy of a particular therapy.

Despite the high prevalence of studies that use chemical or toxin injuries, efforts have been made to develop patient-relevant injury models that mimic the pathophysiology of tissue damage observed clinically. Athletes commonly endure strains and contusions to their lower limb muscles. Strain injuries usually occur owing to excessive tensile stretching and lead to shear rupture, small haematoma formation, and damage to both the muscle and its associated tendon. It is replicated in animal models typically by electrical stimulation of the tissue or via tissue elongation by pulling on the tendon/muscle using weights.[65] In contrast, contusions occur owing to a rapid and high-impact compressive force, which causes haematoma formation in the muscle tissue. This limits mobility and causes pain and soreness to the patient. The blunt, non-penetrating impact model has been widely used to mimic contusion injuries and involves the dropping of a metallic object (usually spherical or cylindrical) of a defined mass from a certain height guided by a hollow tube directly onto the exposed muscle tissue.[66,67] Laceration is another type of muscle injury that is conveniently replicated in animal models.[68,69] A laceration injury occurs owing to a direct, penetrating trauma to the tissue by a sharp object and is usually associated with accidents, collisions, and military injuries.[70] This injury essentially splits the muscle tissue, causing damage to myofibers, blood vessels, nerves, and connective tissue and is accompanied by a large haematoma formation and substantial fibrosis. Clinical situations involving severe trauma associated with surgical interventions often lead to irreversible fatty degeneration and fibrosis in the muscle, and any new therapy for this indication must use a model that mimics this situation. The crush trauma model was developed to mimic the characteristics (no spontaneous regeneration, gradual decline in muscle function, and persistent fibrosis) of clinically encountered iatrogenic muscle injuries.[71] Fatty degeneration is also typically observed in patients with rotator cuff injuries, which affects the muscles that surround and dynamically stabilize the shoulder joint.[72,73] Surgical repair of such injury is advised, even though it is increasingly acknowledged that this procedure not only is unable to restore normal function and strength in most cases[74] but also causes further damage to the muscle fibres.[75]

Molecular therapies

Molecular approaches to treat skeletal muscle injuries predominantly consist of growth factor therapy. Growth factors secreted by cells or liberated from their sequestered state from the damaged ECM play important signalling roles during the endogenous phases of healing after trauma.[76] In acute injuries, weak or muted growth factor signalling can either disrupt or aggravate downstream cellular events that direct the muscle tissue away from regeneration and towards scarring.[77] Several growth factors have been delivered intramuscularly, systemically, or via biomaterial carriers to aid muscle healing. These include among others, hepatocyte growth factor (HGF), insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), and fibroblast growth factor.[78–80] HGF is known to stimulate the activation of quiescent SCs,[81,82] promoting their proliferation and migration while inhibiting their premature differentiation.[83] Grasman *et al*. reported that the rapid but prolonged release of HGF from a fibrin-based biomaterial improves muscle contractility

DOI: 10.1002/jcsm.12416

and remodelling in a volumetric muscle loss injury model.[84] Some muscle injuries such as contusions and lacerations may also lead to ischaemia in parts of the tissue. Therefore, the delivery of angiogenic factors such as VEGF has also been investigated. Shvartsman and colleagues reported that VEGF promotes innervation and re-perfusion of ischaemic limbs via nerve growth factor signalling.[85] Using biomaterials to provide temporal release of VEGF can retain the bioactivity, ensure targeted delivery, and enhance the potency of the growth factor.[86] IGF-1 is perhaps the most relevant growth factor that acts directly on muscle cells to stimulate hypertrophy *in vivo* and myogenic differentiation *in vitro*.[87,88] Owing to the complexity of the healing cascade and its precise spatiotemporal regulation, it is unlikely that a single molecular factor is able to rescue a tissue from degeneration. Thus, the timed delivery of multiple growth factors could be required.[89] In this regard, Borselli *et al*. have shown that the simultaneous delivery of myogenic (IGF-1) and angiogenic growth factors (VEGF) can promote functional regeneration of ischaemic muscle tissues.[90] However, it is pertinent to note that while this combination of growth factors demonstrated significant benefit in ischaemia, no apparent benefit was observed with the same dose of growth factors in a crush muscle injury model.[91] This underlines the importance of choosing the animal model according to the human pathology and thus to consider the distinct complexities among the different types of muscle injuries. In fact, very few studies have investigated the efficacy of growth factor delivery in acute and severe models of muscle injury or have reported negative results, which limits the potential clinical application of these approaches. A further drawback is that growth factors have short half-lives and are rapidly cleared by the circulatory system. These issues can be circumvented by using sophisticated biomaterials that enable tunable release of several growth factors, but receiving the relevant regulatory approval for the biomaterial is an additional factor that may limit growth factor-based approaches from clinical translation.[92]

Growth factors can be potent mediators of biological processes that constitute tissue regeneration; their spatiotemporal release via biomaterials is challenging and yet to be optimized. However, in recent years, cells have been used as vehicles to deliver growth factors, either by genetically overexpressing one or several proteins or by employing cells that naturally secrete numerous cytokines and growth factors in response to environmental cues. These novel approaches have further propelled the field of cell therapy.

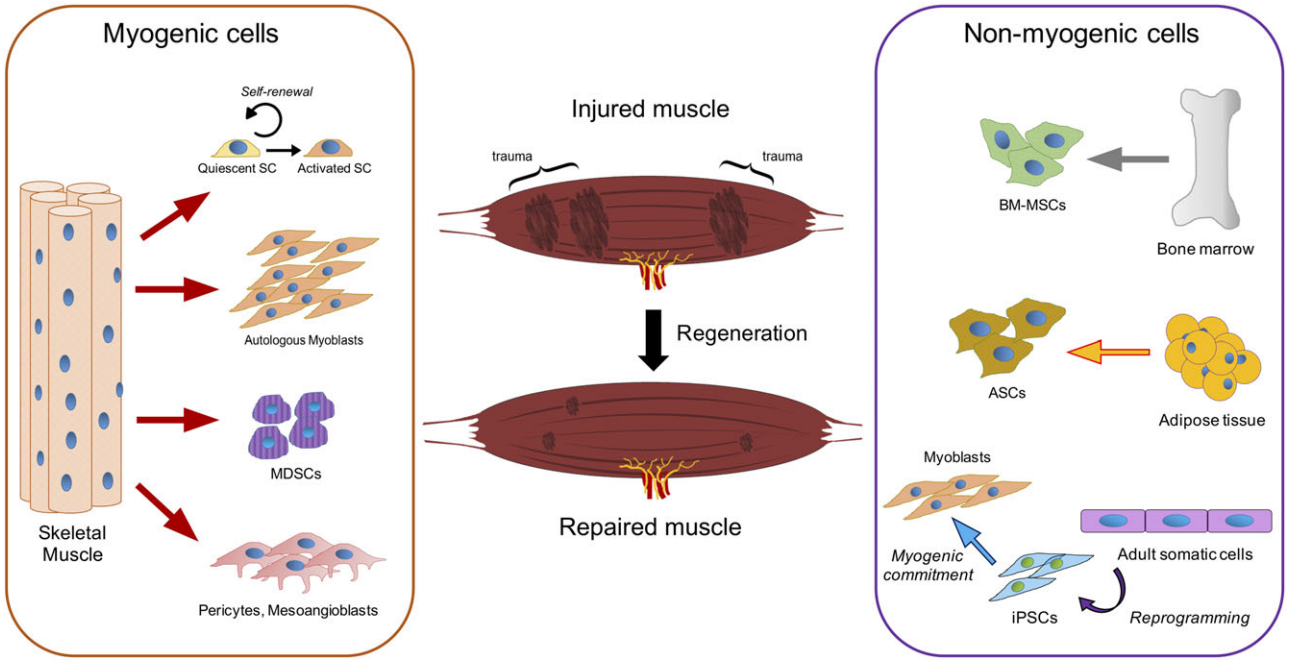
Cellular therapies

A central goal of all approaches for muscle regeneration is to re-establish the structural integrity and functionality of the tissue. This includes stimulating the formation of contractile muscle fibres, re-populating the SC niche, and promoting vascularization of the injured area. In this context, soluble cues such as growth factors play only a supporting role to the various cellular populations that are of central importance to muscle regeneration. Several cellular candidates with myogenic or non-myogenic origins have been proposed for skeletal muscle regeneration, and their transplantation has therefore been a widely investigated therapeutic strategy (*Figure* 2).

