Published in final edited form as:

Expert Opin Biol Ther. 2010 April ; 10(4): 505–517. doi:10.1517/14712591003610606.

Potential therapeutic applications of muscle-derived mesenchymal stem and progenitor cells

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Abstract

Importance of the field—Mesenchymal adult stem cells have properties that make them attractive for use in tissue engineering and regenerative medicine. They are inherently plastic, enabling them to differentiate along different lineages, and promote wound healing and regeneration of surrounding tissues by modulating immune and inflammatory responses, promoting angiogenesis and secreting other trophic factors. Unlike embryonic stem cells, clinical uses of mesenchymal stem cells are not encumbered by ethical considerations or legal restrictions.

Areas covered in this review—We discuss skeletal muscle as a source of mesenchymal stem and progenitor cells by reviewing their biology and current applications in tissue engineering and regenerative medicine. This paper covers literature from the last 5 – 10 years.

What the reader will gain—Skeletal muscle is a plentiful source of mesenchymal stem and progenitor cells. This tissue may be obtained via routine biopsy or collection after surgical debridement. We describe the biology of these cells and provide an overview of therapeutic applications currently being developed to take advantage of their regenerative properties.

Take home message—There is potential for stem and progenitor cells derived from skeletal muscle to be incorporated in clinical interventions, either as a cellular therapy to modify the natural history of disease or as a component of engineered tissue constructs that can replace diseased or damaged tissues.

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Declaration of interest

LJ Nesti has received funding from the Military Amputee Research Program #PO5-A011 and Comprehensive Neurosciences Program #CNP-2008-CR01. RS Tuan received NIH intramural Support (Z01 AR41131).

Keywords

MPCs; MSCs; regenerative medicine; skeletal muscle stem cells; tissue engineering

1. The promise of mesenchymal stem and progenitor cells

Mesenchymal stem cells (MSCs) are a population of adult stem cells with many properties that make them attractive for use in the fields of tissue engineering and regenerative medicine [1–4]. These cells are inherently plastic, enabling them to differentiate along different lineages under the appropriate induction conditions. They also appear to exhibit a number of trophic properties that promote regeneration in the surrounding tissue [5]. MSCs can be harvested from a variety of adult tissues, and unlike embryonic stem cells, their use is not encumbered by ethical considerations or legal restrictions. As a result, there is considerable hope that MSCs will be incorporated in a variety of clinical interventions, either as a cellular therapy to modify the natural history of diseases or as a component of engineered tissue constructs that can replace diseased or damaged tissues. This paper reviews skeletal muscle as a potential source of MSCs, highlights a variety of current applications for muscle-derived MSCs, and suggests some potential advantages of harvesting MSCs from skeletal muscle.

MSCs were originally discovered as adherent, fibroblastic cells in bone marrow aspirates that were capable of multilineage differentiation [6,7]. Following this discovery, the differentiation capacity of MSCs has been verified *in vivo* by inducing the cells to form ectopic bone [7]. Given their ability to differentiate, an obvious application for the cells was to incorporate them into a suitable biomaterial scaffold that would encourage them to form tissues *de novo*, either *in vivo* or *in vitro* [8,9]. In the last decade, there have been substantial developments in the field of tissue engineering [10,11]. Although it is not yet possible to grow replacement tissues and organs in the laboratory for widespread clinical use, MSCs continue to be considered as an important component in a variety of tissue engineering applications.

More recently, it has been noted that the regenerative benefit of MSCs does not appear to correlate solely or directly with their ability to differentiate into the diseased tissue type [12]. In a number of studies, MSCs have been injected into diseased tissue, such as the heart [13,14] and brain [15,16]. Although there was functional improvement to these tissues following MSC injection, there was little evidence of the MSCs differentiating into the surrounding cell types, as was expected. Instead, these studies have led to the discovery that MSCs promote wound healing and regeneration in the surrounding tissues by modulating the local inflammatory responses [17,18] and by limiting fibrosis of the functional tissues [19]. It has since been shown that MSCs also promote angiogenesis and secrete trophic (i.e., pro-growth and pro-survival) factors that augment the endogenous regeneration process [20,21]. Although substantial investigation is still needed to elucidate fully the mechanisms of their trophic behavior, MSCs are an important cell type used in many strategies for cell-based therapy.

