# REVIEW

*Medicina (Kaunas) 2011;47(9):469-79*

# Skeletal Muscle-Derived Stem Cells: Implications for Cell-Mediated Therapies

**Arvydas Ūsas[1, 2], Justinas Mačiulaitis[3], Romaldas Mačiulaitis[2, 4], Neli Jakubonienė[5], Arvydas Milašius[2], Johnny Huard[1]**

*[1]Stem Cell Research Center, Department of Orthopaedic Surgery, University of Pittsburgh, Pittsburgh, Pennsylvania, USA,*
*[2]Institute of Physiology and Pharmacology, Medical Academy, Lithuanian University of Health Sciences, Lithuania,*
*[3]Department of Orthopedics and Surgery, Medical Academy, Lithuanian University of Health Sciences, Lithuania,*
*[4]Department of Nephrology, Medical Academy, Lithuanian University of Health Sciences, Lithuania,*
*[5]Department of Endocrinology, Medical Academy, Lithuanian University of Health Sciences, Lithuania*

***Key words:*** *skeletal muscle; stem cells; cell therapy; tissue engineering.*

***Summary.*** *Current advances in stem cell research and innovative biological approaches in the field of tissue engineering and regenerative medicine could eventually translate into prospective clinical applications. Various adult organs and tissues harbor stem and progenitor cells that could potentially be used to repair, regenerate, and restore a variety of different tissues following acute injury or tissue destructive diseases. Skeletal muscle is a very convenient and plentiful source of somatic stem cells. It contains several distinct populations of myogenic stem cells including satellite cells that are mainly responsible for muscle growth and regeneration, and multipotent muscle-derived stem cells (MDSCs). Although both cell populations share some phenotypic similarities, MDSCs display a much greater differentiation potential in vitro and are capable of regenerating various tissues in vivo. Furthermore, these cells not only participate in the regeneration process by differentiating into tissue-specific cell types, but also promote endogenous tissue repair by secreting a multitude of trophic factors. In this article, we describe the biological aspects of MDSC isolation and characterization and provide an overview of potential therapeutic application of these cells for the treatment of cardiac and skeletal muscle injuries and diseases, urological dysfunction, and bone and cartilage defects. We also discuss major challenges and limitations currently faced by MDSC-based therapies that await resolution before these techniques can be applied clinically.*

## Introduction

Cell transplantation and stem cell-based therapies are rapidly emerging as a potential strategy for tissue repair and regeneration in virtually every field of medicine. The aim of cellular therapy is to replace, repair, or enhance the biological functions of the targeted damaged tissues or organs. Stem cells can be defined as unspecialized cells that are capable of continuous self-renewal, while maintaining the ability to differentiate into multiple different cell types. In general, there are two major categories of stem cells: 1) undifferentiated embryonic stem cells that are present during embryonic development and possess pluripotent differentiation capacity (i.e., they are capable of differentiating into cells of all three germ layers); and 2) adult stem cells that can be isolated from postnatal tissues and are multipotent (i.e., they can differentiate into multiple cell types that are restricted to a given tissue). Stem cells can also be isolated from fetal tissues, placenta, as well as the umbilical cord and amniotic fluid. These cells are considered more primitive than adult stem cells and have been shown to have greater multilineage differentiation capacities. Recent breakthroughs in research have identified a set of genes that when introduced into terminally differentiated cells (e.g., epithelial cells) revert these cells into an embryonic stem cell-like state. These reprogrammed adult cells are known as induced pluripotent stem (iPS) cells.

The use of embryonic stem cells is currently limited due to ethical issues and problems with regulating the behaviors of cell proliferation and differentiation, which can lead to the development of tumors (1). In contrast, adult stem cells residing in multiple tissues including bone marrow, skeletal muscle, adipose tissue, skin, liver, and brain have no significant ethical considerations related to their use. It is relatively easy to obtain large pools of autologous cells that possess multipotent differentiation capacities and bear no risk of immune rejection. Ideally, stem cells that are used for regenerative medicine applications should meet several criteria: 1) they should be present in plentiful quantities; 2) they should be able to be collected and harvested by minimally

Correspondence to J. Huard, 450 Technology Drive, Suite 206, Pittsburgh, Pennsylvania, USA. E-mail: jhuard@pitt.edu

Adresas susirašinėti: J. Huard, 450 Technology Drive, Suite 206, Pittsburgh, Pennsylvania, USA. El. paštas: jhuard@pitt.edu

invasive procedures; 3) they should be capable of differentiating along multiple cell lineage pathways in a reproducible manner; and 4) they should be capable of being safely and effectively transplanted either autologously or allogeneicly.

