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Chapter 8

Trends in Mesenchymal Stem Cells' Applications for Skeletal Muscle Repair and Regeneration

A.R. Caseiro, T. Pereira, P.J. Bártolo, J.D. Santos, A.L. Luís and A. C. Maurício

Additional information is available at the end of the chapter

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Abstract

Skeletal muscle injuries are quite frequent in traumatic scenarios, such as war injuries or road- or work-related accidents. The skeletal muscle has good regenerative ability, but the extent or recurrence of muscle injury might impair complete structural and functional recovery. Severe tissue loss overwhelms skeletal muscle´s intrinsic regenerative capabilities and culminates in the development of noncontractile fibrous tissue scar. Conservative RICE -based and surgical treatments show limited efficacy in terms of improving these severe cases outcomes, pressing the need for new approaches on skeletal muscle's therapy. Since the first suggestions of the potential of mesenchymal stem cells for regenerative medicine and tissue engineering, many applications have been explored for a variety of tissues and diseases, including the skeletal muscle, which is the focus of this literature review.

Current research has focused on the influence of nonmuscular MSCs on promoting tissue healing and limiting fibrotic scar formation, as well as on the modulation of the inflammatory response to injury. The most popular source of MSCs is, without a doubt, the bone marrow. However, MSC populations are present in virtually all body tissues, and alternative sources have been proposed, such as the adipose tissue, synovial membrane, dental pulp, and even umbilical cord tissue.

MSCs from various sources have been demonstrated as capable of *in vitro* differentiation into myogenic lineages, through adequate stimuli, displaying phenotypical markers of native skeletal muscle cells. In addition, *in vivo* applications suggest they are capable of integrating host muscular tissues, even when delivered systemically.

MSCs are capable of secreting a wide range of active molecules to their surrounding media, including growth factors, chemokines, and cytokines. Most of these growth factors have been associated to the skeletal muscle's regenerative process, and their efficiency has been demonstrated to increase when applied in spatial and temporal coordination. Hence, the combination of molecules secreted by MSCs gained interest as modulator of inflammatory, fibrotic, and regenerating events. This has been proposed as possibly the primary mode of action of undifferentiated MSCs into a lesion site, providing controlled release of such components. Concurrently to the implantation of undifferentiated cells, it has also been hypothesized that the application of secretion products alone (termed as Conditioned Medium) display similar if not improved effects on skeletal tissue regeneration, as it does in other damaged tissues.

Keywords: Skeletal muscle, skeletal muscle regeneration, cellular therapies, mesenchymal stem cells, biomaterials, secretome

1. Introduction

Skeletal muscle accounts for nearly half of the human body mass [1], and inherited and acquired pathologies are often observed in clinical practice.

Given their impact on quality and life expectancy of patients, severe forms of degenerative muscular diseases, such as Duchenne muscular dystrophy (DMD), have been one of the hot topics of skeletal muscle regeneration research, and encouraging results have been obtained through the application of mesenchymal stem cells (MSCs), giving hope for the development of new therapies that can effectively improve the quality of life of affected patients [2–7].

Acquired muscle affections are seemingly more common in active humans, greatly associated to sports practice, but also quite frequent in other traumatic sceneries, such as road or work-related accidents or war injuries [8–11]. Muscle damage can result from ischemia and denervation, to contusion, sprain damage, laceration, avulsion, and other severe tissue losses.

Skeletal muscle has a good regenerative ability, but the extent or recurrence of these insults might impair complete myofibers regeneration, limiting structural and functional recovery of the affected muscle groups. Severe tissue loss usually supplants skeletal muscle's intrinsic regenerative capabilities [8] and culminates in the development of noncontractile fibrous tissue scar [12]. Other well-known factor to impact the intrinsic capacity of skeletal muscle to respond to injury events is the age of the patient [13], affecting both intrinsic cellular mechanisms and their involving niche, hindering their effectiveness upon activation [14]. The regeneration potential of skeletal muscle depends on a multitude of cell types that, upon exposure to specific cues, cooperate to regenerate the damaged tissue, generating a coordinated tissue response [15]. Under particular conditions, such as chronic diseases and aging, the ability of these cells to support the regenerative response declines, leading to maladaptive responses, e.g., the formation of fibrotic scars and fatty infiltration [15].

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Current recommendations for skeletal muscle lesions management rely on empirical application of conservative RICE-based (rest, ice/cold, compression and elevation) and surgical treatments [10, 16] but show limited efficacy in terms of improving severe cases outcomes, pressing the need for new approaches on skeletal muscle´s therapy.

Presently, biomedical research is working in various fronts toward complete restoration of structure and function of damaged muscles, converging efforts in the areas of biomaterial development, cell systems applications, and bioactive molecules aiming at filling the defect and recovering the esthetics of the body part, as well as its function.

One of the strategies being intensely explored involves the application of muscle resident and nonmuscular stem cells in search for faster and more effective recovery from severe injuries, restoring both tissue structure and function [17, 18].

2. Skeletal muscle structure and intrinsic healing mechanisms

The basis of skeletal muscle structure and regeneration have been extensively revised in literature, and only a brief description and emphasis to strategic "key points" will be given herein [9, 16].

Skeletal muscle is composed of a mixture of muscle-specific cells, nerves, blood vessels, and connective tissue support matrix. Skeletal muscle tissue-specific cells are multinucleated structures holding complex and highly organized contraction machinery enclosed within the plasma membrane (sarcolemma), and a single cell is termed as myofiber. According to their contractile properties, myofibers can be classified into three types. Type 1 myofibers are slow contracting and fatigue resistant, type 2A myofibers are fast contracting and have intermediate resistance to fatigue, and type 2B myofibers are fast contracting and have poor fatigue resistance. The function and training of a specific muscle or muscle group determine their composition in terms of fiber type content.

The extracellular matrix (ECM) supporting the myofibers (basal lamina or basement membrane) is composed of a vast set of proteins, such collagen, fibronectin, laminin, and other glycoproteins [9]. Myofibers are bound together by connective tissue sheaths (the endomysium, perimysium, and epimysium) associating them at three upscaling levels from involving a single myofiber to the whole muscle belly [16].

The healing process following skeletal muscle injury is classically divided into three interrelated and time-dependent phases, conveying the destruction, repair, and remodeling of the affected tissue. The first phase (destruction phase) is defined by the rupture and necrosis and degeneration of the myofibers (mainly mediated by alterations of the sarcolemma and loss of calcium homeostasis) and associated neurovascular structures and ECM, by the formation of a hematoma (between the damaged/ruptured and retracted muscle cells) and the initiation of the inflammatory cells response [9, 16]. Other authors distinguish a primary hemostatic stage, preceding the inflammatory reaction [19]. The inflammatory phase becomes evident within 24 hours after injury and comes up until approximately 3 days after the event. It is defined by a

sequential influx of neutrophils and macrophages to the site, engaging in the phagocytosis of the debris on site and the release of a cascade of mediatory cytokines [9, 20].

The following phase (repair phase) includes the phagocytosis of the debris resulting from the damaged/necrotized tissue, the kickoff of the regeneration of the myofibers, and the production of a connective-tissue scar by migrating fibroblasts and neurovascular regrowth [16].

At this stage, satellite cells (SCs) assume a preponderant role. These cells, firstly identified in the early 1960s [21], constitute a population of myogenically committed but undifferentiated cells, residing between the basal lamina and the muscle fiber, assuming functions of maintenance of tissue homeostasis and regeneration. Muscle-specific paired box 7 (Pax7) is a hallmark of postnatal myogeneis capacity and commonly characterize SCs populations [15]. Upon injury, SCs are activated and undergo one of two faiths: differentiation into myogenic cells or "stem-like" division, maintaining the pool of available cells for intervention in future events of injury. These two courses of the SCs population relate to their Myf5 transcription factor expression: the dominant population $Pax7^{+}/Myf5^{+}$ undergoes myogenic differentiation while the minor $Pax7^{+}/Myf5^{-}$ population remains undifferentiated replenishing the SCs niche [22]. Other populations with stem cells' characteristics have been identified within the muscle tissue, such as mesangioblasts and PICs ($PW1^{+}/Pax7^{-}$ interstitial cells). Further details on intrinsic regenerative populations associated to skeletal muscle and SCs origins and dynamics can be found elsewhere [23–27].