Despite the potentially far-reaching promise of MSCs in many aspects of regenerative medicine and tissue engineering, any approaches using these cells are limited by the availability of a suitable MSC population in a clinical setting. The most common source of MSCs for clinical use is the bone marrow. However, the low concentration of MSCs in the bone marrow necessitates the application of specialized equipment to concentrate the MSCs prior to use [22], and may still yield too few cells for many tissue engineering applications [23]. Furthermore, in many countries, including the USA, there is a substantial regulatory

burden associated with *ex vivo* expansion and maintenance of these cells for clinical use [24]. Similar limitations also exist for MSCs derived from adipose tissue, which is another commonly used source of MSCs [25]. As a result, there is considerable research into alternative sources of MSCs that may be able to overcome these clinical limitations. This review will focus on skeletal muscle as an alternative source of MSCs and on the potential clinical advantages of using the cells derived from this source.

2. Skeletal muscle-derived mesenchymal stem and progenitor cells

MSCs were first identified in the adherent fraction of cells in a bone marrow aspirate when it was observed that they can be induced to differentiate into osteoblasts and adipocytes *in vitro* [26]. Although these are important characteristics of MSCs, there is no definitive, agreed-upon marker to positively identify a population that is capable of these functions [27]. Instead, there is a set of three minimal criteria that must be met by an MSC population, which are designed to encourage consistency between investigators [28]: i) the population must be adherent to tissue culture plastic (TCP) and capable of *in vitro* expansion on TCP; ii) the cell surface epitope profile of the population must meet specific requirements (Table 1) to ensure a uniform cell type and minimal contamination by leukocytes and hematopoietic progenitor cells; and iii) the cells must be capable of differentiating into osteoblasts, adipocytes and chondrocytes *in vitro*. These criteria were developed for cell populations harvested from the bone marrow, and although applicable to MSCs harvested from other tissues, additional tissue-specific criteria may also apply to these other MSC populations.

In addition to bone marrow aspirate, MSCs have been isolated from a variety of other adult tissues. In particular, the discovery of MSCs in adipose tissue aspirates was exciting from a clinical standpoint because it provided an additional source of tissue that is clinically available and can be processed to yield MSCs [25,29]. Adipose-tissue-derived MSCs could also yield a viable allogeneic source by harvesting cells from tissue collected during liposuction procedures [30]. MSCs have been isolated from other connective tissues, such as the marrow space of long bones [31,32], trabecular bone chips [33–35], periosteum [36,37], synovial fluid [38–40], periodontal ligament [41,42], palatine tonsil [43], parathyroid gland [44] and fallopian tube [45]. Cells derived from these tissues are of scientific interest; however, due to the limited clinical availability of these tissues, applications for their use as a source of cells for biological therapy are not obvious. Finally, MSCs have been harvested from tissues that are lost as a result of development, such as umbilical cord [46,47], umbilical cord blood/Wharton's Jelly [48,49] and primary tooth dental pulp [50,51], and these have been identified as potential sources of MSCs for cell banking [18].

Recently, there have been several reports of harvesting human MSC-like cells from adult skeletal muscle. The muscle tissue used to harvest the cells was obtained from healthy muscle tissue biopsies [52], surgical waste tissue from orthopaedic reconstructions ([53] and unpublished observations: Nesti *et al.*), or surgically debrided muscle tissue following orthopaedic trauma [53]. Given that these cells can be obtained from surgical waste tissue or with minimally invasive biopsy procedures, there is growing evidence that skeletal muscle may be an important clinical source of MSCs for use in therapeutic applications. The purpose of this review is to highlight the work that has been done to characterize the muscle-derived MSCs and the use of these cells in tissue engineering and regenerative medicine.

3. Origins of stem and progenitor cells in muscle tissue

Several cell populations with the properties of mesenchymal stem cells have been previously isolated from the skeletal muscle. One of the best characterized populations are the muscle derived stem cells (MDSCs) harvested from murine skeletal muscle [54,55]. MDSCs are isolated from the muscle homogenate using a pre-plating technique, which enriches the

population of MDSCs by eliminating the contaminating populations of more adherent cell types [56]. The more slowly adherent MDSCs have demonstrated enhanced differentiation potential, and can be readily induced to become osteoblasts, adipocytes and chondrocytes *in vitro*. An additional feature of this cell type is their ability to differentiate into myoblasts *in vitro* and to promote muscle regeneration *in vivo* [57].