Cells with myogenic origin

Cells utilized for regenerating skeletal muscle either directly participate in the formation of new myofibers or indirectly support this process by signalling mechanisms. SCs have been widely used owing to their central role in muscle endogenous repair, regeneration, and commitment to the myogenic lineage. As already mentioned, SCs make up only a small proportion of the muscle cell population (2–7%) and can be isolated using defined surface markers in combination with fluorescent activated cell sorting.[93,94] Once transplanted, SCs can give rise to a large number of progeny, which can form myofibers and engraft into the defective region.[95] SC transplantation has been shown to improve contractile function and repopulate the SC niche in host muscles.[96] A further advantage of using SCs is the relatively low dose required to achieve a desirable regenerative response. Collins *et al*. showed that the transplantation of as few as seven functional SCs can directly contribute to the formation of a hundred multinucleated myofibers, while also undergoing self-renewal to sustain the endogenous stem cell pool.[97] Sacco and colleagues further strengthened this observation by reporting that a single transplanted SC can undergo vigorous proliferation, contribute to myofiber formation, and populate the *in vivo* niche.[98] This finding has been encouraging for a number of reasons. First, freshly isolated SCs mount a more potent response *in vivo* than do culture-expanded cells.[99] Second, *ex vivo* expansion of SCs on non-physiologically stiff and inert plastic flasks causes spontaneous differentiation of Pax7+ SCs into committed myoblasts that have a lower regenerative potential.[100] However, even with improved sorting techniques, heterogeneity in regenerative potential and proliferation kinetics exists within the SC population with reports of only a subpopulation exhibiting stem cell-like properties.[101,97] Despite experimental evidence of SC activation and subsequent repair of injured muscles after contusion injuries,[102] there has been a lack of studies involving SC transplantation in clinically relevant muscle injury models. Rossi *et al*. reported a muscle laceration injury model that benefited from the transplantation of culture-expanded SCs encapsulated in a hydrogel.[103] The dearth of studies with SCs may be explained by the fact that autologous transplantation is not possible owing to tissue harvesting and cell isolation protocols, making it an unfeasible option for clinical translation in patients. An alternative approach could be the

Figure 2 Cell therapy for muscle regeneration has involved the use of several cell types with myogenic or non-myogenic origins. Regeneration of injured muscle ultimately requires the formation of contractile muscle fibres, which makes myogenic cells obvious candidates for cell therapy. These include SCs, which can be isolated in a quiescent or activated state and can simultaneously replenish the host tissue niche and give rise to committed progeny. Myoblasts, already committed to differentiating down the myogenic lineage, are another cell type that has been isolated from autologous muscle tissues and re-applied to injured sites after *ex vivo* expansion. Muscle-derived stem cells (MDSCs) can be useful if the injury affects the myotendinous junction and the muscle associated tendon, as these cells are multipotent and can potentially differentiate into fibroblasts or tenocytes. Pericytes and mesoangioblasts are associated with vasculature running through the muscle tissue and can differentiate into muscle fibres as well as act via paracrine mechanisms. Cells with a non-myogenic origin can be equally beneficial for muscle regeneration. Mesenchymal stromal cells from bone marrow (BM-MSCs) or adipose tissue (ASCs) can stimulate regeneration via paracrine signalling and/or immune modulation, whereas induced pluripotent stem cell (iPSC) technology has enabled the reprogramming of adult somatic cells and their subsequent commitment towards the myogenic lineage.



generation of myogenic progenitor cells from other types of adult cells via genetic approaches. In a recent study, Bar-Nur *et al*. reported a robust approach for the direct conversion of adult mouse fibroblasts to myogenic progenitor cells via ectopic overexpression of the transcription factor MyoD.[104]

Satellite cells are committed to the myogenic lineage, but other populations of muscle resident cells with multi-lineage potential have been identified that can contribute to muscle repair.[105] Among these are muscle-derived stem cells (MDSCs) and CD133+ mesoangioblasts that closely resemble pericytes.[106,107] MDSCs are not terminally committed to the myogenic lineage and can differentiate into the mesodermal lineages as evidenced by their myogenic, osteogenic, and chondrogenic potential.[108–110] Transplantation of MDSCs into severe muscle injury models has improved muscle repair and reduced fibrosis,[111,110,112] with some of the observed effects being attributed to their secretome.[113,114] While MDSCs have demonstrated clear benefit in models of muscular dystrophy, there is still a lack of studies using acute muscle injury models. Unless these are performed in sizeable numbers, it is difficult to regard any cell type as a reliable and potent source for different muscle injuries. For example, MDSCs have stimulated muscle regeneration in hindlimb muscles[115] yet have failed to replicate this effect in a cryoinjury of the external anal sphincter muscles in rats despite using a higher dose.[116] One area of application where multipotent myogenic cells could be useful is injury that affects two adjacent tissues, as is the case with myotendinous junction injury. Indeed, Hashimoto and colleagues reported that the structure and function of myotendinous junction could be reconstituted in an acute mice hindlimb injury model after the application of multipotent MDSCs.[117] Interestingly, the cells were applied as sheet-like structures that contained cell-secreted ECM, and multiple vasculogenic and neurotrophic factors formed over 7 days of *in vitro* culture. The authors observed the engraftment of the cells and subsequent differentiation into various lineages, along with formation of connective tissue that bridges the muscle–tendon interface.

Pericytes or mesoangioblasts are cells that are closely associated with blood vessels in tissues including cardiac and skeletal muscle and are believed to play key roles in tissue

DOI: 10.1002/jcsm.12416

homeostasis.[118,119] Dellavalle *et al*. reported that isolated pericytes not only can be expanded *ex vivo* while retaining their potency but also can spontaneously differentiate into multinucleated myotubes. However, their kinetics of myogenic differentiation vary significantly than that of SCs, only expressing myogenic markers at the onset of terminal differentiation.[120] In a mouse cryoinjury model, intramuscular transplantation of muscle-derived CD133+ cells re-populated the SC niche, and these donor cells were observed to mount a potent regenerative response after subsequent re-injury.[121]

Cells with non-myogenic origin

Given that severe injury disrupts the endogenous cascade of muscle repair, it has been hypothesized that the transplantation of cells that naturally secrete bioactive factors can locally augment and stimulate these biological processes via signalling mechanisms. Therefore, various types of cells have been investigated that do not necessarily possess myogenic differentiation capacity or are a muscle-specific population but which can nevertheless indirectly support regeneration. Among these, mesenchymal stem/stromal cells (MSCs) have been the most abundantly used. MSCs have multi-lineage differentiation potential and can be isolated from various tissue sources including bone marrow, adipose tissue, and umbilical cord.[122,123] In addition to their osteogenic, chondrogenic, and adipogenic differentiation potential, MSCs can orchestrate the function of other cells by paracrine signalling or even endocrine mechanisms.[124] MSCs are known to secrete a variety of cytokines and growth factors that can promote angiogenesis, cell recruitment, migration, proliferation, and differentiation. MSCs are also known to be immunomodulatory, which may allow it to exert beneficial effects on the local immune cell population at the site of muscle injury.[125] A further advantage is that MSCs can easily be harvested without destroying source tissue and can rapidly be expanded in culture. *In vitro*, MSCs from the bone marrow can modulate the function of myoblasts such as their fusion into myotubes, and their migration and proliferation kinetics.[126] A few *in vivo* studies have been performed using MSCs from different sources to treat various muscle injury models. For example, intramuscular as well as intra-arterial transplantation of autologous bone marrow-derived MSCs (BM-MSCs) improved contractile muscle function after severe crush trauma, without any teratoma formation.[127–129] BM-MSCs also improved muscle contractility by promoting new myofiber formation in a sphincterotomy injury model in rats, indicating their potential utility in treating incontinence.[130,131] In separate studies, Oh *et al*.[132] and Gumucio *et al*.[133] have reported the potential efficacy of adipose-derived MSCs in repairing supraspinatus and subscapularis muscle tears and attenuating fibrosis in models of rotator cuff injury. In injury models of volumetric muscle loss, improved muscle function (contractility) and structure (myofiber formation, reduction of scar tissue, increased blood vessel density) have been observed using MSCs from adipose tissue,[134,135] bone marrow,[136] cranial neural crest,[137] and tonsil.[138] The consensus on the mode of action of MSCs is on their paracrine/endocrine properties, but it is unclear whether the secreted factors act directly on muscle cell populations or locally/systemically modulate the immune environment.[139]

Drawbacks and limitations

Although cell therapy has clearly demonstrated its potential for treating traumatic skeletal muscle injuries, overcoming existing limitations and optimizing variables will be key for clinical translation. In general, pre-clinical work has been limited to a few specific muscle groups and have almost exclusively been carried out in small rodent animal models. Muscles of the hindlimb including the gastrocnemius, tibialis anterior, and soleus have been the most widely studied, whereas some studies also focus on the external sphincter muscles. Large variability exists in these muscles with regard to fibre-type composition, size, and cellular populations. Discrepancies in reported results from groups that use the same cell population in different injury models likely arise owing to these inherent biological peculiarities. Determining the optimal cell dosage needs to be addressed and will be dependent on the extent of trauma and size of the muscle group. This can have important implications for cell source and preparation protocols. For example, although MDSCs and SCs have demonstrated outstanding engraftment efficacy and improvement in muscle function, one major limitation of their clinical application will be the availability and sparse presence in muscle tissues.[140] Harvesting these cells requires the extraction and enzymatic or mechanical degradation of the muscle tissue, which automatically disqualifies autologous use especially in aged or frail patients who have the highest need for such therapies. Even if donor tissue is available, the *ex vivo* proliferation of SCs is currently problematic owing to their well-known mechanical and chemical sensitivity to the *in vivo* niche.[141]

The same hurdle affects the application of non-myogenic cells, which are also needed in large quantities to produce significant biological effects. For example, MSCs show a dose-dependent response in stimulating muscle contractile function after severe crush trauma in rat soleus muscles (up to 1×10^6 cells), with no additional benefit occurring at higher doses (up to 10×10^6 cells). Even though MSCs can be efficiently harvested from various tissues, their clinical application requires significant expansion under laboratory conditions, which precludes autologous approaches for the treatment of acute muscle injuries. Allogeneic strategies, such as off-the-shelf cell products,[142,143] can evade the time problem, but the expansion process itself has been shown to induce senescent behaviour and loss of phenotype.[144] Thus, appropriate protocols for cell isolation, expansion, and cryopreservation, ensuring the maintenance of cellular properties, are mandatory for all cell-based strategies and their

DOI: 10.1002/jcsm.12416

translation into the clinic.[145–147] Successful first-in-human trials with allogeneic MSCs demonstrate the general feasibility of cell therapy approaches under clinical conditions.