In the final remodeling phase, the regenerated tissue matures and the formed fibrous connective tissue reorganizes and contracts [16]. This stage is highly significant for the outcome of the whole process and fine regulation of the late fibrotic events turns essential [28–30]. Especially after severe tissue loss, the fibrin matrix derived from the clotting process and inflammatory response requires remodeling into collagen type 1 network, produced by fibroblastic cells [31]. The development of definitive fibrosis at a lesion site begins at approximately 2 weeks after injury and progresses over time. Exacerbated fibrosis prejudices the repair and remodeling phases hindering muscle regeneration and full functional recovery [32].

Although fibrosis is mostly referred to as a negative aspect of the healing process, evidence suggests that a certain level of fibrosis acts as support matrix to new tissue ingrowth, promoting proper realignment of the myofibers and the myofibrils, and maintaining a degree of mechanical properties on the regenerating tissue [32]. Also, reports of "functional fibrosis" support its importance to the process, by contributing to a certain stance to the force distribution along the muscle or muscle group, preventing continued overload of the remaining skeletal muscle tissue, and contributing to functional recovery unrelated to effective skeletal muscle tissue regeneration [33].

3. MSCs' sources for skeletal muscle regeneration

In the ever-growing field of regenerative medicine and tissue engineering, stem cells are posing as one of the main characters in the most recent therapeutic strategies [17, 18].

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Given the presence of resident stem and SCs within the skeletal muscle tissue, native tissue skeletal muscle-derived MSCs (MDSCs) would appear as the favorite source for regeneration therapies [34]. In response to muscle damage, the SCs population is activated by the released biomolecules and begins to proliferate and originates large numbers of muscle progenitor cells (MPCs), which will in turn contribute to skeletal muscle structure reconstruction. At the same time, not all SCs derive into MPCs but rather self-renew, contributing to the replenishment of the quiescent cells within the muscle tissue [35, 36].

Many authors have explored skeletal muscle tissue-specific cells for repair and regenerative purposes, as summarized by Koning et al. [37]. Despite of their demonstrated benefits in several settings [38], the practical use of postnatal skeletal muscle progenitors or SCs is limited due to decreased cell availability (requiring the harvest of large volumes of healthy tissue for adequate numbers) [31, 39]. The expansion of MDSCs is possible, but as it has long been known, it leads to dedifferentiation of early committed myogenic cells [40] and loss of potential. As little as 1 day in culture following isolation and sorting hinders its engraftment potential and contribution to regeneration events, hence turning it difficult to attain relevant cell numbers for implantation [34]. Confirming this loss of potential, the implantation of freshly isolated SCs in numbers as low as 250 cells outperforms the use of as many as 1.5×10^5 MPCs of first passage derived from SCs expansion [35]. This initial boost provided by seldom expanded MPCs, however, does not seem to sustain for long term effects [41].

Consequently, current research has focused on the influence of nonmuscular MSCs on promoting tissue healing and limiting fibrotic scar formation, as well as on the modulation of the inflammatory response to injury.

From the first suggestion of MSCs' potential for regenerative medicine and tissue engineering, many applications have been explored for a variety of tissues and diseases [42], including the skeletal muscle, which is the focus of this literature review. Our research group has dedicated to the development of MSC-based cellular therapies for application on several body tissues, from peripheral nerve to blood vessels and skin wounds [43–53], including for skeletal muscle volumetric loss lesions [54].

Since their first descriptions as a specific cell population in the late 1960s [55–57], knowledge on MSCs' features and potential has grown exponentially [58], as have the effective medical applications in which these are beneficial. The MSC population from the bone marrow (BM-MSCs) was the first to be characterized [55], and at the time, stem cells were thought to be exclusive to organs with fair regenerative capacity, such as the blood, intestine, bone, and skin. Nowadays, we are aware that they are present in virtually all the body tissues, in variable numbers, mostly remaining in a quiescent state until activated by significant events, ensuring a certain degree of defense against damage and disease [56, 59, 60].

The most significant features of MSCs are their clonogenic and proliferative capacities, while remaining genetically stable and in undifferentiated state, and their differentiation abilities [58], into various mesodermal, ectodermal, and endodermal cell types [61].

Through the years, significant progress has been made toward MSC characterization, and in an effort to standardize and unite the scientific community, the Mesenchymal and Tissue Stem

Cell Committee of the International Society for Cellular Therapy (ISCT) gathered a series of recommendations regarding the acceptable criteria for the definition of "mesenchymal stem cell" populations. Specifically, MSCs are determined to be characterized by (i) plastic adherent ability; (ii) absence of definitive hematopoietic lineage markers, such as CD45, CD34, CD14, CD11b, CD79-α, CD19, and class II major histocompatibility complex (MHC) molecules, specially human leukocyte antigen (HLA)-DR, and expression of nonspecific markers CD105, CD90, and CD73; and (iii) ability to differentiate into mesodermal lineage cells, osteocytes, chondrocytes, and adipocytes [62].

Another appealing point on MSC research is their immune features. Unlike terminally differentiated cells, MSCs are somehow immunologically privileged, avoiding the use of additional immunosuppressive supplements during the treatments, which are mostly (although not exclusively) deleterious for intrinsic regeneration mechanisms [42, 63]. One of the main mediators of immune responses is the HLA-II, of which MSCs present only neglectable levels, deeming them immunologically privileged [64]. This is a key point, considering the difficulty of finding matching donors among the human population and the challenges of harvesting sufficient numbers of cells from one patient upon necessity [65]. Hence, the lack of HLA-II opens the possibility of using directly obtained or banked cells from consenting healthy donors from the same species, designated as allografts [66]. Given their peculiar immune features, the xenogenic implantation of human-derived cells in appropriate nonimmunosuppressed animal models is feasible [52, 54, 67] and provides valuable information on their behavior and effect on experimental stages that more closely mimic clinical practice reality [63].

In addition, immunomodulatory actions have also been attributed to MSCs, by controlling and modifying host immune response, either locally (by blunting the tissue response at the implantation site [54]) or systemically (ameliorating signals of severe immunological disturbances, such as chronic inflammatory, autoimmune diseases or graft-versus-host-disease) [63, 68].

The bone marrow is without a doubt the most widely explored source for MSCs for therapeutic purposes. The bone marrow is harvested from the patient or consenting donor, and the adherent MSCs are isolated and expanded until desired numbers are attained for the intended application. The harvesting procedure is however highly invasive and potentially painful, motivating the search for more easily accessible sources. Furthermore, the "quality" of the isolate cells strongly depends on the age, gender, and health status of the patient or donor [65]. Adipose tissue and synovial membrane are also valid sources, and harvesting tissue for cell isolation is mostly associated to primary interventions for esthetical and/or medical reasons. [2, 39]. Other sources of MSCs are gaining ground for the minimally invasive nature of their harvest, as well as for the lesser ethical concerns surrounding their tissues of origin, namely, the stromal tissue of the umbilical cord [66] and the dental pulp [69] (Figure 1). The collection of the tissue sources for these implicates lesser ethical and technical issues since they were mostly discarded as medical waste following birth or dental procedures [66, 67, 69, 70]. Another alternative method could be the collection of MSCs from postmortem tissues. MSCs have been successfully isolated from the bone marrow, skeletal muscle, neural tissue, and dental pulp of deceased donors [71, 72]. These options, however, comply with similar if not aggravated ethical, legal, and even social and religious concerns to conventional MSC sources [71].