Interestingly, this population of MDSCs has not been harvested from human muscle tissue solely on the basis of their adhesion characteristics. Instead, a population of cells with similar *in vitro* characteristics has been identified in human skeletal muscle using FACS to isolate the cells that are positive for CD34, CD56 and CD144 [52,58]. These cells, called myoendothelial cells, express surface markers of both endothelial (CD34 and CD144) and satellite cells (CD56), which are skeletal muscle stem cells. Typically, satellite cells are quiescent myoblast precursors that lie adjacent to the myofibers beneath the basal lamina, and divide asymmetrically in response to muscle injury [59]. Although most satellite cells are committed to the myogenic lineage, the small subset of satellite cells that co-express the endothelial makers (less than 0.5% of all satellite cells) are associated with the vasculature *in vivo* and capable of osteogenic, adipogenic and osteogenic differentiation [52]. Myoendothelial cells appear to have enhanced myogenic potential *in vivo* compared with satellite cells. There is also evidence that these cells can be induced to differentiate into endothelial cells under the appropriate conditions. However, these are not the only multipotent stem cell type in skeletal muscle with an anatomical affiliation to the vasculature.

Pericytes are cells intimately associated with capillaries and microvessels. They play several roles in the maintenance of the vasculature [60], and recent experiments suggest that these cells may have other important functions for tissue regeneration. Isolated pericytes are capable of regenerating muscle tissue *in vivo* [61], and they can differentiate into myocytes, osteoblasts, adipocytes and chondrocytes *in vitro* [62,63]. Given that pericytes are present in almost every tissue in the body, it has been suggested that MSCs harvested from various tissues were in fact pericytes, or a similar cell type, which originated in the vasculature of those tissues [64]. Recently, there has been compelling evidence to support this hypothesis by the demonstration that a sub-population of pericytes express the markers used to identify MSCs and exhibit the *in vitro* differentiation characteristics of MSC populations [65]. Based on these observations, it has been proposed that pericytes serve as a reservoir of multipotent cells that can be recruited from the vasculature as needed to repair the tissue in response to injury.

Another distinct population of multipotent cells has been harvested from skeletal muscle following traumatic injury [53]. One important distinction that has been made about the traumatized-muscle-derived progenitor cells is that they are rapidly adherent during the harvesting procedure, as opposed to the MDSCs, which are selected on the basis of their slow adherence. The MPCs are also present in substantial numbers at the time of harvest. Multipotent cells in uninjured muscle are rare, whereas approximately one million cells per gram of tissue can be isolated from injured muscle [66]. The debrided muscle tissue from which MPCs are harvested was in the process of wound healing and tissue remodeling in response to a traumatic injury [67]. These observations support the hypothesis that multipotent cells are recruited from their niche following injury, and that they proliferate in the tissue to fulfill their regenerative function [64,68]. As a result, the cells harvested from traumatized muscle are referred to as mesenchymal progenitor cells (MPCs) to indicate that these cells may not have been in a quiescent, stem cell state when they were harvested (Figure 1). However, except that they are available in greater numbers in the tissue, there do not appear to be any major differences between the traumatized-muscle-derived MPCs and a typical MSC population [53,66].

Traumatized muscle-derived MPCs also exhibit several of the trophic properties that are associated with MSCs. *In vitro* studies have shown that MPCs can modulate local inflammatory responses [69], promote angiogenesis by increasing the rate of endothelial cell proliferation and inhibit apoptosis of nearby cells [70,71]. The MPCs also appear to be intrinsic to severely injured muscle that has compromised tissue architecture [67]. These observations suggest that MPCs play a role in promoting the functional regeneration of skeletal muscle following traumatic injury. By providing a biochemical environment that favors tissue growth, the MPCs could augment the ability of committed myogenic progenitor cells to remodel the tissue and generate new skeletal muscle.