Bone marrow has long been recognized as a common source of mesenchymal stem cells capable of differentiating into adipogenic, osteogenic, chondrogenic, and myogenic cells. When bone marrow becomes compromised because of disease or in aged individuals, the other tissues of the human body including skeletal muscle, adipose tissue, and skin may become a particularly useful source of progenitor cells, especially when patients do not wish to have bone marrow aspiration. Skeletal muscle comprises the largest percentage of total body mass and represents an alternative source of stem cells, which can be easily obtained in large quantities through a relatively painless biopsy procedure performed in an outpatient clinic. Skeletal muscle is a highly dynamic tissue that possesses an inherent ability to regenerate following damage caused by injury or exercise. This regenerative capacity is due to the presence of a tissue-specific population of myogenic stem cells called satellite cells. The satellite cells, muscle precursors committed to the myogenic lineage, were originally identified based on their location beneath the basal lamina of muscle fibers, and were found to have the potential to proliferate, self-renew, and repair the damaged muscle (2–3). Although satellite cells are capable to undergo multipotent differentiation in vitro, their phenotypic characteristics indicate that these cells are committed to the myogenic lineage (4).

The presence of muscle-derived stem cells (MDSCs), a possible predecessor of satellite cells, has been demonstrated (5–6). MDSCs are distinct from satellite cells because they are not limited to the myogenic lineage and capable of differentiating into other lineages (osteogenic, chondrogenic, adipogenic, neural, endothelial, and hematopoietic) in vitro and in vivo (7–9). These cells display capacities of long-term proliferation, high self-renewal, immune-privileged behavior, and a superior capacity to regenerate skeletal muscle (6, 10). In addition, MDSCs can readily be transduced with a variety of different genes using viral vectors, which is a very important attribute in the development of tissue-engineering applications where the secretion of specific proteins is desired to aid in the regeneration of specific tissues. Several other types of muscle-derived stem cells including side population (SP) cells, mesangioblasts, and perivascular cells have been recently identified and purified by flow cytometry from adult skeletal muscle. A human counterpart to MDSCs has been recently isolated from human skeletal muscle using fluorescence-activated cell sorting (FACS) to select cells coexpressing myogenic and endothelial markers. These cells can retain the expression of surface markers and capacity of myogenic differentiation during long-term culture and exhibit multilineage developmental potential in vitro and in vivo at the clonal level (11).

Here, we review recent progress in MDSC biological characterization and its therapeutic application in preclinical animal models and clinical trials for the treatment of cardiovascular, urological, and musculoskeletal disorders. The major issues, limitations, and challenges of cell-based therapies are also revealed and briefly discussed.

**Isolation and Characterization of MDSCs**

Different methods have been used to separate distinct muscle-derived cell populations by means of cell adhesiveness, their proliferation behaviors, and stem cell marker expression profiles. These methods include density-gradient fractionation, preplating culture series, repeated culture using a freeze-thaw technique, magnetic cell sorting, and FACS. Muscle-derived stem and progenitor cell populations are defined by cell markers, some of which are restricted to these populations, whereas other markers are shared with other cell populations. The marker profile-based isolation methods are limited because they rely exclusively on the expression of cell surface markers that are variable and may change under cell culture conditions. Currently, the most prevalent method for isolating MDSCs is a marker profile-independent technique known as the modified preplate technique, which is based on variable adherence of freshly dissociated muscle cells to collagen-coated flasks (12). Skeletal muscle biopsy specimens are digested using several different enzymes, and the resulting single cell suspension is plated onto a collagen-coated flask and then serially transferred to new flasks daily over a period of 6–7 days. Among the cells that adhere within 12–96 hours after plating, there are rapidly adhering cells (RACs), which are comprised of fibroblast-like cells, and slowly adhering cells (SACs), which contain myoblasts already committed to the myogenic lineage. The SACs that are slowest to adhere contain a population of early myogenic progenitor cells that can be further purified by continuing to passage the cells until the long-term muscle progenitor cells, MDSCs, are obtained (6, 12). Several modifications of the isolation technique to obtain the MDSCs from various species have been reported using different types of enzymes to digest the tissue, different types of materials to coat the tissue culture flasks, and different intervals of culture time between each serial preplate (13).