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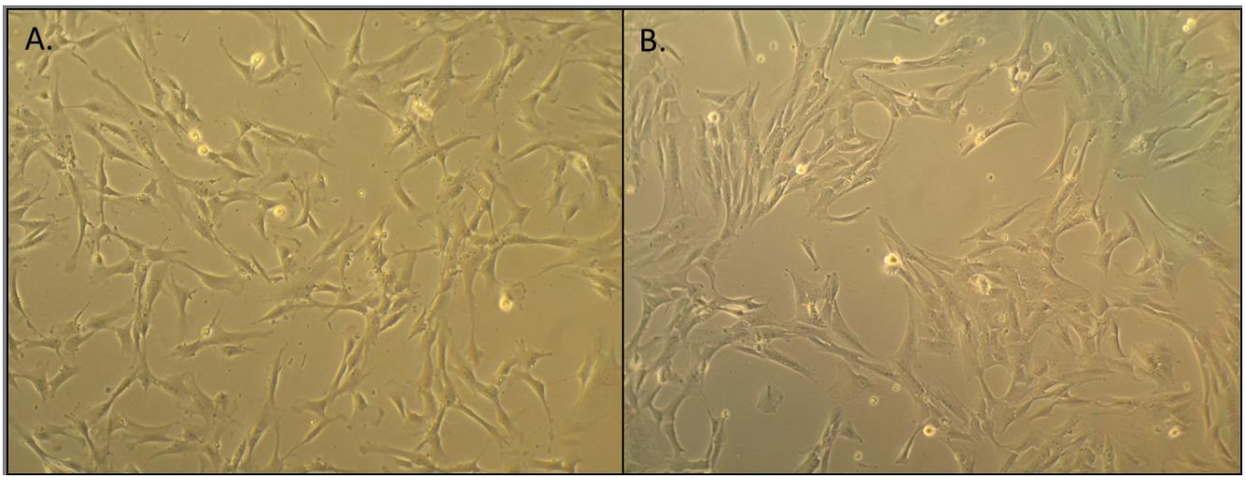

Figure 1. Morphological similarities between (A) DPSCs and (B) UC-MSCs (magnification: ×100).

Focusing on the skeletal muscle repair and regeneration, and apart from muscle-derived cells, the most popular source of MSCs is still the bone marrow. However, MSC populations are present in virtually all body tissues, and alternative sources have been proposed, such as adipose tissue, synovial membrane [2, 73, 74], dental pulp [4, 69, 75], and even umbilical cord tissue [76, 77]. Cells from these sources display comparable phenotypical features regarding their "stemness" potential (they are plastic adherent, positive for markers of the mesenchymal and negative for the hematopoietic lineage markers). As previously referred, they are similarly amenable of differentiation into mesodermal and endodermal cell lineages, including adipocytes, chondrocytes, osteoblasts, endothelial cells, and hepatocytes [61]. Nonetheless, MSCs from distinct sources are not completely identical, whether regarding phenotypical markers expression, proliferation rates, or even multilineage differentiation aptitudes [74].

3.1. Evidence of the *in vitro* myogenic differentiation potential of MSCs

MSCs from various nonmuscular sources have been demonstrated as capable of *in vitro* differentiation into myogenic lineages, through adequate stimuli, displaying phenotypical markers of native skeletal muscle cells [61]. However, nonmuscular MSCs depict hardly any spontaneous tendency toward myodifferentiation (0.001%) [6], unlike the "MSC-like" populations residing in the muscle tissue that show a degree of lineage commitment to myogenic formation, undergoing spontaneous differentiation in standard culture [31]. Not all MSC sources seem to show the same tendency or predisposition toward this differentiation pathway upon stimuli. As an example, the myogenic potential of MSCs from the synovial membrane (SM-MSCs) seems more limited than for chondrogenesis, osteogenesis, or adipogenesis since only limited number of individually expanded clones presented myotube formation capacity, suggesting subpopulations with specific tendencies for lineage commitment [73]. MSCs isolated from the umbilical cord stromal tissue display limited intrinsic tendency toward myogenic lineage differentiation, expressing diminished levels of pluripotency and specific myogenic markers involved in such process (such as Oct-4, Nanog, Pax-7, MyoD and myogenin, and M-cadherin), and they do not seem to spontaneously differentiate toward this lineage

[76]. Conversely, when cocultured with differentiating myoblasts, differentiation can be observed [76].

Cells undergoing myodifferentiation paths sequentially express characteristic transcription factors belonging to the myogenic regulatory factors (MRFs) family, such as MyoD and myosin (early a terminal differentiation markers) that are expressed with a well-defined time course depending on how far long the process has progressed [39, 78], replicating the embryogenesis of skeletal muscle tissue [15].

MSCs from the bone marrow, adipose tissue (AT-MSCs), synovial membrane, and dental pulp (DPSCs) are also capable of fusing to myoblasts in coculture systems. Although multinucleated hybrid myotubes generally appear only at low frequencies in the total population [6, 38, 74, 75] t results in the detection of muscle-specific gene expression in stromal cells, which is turned on through myogenic fusion [79].

In alternative to direct contact coculture, trans-well settings can also efficiently induce differentiation into skeletal muscle of AT-MSC subpopulations, via a fusion-independent mechanism, leading to the expression of aforementioned differentiating myotubes markers. This suggests that the differentiating myoblast can promote MSC myogenesis through secreted biomolecules that can effectively cross the trans-well filter and exert action on the MSC receptors. Nonetheless, the differentiation efficiency did not match the direct contact settings, deeming cell-to-cell direct interaction a key factor and suggesting that these two mechanisms act in complementary ways [6].

Further away from the coculture system, the supplementation of MSCs with conditioned medium (CM) from both mature muscle cells and primary precursors induced differentiation toward myogenic phenotypes [80]. CM from injured skeletal muscle also influences MSC proliferation, in a dose-dependent manner, and promotes myogenic lineage differentiation into the characteristic morphologies and transcription factors sequential expression. The medium from undamaged muscle did not elicit such responses, demonstrating that the injury event triggers the secretion of essential signaling biomolecules that modulate intervenient cells' fate *in situ* and are capable of modulating exogenous cells, such as BM-MSCs [78].

In 5-azacytidine (5-Aza)-induced differentiation, enriched umbilical cord stroma (UC-) MSCs, adherent fraction of umbilical cord blood (UCB-MSCs), periodontal ligament-derived MSCs (PDL-MSCs), SM-MSCs, AT-MSCs, BM-MSCs, and skeletal muscle-derived MSCs (SkM-MSCs) also begin displaying suggestive myoblast-like shape and fusing into multinucleated immature myofibers, expressing early muscular markers, such as Myf5 and then MyoD [2, 39, 67, 70, 77, 81]. The spontaneous twitching of multinucleated fused differentiating myotubes has also been described after 9–10 days culture [78]. Although classical myodifferentiation protocols rely on the pathway triggering by 5-Aza-induced DNA methylation, this is known to cause epigenetic changes, possibly precluding further advancements into clinical applications. In an alternative approach, differentiation can be successfully induced using a more "physiological" induction medium, composed of defined growth factors, such as bFGF, VEGF, and IGF-1, and it can successfully induce BM-MSCs into multinucleated myotube-like structures, with striated cytoplasm and replicating specific expression patterns of the myogenic pathway,

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similarly to other induction techniques [61]. Similar behavior is also observed in MSCs cultured in promyogenic medium containing dexamethasone and hydrocortisone [82].

3.2. Evidence on the application of MSCs for *in vivo* skeletal muscle regeneration

Further, *in vivo* applications suggest that, to variable extent, MSCs are capable of integrating host muscular tissues, being identifiable at the lesion site shortly after implantation (Figure 2) [54, 67, 75–77, 83–85]. The long time observation of administered MSCs in host muscle has also been reported, even when delivered systemically [2, 4]. Intra-arterial delivery appears more adequate for systemic MSC delivery, in detriment of intravenous routes. Keeping in mind the circulatory anatomy, this was an expected observation since venous routes implicate increased systemic dilution effects, as well as significant entrapment of cells within the pulmonary capillary bed [86].