The origin of these proliferating cells is not currently known, although it is possible that the population of pericytes serves as a cellular reservoir for MPCs as well as the myoendothelial and satellite cell types. A model for pericyte recruitment can be constructed in which the local tissue architecture determines the pericyte fate (Figure 2). In response to minor, routine muscle damage, pericytes may be recruited from their vascular niche and travel along the architecture of the skeletal muscle to the site of muscle damage where they can augment satellite cell regeneration of the myofibers and may differentiate into satellite cells after the myofiber has been repaired [52]. Alternatively, following severe injury, the architecture of the skeletal muscle may be disrupted such that the recruited pericytes have no orientation in the tissue. In this case, they are free to proliferate in the cellular milieu, which includes inflammatory leukocytes and other wound healing cell types (e.g., fibroblasts and myofibroblasts), and assume the MPC phenotype. According to this model, skeletal muscle contains several stem cell types, which can respond to the severity of injury and repair minor muscle damage or remain as a more plastic stem cell type to promote regeneration following major tissue damage. The distinctions made between the MDSCs, myoendothelial cells, pericytes and traumatized-muscle-derived MPCs are based on their *in vitro* characteristics and species of origin, which are summarized in Table 2.

4. Muscle-derived MSCs and MPCs for tissue engineering

Muscle-derived stem cells have been used in various tissue engineering strategies to generate replacement structures for tissue that has damaged or diseased. The general approach to tissue engineering is to design a scaffold to support a stem cell population, and frequently at least one biochemical factor is used to promote and guide differentiation of the MSCs into the desired cell type. The engineered device can be implanted to replace the damaged tissue of the host, and over time, it will be remodeled and incorporated into the adjacent tissue. There has been exhaustive work to develop such tissue engineered devices using MSCs derived from bone marrow (reviewed in [1,32]), adipose tissue (reviewed in [72]) and other tissues, but recent work suggests that muscle stem cells could be an alternative cell source for several of these engineered tissues.

4.1 Bone

Following a complex fracture or traumatic injury, a gap or segmental defect may occur in the bone. A segmental defect can also be generated during the repair of a non-union fracture or when a tumor must be removed from the bone. Once formed, surgeons have limited options to repair the bone to its original length. A tissue engineered bone graft would provide an optimal solution for repairing the segmental defect.

Several populations of muscle-derived stem cells have osteogenic potential, including MDSCs [55], MPCs [53], pericytes [65] and myoendothelial cells [52]. Considerable work has been done to determine the optimal conditions for the MDSC cell type to generate bone tissue *in vivo* [73,74]. By pre-transfecting MDSCs with the gene for bone morphogenetic protein-4 (BMP-4), the MDSCs produced this osteoinductive factor *in vivo* upon

implantation and then differentiated into osteoblasts [75]. When these cells were loaded onto an implantable scaffold, the MDSCs were able to fill a critical sized femoral defect with bone in a rat model. MDSCs expressing BMP-4 have also been seeded in both collagen and gelatin sponges and were sufficient to repair a critical sized calvarial defect in mice [73,76]. These cells are being evaluated for use with fibrin and collagen gels to fill bone defects in the craniofacial skeleton [77].

4.2 Articular cartilage

Osteoarthritis is a disease characterized by degeneration of the joint articular cartilage. Once a defect is initiated in the joint surface, for example as a result of trauma or degenerative joint disease such as osteoarthritis, the damaged articular cartilage cannot be regenerated by endogenous repair mechanisms [78]. The damaged region can be surgically removed to alleviate pain associated with the disease, but this procedure results in a reduction in the articular surface. Substantial effort has been made to tissue engineer a cartilage plug, which could replace the damaged tissue and restore full function to the joint [79].

MDSCs, pericytes and traumatized-muscle-derived MPCs have chondrogenic potential and might be applicable for the engineering of a cartilage tissue construct. The ability of these cell types to differentiate into chondrocytes has been evaluated based on their ability to express collagen type II and an extracellular matrix rich in sulfated proteoglycans [62,66,80]. Furthermore, MDSCs transfected with the gene for BMP-4 adopt a chondrogenic phenotype *in vitro*. Implantation of these cells into a full thickness osteochondral defect in the articular cartilage of rat knees using fibrin glue resulted in persistent repair at the defect site after 24 weeks [81]. In a similar experiment, MDSCs were seeded in bovine collagen type I gels [82], and after culturing for 3 weeks *in vitro*, the constructs were implanted into full thickness osteochondral defects in the knees of New Zealand white rabbits. After 24 weeks, the implanted MDSCs provided improved regeneration of the articular surface, based on expression of collagen type II and construct integration, compared with control constructs containing chondrocytes. These experiments demonstrate the potential of muscle-derived stem and progenitor cells for cartilage tissue engineering.