Immunostaining and FACS are the most common methods used to characterize MDSCs based

on their marker profile. Although MDSCs are similar to satellite cells in their regeneration abilities in skeletal muscle, their marker expression is slightly different (6) (Table 1). Satellite cells, whether active or quiescent, typically express the paired box gene 7 (Pax-7), whereas MDSCs are more heterogenous but express stem cell antigen 1 (Sca-1) consistently and often express the cluster of differentiation 34 (CD34) (24, 26). Pax-7- and Sca-1-positive cells have not been found to colocalize in skeletal muscle, providing further evidence that satellite cells and MDSCs are distinct subpopulations (27). MDSCs also appear to be more primitive than satellite cells, as they have the ability to differentiate into a broader number of cell types, thus displaying greater plasticity (5, 28). It is likely that MDSCs are situated hierarchically upstream of the Pax7+ cells and constitute the population of cells that are early in the myogenic lineage cells (6, 29). MDSCs are a unique cell population whose characteristics, including marker profile, proliferation and differentiation kinetics, and regenerative capacity, are distinct from myoblasts (10, 30).

In vivo studies provide additional evidence for the stem cell nature of MDSCs. It has been demonstrated that MDSCs can self-renew and differentiate into multiple lineages, and have the potential to regenerate various adult tissues (Table 2). In fact, a recent study has revealed that MDSCs have an extended replicative lifetime and substantial self-renewal capacity comparable to that exhibited by embryonic stem cells (10). MDSCs retained their phenotype and ability to promote muscle regeneration in vivo for up to 200 population doublings and can therefore yield a large number of cells, which is necessary for appropriate clinical application. These cells display a superior regenerative capacity relative to satellite cells following transplantation into dystrophic muscle in a murine model of muscular dystrophy (*mdx*) (6, 10, 30). MDSCs also are at least partially immune-privileged, as the transplantation of MDSCs results in robust dystrophin expression in *mdx* mice (dystrophin-deficient) over 3 months after injection (6). It has also been reported that stem cells exhibit enhanced resistance against oxidative stress, which helps these cells avoid oxidative damage (47). In fact, MDSCs have been found to contain higher levels of antioxidants and to be more resistant to oxidative stress-induced apoptosis and ischemia compared to myoblasts (48–49).

Although MDSCs possess the capacity of multilineage differentiation and can participate in tissue regeneration, it has been proposed that terminal differentiation of these cells is not a major determinant for successful tissue repair. In fact, very few donor cells are typically seen within the transplanted regenerated tissues with the vast majority of the restorative cells being host-derived. Most likely MDSCs act as trophic mediators by releasing angiotrophic, neurotrophic, and other growth factors, and therefore can enhance endogenous mechanisms of regeneration through a paracrine effect. While tissue-engineering strategies based on the induction of MDSC differentiation necessitate more basic investigation, strategies based on the paracrine effects of cells may present greater opportunities for clinical application in the immediate future.

*Table 1.* Phenotypic Markers of Murine Satellite Cells and Muscle-Derived Stem Cells (MDSCs)

| Cell Marker | Satellite Cells | References | MDSCs | References |
|---|---|---|---|---|
| CD34 | + | (14) | + | (6) |
| Sca-1 | – | (15) | + | (5) |
| Bcl-2 | +/– | (6) | +/– | (5) |
| CD56 | +/– | (16) | + | (17) |
| Flk-1 | – | (18) | + | (5) |
| CD45 | – | (18) | – | (5) |
| C-kit | – | (6) | – | (6) |
| Desmin | + | (19) | +/– | (5) |
| c-Met | + | (20) | + | (5) |
| MNF | + | (21) | + | (5) |
| MyoD | + | (22) | +/– | (5) |
| M-cadherin | + | (23) | – | (6) |
| Pax7 | + | (24) | | |
| Pax3 | + | (25) | | |
| Myf5 | + | (14) | | |

*Table 2.* Multilineage Differentiation of Muscle-Derived Stem Cells In Vitro and In Vivo

| Lineage | In Vitro (References) | In Vivo (References) |
|---|---|---|
| Myogenic | (6, 28, 31) | (5–6, 31) |
| Osteogenic | (5, 32) | (5, 32) |
| Chondrogenic | (33-34) | (33, 35) |
| Adipogenic | (31, 36) | (37–38) |
| Hematopoietic | (8, 28) | (31, 39) |
| Endothelial | (6, 31) | (42) |
| Neural | (6, 40–41) | (45) |
| Cardiac | (43–44) | |
| Hepatocyte | (46) | |