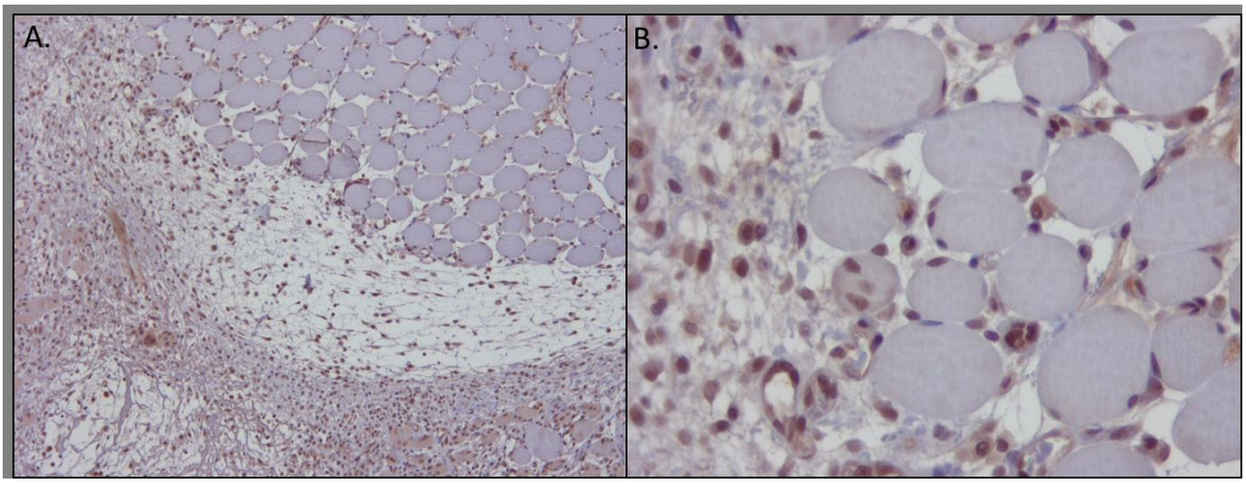

Figure 2. Human-derived UC-MSCs engrafted within injured skeletal muscle tissue 4 days after implantation. Immunohistochemistry staining for human nuclei (hNu) antigen (blue stained cells). Magnification: (A) ×100, (B) ×400.

It is set that the engraftment potential of MSCs into a damaged tissue is not absolute. The numbers or percent of cells identified at different time points following injection invariably decreases [76, 85, 87, 88], down to nearly as little as 10% of the initially delivered numbers in a couple of months [88].

MSCs positively influence recovery of chemically induced muscle damage [76, 77], nonvolumetric laceration [89] as well as in crush injuries [90, 91]. Although on occasions the delivered cells could not be identified on site as differentiated entities or fused to host cells or, when so, only to low degrees, the observed benefits further suggest that their contribution to the regeneration of skeletal muscle might rely on mechanisms other than fusion to myofibers after differentiation [89, 90].

UC-MSCs also engraft in the resident skeletal muscle tissue and are identifiable up to 14 days after administration and in some extent differentiate into cells expressing sarcomeric tropomyosine antigens [67]. When administered in undifferentiated state MSCs seem to replicate

embryonic myogenesis events, as they do following *in vitro* induction [67], triggering gene expression characteristic of myogenic differentiation pathways [2].

SM-MSCs are detectable integrated within host tissue for up to 6 months after either local or systemic delivery, demonstrating their preferential homing ability to the injured tissues since greater numbers were harbored by the injured muscle, although they could seldom be identified in other body systems [2]. At longer terms after BM-MSC [88] or UC-MSC [76] administration, about 5% of myofibers were of hybrid nature and could be identified along the whole muscle length [88]. The hybrids' formations seem to be a progressive process since hybrid myofibers represent a much smaller fraction (under 1%) in the regenerating muscle at shorter time points [74]. The administration of these cells granted increased muscle mass and mature fiber formation when compared to untreated muscles [76]. AT-MSCs also contribute to enhanced regeneration, reducing fibrosis and improving histological and functional features after only 4 weeks. However MSCs alone did improve the process, their association to a biomaterial vehicle and bioactive cues further enhanced those results, as detailed later in this section [83].

In the last decade, the question whether the seldom identified donor-derived cells resulted from trans-differentiation events of MSCs into muscle cells or from fusion to host cells raised significant controversy [79]. Today, based on the acquired evidence, the scientific community tends toward the fusion theory. As mentioned earlier, MSCs have been demonstrated to successfully fuse to host cells and contribute with genetic information, leading to the expression of human-derived genes and gene products [2, 79, 88]. Fusion efficiency seems to differ among MSCs sources, and AT-MSCs appear more prone to *in vivo* formation of hybrid myotubes, surpassing BM-MSCs and SM-MSCs [74].

Delivered cells also seem to interact with the resident tissue's satellite pool. Skeletal muscle SCs delivered to a regenerating muscle effectively contribute to the replenishment of the resident satellite pool, migrating to locations far from the lesion site within the muscle. These freshly isolated native skeletal muscle stem cells contribute in high extents to the total $Pax7^+$ population (about 38%), unlike expanded MPCs, that only account for about as little as 12% [35], but can still be identified within the muscle tissue and in association to connective structures [31]. Nonmuscular sources seem to behave closer to MPCs in terms of niche replenishment. Based on the location of the remaining delivered cells not associated to hybrid fibers, some authors suggest that these residual donor-derived cells might have also contributed to the resident SCs pool, and that they can effectively contribute to skeletal muscle regeneration in the events of a new injury [2, 35, 76, 88]. Alike exogenous SCs and MPCs integrated into the resident quiescent pool, other sources of engrafted cells are amenable of activation upon reinjury of the muscle [2, 35] and can be reisolated [35] and also originate primary myoblast cultures *in vitro* [2]. These expanded myoblast cultures originating from human-derived isolated SM-MSCs could further engraft a second recipient's regenerating muscle [2].

As suggested by some authors, the different engraftment potential might be due to the distinct MSC sources reported [35, 74], but it may also relate to the chosen disease model: dystrophic muscle models seem to better adopt exogenous cells than non-dystrophic-induced lesion

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models [88]. Although some disease models, especially of degenerative nature, seem to display sex-related differences, physical models of skeletal muscle lesions appear to respond similarly, at least in terms of functional recuperation. As for fibrosis development, male specimens might display a decreased tendency for the event [90]. The gender, age, and health status of the MSC donor is also a factor known to influence cell quantity, quality and general performance [65].

In line with the low engraftment potential and low contribution to *de novo* myotube formation and the solid observations that nevertheless MSCs tend to consistently lead to structural and functional improvement in skeletal muscle repair and regeneration models, the paradigm on the mode of action of MSCs started to shift. Evidence supports that their primary mechanism of action may rely on their secretory abilities rather than on their differentiation capacities, as discussed in detail further ahead [89, 90].

3.3. MSC-biomaterial systems for *in vivo* skeletal muscle regeneration

Most of the above-mentioned diseases and disease models involve severe affection of skeletal muscle tissue function (laceration or chemically induced damage) but, generally, the structural integrity of the tissue is maintained, preserving the blood and neural supply to the muscles as well as the resident SCs population [67, 88]. Although skeletal muscle detains a fair capacity for regeneration, severe injuries involving the loss of extensive volumes of muscle, termed as volumetric muscle loss (VML), mostly overwhelm this intrinsic response [12]. To date, these situations pose a relevant therapeutic challenge.

The gold standard for the surgical management is the creation of muscle flaps filling the defective area. However, these autoflaps depend on the maintenance of an adequate blood supply and involve the damage of a neighboring muscle. Therefore, the donor site morbidity and limited success of such approaches push toward the development of new treatment options [92].