5. Muscle-derived MSCs and MPCs as trophic mediators of tissue regeneration

An alternative therapeutic approach using muscle-derived MSCs takes advantage of their trophic properties to enhance the endogenous mechanism of regeneration [83]. For this strategy, it is not necessary for the muscle-derived cells to differentiate in order for tissue repair to occur. Instead, muscle-derived cells will act to promote the surrounding cells to regenerate the desired tissue, and thus modify the natural history of some disease mechanisms. Although similar studies have been performed in MSCs harvested from other tissues (reviewed in [84–87]), there are several applications for tissue regeneration using muscle-derived MSCs and MPCs.

5.1 Bone and cartilage

An alternative approach to regenerating bone tissue with mesenchymal stem and progenitor cells may not require the differentiation of these cells into osteoblasts. In a recent study, co-transfection of MDSCs with the gene coding for BMP-4 and the gene for VEGF led to enhanced endochondral ossification to form bone tissue compared to MDSCs that were transfected with only BMP-4 [74]. VEGF is an important mediator of fracture healing [88], and the synergistic interaction of VEGF with BMP-4 appeared to enable greater recruitment of local MSCs from the bone marrow, promoted angiogenesis of the regenerating tissue and accelerated the process of cartilage tissue resorption [89]. A similar synergistic relationship

has been observed in terms of VEGF and BMP-2 expression. MPCs derived from traumatized muscle tissue express high levels of VEGF [70], suggesting that they could provide a similar regenerative benefit to regenerating bone tissue without the need for gene transfection prior to implantation. These cells could be implanted on a scaffold to promote conduction of endogenous MSCs into a segmental defect, or the cells could be injected directly into a fracture callus to improve the rate of healing and reduce the likelihood of a non-union [90]. A similar approach has been used to promote chondrogenesis in a murine model of osteoarthritis by intra-articular injection of MDSCs expressing BMP-4 and VEGF [91].

5.2 Nerve

The trophic properties of muscle-derived stem and progenitor cells may also be useful to promote peripheral nerve regeneration. Peripheral nerve trauma frequently accompanies orthopaedic trauma, and may result in a segmental defect of the nerve that must be bridged to restore function to the distal extremities. The process of nerve regeneration is typically mediated by neurotrophic factors (NTFs) that are expressed by Schwann cells, which are the support cells in peripheral nerves [92]. MPCs derived from traumatized muscle also express many of the NTFs that are important to nerve regeneration, such as brain derived neurotrophic factor (BDNF), nerve growth factor (NGF), ciliary neurotrophic factor (CNTF) and neurotrophin-3 (NT-3), and the factors secreted by the MPCs are sufficient to enhance the rate of axon growth *in vitro* [93]. The ability of these cells to promote nerve regeneration *in vitro* is currently being evaluated using a rat model of sciatic nerve injury. Similarly, MDSCs seeded in a silicone nerve guide have been shown to augment sciatic nerve regeneration similar to a population of neural progenitor cells in nude mice [94].

5.3 Skeletal/cardiac muscle

An obvious therapeutic application for muscle-derived stem and progenitor cells is to promote the regeneration of skeletal muscle. Skeletal muscle has substantial capacity to regenerate itself by activating the reservoir of muscle-specific stem cells and other mechanisms discussed earlier in this review. However, following severe injury in the muscle tissue, the regenerative activities of the satellite cells are in competition with the pro-fibrotic activities of fibroblasts that also proliferate in the region of injury [95,96]. One factor that determines which cell type can dominate the wound healing process is the ratio of TGF- β 1 to TGF- β 3 [97]. When this ratio is high, the excess amount of TGF- β 1 stimulates the fibroblasts to secrete disorganized extracellular matrix proteins and leads to fibrosis in the tissue [98,99]. The fibrotic tissue physically impedes the ability of the satellite cells to repair the muscle tissue.