## Cell Therapy for Cardiac Diseases

The idea that myoblasts (satellite cell progeny) may repopulate postinfarction scar occurred around the mid-1990s and was based on several clinically relevant advantages that made skeletal myoblasts the first generation of cells attempted to be used for the treatment of heart failure. These advantages include the following: 1) their autologous origin; 2) their high expansion capacity; 3) their commitment to a myogenic lineage; and 4) their resistance to ischemia (50). Numerous research studies have demonstrated that myoblasts differentiate into myotubes and retain skeletal muscle properties when transplanted into infarcted myocardium (51). Even though myotubes do not couple with resident cardiomyocytes, myoblast transplantation was found to be able to augment left ventricular function in ani-

mal models of myocardial infarction (52). Although the transplanted myoblasts retained their proliferation ability, very few of these cells were detectable in the heart after several weeks. The low survival rate of intracardially transplanted myoblasts is most likely due to inefficient engraftment and their limited regenerative capacity in vivo. In fact, the extent of myoblast engraftment has been shown to influence functional outcome (53–54); therefore, repetitive myoblast transplantations have been proposed as a feasible and more effective cell delivery method (55). Recently, the innovative approach of using an autologous myoblast sheet has demonstrated the capacity to improve cardiac function by attenuating cardiac remodeling and metabolic recovery in the impaired myocardium (56). Although myoblasts do not integrate electrically with the native myocardium, a recent report indicates that skeletal myoblasts could be genetically engineered to express the gap-junction protein connexin 43, and this modification was able to improve electrical coupling between the infarct region and the surrounding myocardium (57).

Consequently, autologous skeletal myoblasts have proved to be the cells of choice for cardiac repair in the clinical scenario (50, 58). The early results of phase 1 clinical trials established both the feasibility and safety of this procedure and demonstrated improvements in cardiac function (58); however, sustained ventricular tachycardias, which occurred within the first weeks following myoblast transplantation, have been reported (59–60). The variable incidence of arrhythmias has therefore prompted the prophylactic use of an implantable cardioverter-defibrillator in the following trials (61). Clinical studies also revealed the major issues that may have hampered the efficacy of myoblast transplantation and cell therapy overall, including limited retention of injected cells and poor survival of engrafted cells.

In fact, skeletal myoblasts may not be the most appropriate cells for cardiac muscle regeneration because of their myogenic commitment that prevents them from "transdifferentiation" into cardiomyocytes. Recent studies indicate that skeletal muscle harbors a subpopulation of MDSCs that is less committed to the myogenic lineage than satellite cells, displays stemness markers, and is capable of acquiring a cardiac phenotype (62). In comparison with the myoblast population, MDSCs implanted into infarcted hearts displayed greater and more persistent engraftment, enhanced neoangiogenesis, prevented cardiac remodeling, and significantly improved cardiac function. MDSCs were also found to be more resistant to oxidative stress-induced apoptosis and ischemia in comparison with myoblasts and cardiomyocytes that may explain their increased chances of survival and engraftment when transplanted into the compromised areas of the heart (62–63). The recent report indicates that purified human skeletal muscle-derived myoendothelial cells exhibit superior engraftment within the infarcted myocardium and improve left ventricular function to a greater extent than myoblasts or endothelial cells (64).

### Cell Therapy for Muscular Dystrophies

Cell-based therapies have been largely considered a promising therapeutic strategy for the treatment of muscular dystrophies. Myoblast transplantation has been investigated as a means for improving regeneration in injured skeletal muscle and, in particular, dystrophic muscle under progressive muscle-wasting conditions caused by disorders such as Duchenne muscular dystrophy (DMD). It has been shown that myoblasts can contribute to the formation of dystrophin-positive myofibers within dystrophic muscle (65). Normal myoblasts fuse with dystrophic myoblasts to form hybrid myotubes resulting in dystrophin expression at the muscle fiber plasma membrane in the injected dystrophic muscle. Although this method has shown the capacity to restore normal histology and improve skeletal muscle strength, only transient effects have been observed due to immune rejection, poor cell survival, and the limited distribution of the transplanted cells (66).