Other approaches to overcome problems associated with expansion to large cell numbers could be the usage of myogenic and non-myogenic cells derived from embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs).[148–151] These cells can be maintained in their unspecialized states for virtually indefinite periods and maintain their ability to differentiate into functional cells of all three germ layers *in vitro* and *in vivo*.[152] Whereas ethical concerns might restrict the use of ESCs, iPSCs can be derived via reprogramming from any adult tissue cell from human donors of any age.[152] Somatic cell reprogramming using the induction factors Oct4, Klf4, Sox2, and c-Myc enables the generation of patient-specific cells in large quantities for autologous transplantation.[153,154] However, it should be noted that the pluripotency of these cells implies that their allogeneic use inevitably entails the risk of teratoma formation. Therefore, a variety of protocols have been developed to differentiate these cells under controlled conditions. For example, transient induction of Pax3 and Pax7 can lead to the generation of a large number of muscle progenitors from iPSCs that can engraft into host tissue and improve contractile function after injury.[155,156,151,157] Despite the fact that the usage of iPSC-derived cells to improve the healing of traumatic muscle injuries is still far from clinical application, these initial results are very encouraging.

Beyond the importance of the cell type, technical considerations for optimization of cell therapy include timing, mode, and location of delivery. Studies with MSC transplantation have indicated the existence of a relatively wide time window of 7 days for cell delivery to still be beneficial.[158,159] This may simultaneously dispel doubts about administering cells intraoperatively in case of iatrogenic injury and allow surgeons to carefully consider their treatment options. In the vast majority of pre-clinical and clinical studies, cells have been administered by intramuscular injection. There has been much debate surrounding the shear pressures involved in injecting a viscous suspension of cells through a narrow needle that may cause irreversible cell damage, and the secondary injury caused by the needle puncturing the tissue.[160] In case of large haematomas or widespread injury, cells may need to be injected at multiple points. Another drawback of cell delivery via injection is substantial cell death that occurs owing to anoikis (lack of engraftment)[161] or the harsh immune environment that may either cause immune rejection or cause inflammatory cytokine-mediated cell death.[162] The failure of early clinical trials with myoblasts was partly caused by massive cell death after transplantation,[163,164] and this has been attributed to host immune cells such as CD8+ T lymphocytes.[165] The regular use of immunosuppressive drugs such as cyclosporine in subsequent trials bypassed this problem, although a big drawback is their potentially harmful side effects.[166,167] Immunosuppressants may not be required if transplanted cells are either immune privileged or autologous or have inherent immunomodulatory potential such as MSCs.[168]

Instructing cells: a new paradigm in cell therapies

While the choice of cell type may primarily be dictated by its inherent regenerative potential, some cell types are known to be responsive to external physical and chemical cues. This has enabled studies on how external factors can enhance cell function and guide their regenerative response. It is known that SCs are sensitive to physicochemical cues. Their engraftment and subsequent *in vivo* function can be impaired or improved depending on the properties of *ex vivo* culture substrates.[169,170] Davoudi *et al*. reported that using an injectable hyaluronan-based and methyl cellulose-based hydrogel for intramuscular delivery significantly improved proliferation of MDSCs, retained their presence at the site of injury, and showed a significant benefit over injection-based administration.[171] In another study, Sleep and colleagues utilized a nanofiber-containing self-assembled hydrogel to guide the alignment and engraftment of SCs into injured tissue.[172] External stimulation of cell function especially holds true for MSCs, which respond to a variety of biophysical cues.[173] For instance, a three-dimensional culture of MSCs on biomaterials that promote cell–cell interactions was shown to enhance the paracrine effects of MSCs on cultured myoblasts.[174] MSCs also enhance their secretory properties when stimulated with growth factors such as IGF-1 and VEGF.[175] When delivered *in vivo* using a biomaterial scaffold that released these factors, MSCs showed a tremendous potential to resolve severe muscle injury by reducing scar tissue, promoting angiogenesis, and stimulating the formation of new myofibers.[91]

Enhancing cellular function via physical, chemical, biological, or structural cues is a relatively new paradigm in cell therapy. Basic research on understanding cell–matrix interactions that either impair or stimulate vital signalling pathways in cells to boost their function have led to the design and development of synthetically modified materials that act as instructive niches to the cells. In the future, these developments are expected to reduce cell doses required for regeneration, lower the costs of therapeutic interventions, and accelerate muscle regeneration even after severe injuries.

Translating pre-clinical promise into clinically successful applications

The number of completed or planned clinical trials for cell-based approaches to treat Duchenne muscular dystrophy is justifiably high owing to its wide prevalence, socio-economic

DOI: 10.1002/jcsm.12416

implications, and fatal nature.[176–178] In comparison, traumatic skeletal muscle injuries may not be perceived by the general population as being a major health care issue, but as we have described in this review, they frequently occur directly (sports, military, accidents) or indirectly (iatrogenic, invasive surgeries) across all age groups. Currently, makes it a major unmet clinical need and one that demands the development of new regenerative strategies. Cell therapy, with its demonstrated pre-clinical success and promise for further optimization, is a frontrunner for clinical translation and success. However, only a handful of clinical trials have been performed to assess its benefits in human muscle injuries to date (*Table* 1). Surprisingly, it is the urologists who have performed the majority of trials on patients suffering from urinal or faecal incontinence as a result of trauma to the external sphincter skeletal muscles. It should be noted that incontinence can also be caused in the absence of traumatic injury to the external sphincter muscles. Here, we have only included studies where the regeneration of the external skeletal muscle promised restoration of continence to patients. Two types of cells—muscle-derived myoblasts and adipose tissue-derived MSCs—have been used. Park *et al*. reported the safety and tolerability of allogeneic adipose tissue-derived MSCs with doses of 30–90×10^6 cells after injection into the anal sphincter muscles but did not report whether any functional or structural benefits were observed.[179] The group of Sarveazad and colleagues reported no significant functional improvement but observed structural replacement of scar tissue with muscle fibres in faecal incontinence patients.[180] The lack

Table 1 List of clinical trials involving cell therapy to treat skeletal muscle injury

Clinical trial identifier/phase	Status	Cell type	Indication	Application	Dosage	Observations
NCT03332238 Phase II	Planned	Autologous stromal vascular fraction cells	Rotator cuff tear	Injection into supraspinatus muscle and tendon	Not available	Not available
NCT03068988 Phase I	Planned	Mesenchymal stem cells	Rotator cuff tear and rupture	Injection into supraspinatus muscle and tendon	Not available	Not available
NCT03451916 Phase III	Planned	Allogeneic human placenta-derived stromal cells (PLX-PAD)	Muscle injury after hip fracture arthroplasty	Injection into gluteus medius muscle	150×10^6	Not available
NCT02384499 Phase I	Completed	Allogeneic adipose derived mesenchymal stem cells	Faecal incontinence	Injection into anal sphincter	30×10^6 60×10^6 90×10^6	Safe. Efficacy of therapy not reported.[179]
IRCT20160228263 16N2	Completed	Human adipose tissue derived stromal/stem cells (hADSCs)	Faecal incontinence due to injured sphincter	Injection into external anal sphincter muscle	6×10^6	Replacement of fibrous tissue with muscle tissue. No significant improvement in Wexner score.[180]
NCT01523522 Phase II/III	Completed	Autologous myoblasts	Faecal incontinence due to injured sphincter	Injection into external anal sphincter muscle	100×10^6	Clinical benefit after 12 months. Reduction of Cleveland Clinic Incontinence score. Safe, well tolerated.[181]
NCT00847535 Phase II NCT01008943 Phase II NCT01382602 Phase III	Completed	Autologous muscle-derived cells (AMDC-USR)	Stress urinary incontinence	Injection into external striated sphincter	10×10^6 50×10^6 100×10^6 200×10^6	Statistically significant reduction of stress leaks in all dose groups compared with baseline between 1 and up to 12 months.[182,183]
NCT01525667 Phase I/II	Completed	Allogeneic human placenta-derived stromal cells (PLX-PAD)	Muscle injury after total hip arthroplasty	Injection into gluteus medius muscle	150×10^6 300×10^6	Increase in muscle volume after cell therapy. Increase in muscle contraction force. Better outcomes with lower dose.[184]

DOI: 10.1002/jcsm.12416

of any functional improvement may be attributed to the relatively low dosage (6×10^6 cells) administered. Boyer *et al*. reported the functional efficacy of injecting 100×10^6 autologous myoblasts in patients suffering from faecal incontinence in a Phase II study.[181] Interestingly, there was no difference in functional outcome between the myoblast and placebo groups at the 6 month follow-up, but a significant difference was observed after 12 months. This may either be indicative of time taken for engraftment and maturation of myofibers into contractile units or suggest that the bulking effect due to injection also aids in ameliorating continence in the placebo group for a short time period (6 months). The group of Peters and colleagues also reported the safety and significant efficacy of autologous muscle-derived cells in a dose escalation study (range, $10–200 \times 10^6$ cells) in patients with stress urinary incontinence.[182] A statistically significant reduction of stress leaks in all dose groups was observed compared with baseline.[183] In the first clinical study of its kind, skeletal muscle injury associated with hip arthroplasty surgeries was treated by local administration of allogeneic placenta-derived MSCs in a small cohort of patients.[184] Cells were delivered intraoperatively after thawing. Interestingly, patients who received the lower dose (150 vs. 300×10^6) showed a significant improvement in contractile function and muscle volume, which was in part attributed to a systemically observable immunomodulatory effect of the cells. A Phase III, multi-centre trial has been planned to further investigate the efficacy of these cells in a larger cohort of patients.