The advent of tissue engineering and regenerative medicine research, focusing on both biomaterials, cells, and bioactive molecules, has boosted the search for new possibilities for the development of effective clinical treatment of affected patients [11, 92–94].

These cases are mainly related to traumatic or surgical events and result in complete tear of the myofibers or even significant loss of skeletal muscle tissue portions, VML, in large and relevant active muscle groups. Here, no support structures remain on the lesion site, nor do blood vessels, neural structures, or cell populations with capacity to repair and restore the lost tissue. The loss of 10% to 20% of the mass of a weight-bearing muscle represents a critical loss that will not fully regenerate even after long periods [8] (Figure 3).

The healing of these severe injuries can be improved by the sole administration loose of cells [34], but for the most cases, complete repair of such defects remains dependent on the ability of bridging the gap between the transected muscle segments. For VMLs, this point presents an impending challenge.

The research field of biocompatible biomaterials has opened a possible strategy to address previously irreparable lesions. These materials aim at providing a physical support to the

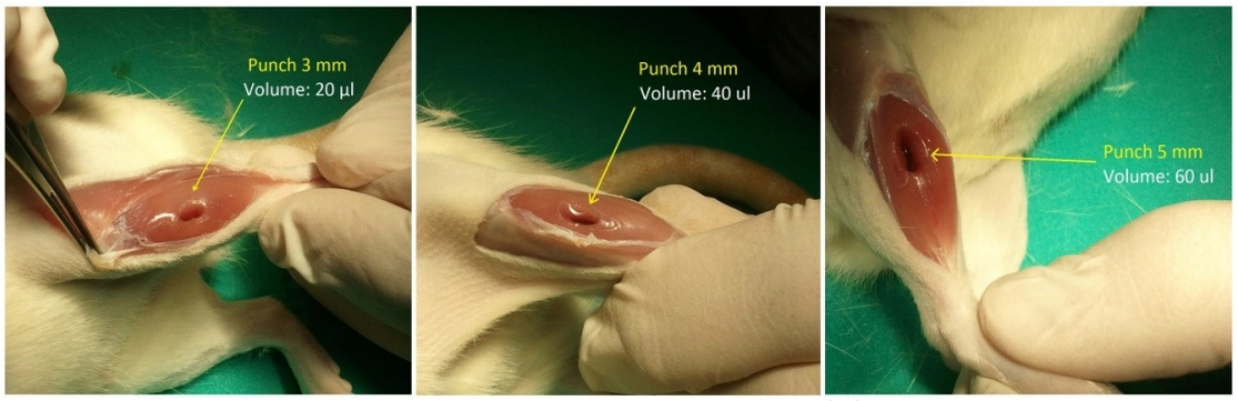

Figure 3. VML lesion model development: myectomy lesions in the tibialis anterior muscle of the rat's hindlimb. Different volumes (20–60 µl) in the defects produced by biopsy punch blades of different sizes (3–5 mm) [51].

regenerating myofibers on both ends, promoting their development and proper orientation, a key point for structural as well as functional recovery [8]. These biomaterial scaffolds are also valuable in recreating an advantageous mechanical and chemical microenvironment for the proliferation and differentiation of resident or delivered progenitors [8]. In addition, these matrices may act as (as in the case of ECM) [93] or be modified/loaded with bioactive signaling molecules participating in the repair process [83].

Here, decellularized ECMs appear as the most widely explored scaffold material for skeletal muscle tissue engineering, with commercially available products ready for clinical use and amenable of application in skeletal muscle repair and regeneration [33].

Decellularized skeletal muscle ECM has been demonstrated to adequately fill a critical muscle defect, and to benefit structural recovery. Although structural improvement was determined, functional outcomes did not significantly differ between the bridged and the unbridged control defects [8]. Other study reported functional indexes recovery comparing to the unimplanted group, but still insufficient to match undamaged muscles' response [94]. Scaling up to a larger preclinical canine model, these implants were capable of promoting endogenous progenitors migration into the regenerating area [95].

On a clinical setting application, commercially available ECM products have been successfully applied to restore chronic volumetric muscle injuries [11, 94, 96], reporting esthetic and, to some degree, functional improvements.

Besides large voids in the muscle tissue, biomaterials can also be applied in smaller defects. A gelatin-based hydrogel could enhance regeneration after laceration injury. This *in situ* forming gel was loaded with a prosurvival growth factor (bFGF), sustaining controlled release for up to 3 weeks. *In vivo*, the loaded hydrogel significantly improved contractile force of healing muscles, reduced local fibrosis, and increased multinucleated regenerating myofibers and neurovascular structures on site [83].

Stem cell implantation is a possible strategy to enhance the recovery rates whether they are delivered alone or in association to biomaterial scaffolds. Their inclusion has been demonstrated beneficial in skeletal muscle injury.

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As a first approach, SCs and other muscle-derived progenitors were investigated toward the optimization of the healing process of biomaterial treated defects [41, 97].

The performance of fibrin-based [31] and hyaluronan-based hydrogels [35] was significantly improved by the association of cellular systems. MPCs were able to reduce inflammatory infiltration and scar formation and regeneration events were improved, with reinnervation and revascularization of the area as well as increasing the regenerating myofibers content. The inclusion of freshly isolated SCs instead of expanded MPCs presented even better results with functional parameters closely meeting up to controls after 6 months of recovery [35].

Given the pros and cons earlier discussed regarding native tissue-derived cells and the effects of undifferentiated nonmuscular MSCs on other lesion models, some focus was given to their potential role in cellular-biomaterial systems.

The benefits of BM-MSCs in a severe VML models through the inclusion of the cell system into a decellularized ECM frame boosted both structural and functional recovery, with increased muscle tissue, blood vessels and nervous supply ingrowth into the defect area and improved muscle functional performance, when compared to the cell-free systems [8, 98].

The potential of AT-MSCs was also tested on a previously described gelatin-based hydrogel vehicle. Although the bFGF-loaded hydrogel alone performed satisfactorily, the addition of hAT-MSCs granted further improvements. The most striking result was the reduction of fibrosis to only roughly 20% and the recovery of functional parameters reaching 89% of uninjured muscles. Indicators of regenerative events (immature myofibers, reinnervation, and neovascularization) were also significantly improved [83]. When associated to Matrigel™, early improvements were observed. However, such differences were no longer evident in a 4-week time point [87].

Besides the obvious impact on the overall regenerative milieu, the exogenous MSCs are also strong modulators of tissue reaction to biomaterials implanted within the muscle tissue.

Using a volumetric loss rat model [51], UC-MSCs demonstrated their potential in modulating early inflammatory responses to a gelatin/thrombin-based matrix [52]. It was further confirmed in response to other biomaterial systems in terms of both the inflammatory response and the collagen type I deposition. The results from the sole application of a good vehicle (fibrin-based) were further improved, and the reaction to vehicles deemed less adequate (gelatin/thrombin-matrix and hyaluronan/alginate-based) was attenuated by the presence of UC-MSCs [54].

As described in an earlier section, one of the challenges faced on the use of cell systems is the success in engrafting the lesion site. The preconditioning of BM-MSCs to the myogenic lineage seems to improve integration in host muscles [61], but the pretreatment of UC-MSCs with SDF-1 does not seem to affect engraftment efficiency, despite the fact that SDF-1 is a known promoter of migration of transplanted and host cells to active lesion sites [76]. By contrast, the combination of bFGF, VEGF, and IGF-1 positively impacted engraftment, with increased donor cells' numbers identified forming mature hybrid myotubes [61].

The delivery of the intended MSCs within a scaffold/vehicle also contributes to prolonged maintenance on site, increasing engrafted cells number comparing to loosely delivered cells

[83]. Hence, the mode of delivery of cells or other regenerative cues is of vital importance [54]. Loosely delivered cells tend to show poor survival and engraftment and inadequate interaction with the host tissue. These drawbacks seem to be counteracted by their association to delivery vehicles [35] that also seem to positively influence cell survival and myodifferentiation, as well as neovascularization of the lesion sites when implanted [83].