In comparison to satellite cells, MDSCs have been able to overcome some of these hurdles by displaying a superior regenerative capacity following transplantation (6, 30) as demonstrated by their restoration of dystrophin expression in the MDX mouse (a mouse model of Duchenne muscular dystrophy) more effectively than myoblasts during an extended period (5, 37). Along with their strong capacity for self-renewal, multipotent differentiation, and immune privileged behavior (6), MDSCs have been able to proliferate and regenerate skeletal muscle due to their high resistance to oxidative and inflammatory stressors (62). Indeed, lower rates of oxidative and inflammatory stress-induced cell death may relate to their increased regenerative capacity and to some extent justify the sex-related differences in myogenic differentiation and skeletal muscle regeneration observed between male and female MDSCs (48).

Myogenic progenitors derived from skeletal muscle are typically a heterogenous population that displays unique properties depending on the cell isolation methods and culture conditions utilized to isolate them (3, 67). Alternative multilineage progenitor cell populations, including SP cells, CD $133^{+}$ progenitor cells, and mesoangioblasts, recently isolated from adult skeletal muscle, have shown a great potential for the regeneration of dystrophic muscle (29). These stem cell populations may have a great promise for the treatment of muscular dystro-

phies because of engraftment and their potential to regenerate dystrophic muscle through intravascular systemic delivery (68).

Clinical trials using allogenic myoblasts to deliver dystrophin to DMD patients without immunosuppressive treatment resulted in a safe, transient restoration of the protein; however, serum antibodies against the injected cells were detected, and the poor transplantation success was attributed, at least in part, to immune rejection. A recent clinical trial on immunosuppressed DMD patients demonstrated that donor-derived dystrophin transcripts could be detected after myogenic cell transplantation (69). The feasibility of intramuscular delivery of cells has been shown in these studies; however, systemic delivery remains a major concern for the therapy of DMD given the fact that the direct injection of cells will be unlikely capable of restoring dystrophin in the diaphragm and intercostal muscles, which are the most affected by the disease and indeed critical for patient survival. This issue remains an important area of basic investigation before it can be translated into clinical application.

### Cell Therapy for Urological Disorders

Muscle-derived cell transplantation has been utilized for the treatment of urinary incontinence, a disorder that results from sphincter muscle deficiency and impaired contractility. The implantation of myoblasts into the urethral and bladder walls has been found feasible and resulted in the formation of myotubes and myofibers in the smooth muscle layer of the lower urinary tract in rats (70). Primary skeletal muscle-derived cells isolated from normal mice demonstrated long-term persistence (up to 70 days) and were able differentiate into myofibers after injection into the bladders of severe combined immunodeficient mice. Highly purified muscle-derived cells (including a clonal population of cells with stem cell-like characteristics) exhibited not only myogenic differentiation within the injected bladder wall, but also differentiated toward $\alpha$-smooth muscle actin-positive muscle cells (71). Furthermore, this study revealed that some donor cell-derived myofibers in the injected bladders became innervated and expressed acetylcholine receptors, usually located at the endplate of neuromuscular junctions, which is believed to be the most likely reason for the improved urinary bladder contractility after cryoinjury observed in this study (71). Periurethral transplantation of allogenic MDSCs did not trigger an immune response and significantly improved leak point pressure in female rats after sciatic denervation (72). The comparison of MDSCs with fibroblasts, with regard to efficacy in restoring urethral function, revealed no adverse events in the former group, whereas the injection of a high number of fibroblasts led to urinary retention (73).

Preliminary results of the first clinical studies in patients with stress urinary incontinence are very encouraging. Autologous fibroblasts mixed with collagen were injected into the urethral submucosa to treat mucosal atrophy, and a myoblast-collagen mixture was delivered into the rhabdosphincter to reconstruct the muscle. At the 12-month follow-up, 85% of the patients in the cell treatment group were cured of incontinence. The mean quality-of-life score, thickness of urethra and rhabdosphincter, as well as sphincter contractility were increased postoperatively (74). The results of the first North American clinical trial using purified autologous MDSCs for the treatment of stress urinary incontinence have been reported (75). One-year follow-up revealed a modest improvement in 5 of the 8 women injected with 18–22 million MDSCs obtained from lateral thigh muscle biopsies with no short- or long-term adverse events observed.