Despite the broad success of cell therapies in relevant muscle injury models, there is a large gulf between preclinical work and translating these therapies to humans. We propose that this may be due to a multitude of potential reasons including (i) discrepancy in outcomes with different injury models, (ii) lack of sufficient studies in large animal models, (iii) difficulties in the selection of appropriate patient cohorts, (iv) indecision on cell dosage and associated issues in cell expansion, (v) funding and regulatory issues, or (vi) a lack of confidence in the translational potential of the therapy. Nevertheless, a number of successful clinical trials have been performed in recent years, and more are planned in the near future—encouraging signs in the quest to bring efficacious cell therapies to the clinical routine.

Conclusions

Severe traumatic injuries of skeletal muscles are responsible for discomfort, degeneration, dysfunction, and even disability in patients. Owing to the wide prevalence of these injuries and the associated socio-economic implications, muscle regeneration has been a topic of scientific and clinical interest. While some injuries can be effectively managed by conservative treatments, many severe ones show signs of permanent structural and functional degeneration (*Figure* 1). The most pressing issues relate to remodelling scar tissue, promoting myofiber regeneration, and reversing fatty deposits that plague the muscle after severe injury. A strategy to address all three challenges at once is likely to result in the most benefit. Another challenge that has not received enough scientific attention is the repair of myotendinous junctions and composite muscle–tendon injuries that are quite commonly damaged in strain injuries. Molecular therapies such as growth factor delivery may have utility in repairing ischaemic muscle tissues but have not been as effective in severe injury models. Sustained delivery of several growth factors over well-defined periods is challenging and thus an area of ongoing research in the field of controlled drug release. Cell therapy has been, by far, the most promising approach to treat skeletal muscle injuries in pre-clinical settings. Several cellular candidates have been identified that operate via distinct modes of action and contribute to the restoration of muscle structure and function. Because optimal dosage is still widely debated, the *ex vivo* expansion of cells is a technical issue that is yet to be optimized, especially with regard to maintenance of their potency. Allogeneic cells, such as MSCs, mount a potent regenerative effort and can be purchased off the shelf from industrial partners with the resources to expand cells in large batches. Recent clinical trials have demonstrated that allogeneic cell therapy can be useful, but unforeseen observations warrant a deeper unravelling of the mechanism of action. Recently, innovative approaches have been proposed to further enhance the efficacy of cell-based therapy. These include re-engineering a sufficient niche, improving biomaterial design to deliver and retain viable cells near the site of injury in a minimally invasive manner, and modifying physical and chemical properties of biomaterials to improve cellular function. Continued innovations along with the improvement of preclinical study design, including larger patient cohorts, make cell therapies a promising candidate for traumatic muscle injury treatment.

Acknowledgments

The authors gratefully acknowledge the German Research Foundation and German Federal Ministry of Education and Research (BMBF) for funding S. G. (GE2512/2-2, DIMEOS:01EC1402B) and G. N. D. (FOR2165) and the Berlin-Brandenburg Center for Regenerative Therapies (BCRT) for funding T. H. Q. This project has received funding from the European Union's Horizon 2020 research and innovation programme (Horizon 2020 Framework Programme) under the

grant agreement no. 779293 (HIPGEN). The authors certify that they comply with the ethical guidelines for authorship and publishing of the *Journal of Cachexia, Sarcopenia and Muscle*.[185]

Conflict of interests

The authors declare no competing financial and non-financial interests.

References

1. Frontera WR, Ochala J. Skeletal muscle: a brief review of structure and function. *Calcif Tissue Int* 2015;**96**:183–195.
2. Ekstrand J, Hägglund M, Waldén M. Epidemiology of muscle injuries in professional football (soccer). *Am J Sports Med* 2011;**39**:1226–1232.
3. Taneja AK, Kattapuram SV, Chang CY, Simeone FJ, Bredella MA, Torriani M. MRI findings of rotator cuff myotendinous junction injury. *AJR Am J Roentgenol* 2014;**203**:406–411.
4. Ekstrand J, Hagglund M, Walden M. Injury incidence and injury patterns in professional football: the UEFA injury study. *Br J Sports Med* 2011;**45**:553–558.
5. Gates C, Huard J. Management of skeletal muscle injuries in military personnel. *Oper Tech Sports Med* 2005;**13**:247–256.
6. Bharucha AE, Dunivan G, Goode PS, Lukacz ES, Markland AD, Matthews CA, et al. Epidemiology, pathophysiology, and classification of fecal incontinence: state of the science summary for the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) workshop. *Am J Gastroenterol* 2015;**110**:127–136.
7. Aragon IM, Imbroda BH, Lara MF. Cell therapy clinical trials for stress urinary incontinence: current status and perspectives. *Int J Med Sci* 2018;**15**:195–204.
8. Miner PB Jr. Economic and personal impact of fecal and urinary incontinence. *Gastroenterology* 2004;**126**:S8–S13.
9. Xu X, Menees SB, Zochowski MK, Fenner DE. Economic cost of fecal incontinence. *Dis Colon Rectum* 2012;**55**:586–598.
10. Garrett WE Jr, Swiontkowski MF, Weinstein JN, Callaghan J, Rosier RN, Berry DJ, et al. American board of orthopaedic surgery practice of the orthopaedic surgeon: part-II, certification examination case mix. *J Bone Joint Surg Am* 2006;**88**:660–667.
11. Maffulli N, Oliva F, Frizziero A, Nanni G, Barazzuol M, Via AG, et al. ISMuLT guidelines for muscle injuries. *Muscles Ligaments Tendons J* 2013;**3**:241–249.
12. Goutallier D, Postel JM, Bernageau J, Lavau L, Voisin MC. Fatty muscle degeneration in cuff ruptures. Pre- and postoperative evaluation by CT scan. *Clin Orthop Relat Res* 1994;78–83.
13. Grogan BF, Hsu JR. Volumetric muscle loss. *J Am Acad Orthop Surg* 2011;**19**: S35–S37.
14. Sanes JR. The basement membrane/basal lamina of skeletal muscle. *J Biol Chem* 2003;**278**:12601–12604.
15. Charge SB, Rudnicki MA. Cellular and molecular regulation of muscle regeneration. *Physiol Rev* 2004;**84**:209–238.
16. Pallafacchina G, Blaauw B, Schiaffino S. Role of satellite cells in muscle growth and maintenance of muscle mass. *Nutr Metab Cardiovasc Dis* 2013;**23**:S12–S18.
17. Starkey JD, Yamamoto M, Yamamoto S, Goldhamer DJ. Skeletal muscle satellite cells are committed to myogenesis and do not spontaneously adopt nonmyogenic fates. *J Histochem Cytochem* 2011;**59**:33–46.
18. Mauro A. Satellite cell of skeletal muscle fibers. *J Biophys Biochem Cytol* 1961;**9**:493–495.
19. Rocheteau P, Vinet M, Chretien F. Dormancy and quiescence of skeletal muscle stem cells. *Results Probl Cell Differ* 2015;**56**:215–235.
20. Yin H, Price F, Rudnicki MA. Satellite cells and the muscle stem cell niche. *Physiol Rev* 2013;**93**:23–67.
21. Lepper C, Partridge TA, Fan CM. An absolute requirement for Pax7-positive satellite cells in acute injury-induced skeletal muscle regeneration. *Development* 2011;**138**:3639–3646.
22. Sambasivan R, Yao R, Kissenpfennig A, Van Wittenberghe L, Paldi A, Gayraud-Morel B, et al. Pax7-expressing satellite cells are indispensable for adult skeletal muscle regeneration. *Development* 2011;**138**:3647–3656.
23. Tidball JG. Inflammatory cell response to acute muscle injury. *Med Sci Sports Exerc* 1995;**27**:1022–1032.
24. Toumi H, Best TM. The inflammatory response: friend or enemy for muscle injury? *Br J Sports Med* 2003;**37**:284–286.
25. Rybalko V, Hsieh P-L, Merscham-Banda M, Suggs LJ, Farrar RP. The development of macrophage-mediated cell therapy to improve skeletal muscle function after injury. *PLoS One* 2016;**10**:e0145550.
26. Novak ML, Weinheimer-Haus EM, Koh TJ. Macrophage activation and skeletal muscle healing following traumatic injury. *J Pathol* 2014;**232**:344–355.
27. Arnold L, Henry A, Poron F, Baba-Amer Y, van Rooijen N, Plonquet A, et al. Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. *J Exp Med* 2007;**204**:1057–1069.
28. Kaariainen M, Jarvinen T, Jarvinen M, Rantanen J, Kalimo H. Relation between myofibers and connective tissue during muscle injury repair. *Scand J Med Sci Sports* 2000;**10**:332–337.
29. Wang H, Melton DW, Porter L, Sarwar ZU, McManus LM, Shireman PK. Altered macrophage phenotype transition impairs skeletal muscle regeneration. *Am J Pathol* 2014;**184**:1167–1184.
30. Kharraz Y, Guerra J, Mann CJ, Serrano AL, Munoz-Canoves P. Macrophage plasticity and the role of inflammation in skeletal muscle repair. *Mediators Inflamm* 2013;**2013**:9.
31. Davies BM, Morrey ME, Mouthuy PA, Baboldashti NZ, Hakimi O, Snelling S, et al. Repairing damaged tendon and muscle: are mesenchymal stem cells and scaffolds the answer? *Regen Med* 2013;**8**:613–630.
32. Jarvinen TA, Jarvinen TL, Kaariainen M, Aarimaa V, Vaittinen S, Kalimo H, et al. Muscle injuries: optimising recovery. *Best Pract Res Clin Rheumatol* 2007;**21**:317–331.
33. Delos D, Maak TG, Rodeo SA. Muscle injuries in athletes: enhancing recovery through scientific understanding and novel therapies. *Sports Health* 2013;**5**:346–352.
34. Jarvinen MJ, Lehto MU. The effects of early mobilisation and immobilisation on the healing process following muscle injuries. *Sports Med* 1993;**15**:78–89.
35. Bayer ML, Magnusson SP, Kjaer M, Tendon Research Group B. Early versus delayed rehabilitation after acute muscle injury. *N Engl J Med* 2017;**377**:1300–1301.
36. Almekinders LC. Anti-inflammatory treatment of muscular injuries in sport. An update of recent studies. *Sports Med* 1999;**28**:383–388.
37. Thorsson O, Rantanen J, Hurme T, Kalimo H. Effects of nonsteroidal antiinflammatory medication on satellite cell proliferation during muscle regeneration. *Am J Sports Med* 1998;**26**:172–176.
38. Obremsky WT, Seaber AV, Ribbeck BM, Garrett WE Jr. Biomechanical and histologic assessment of a controlled muscle strain injury treated with piroxicam. *Am J Sports Med* 1994;**22**:558–561.
39. O'Grady M, Hackney AC, Schneider K, Bossen E, Steinberg K, Douglas JM Jr, et al. Diclofenac sodium (Voltaren) reduced exercise-induced injury in human skeletal muscle. *Med Sci Sports Exerc* 2000;**32**:1191–1196.
40. Mishra DK, Friden J, Schmitz MC, Lieber RL. Anti-inflammatory medication after muscle injury. A treatment resulting in