In summary, both biomaterials and cells alone can aid the healing process, but their association seems to boost their individual actions. Cells help in functionalizing biomaterials while biomaterial provided beneficial microenvironments for the survival and action of the encapsulated MSCs.

From the currently available data, biomaterials alone are capable of providing fair benefits to volumetric lesions, but longer periods of recovery might be required (over 6 months). The coordination of those with cellular systems is likely to speed up the process, providing evidence of functional recovery earlier after the treatments [97].

The type and the magnitude of the contribution of the seeded population to the regenerative process also seem to relate to their differentiation state. Cells closer to undifferentiated state seem to elicit boosted initial responses, accelerating the onset of the process [87, 97]. By contrast, specialized cells tend to provide a more gradual but sustained response. The combination of the two populations may provide the key for additive effects and magnified recoveries [41].

One of the most striking observations is that the application of lineage committed or undifferentiated cells correlates to increased vascularization (and also innervation) at interface and core areas of implanted materials, which is known to be a vital factor for cell survival and function and tissue regrowth into volumetric matrices [98], since three-dimensional scaffolds easily exceed the diffusion capacity for nutrient and other essential components toward the inner parts of the constructs [99]. Seeding with potentially vasculogenic cells and/or prevascularization of constructs via *in vitro* culture seem viable options for improved results upon implantation into critical defects [100, 101]. The association of seeding strategies to surgical vascularization (i.e., connection to hosts vessels) is also described as boosting regeneration and recovery from large defects [102].

The timing of administration might also impact on MSCs engraftment and function. Most authors describe the existence of delivered cells for weeks following implantation [67, 76, 77, 83], when delivered up to 24 hours after injury. This topic remains highly debatable. The delivery of MSCs into a crush injury model either immediately or 1 week after the event did not lead to significant differences in functional recovery, indicating the possibility of a fairly large time window for the application of these therapies [84]. However, others report that the delivery of MSCs 1 week after injury (in attempt to escape initial inflammatory reaction) seems to impair their engraftment since no trace was detected after only 2 weeks. Most surprisingly, the cells were delivered on a hydrogel vehicle [87] what had been described to positively influence the permanency of delivered cells at the lesion site [83]. It might suggest that the early inflammatory microenvironment modulates MSCs function and maintenance on site or that their modulatory effects on the inflammatory milieu affect their engraftment.

Another critical factor under investigation is the most adequate number of MSCs to be delivered to the defect site, associated or not to a biomaterial vehicle. Winkler and colleagues

demonstrated a dose-dependent response to BM-MSCs administration in functional recovery from 0.1 to 10 million cells administered to a crush injury site [103]. Irrespectively, the number of cells administered regardless of the disease model or cell source or vehicle depicts no consensus among research groups, ranging from few thousands to several millions [52, 54, 67, 76, 77, 88, 98, 103].

Cell Source	Lesion Model	Delivery Mode/ Vehicle	Reference
hBM-MSCs	CTX TA mice	local injection	[88, 74, 79, 61]
rBM-MSCs	VML GTN rat	ECM	[98]
rBM-MSCs	CR SL rat	local injection	[103, 90, 91, 84, 85]
rBM-MSCs	CR SL rat	systemic delivery	[86]
hUC-MSCs	VML TA rat	thrombin-based matrix	[51, 52, 54]
hUC-MSCs	VML TA rat	fibrin-based matrix	[54]
hUC-MSCs	VML TA rat	hyaluronan/ alginate-based hydrogel	[54]
hUC-MSCs (enriched)	BVC TA rat	local injection	[67]
hUC-MSCs	CTX GTN mice	local injection	[76]
hAT-MSCs (+ bFGF)	LAC GTN mice	hyaluronic acid- based hydrogel	[83]
rAT-MSCs	LAC SL rat	Matrigel ™	[87]
hAT-MSCs	CTX TA mice	local injection	[74]
hUCB-MSCs	CTX GTN mice	local injection	[77]
hSM-MSCs	CTX TA mice	local injection systemic delivery	[2]
hSM-MSCs	CTX TA mice	local injection	[74]
hDPSCs	CTX TA mice	local injection	[75]

Table 1. Examples of non-muscular MSCs sources for *in vivo* skeletal muscle repair and regeneration; r: rat or mice derived cells; h: human derived cells; CTX: Cardiotoxin chemical model; VML: Volumetric Muscle Loss model; CR: Crush Injury model; BVC: Bupivacaine chemical model; LAC: Laceration injury model; TA: *Tibialis anterior* muscle; GTN, *Gastrocnemius* muscle; SL: *Soleus* muscle; ECM: Extracellular Matrix.

Since the core topic of this section is *in vivo* skeletal regeneration of acquired lesions models, an important remark must be made. Although the general intrinsic regenerative response of the skeletal muscle tissue to an injury event (approached in detail earlier in this chapter) follows a mostly constant sequence of events, the source and the magnitude of the induced injury lead to distinct native healing efficiencies and consequently distinct responses to implemented therapies [27, 104]. It results into an additional challenge when trying to understand the true magnitude of effects, especially in attempts of comparative analysis on the available literature.

Besides the selected defect model, the animal species/strain also assumes relevance. Different animal strains within the same species depict distinct tissue and systemic response profiles to

a similar injury [105]. Also, the use of immunocompromised [31] or non-immunocompromised [54] animals may also contribute to the inflammatory responses obtained when biomaterials and xenogenic cell sources are applied.

Other consideration possibly precluding the translation of developed therapies to the clinical practice is the character of the lesion site. In research scenarios, therapies are applied to recently injured sites. However, on a clinical setting, the most expectable situation is a chronic irreparable wound that underwent multiple surgical repair attempts though the course of several months or years [11, 94, 96]. Hence, as pointed out by Vigodarzere and Mantero [92], the homeostasis of such extensively injured and remodeled sites is significantly distinct from freshly induced insults; thus, the predictive value of the currently used animal models turns questionable.

4. Secretory potential of MSCs and impact on skeletal muscle regeneration

As evidenced by several authors, the beneficial action of MSCs on regenerating skeletal muscle might not solely depend on their differentiation capabilities, especially in nondegenerative lesion models, where their engraftment capacities seem fairly limited [76, 84, 88–90, 103]. Other proposed action mechanisms involve the secretion power of those cells [42, 76] since relevant growth factors and cytokines have been identified in various MSCs' sources secretome profiles [106, 107].

4.1. The role of growth factors and cytokines in skeletal muscle regeneration

The basis for this approach rests on the evidence that specific growth factors influence skeletal muscle regenerative response [108, 109]. In injured tissues, these factors are secreted into the surrounding microenvironment, exerting effect on, as an example, quiescent progenitor cells or delivered MSCs [78]. Thus, upon injury, the skeletal muscle itself releases a cascade of modulatory and signaling biomolecules, aiming at the recruitment and activation of essential characters to the regenerative process and triggering cell-type-specific programs [15]. These secretory capacities can inclusively modulate *in vitro* cell cultures and induce undifferentiated MSCs toward myoregeneration. In contrast, undamaged muscle seems to remain in a quiescent state, devoid of active stimulatory or differentiating factors [78].

Relevant growth factors are secreted by the remaining tissue but also by the invading immune cells participating in the intrinsic inflammatory response. Some of these molecules act as chemoattractant to additional inflammatory infiltration to the lesion site, such as MCP-1, IL-17, TNF-α, and TGF-β, among many others (a comprehensive table on the normalized nomenclature for some growth factors and cytokines is available as supplementary material on [107]) [110]. Macrophages are a grand character of skeletal muscle inflammatory response and accompany the full process of recovery [111], modulating their phenotype and secretory abilities and interaction with neighboring cells. They are chemoattracted to the site by molecules deriving from damaged muscle cells and other populations, such as neutrophils. They primarily secrete TNF-α and INF-γ then shift to increased levels of IL-4 and IL-10,

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promoting initial SCs division in undifferentiated state and, later on, their differentiation toward myogenesis [110]. IL-4 is also actively secreted by eosinophils active in the early stages of response to muscle insult. At this time point, this cytokine is essential to the activation and action of resident cell populations fibro/adipogenic precursors, promoting their proliferation while inhibiting differentiation into adipogenic lineages, contributing to the formation of essential support structures to aid myotube regeneration and to further secretion of bioactive factors [112].