### Cell Therapy for Orthopedic Applications

Skeletal muscle has been extensively investigated as a potential source of progenitor cells for bone and cartilage repair. Several studies have demonstrated that muscle contains osteoprogenitor cells that exhibit the potential to differentiate toward the osteogenic lineage and induce endochondral bone formation on stimulation with bone morphogenetic proteins (BMPs) (76–77). Similar results were observed with cells isolated from human skeletal muscle (78). Subsequent studies using a clonal population of purified mouse MDSCs demonstrated an enhancement of bone healing, which suggests that these cells may be better cellular vehicles for BMP4 gene delivery compared to primary isolated muscle cells (5, 32, 79). Simultaneous delivery of BMP4 and vascular endothelial growth factor (VEGF) by MDSCs produced a synergistic effect on bone formation by promoting both osteogenesis and angiogenesis when the proper ratio of protein-expressing cells was used (80). It also appeared that the sex of cell donor plays an important role in the osteogenic differentiation of murine MDSCs. In fact, male cells have been found to be more osteogenic and exhibited a greater mineralization capacity in vitro compared with their female counterparts (81). Not surprisingly, the male hosts promoted more ectopic bone formation than female hosts, suggesting that male skeletal muscle may contain more cells with osteogenic potential (81).

In search of new ways to optimize MDSC-mediated bone growth and circumvent the adverse effects associated with overexpression of growth factors, several new strategies have been recently employed. One approach, development of a self-inactivating tet-on retroviral vector system, enables the regulation of therapeutic gene expression by transduced

cells via the addition or withdrawal of doxycycline. Using this system, the modulation of BMP4 (82) and VEGF (unpublished data) expression by MDSCs in vitro and in vivo has been found feasible. Another strategy has been designed to mimic concomitant expression of growth factors and their specific antagonists during normal fracture healing. The coimplantation of MDSCs expressing Noggin, a specific BMP antagonist, and MDSCs expressing BMP4 was capable of reducing hypertrophy of regenerated bone in a mouse calvarial defect (83).

The use of muscle-derived cells for articular cartilage repair also has been widely explored. Muscle-derived cells transplanted into an osteochondral defect created in a rabbit femur were able to repair cartilage with the same efficiency of chondrocyte transplantation (84). Retrovirally transduced MDSCs expressing BMP4 were able to undergo chondrogenic differentiation in vitro and in vivo after their transplantation into a cartilage defect created in an immunodeficient rat knee (33). The repaired hyaline-like cartilage remained well integrated and showed no signs of calcification up to 24 weeks after transplantation. Recent studies indicate that the blockade of angiogenesis can further improve the MDSC-mediated regeneration of articular cartilage (35, 85). BMP4 treatment combined with soluble fms-like tyrosine kinase 1 (sFlt-1) treatment to block VEGF signaling improved the chondrogenic potential of MDSCs and quality of articular cartilage repair (85). Although no attempt has been made yet to apply MDSC therapy for bone and cartilage regeneration in a clinical setting, the cells isolated from skeletal muscle may soon become an adjunct to traditional surgical intervention (86).

## Challenges and Limitations of MDSC-Based Therapies

The rising enthusiasm regarding clinical applications of autologous MDSCs is based on promising results obtained during experimental-preclinical studies and early phase clinical trials; however, before embracing clinical applications, several essential precautions must be properly addressed, including basic science issues and practical inquiries regarding the safety, efficacy, and cost-effectiveness of the use of these cells. Similar to other cell-based approaches in regenerative medicine, MDSC therapy faces several challenges including cell isolation and expansion, effective delivery, immune response, long-term survival, efficacious engraftment, proper differentiation, and functional incorporation into the new, unique microenvironment. It is necessary to define pharmacologic characteristics of living cells that secrete variable and largely unknown amounts of bioactive molecules. The definition of the cellular product, mechanism of action, pharmacokinetics, toxicity, and efficacy assessment represent challenges never previously faced by traditional pharmacology (87).

Defining the cellular product is one of the major challenges for the development of cell-based therapies. Cells need to be well-characterized, isolated, and expanded under well-controlled manufacturing processes. In order to define cell populations by the presence or absence of certain cell surface markers, the assays for cell characterization have to be standardized. The cell preparations frequently are heterogeneous, although they are typically enriched with certain subpopulations of cells; thus, there is a need to develop and evaluate the biological properties of more homogeneous populations of cells with regard to the contribution of given subpopulations of cells for tissue repair. Preferably, the characterization of cellular products should be linked to specific biological functions suitable to the desired clinical application.