There have been several studies supporting the possibility that muscle-derived stem cells can augment wound healing following injury. MPCs derived from traumatized muscle express high levels of TGF- β 3 (unpublished observations: Jackson *et al.*), which may help maintain a balance of cytokines to promote muscle regeneration. Furthermore, using models of skeletal muscle injury in mice, treatment with MDSCs results in improved muscle regeneration, which is attributed to ability of the MDSCs to recruit capillaries and nerves into the region of muscle regeneration [100]. The MDSCs can also differentiate directly into endothelial cells and cell types with neuronal characteristics [55]. As a result, treatment with MDSCs results in superior muscle regeneration compared with treatment with satellite cells. MDSCs may also provide some benefit to patients suffering from Duchenne Muscular Dystrophy, as *mdx* mice, which are a model of dystrophic muscle, exhibit an improved muscle structure following treatment with MDSC [101].

Stem and progenitor cells from skeletal muscle may also promote the regeneration of cardiac muscle tissue [102,103]. Ischemic or infarcted tissue that results from a coronary episode can result in a reduction in cardiac function. Injection of MDSCs into the infarcted region of cardiac tissue resulted in improved function compared with injection with myoblasts [104]. The improvement in function was attributed to enhanced engraftment of the injected MDSCs and fusion of these cells with the host cardiomyocytes [105]. Furthermore, there was evidence that MDSCs mediated a secondary mechanism of regeneration by promoting angiogenesis in the tissue [106]. Similar results have been observed after injection of myoendothelial cells into infarcted myocardium [107]. As a result, MDSCs and myoendothelial cells are a promising candidate cell type for cell transplantation therapies to improve cardiac function following infarction.

5.4 Other tissues

There are two other notable examples of therapeutic applications that are based on the trophic properties of muscle-derived stem and progenitor cells. First, muscle-derived stem and progenitor cells are currently being used in vascular regeneration. MDSCs seeded on a compound polyester urethane urea scaffold have been used to promote the proliferation and migration of smooth muscle and vascular endothelial cells into the luminal space of the construct *in vivo* [108]. As a result, regenerating blood vessels that were exposed to MDSC trophic factors exhibited greater patency, less fibrosis and greater mechanical strength than the acellular controls. MPCs derived from traumatized muscle also express many of the growth factors associated with MSC-mediated maintenance of vascular endothelial cells [70]. MPCs can also promote the proliferation of endothelial cells *in vitro*, and these cells are currently being vetted for use in a vascular graft device.

A variety of MSC populations have also been used to correct urological disorders (reviewed in [109]), including those derived from skeletal muscle tissue. MDSCs have been used as a cellular therapy to overcome stress urinary incontinence. In this disorder, a weakening of the urethral sphincter results in involuntary urine release as a result of increased intra-abdominal pressure. Using an animal model of stress urinary incontinence, periurethral injection of MDSC resulted in improved urological function [110]. In addition to differentiation into smooth muscle to enhance contractility in the sphincter muscle, these cells also appeared to promote innervation of the myofibers, which provided further enhancement to the urethral sphincter function [111]. MDSCs seeded on intestinal submucosa and implanted into a rat model to have also been shown to promote the repair of vaginal tissue *in vivo* [112].

6. Expert opinion

In recent years, skeletal muscle has emerged as a promising tissue source for mesenchymal stem and progenitor cells that can be used in a variety of therapeutic applications. Skeletal muscle is one of the most plentiful tissues in the body, accounting for approximately one third of body weight in a healthy individual [23]. The high capacity of muscle to repair itself after injury suggests that it serves as a reservoir for cells that participate in tissue regeneration processes [113]. Several research groups have characterized different muscle-derived stem cell populations that exhibit the ability to differentiate into multiple cell types, including osteoblasts, adipocytes, chondrocytes, myoblasts and endothelial cells. In addition, there is evidence that these cells exhibit the same trophic properties (i.e., pro-growth, anti-inflammation and anti-apoptotic) that are attributed to the pro-regeneration effects of bone marrow-derived MSCs. These cells, which can be obtained via minimally invasive muscle biopsy, may provide surgeons with clinically versatile populations of stem cells.