Parallel to the types of growth factors and cytokines involved, it is essential to bear in mind that release/delivery dynamics is also of vital importance [27]. The intrinsic regenerative mechanisms following skeletal muscle damage does rely on the sequential and coordinated interaction of molecules [113], and the key to the development of improved strategies might come from contemplating and replicating these facts. Therefore, besides the growth factors content in a lesion site, the strict patterns of interaction between those play a crucial role in the outcome of the regenerative process. For example, HGF and bFGF activity after crush injury increases during the early regeneration period (first week), while TGF-β3 only significantly increased later in the process (after 12 days postcrush) [114].

HGF is a potent mitogen for quiescent SCs, inducing their activation and increasing the numbers of proliferating MPCs while preventing their differentiation [115, 116]. The effects of HGF in SCs quiescence appears to be the work of a concentration-dependent negative-feedback mechanism, promoting activation and proliferation at low concentrations, while rebooting SCs to quiescence and promoting muscle-specific proteins expression in increasing concentrations [117]. It is present in the undamaged muscle and is released upon injury [118], mainly of physical/mechanical nature [117], and it is also released from other organs, such as the liver and spleen, acting on skeletal muscle tissue in an endocrine way [119]. Its effects are observed in a restricted time window, peaking for the first days following injury and then decreasing. Given its inhibitory effect in myodifferentiation, its role in later stages of the regenerative process turns deleterious [116], if low expression is maintained [117].

Basic-FGF and IGF-I have also been reported to positively influence muscular cell populations in both *in vitro* and *in vivo* settings. In myoblast cultures, bFGF, IGF-I, and NGF effectively promoted myoblast proliferation and fusion into multinucleated myofibers, while other factors such as PDGF-AA, EGF, TGF-α, and TGF-βs seemed detrimental in that specific setting [120]. *In vivo* application into a laceration injury confirmed the beneficial effects of bFGF and IGF-I [120]. Basic-FGF sustained release at a lesion site (i.e., via hydrogel delivery) also elicits increased revascularization and reinnervation of the regenerated tissue, reducing the development of fibrosis [83]. IGFs are associated to both muscle cell proliferation and differentiation and play a key role in muscle regeneration and hypertrophy, with different isoforms affecting different stages of the process. IGF-IEc/MGF is expressed early in events, associated to SCs and proliferating myoblasts, while IGF-Ia and IGF-II expression occurs later in myogenic differentiation and muscle fiber formation [121]. IGF-I also modulates the local inflammatory response by down-regulating inflammatory cytokines on site, and thus limiting fibrosis development [122]. The combination of anti-fibrotic agents to IGF-I administration exerts

additive effects on muscle regeneration [32]. Sustained IGF-I delivery enhances ischemic muscle fiber regeneration, and beneficial effects are potentiated by combination with other growth factors, such as VEGF, resulting in synchronized angiogenesis, reinnervation, and myogenesis [123].

Other members of the FGF family interfere with skeletal muscle regeneration. FGF-6 is deemed muscle specific and is up-regulated during regenerative events, and its absence has been reported to relate to regenerative defects [124]. High concentrations of FGF-6 stimulate the proliferation of the myogenic stem cells, while while lower concentrations regulate muscle differentiation. It is also a determining factor for skeletal muscles' fiber type content [125, 126].

Vascular endothelial growth factor (VEGF) is also an important factor in muscle regeneration. In damaged tissue, VEGF and its receptors are detected in SC and in regenerating muscle fibers, as well as in cultured SC and myoblasts. VEGF acts by stimulating myoblast migration and survival, preventing apoptosis, and promoting myogenic cell growth. Furthermore, VEGF may have a relevant role in the homing of circulating progenitor cells to specific muscle location and/or in regulating the SC pool [127]. The local administration of VEGF has also been associated with reduced scaring and improved muscle regeneration and strength recovery after acute trauma [128]. Sustained VEGF delivery promotes neo-angiogenesis and tissue perfusion recovery, as well as conferring protection from hypoxia and tissue necrosis in ischemic limbs [123], but it may derive into aberrant ECM deposition and undesired fibrosis [129].

Granulocyte colony-stimulating factor (G-CSF) also exerts beneficial effects in skeletal muscle healing, promoting both structural and functional recovery in damaged muscles [130, 131], and is a crucial factor for skeletal muscle development [130]. It promotes myoblasts proliferation *in vitro*, in a dose-dependent manner, and although it is a recruiting factor for hematopoietic stem cells from the bone marrow, it does not influence the recruitment of BM-MSCs for skeletal muscle regeneration purposes [130].

As mentioned before, not all bioactive molecules and interactions have solely positive effects. Increased TGF-β1 levels are observed at injury sites [132]. This particular growth factor is stimulatory to collagen and ECM deposition that can be detrimental to the skeletal muscle regeneration process, contributing to exacerbated fibrosis and loss of contractile properties [32]. When TGF-β1 activity is inhibited by the action of decorin, regeneration indexes significantly improve and fibrosis development decreases by 50% in laceration injuries [133], conveying toward *in vitro* observations [132]. TGF-β1 acts on myoblasts, leading to the overexpression of fibrosis-related proteins and the down-regulation of myogenic proteins (desmin, MyoD, and myogenin). Furthermore, TGF-β1 released by injured muscle stimulates autocrine expression on surrounding myoblasts and inflammatory cells, amplifying its local fibrogenic effects [132].

These and other growth factors and cytokines are well known to guide and modulate tissue response to damage, and their coordinated actions are essential for the timely activation of myogenic cells, revascularization, and reinnervation of the lesion site and ECM deposition and remodeling.

4.2. MSCs secretome and effects on *in vivo* skeletal muscle regeneration

Most of MSCs secretome components are described to exert regulatory functions in both autocrine and paracrine ways [134], and interact both directly and indirectly with other cells, by triggering direct intracellular signaling pathways or by activating molecules production and release by other targeted cell types [42]. These bioactive molecules are deemed to benefit repair and regeneration processes mostly by inhibiting apoptosis and limiting the extent/propagation of injury, by diminishing fibrotic tissue development, by stimulating angiogenesis and revascularization of the regenerating tissue, and by activating/boosting intrinsic tissue-specific stem cell pools [63].

As disclosed in a previous section, the observations of positive effects upon MSCs application regardless of their presence as differentiated entities on site strongly support the assumption that their actions may alternatively depend on their capacity to produce and secrete compounds when in undifferentiated state [41, 89, 90]. This theory is also supported by the fact that exogenous MSCs mostly position themselves in close vicinity to regenerating myofibers, in native SCs/PICs-like locations, providing controlled release of such components [76]. Caplan and Dennis quite accurately described MSCs as "multi-drug delivery vehicles that are injury-site sensitive and/or responsive" [42], also referring to MSCs "homing" capacity (i.e., their ability to respond to signaling chemokines and preferentially migrate and attach close to lesion sites). Hence, the combination of molecules secreted by MSCs gain interest as modulators of inflammatory, fibrotic, and regenerating events [54].

Since the 1990s, considerable efforts have been made toward the comprehension of the secretion potential of MSCs derived from various tissues and exhaustive studies have focused on the detailed composition of their secretome [107, 135–137] and their actions and functions on the modulation of inflammatory and regenerative events, as thoroughly revised in [42, 63, 134, 138–142].