Characterization of the MDSC subpopulation based on cellular surface markers has proven to be very complicated. Several studies indicated that the cells demonstrated an increase in Sca-1 and CD34 expression in the late preplate populations; however, the levels of Sca-1 expression varied between different investigators. Furthermore, Sca-1 is not restricted to expression on muscle-specific cells and the transplantation of either single (Sca-1) or double (Sca-1 and CD34) positive expressing populations was not as effective as predicted (88). It has been demonstrated that slowly adherent MDSCs have variable expression levels of CD34 (25%) and Sca-1 (58%) when cultured in vitro (88); however, the CD34+ sorted cells showed significant differences in proliferation and differentiation that resulted in improved transplantation efficiency in skeletal muscle when compared with CD34- cells isolated from the same population of MDSCs (30).

There is an increasing awareness of the issues related to the altered expression of cell surface markers and changes in cell properties with increasing time in culture; hence, the in vitro expansion of cells without the loss of specific phenotypic characteristics demands the optimization of cell culture conditions. It has been demonstrated that culture conditions may alter myogenic potential (3). Reciprocally, the lack of definitive markers for cell isolation and culture-dependent phenotypic changes can lead to heterogeneity, which makes the correlation of phenotypic and stem cell-functional identification a challenge. Long-term expansion in culture also has the potential for introducing mutations that can transform and immortalize the cells.

Efficacy of cell delivery is a significant limitation in the usefulness of stem cell therapy. Cells need to be delivered either locally to the injury site or

systemically to reach particular organs. The major concern for the use of cell therapy to treat DMD patients will be systemic delivery, given the fact that direct site-specific injection of the cells will be unlikely capable of restoring dystrophin expression in the diaphragm, intercostal, and cardiac muscles. No systemic delivery of committed myogenic cells has ever been successful; however, a few recent reports indicate that myogenic precursors associated with microvascular walls in the human skeletal muscle have the potential to regenerate dystrophic muscle when administered into the blood circulation (29, 89).

Despite an improvement in cell delivery techniques, the majority of cells transplanted into the heart do not survive due to physical stress, inflammatory and hypoxic conditions, anoikis (apoptosis), absence of survival factors, and disruption of intercellular communication (90). The loss of grafted cells most likely hampers the efficacy of the procedure as the improvement of cardiac function seems to be tightly related to the number of injected myoblasts (53). Hence, the development of strategies that promote donor cell survival may enhance the effectiveness of transplantation therapy (91). The addition of prosurvival factors into the cell suspension and use of slowly polymerizing hydrogels or cardiac-specific decellularized matrices as a delivery vehicle to improve cell retention are new approaches to improve cell engraftment that can maximize the potential for the transplanted cells to mediate heart regeneration (92–94). The emphasis on proper cell placement in treating stress urinary incontinence has also been reported, which indicates that the periurethral or transurethral injection route utilizing a longer needle could result in a greater improvement of quality of life measures (75).

The fate of transplanted cells is another major issue. The ability to monitor the fate of the transplanted cells, including both cell tracking and cell differentiation, is critical. A number of approaches have been employed to trace donor cells using fluorescent dyes, metabolic labeling, intrinsic genetic differences (i.e., dystrophin deficiency or possession of the Y chromosome), and strategies of gene transfer (DNA transfection, viral transduction or cells expressing reporter genes from transgenic species) (52). These labeling techniques enable the performance of histochemical, immune cytological, and ultrastructural analyses to monitor cell fate as well as the quantification of the engraftment efficiency. Recently introduced noninvasive imaging techniques, such as magnetic resonance imaging using magnetically labeled cells or positron emission tomography using cells tagged with specific agents, offer the possibility not only to track and quantify cell fate but also determine their functional effects on the host environment (95–96). However, before these agents can be used in clinical trials, researchers need to verify that these substances are nontoxic to the cells; do not alter the phenotypic characteristics and capacities of proliferation and differentiation of a cell; have no impact on cell viability and do not promote apoptosis.

The environmental-predispositional behavior of the cell population is another major challenge that requires careful consideration. After transplantation, the cells respond to the local microenvironment, which includes exposure and stimulation by growth factors, cytokines, oxygen content, and biochemical and mechanical stimuli that may prompt differentiation into various lineages. For example, when MDSCs were transplanted into injured muscle and exposed to transforming growth factor $\beta$1 (TGF-$\beta$1), which is secreted after injury, they differentiated into fibrotic cells and provoked scar tissue formation (97). Similarly, MDSCs treated with BMPs, VEGF, and nerve growth factor can differentiate into osteogenic, endothelial, and neural lineages, respectively. Of note, it has been reported that exposure of specific populations of postnatal MDSCs to concomitant growth/differentiation signals in vivo may evoke the environmental-specific malignant transformation of the cells, suggesting a potential link between somatic stem cells and cancer (98).