In addition to the stem cell populations that can be harvested from healthy muscle, it has also been reported that MPCs can be harvested from muscle tissue following traumatic

injury. Harvesting traumatized-muscle-derived MPCs offers two substantial advantages over untraumatized tissue as a cell source. First, the MPCs can be harvested from tissues at the wound margins of an open fracture, which are surgically debrided as a part of the standard method of care for these injuries. Therefore, there is no need to perform additional procedures to acquire tissue for cell harvesting. Second, the MPCs are present in substantially greater numbers in the traumatized muscle compared with uninjured muscle tissue. It is speculated that the MPCs begin to proliferate (or migrate to the injury site) in response to the injury, and are thus harvested while they are still at the uncommitted progenitor cell state. As a result, the surgeon may have access to greater numbers of mesenchymal stem and progenitor cells for therapeutic applications without requiring a large mass of muscle tissue. A patient who has been exposed to substantial soft tissue damage and/or open fractures would be an ideal candidate for autologous traumatized-muscle-derived MPC therapy, as these cells could be used to regenerate the tissues in these wounds (e.g., bone, muscle, nerve and cartilage). Current research is directed towards identifying and characterizing the cell proliferation and stimulation factors that are associated with traumatic injury, with the goal of applying them to other populations of MSCs derived from uninjured muscle tissue.

One limitation to the use of muscle-derived stem and progenitor cells in therapeutic application is the lack of standards in the cell types that are currently being investigated. There are at least three distinct populations of cells harvested from human skeletal muscle (i.e., pericytes, myoendothelial cells and traumatized-muscle-derived MPCs) and one murine cell population (MDSC) that have the characteristics of an MSC population. Additionally, there are several other stem cell populations (i.e., mesoangioblasts [114], side population cells [115], and CD133⁺ cells [116]) that do not meet the requirements for MSCs, and were therefore not included in this review. Finally, there are muscle-specific stem cells (i.e., satellite cells and myoblasts) that are capable of only myogenic differentiation. Each of these cell types is characterized primarily on the basis of their *in vitro* characteristics after they have been harvested from the body. However, it is evident that the phenotype of these cells may shift *in vivo* as they migrate through different tissues and are exposed to different extracellular and environmental signals. While rudimentary models may be developed to describe the *in vivo* relationship among these stem cell populations (see Figure 2), substantial additional studies are needed to refine and verify these relationships. A better understanding of how the muscle-derived mesenchymal stem and progenitor cells are related will allow us to better predict the regenerative capabilities of cell populations that are harvested by differing methods.

There are two broad strategies for therapeutic applications using muscle-derived stem and progenitor cells (Figure 3). The first is a tissue engineering strategy, whereby the cells are loaded onto a construct (i.e., a scaffold) and induced to differentiate (using biochemical factors) into the appropriate cell type for the tissue that is being engineered. The second strategy takes advantage of the inherently trophic nature of the stem and progenitor cells to promote the endogenous mechanisms of tissue regeneration *in vivo*. While the promise of the former strategy to generate replacement organs and tissues has generated a great deal of investigation, the latter strategy may present more opportunities for clinical success in the immediate future.

There are several factors that limit the clinical success to the current approach of tissue engineering with muscle-derived stem cells. First, the majority of tissue engineering studies have been performed using the murine MDSC population, and the human cell type that is most similar to the MDSCs are the myoendothelial cells, which are rare in skeletal muscle. Substantial *ex vivo* expansion will be required to generate sufficient numbers of cells for tissue engineering applications. Second, in many of the tissue engineering studies using

MDSC, the cells were transfected with genes that would promote differentiation prior to implantation. While this is an excellent system to study the cellular and molecular mechanisms involved in tissue engineering, whether the MDSCs are merely acting as gene delivery vehicles rather than as a stem cell population in the process should be taken into consideration [82]. Furthermore, the safety concerns about gene therapy approaches may limit direct application of these strategies to move from animal models towards clinical translation [117]. It is critical that the efficacy of these tissue engineering studies be tested using stem cell populations that can be harvested from human patients to facilitate the translation of these therapeutic applications into clinical use.