short-term improvement but subsequent loss of muscle function. *J Bone Joint Surg Am* 1995;**77**:1510–1519.
41. Baoge L, Van Den Steen E, Rimbaut S, Philips N, Witvrouw E, Almqvist KF, et al. Treatment of skeletal muscle injury: a review. *ISRN Orthop* 2012;**2012**:689012.
42. Sass FA, Fuchs M, Pumberger M, Geissler S, Duda GN, Perka C, et al. Immunology guides skeletal muscle regeneration. *Int J Mol Sci* 2018;**19**:835.
43. Rahusen FT, Weinhold PS, Almekinders LC. Nonsteroidal anti-inflammatory drugs and acetaminophen in the treatment of an acute muscle injury. *Am J Sports Med* 2004;**32**:1856–1859.
44. Chen MR, Dragoo JL. The effect of nonsteroidal anti-inflammatory drugs on tissue healing. *Knee Surg Sports Traumatol Arthrosc* 2013;**21**:540–549.
45. Ziltener JL, Leal S, Fournier PE. Non-steroidal anti-inflammatory drugs for athletes: an update. *Ann Phys Rehabil Med* 2010;**53**:278–288.
46. Dziki J, Badylak S, Yabroudi M, Sicari B, Ambrosio F, Stearns K, et al. An acellular biologic scaffold treatment for volumetric muscle loss: results of a 13-patient cohort study. *NPJ Regen Med* 2016;**1**.
47. Sicari BM, Rubin JP, Dearth CL, Wolf MT, Ambrosio F, Boninger M, et al. An acellular biologic scaffold promotes skeletal muscle formation in mice and humans with volumetric muscle loss. *Sci Transl Med* 2014;**6**:234–258.
48. Hurtgen BJ, Ward CL, Leopold Wager CM, Garg K, Goldman SM, Henderson BEP, et al. Autologous minced muscle grafts improve endogenous fracture healing and muscle strength after musculoskeletal trauma. *Physiol Rep* 2017;**5**:e13362.
49. Whiteside LA. Surgical technique: gluteus maximus and tensor fascia lata transfer for primary deficiency of the abductors of the hip. *Clin Orthop Relat Res* 2014;**472**:645–653.
50. Suda AJ, Heppert V. Vastus lateralis muscle flap for infected hips after resection arthroplasty. *J Bone Joint Surg Br* 2010;**92**:1654–1658.
51. Kovalak E, Ozdemir H, Ermutlu C, Obut A. Assessment of hip abductors by MRI after total hip arthroplasty and effect of fatty atrophy on functional outcome. *Acta Orthop Traumatol Turc* 2018;**52**:196–200.
52. Memtsoudis SG, Pumberger M, Ma Y, Chiu YL, Fritsch G, Gerner P, et al. Epidemiology and risk factors for perioperative mortality after total hip and knee arthroplasty. *J Orthop Res* 2012;**30**:1811–1821.
53. Maradit Kremers H, Larson DR, Crowson CS, Kremers WK, Washington RE, Steiner CA, et al. Prevalence of total hip and knee replacement in the United States. *J Bone Joint Surg Am* 2015;**97**:1386–1397.
54. Tan J, Chen H, Chen C, Liang X, Huang W. The strength and function of hip abductors following anterolateral minimally invasive total hip arthroplasty. *Chin J Traumatol* 2014;**17**:73–78.
55. Jensen C, Aagaard P, Overgaard S. Recovery in mechanical muscle strength following resurfacing vs standard total hip arthroplasty—a randomised clinical trial. *Osteoarthr Cartil* 2011;**19**:1108–1116.
56. von Roth P, Abdel MP, Wauer F, Winkler T, Wassilew G, Diederichs G, et al. Significant muscle damage after multiple revision total hip replacements through the direct lateral approach. *Bone Joint J* 2014;**96-b**:1618–1622.
57. Whiteside LA, Nayfeh T, Katerberg BJ. Gluteus maximus flap transfer for greater trochanter reconstruction in revision THA. *Clin Orthop Relat Res* 2006;**453**:203–210.
58. Drexler M, Dwyer T, Kosashvili Y, Chakravertty R, Abolghasemian M, Gollish J. Acetabular cup revision combined with tensor facia lata reconstruction for management of massive abductor avulsion after failed total hip arthroplasty. *J Arthroplasty* 2014;**29**:1052–1057.
59. Souza J, Gottfried C. Muscle injury: review of experimental models. *J Electromyogr Kinesiol* 2013;**23**:1253–1260.
60. Garry GA, Antony ML, Garry DJ. Cardiotoxin induced injury and skeletal muscle regeneration. *Methods Mol Biol* 2016;**1460**:61–71.
61. Le G, Lowe DA, Kyba M. Freeze injury of the tibialis anterior muscle. *Methods Mol Biol* 2016;**1460**:33–41.
62. Tierney MT, Sacco A. Inducing and evaluating skeletal muscle injury by notexin and barium chloride. *Methods Mol Biol* 2016;**1460**:53–60.
63. Takagi R, Fujita N, Arakawa T, Kawada S, Ishii N, Miki A. Influence of icing on muscle regeneration after crush injury to skeletal muscles in rats. *J Appl Physiol* 2011;**110**:382–388.
64. Hardy D, Besnard A, Latil M, Jouvion G, Briand D, Thepenier C, et al. Comparative study of injury models for studying muscle regeneration in mice. *PLoS One.* 2016;**11**:e0147198.
65. Carvalho N, Puntel G, Correa P, Gubert P, Amaral G, Morais J, et al. Protective effects of therapeutic cold and heat against the oxidative damage induced by a muscle strain injury in rats. *J Sports Sci* 2010;**28**:923–935.
66. Ambrosio F, Ferrari RJ, Distefano G, Plassmeyer JM, Carvell GE, Deasy BM, et al. The synergistic effect of treadmill running on stem-cell transplantation to heal injured skeletal muscle. *Tissue Eng Part A* 2010;**16**:839–849.
67. Minamoto VB, Bunho SR, Salvini TF. Regenerated rat skeletal muscle after periodic contusions. *Braz J Med Biol Res* 2001;**34**:1447–1452.
68. Lebaschi A, Deng XH, Zong J, Cong GT, Carballo CB, Album ZM, et al. Animal models for rotator cuff repair. *Ann N Y Acad Sci* 2016;**1383**:43–57.
69. Valencia Mora M, Ruiz Iban MA, Diaz Heredia J, Barco Laakso R, Cuellar R, Garcia AM. Stem cell therapy in the management of shoulder rotator cuff disorders. *World J Stem Cells* 2015;**7**:691–699.
70. Turner NJ, Badylak JS, Weber DJ, Badylak SF. Biologic scaffold remodeling in a dog model of complex musculoskeletal injury. *J Surg Res* 2012;**176**:490–502.
71. Winkler T, von Roth P, Matziolis G, Schumann MR, Hahn S, Strube P, et al. Time course of skeletal muscle regeneration after severe trauma. *Acta Orthop* 2011;**82**:102–111.
72. Bedi A, Dines J, Warren RF, Dines DM. Massive tears of the rotator cuff. *J Bone Joint Surg Am* 2010;**92**:1894–1908.
73. Mendias CL, Roche SM, Harning JA, Davis ME, Lynch EB, Sibilsky Enselman ER, et al. Reduced muscle fiber force production and disrupted myofibril architecture in patients with chronic rotator cuff tears. *J Shoulder Elbow Surg* 2015;**24**:111–119.
74. Gumucio JP, Davis ME, Bradley JR, Stafford PL, Schiffman CJ, Lynch EB, et al. Rotator cuff tear reduces muscle fiber specific force production and induces macrophage accumulation and autophagy. *J Orthop Res* 2012;**30**:1963–1970.
75. Davis ME, Stafford PL, Jergenson MJ, Bedi A, Mendias CL. Muscle fibers are injured at the time of acute and chronic rotator cuff repair. *Clin Orthop Relat Res* 2015;**473**:226–232.
76. Smith C, Kruger MJ, Smith RM, Myburgh KH. The inflammatory response to skeletal muscle injury: illuminating complexities. *Sports Med* 2008;**38**:947–969.
77. Karalaki M, Fili S, Philippou A, Koutsilieris M. Muscle regeneration: cellular and molecular events. *In Vivo* 2009;**23**:779–796.
78. Syverud BC, VanDusen KW, Larkin LM. Growth factors for skeletal muscle tissue engineering. *Cells Tissues Organs* 2016;**202**:169–179.
79. Pawlikowski B, Vogler TO, Gadek K, Olwin BB. Regulation of skeletal muscle stem cells by fibroblast growth factors. *Dev Dyn* 2017;**246**:359–367.
80. Menetrey J, Kasemkijwattana C, Day CS, Bosch P, Vogt M, Fu FH, et al. Growth factors improve muscle healing in vivo. *J Bone Joint Surg Br* 2000;**82**:131–137.
81. Rodgers JT, King KY, Brett JO, Cromie MJ, Charville GW, Maguire KK, et al. mTORC1 controls the adaptive transition of quiescent stem cells from G0 to G(Alert). *Nature* 2014;**510**:393–396.
82. Allen RE, Sheehan SM, Taylor RG, Kendall TL, Rice GM. Hepatocyte growth factor activates quiescent skeletal muscle satellite cells in vitro. *J Cell Physiol* 1995;**165**:307–312.
83. Miller KJ, Thaloor D, Matteson S, Pavlath GK. Hepatocyte growth factor affects satellite cell activation and differentiation in regenerating skeletal muscle. *Am J Physiol Cell Physiol* 2000;**278**:C174–C181.
84. Grasman JM, Do DM, Page RL, Pins GD. Rapid release of growth factors regenerates force output in volumetric muscle loss injuries. *Biomaterials* 2015;**72**:49–60.
85. Shvartsman D, Storrie-White H, Lee K, Kearney C, Brudno Y, Ho N, et al. Sustained delivery of VEGF maintains innervation and promotes reperfusion in