Briefly, and according to their prospective effects on regenerative processes in general, these factors and chemokines can classically be classified as anti-apoptotic, immunomodulatory, anti-scaring, supportive, angiogenic, and chemoattractant [138]. Factors including HGF, IGFs, FGFs, CSFs, PDGFs, and TGFs as well as cytokines such as IL-6, IL-8, and IL-10 have been identified in different magnitudes in MSCs culture supernatants [106, 107, 142]. Other performers in the skeletal muscle regenerative process seem to be absent, such as IL-4 [107].

The array of secreted molecules is related to the microenvironment accommodating the active MSCs, displaying consistent patterns of secretions in response to their local microenvironment, as well as to their functional status [42]. This responsiveness of MSCs to a variety of microenvironmental cues can be availed as to enhance their therapeutic potential from the amount of secreted factors [134] up to incrementing the engraftment success when implanted at a lesion site [61]. Inflammatory cues alter the expression patterns of MSCs, resulting in increased secretion of selected growth factors and other cytokines [135, 142]. MSCs can be exposed to controlled stimuli before application, such as hypoxia and mechanical stimulation, leading increased expression of growth factors such as bFGF, IGF-I, HGF and, with particular empha-

sis, VEGF [137]. These observations are of particular interest since severely damaged muscles present hypoxic milieus due to the impairment or loss of blood supply.

Nevertheless, most of the knowledge available on the MSC secretome derives from *in vitro* settings, and despite studies focusing on the effects of biomolecules on its profile, it hardly replicates the exact inflammatory scenario within a lesion.

Concurrently to the implantation of undifferentiated MSCs, it has also been hypothesized that the application of secretion products alone (termed as conditioned medium [CM]) display similar if not improved effects on skeletal tissue regeneration (Figure 4) [54], as it does in other damaged or degenerated tissues, such as the central nervous system [141, 143, 144].

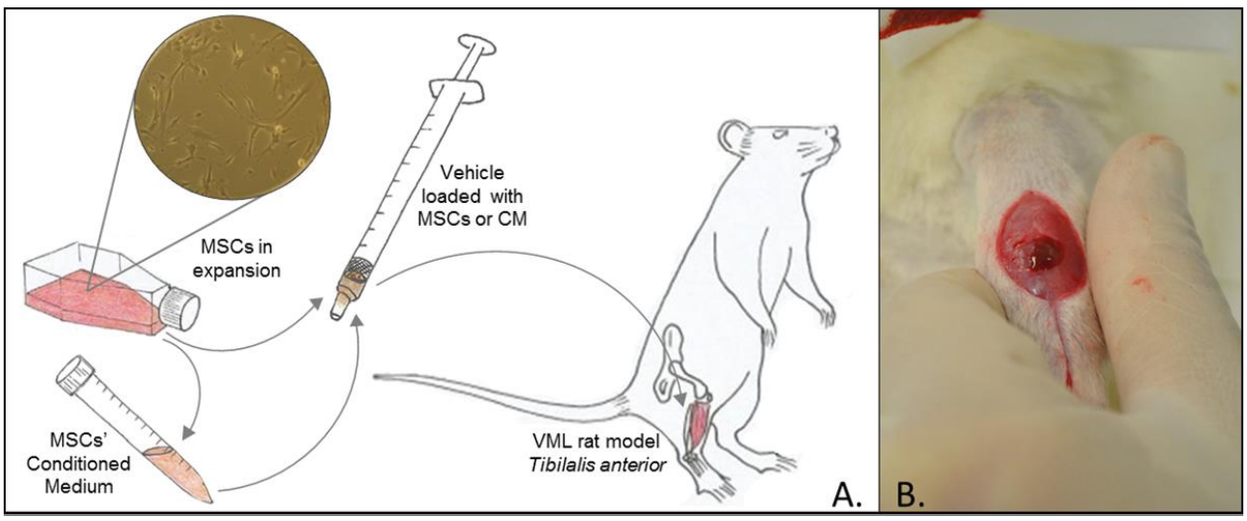

Figure 4. Schematic on the application of MSCs or their CM on hydrogel vehicles for the regeneration of critical muscle defects in a rat model (A) as described in [54]; VML lesion filled with loaded vehicle.

When comparing the skeletal muscle inflammatory response to implanted biomaterials of a severe tissue loss model, the association of either undifferentiated Wharton's jelly MSCs or CM obtained from their *in vitro* culture seems to consistently blunt the inflammatory response at early to medium stage of events (day 15 postlesion) [54]. Besides the reduced afflux of inflammatory cells to the implant sites on these early stages, we have also observed that the presence of MSCs resulted in accelerated progression to chronic inflammatory stages, that would resolve faster than unloaded subcutaneous implants (unpublished data). Therefore, although MSCs secrete a wide range of chemoattractive chemokines, their coordinate effects result in effective immunomodulation and in the control and containment of the local inflammatory response. Similarly, the addition of CM from UC-MSCs significantly improved the local response to different biomaterials as well as promoted improved muscle regeneration, revascularization, and reinnervation at the site of severe VML [54]. These results are supported by the detection of fair levels of immunomodulatory cytokines, such as IL-10 and MCP-1, as well as proregenerative growth factors, such as bFGF and VEGF [107]. Recently, we have observed similar behavior regarding the applications of DPSCs' CM in the same VML model. It similarly reduced the early to medium stages of inflammatory response to implanted

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biomaterial, but slight differences were observed. These differences might be attributed to slight differences in the secretome profile of UC-MSCs and DPSCs, and research is ongoing on this topic (unpublished data).

5. Conclusions and final remarks

The following are brief conclusions on the topics discussed along this chapter:

The skeletal muscle is frequently exposed to severe trauma that overwhelms its intrinsic healing mechanisms. To date, conservative and surgical treatment options often fail to restore the structure and function of the affected muscle.

The expansion of the regenerative medicine research field enlightened scientific community on some possible strategies to improve those clinical outcomes. MSCs appear as a promising source for the development of cellular therapies for skeletal muscle and other body systems. Significant achievements have been made toward their isolation from viable tissue sources, with sources like the umbilical cord or adipose tissue gaining ground over the classical bone marrow.

The recognition of nonmuscular MSCs potential for skeletal muscle regeneration lays on the observations that they can (i) assume skeletal muscle cells phenotypes (differentiate) and (ii) fuse to native muscle cells, that (iii) they can integrate living host tissues as differentiated and undifferentiated entities, and finally (iv) that they secrete a wide range of bioactive molecules with impact on the skeletal muscle regeneration milieu.

There is still great ground to cover in search for definitive therapies, but great promise holds on the development and refinement of tissue engineering strategies, combining the use of structural and active biomaterials, nonmuscular MSCs, and their secretion products in order to aid and guide the body's efforts to heal severe volumetric muscle lesion, aiming at the full recovery of the muscles' structure and function that greatly affect patients quality of live and well-being.

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Author details

A.R. Caseiro[1,2,3,4], T. Pereira[1,2], P.J. Bártolo[3,5], J.D. Santos[4], A.L. Luís[1,2] and A. C. Maurício[1,2*]

*Address all correspondence to: ana.colette@hotmail.com, acmauricio@icbas.up.pt

1 Departamento de Clínicas Veterinárias, Instituto de Ciências Biomédicas de Abel Salazar (ICBAS), Universidade do Porto (UP), Rua de Jorge Viterbo Ferreira, Porto, Portugal

2 Centro de Estudos de Ciência Animal (CECA), Instituto de Ciências e Tecnologias Agrárias e Agro-Alimentares (ICETA), Rua D. Manuel II, Apartado Porto, Portugal

3 CDRsp-Centro para o Desenvolvimento Rápido e Sustentado de Produto, Instituto Politécnico de Leiria, Rua de Portugal, Zona Industrial, Marinha Grande, Portugal

4 School of Mechanical, Aerospace and Civil Engineering & Manchester Institute of Biotechnology, University of Manchester, Manchester, UK

5 CEMUC, Departamento de Engenharia Metalúrgica e Materiais, Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, Porto, Portugal

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