In comparison, the use of stem and progenitor cells as trophic mediators may present a more promising immediate future. In principle, this strategy requires substantially less development, as it chiefly takes advantage of the intrinsic trophic or pro-regenerative properties of the MSC populations. The regenerative benefits of several stem cell populations have already been demonstrated. In this review, we have described several applications using stem cells derived from human skeletal muscle to regenerate a variety of tissue types, many of which are nearing clinical translation. Taken together, these findings strongly illustrate the great potential of this approach for the development of therapeutic applications in the near-term, and underscore the critical importance of research efforts directed at elucidating and harnessing the regenerative properties of skeletal-muscle-derived stem and progenitor cells.

Article highlights

- Skeletal muscle is a reservoir for cells with properties that are similar to those of mesenchymal stem cells.
- Several therapeutic cell types have been isolated from muscle tissue and are distinguished on the basis of their *in vitro* behavior. The relationship between these cells *in vivo* is unclear, but they are probably related to pericytes.
- Mesenchymal stem and progenitor cells may be harvested from muscle tissue and used in a variety of therapeutic and regenerative medicine applications.
- These cell types can be differentiated and delivered via seeding onto biomaterial scaffolds for tissue engineering applications designed to replace diseased or damaged tissues.
- Muscle-derived mesenchymal stem and progenitor cells also secrete trophic factors that can promote and regulate endogenous mechanisms of tissue regeneration.

This box summarizes key points contained in the article.

Acknowledgments

Figures were produced using Servier Medical Art.

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Figure 1. Origin and characteristics of muscle-derived MSCs

Traumatized-muscle-derived MPCs are undifferentiated progenitor cells that have been activated in response to the injury and are proliferating in the tissue. These cells have not yet terminally differentiated and maintain the ability to differentiate into osteoblasts, adipocytes or chondrocytes.

Figure 2. Functional characteristics of pericytes

Pericytes may respond to injury by leaving their vascular niche and migrating into the damaged muscle tissue. In an environment where there is sufficient muscle fiber architecture, they will retain a muscle stem cell phenotype. However, if the tissue damage is extensive, the pericytes may not have sufficient environmental signals and will instead display a functional phenotype that resembles that of MSCs or traumatized-muscle-derived MPCs.

Figure 3. Schematics of two general strategies in the application of muscle-derived mesenchymal stem and progenitor cells in regenerative medicine

(1) The cells can be differentiated into a particular cell type that is needed to replace damaged tissues, or (2) the cells can be used as trophic mediators that will enhance endogenous tissue repair mechanisms.

Table 1

Required cell surface epitope profile for a population of MSCs [28].

Surface marker	Protein name	Indicator for HSC [*]
≥95% of the cell population must be positive for		
CD73	ecto-5'-nucleotidase (SH3 and SH4)	Non-specific
CD90	thy-1	Non-specific
CD105	endoglein (SH2)	Non-specific
≤98% of the cell population must be negative for		
CD34	Unknown antigen	Primitive hematopoietic progenitors and endothelial cells
CD45	Protein tyrosine phosphatase receptor type C	Pan-leukocyte marker
CD14 or CD11b [‡]	Unknown antigen or integrin α M	Monocytes and macrophages
CD79 α or CD19 [‡]	Immunoglobulin-associated α or unknown antigen	B-cells
HLA-DR [§]	MHC class II (heterodimer)	Immunologically simulated cells

^{*} HSC: Hematopoietic stem cell.

[‡] Either marker is sufficient for the given requirement.

[§] Cells positive for HLA-DR may still be considered MSCs, but this marker indicates that they have been stimulated (e.g., by IFN- γ), which should be indicated when describing the cell type (i.e., as 'stimulated' MSCs).

The proteins associated with CD34, CD19 and CD19 are currently unknown.

Table 2

Mesenchymal stem and progenitor cells identified in skeletal muscle tissue.

Cell type	Species	In vitro differentiation *						Trophic?
		OB	A	C	EC	MB	N	
Muscle derived stem cells (MDSCs)	Mouse	+	+	+	+	+	+	Yes
Myoendothelial cells	Human	+	+	+	+	+	+	Yes
Pericytes	Various (including mouse and human)	+	+	+	+	+	+	Yes
Mesenchymal progenitor cells (MPCs)	Human (traumatized muscle)	+	+	+	+	+	+	Yes

* A: Adipocytes; C: Chondrocytes; EC: Endothelial cells; MB: Myoblasts; N: Cell types with neurogenic characteristics; OB: Osteoblasts.