ischemic skeletal muscles via NGF/GDNF signaling. *Mol Ther* 2014;**22**:1243–1253.
86. Silva EA, Mooney DJ. Spatiotemporal control of vascular endothelial growth factor delivery from injectable hydrogels enhances angiogenesis. *J Thromb Haemost* 2007;**5**:590–598.
87. Schiaffino S, Mammucari C. Regulation of skeletal muscle growth by the IGF1-Akt/PKB pathway: insights from genetic models. *Skeletal Muscle* 2011;**1**:4.
88. Tureckova J, Wilson EM, Cappalonga JL, Rotwein P. Insulin-like growth factor-mediated muscle differentiation: collaboration between phosphatidylinositol 3-kinase-Akt-signaling pathways and myogenin. *J Biol Chem* 2001;**276**:39264–39270.
89. Cezar CA, Arany P, Vermillion SA, Seo BR, Vandenburgh HH, Mooney DJ. Timed delivery of therapy enhances functional muscle regeneration. *Adv Healthc Mater* 2017;**6**.
90. Borselli C, Storrie H, Benesch-Lee F, Shvartsman D, Cezar C, Lichtman JW, et al. Functional muscle regeneration with combined delivery of angiogenesis and myogenesis factors. *Proc Natl Acad Sci U S A* 2010;**107**:3287–3292.
91. Pumberger M, Qazi TH, Ehrentraut MC, Textor M, Kueper J, Stoltenburg-Didinger G, et al. Synthetic niche to modulate regenerative potential of MSCs and enhance skeletal muscle regeneration. *Biomaterials* 2016;**99**:95–108.
92. Berardi E, Annibali D, Cassano M, Crippa S, Sampaolesi M. Molecular and cell-based therapies for muscle degenerations: a road under construction. *Front Physiol* 2014;**5**:119.
93. Boldrin L, Muntoni F, Morgan JE. Are human and mouse satellite cells really the same? *J Histochem Cytochem* 2010;**58**:941–955.
94. Montarras D, Morgan J, Collins C, Relaix F, Zaffran S, Cumano A, et al. Direct isolation of satellite cells for skeletal muscle regeneration. *Science* 2005;**309**:2064–2067.
95. Webster MT, Manor U, Lippincott-Schwartz J, Fan CM. Intravital imaging reveals ghost fibers as architectural units guiding myogenic progenitors during regeneration. *Cell Stem Cell* 2016;**18**:243–252.
96. Cerletti M, Jurga S, Witczak CA, Hirshman MF, Shadrach JL, Goodyear LJ, et al. Highly efficient, functional engraftment of skeletal muscle stem cells in dystrophic muscles. *Cell* 2008;**134**:37–47.
97. Collins CA, Olsen I, Zammit PS, Heslop L, Petrie A, Partridge TA, et al. Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. *Cell* 2005;**122**:289–301.
98. Sacco A, Doyonnas R, Kraft P, Vitorovic S, Blau HM. Self-renewal and expansion of single transplanted muscle stem cells. *Nature* 2008;**456**:502–506.
99. Kuang S, Kuroda K, Le Grand F, Rudnicki MA. Asymmetric self-renewal and commitment of satellite stem cells in muscle. *Cell* 2007;**129**:999–1010.
100. Gilbert PM, Havenstrite KL, Magnusson KE, Sacco A, Leonardi NA, Kraft P, et al. Substrate elasticity regulates skeletal muscle stem cell self-renewal in culture. *Science* 2010;**329**:1078–1081.
101. Biressi S, Rando TA. Heterogeneity in the muscle satellite cell population. *Semin Cell Dev Biol* 2010;**21**:845–854.
102. Srikuea R, Pholpramool C, Kitiyanant Y, Yimlamai T. Satellite cell activity in muscle regeneration after contusion in rats. *Clin Exp Pharmacol Physiol* 2010;**37**:1078–1086.
103. Rossi CA, Flaibani M, Blaauw B, Pozzobon M, Figallo E, Reggiani C, et al. In vivo tissue engineering of functional skeletal muscle by freshly isolated satellite cells embedded in a photopolymerizable hydrogel. *FASEB J* 2011;**25**:2296–2304.
104. Bar-Nur O, Gerli MFM, Di Stefano B, Almada AE, Galvin A, Coffey A, et al. Direct reprogramming of mouse fibroblasts into functional skeletal muscle progenitors. *Stem Cell Reports* 2018;**10**:1505–1521.
105. Negroni E, Riederer I, Chaouch S, Belicchi M, Razini P, Di Santo J, et al. In vivo myogenic potential of human CD133+ muscle-derived stem cells: a quantitative study. *Mol Ther* 2009;**17**:1771–1778.
106. Fishman JM, Tyraskis A, Maghsoudlou P, Urbani L, Totonelli G, Birchall MA, et al. Skeletal muscle tissue engineering: which cell to use? *Tissue Eng Part B Rev* 2013;**19**:503–515.
107. McCullagh KJ, Perlingeiro RC. Coaxing stem cells for skeletal muscle repair. *Adv Drug Deliv Rev* 2015;**84**:198–207.
108. Usas A, Huard J. Muscle-derived stem cells for tissue engineering and regenerative therapy. *Biomaterials* 2007;**28**:5401–5406.
109. Li H, Lu A, Tang Y, Beckman S, Nakayama N, Poddar M, et al. The superior regenerative potential of muscle-derived stem cells for articular cartilage repair is attributed to high cell survival and chondrogenic potential. *Mol Ther Methods Clin Dev* 2016;**3**:16065.
110. Lorant J, Saury C, Schleder C, Robriquet F, Lieubeau B, Negroni E, et al. Skeletal muscle regenerative potential of human MuStem cells following transplantation into injured mice muscle. *Mol Ther* 2018;**26**:618–633.
111. Matthias N, Hunt SD, Wu J, Lo J, Smith Callahan LA, Li Y, et al. Volumetric muscle loss injury repair using in situ fibrin gel cast seeded with muscle-derived stem cells (MDSCs). *Stem Cell Res* 2018;**27**:65–73.
112. Sarig R, Baruchi Z, Fuchs O, Nudel U, Yaffe D. Regeneration and transdifferentiation potential of muscle-derived stem cells propagated as myospheres. *Stem Cells* 2006;**24**:1769–1778.
113. McCullagh KJ. Can a young muscle's stem cell secretome prolong our lives? *Stem Cell Res Ther* 2012;**3**:19.
114. Lavasani M, Robinson AR, Lu A, Song M, Feduska JM, Ahani B, et al. Muscle-derived stem/progenitor cell dysfunction limits healthspan and lifespan in a murine progeria model. *Nat Commun* 2012;**3**:608.
115. Ota S, Uehara K, Nozaki M, Kobayashi T, Terada S, Tobita K, et al. Intramuscular transplantation of muscle-derived stem cells accelerates skeletal muscle healing after contusion injury via enhancement of angiogenesis. *Am J Sports Med* 2011;**39**:1912–1922.
116. Kang S-B, Lee HN, Lee JY, Park J-S, Lee HS, Lee JY. Sphincter contractility after muscle-derived stem cells autograft into the cryoinjured anal sphincters of rats. *Dis Colon Rectum* 2008;**51**:1367–1373.
117. Hashimoto H, Tamaki T, Hirata M, Uchiyama Y, Sato M, Mochida J. Reconstitution of the complete rupture in musculotendinous junction using skeletal muscle-derived multipotent stem cell sheet-pellets as a "bio-bond". *PeerJ* 2016;**4**:e2231.
118. Murray IR, Baily JE, Chen WCW, Dar A, Gonzalez ZN, Jensen AR, et al. Skeletal and cardiac muscle pericytes: functions and therapeutic potential. *Pharmacol Ther* 2017;**171**:65–74.
119. Sampaolesi M, Torrente Y, Innocenzi A, Tonlorenzi R, D'Antona G, Pellegrino MA, et al. Cell therapy of alpha-sarcoglycan null dystrophic mice through intra-arterial delivery of mesoangioblasts. *Science* 2003;**301**:487–492.
120. Dellavalle A, Sampaolesi M, Tonlorenzi R, Tagliafico E, Sacchetti B, Perani L, et al. Pericytes of human skeletal muscle are myogenic precursors distinct from satellite cells. *Nat Cell Biol* 2007;**9**:255–267.
121. Meng J, Chun S, Asfahani R, Lochmuller H, Muntoni F, Morgan J. Human skeletal muscle-derived CD133(+) cells form functional satellite cells after intramuscular transplantation in immunodeficient host mice. *Mol Ther* 2014;**22**:1008–1017.
122. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999;**284**:143–147.
123. Elahi KC, Klein G, Avci-Adali M, Sievert KD, MacNeil S, Aicher WK. Human mesenchymal stromal cells from different sources diverge in their expression of cell surface proteins and display distinct differentiation patterns. *Stem Cells Int* 2016;**2016**:5646384.
124. Caplan Arnold I, Correa D. The MSC: an injury drugstore. *Cell Stem Cell* 2011;**9**:11–15.
125. Gao F, Chiu SM, Motan DA, Zhang Z, Chen L, Ji HL, et al. Mesenchymal stem cells and immunomodulation: current status and future prospects. *Cell Death Dis* 2016;**7**:e2062.
126. Sassoli C, Pini A, Chellini F, Mazzanti B, Nistri S, Nosi D, et al. Bone marrow mesenchymal stromal cells stimulate skeletal myoblast proliferation through the paracrine release of VEGF. *PLoS One.* 2012;**7**:e37512.
127. Winkler T, von Roth P, Matziolis G, Mehta M, Perka C, Duda GN. Dose-response