## Concluding Remarks

Regenerative therapies, based on muscle-derived stem cells, are a very promising strategy for the treatment of cardiovascular and urological disorders, as well as for the musculo-skeletal injuries and diseases. With respect to the ultimate clinical utility of skeletal muscle stem cell transplantation, it is important to be aware that more work needs to be performed. Besides the basic characterization and phenotypic identification of myogenic precursors, the isolation of these cells from human skeletal muscle, cell expansion to clinically relevant quantities, assessment of proper cell differentiation and functional incorporation after transplantation, there are many other issues related to the safety, toxicity, and tolerability of cell-based therapies that must be thoroughly evaluated in both preclinical and clinical studies.

## Acknowledgments

We would like to thank James H. Cummins (Stem Cell Research Center, Department of Orthopaedic Surgery, University of Pittsburgh) for his editorial assistance in the preparation of this manuscript.

## Statement of Conflicts of Interest

The authors have no conflicts of interest.

# Ląstelinė terapija raumeninės kilmės kamieninėmis ląstelėmis

**Arvydas Ūsas[1, 2], Justinas Mačiulaitis[3], Romaldas Mačiulaitis[2, 4], Neli Jakubonienė[5], Arvydas Milašius[2], Johnny Huard[1]**

*[1]Pitsburgo universiteto Ortopedinės chirurgijos skyriaus Kamieninių ląstelių tyrimų centras, Pensilvanija, Jungtinės Amerikos Valstijos, [2]Lietuvos sveikatos mokslų universiteto Medicinos akademijos Fiziologijos ir farmakologijos institutas, [3]Lietuvos sveikatos mokslų universiteto Medicinos akademijos Ortopedijos ir traumatologijos klinika, [4]Lietuvos sveikatos mokslų universiteto Medicinos akademijos Nefrologijos klinika, [5]Lietuvos sveikatos mokslų universiteto Medicinos akademijos Endokrinologijos klinika*

**Raktažodžiai:** skeleto raumenys, kamieninės ląstelės, ląstelinė terapija, audinių regeneracija.

**Santrauka.** Šiuolaikiniai pasiekimai kamieninių ląstelių tyrimo srityje bei novatoriški biologiniai metodai, naudojami audinių inžinerijoje ir regeneracinėje medicinoje, ilgainiui gali būti pritaikyti klinikinėje praktikoje. Įvairiuose suaugėlių organuose ir audiniuose yra pirmtakių ir kamieninių ląstelių, kurios gali būti naudojamos įvairių audinių atkūrimui ir gijimui po pažeidimo arba ligos. Skeleto raumenys yra labai geras ir gausus somatinių kamieninių ląstelių šaltinis. Juose yra kelios skirtingos raumeninės kilmės kamieninių ląstelių populiacijos – tai satelitinės ląstelės, kurios linkusios dalyvauti raumenų regeneracijoje, ir raumeninės kilmės kamieninės ląstelės (RKKL), kurios gali diferencijuoti ne tik raumenyne, bet ir kitose organizmo sistemose. Nors ląstelių populiacijos turi panašų fenotipą, bet RKKL turi didesnę diferenciacijos gebą *in vitro* ir gali atkurti įvairius audinius *in vivo*. Be gebėjimo diferencijuotis į audiniams specifines ląsteles RKKL dar išskiria įvairius augimo faktorius, kurie skatina endogeninę audinių regeneraciją. Šiame straipsnyje aptariami RKKL išskyrimo ir biologinių charakteristikų aspektai, apžvelgtos potencialios galimybės taikyti ląstelinę terapiją širdies ir skeleto raumenų ligoms, urologiniams sutrikimams, kaulų ir kremzlių regeneracijai. Taip pat nurodytos svarbiausios kliūtys bei apribojimai, kuriuos tenka įveikti, siekiant panaudoti RKKL klinikinėje praktikoje.

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*Received 17 August 2011, accepted 30 September 2011*
*Straipsnis gautas 2011 08 17, priimtas 2011 09 30*