relationship of mesenchymal stem cell transplantation and functional regeneration after severe skeletal muscle injury in rats. *Tissue Eng Part A* 2009;**15**:487–492.
128. Matziolis G, Winkler T, Schaser K, Wiemann M, Krocker D, Tuischer J, et al. Autologous bone marrow-derived cells enhance muscle strength following skeletal muscle crush injury in rats. *Tissue Eng* 2006;**12**:361–367.
129. von Roth P, Duda GN, Radojewski P, Preininger B, Strohschein K, Rohner E, et al. Intra-arterial MSC transplantation restores functional capacity after skeletal muscle trauma. *Open Orthop J* 2012;**6**:352–356.
130. Lorenzi B, Pessina F, Lorenzoni P, Urbani S, Vernillo R, Sgaragli G, et al. Treatment of experimental injury of anal sphincters with primary surgical repair and injection of bone marrow-derived mesenchymal stem cells. *Dis Colon Rectum* 2008;**51**:411–420.
131. Salcedo L, Mayorga M, Damaser M, Balog B, Butler R, Penn M, et al. Mesenchymal stem cells can improve anal pressures after anal sphincter injury. *Stem Cell Res* 2013;**10**:95–102.
132. Oh JH, Chung SW, Kim SH, Chung JY, Kim JY. 2013 Neer Award: effect of the adipose-derived stem cell for the improvement of fatty degeneration and rotator cuff healing in rabbit model. *J Shoulder Elbow Surg* 2014;**23**:445–455.
133. Gumucio JP, Flood MD, Roche SM, Sugg KB, Momoh AO, Kosnik PE, et al. Stromal vascular stem cell treatment decreases muscle fibrosis following chronic rotator cuff tear. *Int Orthop* 2016;**40**:759–764.
134. Pecanha R, Bagno LL, Ribeiro MB, Robottom Ferreira AB, Moraes MO, Zapata-Sudo G, et al. Adipose-derived stem-cell treatment of skeletal muscle injury. *J Bone Joint Surg Am* 2012;**94**:609–617.
135. Rybalko V, Hsieh PL, Ricles LM, Chung E, Farrar RP, Suggs LJ. Therapeutic potential of adipose-derived stem cells and macrophages for ischemic skeletal muscle repair. *Regen Med* 2017;**12**:153–167.
136. Helal MA, Shaheen NE, Abu Zahra FA. Immunomodulatory capacity of the local mesenchymal stem cells transplantation after severe skeletal muscle injury in female rats. *Immunopharmacol Immunotoxicol* 2016;1–9.
137. Nie X, Xing Y, Deng M, Gang L, Liu R, Zhang Y, et al. Ecto-mesenchymal stem cells from facial process: potential for muscle regeneration. *Cell Biochem Biophys* 2014;**70**:615–622.
138. Park S, Choi Y, Jung N, Yu Y, Ryu KH, Kim HS, et al. Myogenic differentiation potential of human tonsil-derived mesenchymal stem cells and their potential for use to promote skeletal muscle regeneration. *Int J Mol Med* 2016;**37**:1209–1220.
139. Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, et al. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. *Cell Stem Cell* 2008;**2**:141–150.
140. Bareja A, Billin AN. Satellite cell therapy—from mice to men. *Skeletal Muscle.* 2013;**3**:2.
141. Wagers AJ. The stem cell niche in regenerative medicine. *Cell Stem Cell* 2012;**10**:362–369.
142. Olsen TR, Kirian R, Lock L, Rowley JA. A xeno-free fed-batch microcarrier suspension bioreactor system for the scalable and economic expansion of hBM-MSCs. *Cytotherapy* 2018;**20**:S48.
143. Andrew C, Thomas B, Lior R, Jon R, Knut N, Harvey B, et al. Concise review: process development considerations for cell therapy. *Stem Cells Transl Med* 2015;**4**:1155–1163.
144. Geißler S, Textor M, Kühnisch J, Könnig D, Klein O, Ode A, et al. Functional comparison of chronological and in vitro aging: differential role of the cytoskeleton and mitochondria in mesenchymal stromal cells. *PLoS One.* 2012;**7**:e52700.
145. Reinke S, Dienelt A, Blankenstein A, Duda GN, Geissler S. Qualifying stem cell sources: how to overcome potential pitfalls in regenerative medicine? *J Tissue Eng Regen Med* 2016;**10**:3–10.
146. Moll G, Geissler S, Catar R, Ignatowicz L, Hoogduijn MJ, Strunk D, et al. Cryopreserved or fresh mesenchymal stromal cells: only a matter of taste or key to unleash the full clinical potential of MSC therapy? *Adv Exp Med Biol* 2016;**951**:77–98.
147. Simaria AS, Hassan S, Varadaraju H, Rowley J, Warren K, Vanek P, et al. Allogeneic cell therapy bioprocess economics and optimization: single-use cell expansion technologies. *Biotechnol Bioeng* 2014;**111**:69–83.
148. Darabi R, Perlingeiro RC. Derivation of skeletal myogenic precursors from human pluripotent stem cells using conditional expression of PAX7. *Methods Mol Biol* 2016;**1357**:423–439.
149. Chan SS-K, Arpke RW, Filareto A, Xie N, Pappas MP, Penaloza JS, et al. Skeletal muscle stem cells from PSC-derived teratomas have functional regenerative capacity. *Cell Stem Cell* 2018;**23**:74–85.e6.
150. Kim J, Oliveira VKP, Yamamoto A, Perlingeiro RCR. Generation of skeletal myogenic progenitors from human pluripotent stem cells using non-viral delivery of minicircle DNA. *Stem Cell Res* 2017;**23**:87–94.
151. Darabi R, Arpke RW, Irion S, Dimos JT, Grskovic M, Kyba M, et al. Human ES- and iPS-derived myogenic progenitors restore dystrophin and improve contractility upon transplantation in dystrophic mice. *Cell Stem Cell* 2012;**10**:610–619.
152. Megges M, Geissler S, Duda GN, Adjaye J. Generation of an iPS cell line from bone marrow derived mesenchymal stromal cells from an elderly patient. *Stem Cell Res* 2015;**15**:565–568.
153. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007;**131**:861–872.
154. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;**126**:663–676.
155. Filareto A, Darabi R, Perlingeiro RC. Engraftment of ES-derived myogenic progenitors in a severe mouse model of muscular dystrophy. *J Stem Cell Res Ther* 2012;**10**.
156. Magli A, Incitti T, Kiley J, Swanson SA, Darabi R, Rinaldi F, et al. PAX7 targets, CD54, integrin α9β1, and SDC2, allow isolation of human ESC/iPSC-derived myogenic progenitors. *Cell Rep* 2017;**19**:2867–2877.
157. Darabi R, Santos FN, Filareto A, Pan W, Koene R, Rudnicki MA, et al. Assessment of the myogenic stem cell compartment following transplantation of Pax3/Pax7-induced embryonic stem cell-derived progenitors. *Stem Cells* 2011;**29**:777–790.
158. Winkler T, von Roth P, Radojewski P, Urbanski A, Hahn S, Preininger B, et al. Immediate and delayed transplantation of mesenchymal stem cells improve muscle force after skeletal muscle injury in rats. *J Tissue Eng Regen Med* 2012;**6**:s60–s67.
159. Brickson S, Meyer P, Saether E, Vanderby R. Mesenchymal stem cells improve muscle function following single stretch injury: a preliminary study. *J Funct Morphol Kinesiol* 2016;**1**:396–406.
160. Aguado BA, Mulyasasmita W, Su J, Lampe KJ, Heilshorn SC. Improving viability of stem cells during syringe needle flow through the design of hydrogel cell carriers. *Tissue Eng Part A* 2012;**18**:806–815.
161. Bouchentouf M, Benabdallah BF, Rousseau J, Schwartz LM, Tremblay JP. Induction of anoikis following myoblast transplantation into SCID mouse muscles requires the Bit1 and FADD pathways. *Am J Transplant* 2007;**7**:1491–1505.
162. Qu Z, Balkir L, van Deutekom JC, Robbins PD, Pruchnic R, Huard J. Development of approaches to improve cell survival in myoblast transfer therapy. *J Cell Biol* 1998;**142**:1257–1267.
163. Huard J, Roy R, Bouchard JP, Malouin F, Richards CL, Tremblay JP. Human myoblast transplantation between immunohistocompatible donors and recipients produces immune reactions. *Transplant Proc* 1992;**24**:3049–3051.
164. Tremblay JP, Malouin F, Roy R, Huard J, Bouchard JP, Satoh A, et al. Results of a triple blind clinical study of myoblast transplantations without immunosuppressive treatment in young boys with Duchenne muscular dystrophy. *Cell Transplant* 1993;**2**:99–112.
165. Skuk D, Tremblay JP. Necrosis, sarcolemmal damage and apoptotic events in myofibers rejected by CD8+ lymphocytes: observations in nonhuman primates. *Neuromuscul Disord* 2012;**22**:997–1005.
166. Law PK, Goodwin TG, Fang Q, Duggirala V, Larkin C, Florendo JA, et al. Feasibility, safety, and efficacy of myoblast transfer

therapy on Duchenne muscular dystrophy boys. *Cell Transplant* 1992;**1**:235–244.
167. Tedesco FS, Dellavalle A, Diaz-Manera J, Messina G, Cossu G. Repairing skeletal muscle: regenerative potential of skeletal muscle stem cells. *J Clin Invest* 2010;**120**:11–19.
168. Zhao Q, Ren H, Han Z. Mesenchymal stem cells: immunomodulatory capability and clinical potential in immune diseases. *Journal of Cellular Immunotherapy* 2016;**2**:3–20.
169. Urciuolo A, Quarta M, Morbidoni V, Gattazzo F, Molon S, Grumati P, et al. Collagen VI regulates satellite cell self-renewal and muscle regeneration. *Nat Commun* 2013;**4**:1964.
170. Quarta M, Brett JO, DiMarco R, De Morree A, Boutet SC, Chacon R, et al. An artificial niche preserves the quiescence of muscle stem cells and enhances their therapeutic efficacy. *Nat Biotechnol* 2016;**34**:752–759.
171. Davoudi S, Chin C-Y, Cooke MJ, Tam RY, Shoichet MS, Gilbert PM. Muscle stem cell intramuscular delivery within hyaluronan methylcellulose improves engraftment efficiency and dispersion. *Biomaterials* 2018;**173**:34–46.
172. Sleep E, Cosgrove BD, McClendon MT, Preslar AT, Chen CH, Sangji MH, et al. Injectable biomimetic liquid crystalline scaffolds enhance muscle stem cell transplantation. *Proc Natl Acad Sci* 2017;**114**:E7919–E7928.
173. Marklein RA, Burdick JA. Controlling stem cell fate with material design. *Adv Mater* 2010;**22**:175–189.
174. Qazi TH, Mooney DJ, Duda GN, Geissler S. Biomaterials that promote cell–cell interactions enhance the paracrine function of MSCs. *Biomaterials* 2017;**140**:103–114.
175. Youssef A, Aboalola D, Han VK. The roles of insulin-like growth factors in mesenchymal stem cell niche. *Stem Cells Int* 2017;**2017**:9453108.
176. Ryder S, Leadley RM, Armstrong N, Westwood M, de Kock S, Butt T, et al. The burden, epidemiology, costs and treatment for Duchenne muscular dystrophy: an evidence review. *Orphanet J Rare Dis* 2017;**12**:79.
177. Bajek A, Porowinska D, Kloskowski T, Brzoska E, Ciemerych MA, Drewa T. Cell therapy in Duchenne muscular dystrophy treatment: clinical trials overview. *Crit Rev Eukaryot Gene Expr* 2015;**25**:1–11.
178. Wilschut KJ, Ling VB, Bernstein HS. Concise review: stem cell therapy for muscular dystrophies. *Stem Cells Transl Med* 2012;**1**:833–842.
179. Park EJ, Kang J, Baik SH. Treatment of faecal incontinence using allogeneic-adipose-derived mesenchymal stem cells: a study protocol for a pilot randomised controlled trial. *BMJ Open* 2016;**6**:e010450.
180. Sarveazad A, Newstead GL, Mirzaei R, Joghataei MT, Bakhtiari M, Babahajian A, et al. A new method for treating fecal incontinence by implanting stem cells derived from human adipose tissue: preliminary findings of a randomized double-blind clinical trial. *Stem Cell Res Ther* 2017;**8**:40.
181. Boyer O, Bridoux V, Giverne C, Bisson A, Koning E, Leroi AM, et al. Autologous myoblasts for the treatment of fecal incontinence: results of a phase 2 randomized placebo-controlled study (MIAS). *Ann Surg* 2018;**267**:443–450.
182. Peters KM, Dmochowski RR, Carr LK, Robert M, Kaufman MR, Sirls LT, et al. Autologous muscle derived cells for treatment of stress urinary incontinence in women. *J Urol* 2014;**192**:469–476.
183. Carr LK, Robert M, Kultgen PL, Herschorn S, Birch C, Murphy M, et al. Autologous muscle derived cell therapy for stress urinary incontinence: a prospective, dose ranging study. *J Urol* 2013;**189**:595–601.
184. Winkler T, Perka C, Roth P, Agres AN, Plage H, Preininger B, et al. Immunomodulatory placental-expanded, mesenchymal stromal cells improve muscle function following hip arthroplasty. *J Cachexia Sarcopenia Muscle* 2018;**9**:880–897.
185. von Haehling S, Morley JE, Coats AJS, Anker SD. Ethical guidelines for publishing in the Journal of Cachexia, Sarcopenia and Muscle: update 2017. *J Cachexia Sarcopenia Muscle* 2017;**8**:1081–1083.