Chapter 6

Synovia-Derived Mesenchymal Stem Cell Application in Musculoskeletal Injuries: A Review

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Additional information is available at the end of the chapter

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Abstract

Musculoskeletal injuries impact millions of people globally and affect their health and well-being as well as of their companion and athletic animals. Soft-tissue injuries represent almost half of these and are associated with unorganized scar tissue formation and long time-depending healing processes. Cell-based therapeutic strategies have been developed in the past decades aiming at the treatment and reversion of such disorders. Stem cells are fairly appealing in the field, being a responsive undifferentiated population, with ability to self-renew and differentiate into different lineages. Mesenchymal stem cells (MSCs) can be obtained from several adult tissues, including the synovial membrane. Synovia-derived MSCs can be found in individuals of any age and are associated to intrinsic regenerative processes, through both paracrine and cell-to-cell interactions, thus, contributing to hosts' healing capacity. Studies have demonstrated the potential benefit of synovia-derived MSCs in these regenerative processes in both human and veterinary medicine. The purpose of this chapter is to review the literature regarding SM-MSC therapies applied to musculoskeletal disorders, in both human and veterinary medicine.

Keywords: musculoskeletal injuries, regenerative medicine, cell-based therapies, mesenchymal stem cells, synovial membrane

1. Introduction

Musculoskeletal injuries represent a major health issue worldwide, compromising society's health and well-being [1]. In osteoarticular and skeletal muscle clinical injuries, tissue self-healing mechanisms are often insufficient and associated to scar tissue formation and long-term

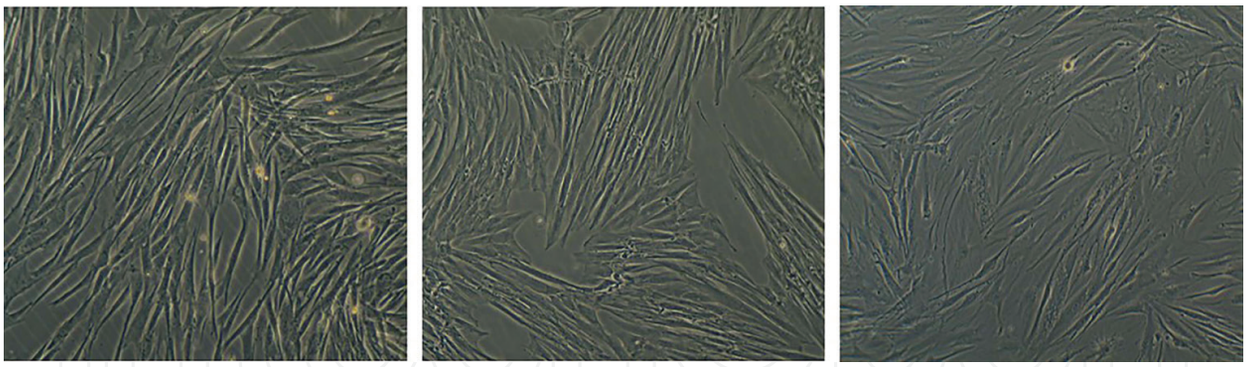

Figure 1. Synovial membrane-derived MSC can be obtained from different species. From the left to the right: canine, equine, and human. Images of cultured cells were obtained from the work developed within our research group.

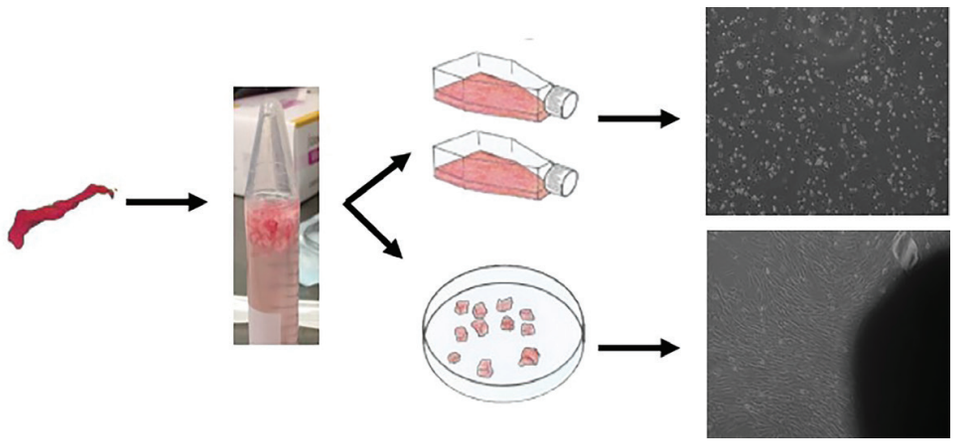

Figure 2. Synovial membrane-derived MSCs obtained from enzymatic digestion and explants technique; images of isolated cells were obtained from the work developed within our research group.

healing processes [2–5]. Different treatment techniques have been developed in the past years, but until now, no ideal regenerative treatment approach has yet been established [2, 4].

The purpose of this chapter is to review on the available literature regarding synovial membrane-derived MSC therapies applied to musculoskeletal disorders, both in human and veterinary medicine. **Figure 1** illustrates synovial membrane-derived MSC from three different species: canine, equine, and human.

MSCs can be obtained from the synovial membrane tissue through two different procedures: enzymatic digestion and explants technique, both illustrated in **Figure 2**.

We will address the musculoskeletal injuries and intrinsic repair mechanism and MSC sources applicable for its treatment, focusing on the advantages of synovial membrane-derived MSCs.

2. Musculoskeletal injuries

2.1. Osteoarticular disorders and regeneration physiology

The articular cartilage (AC) is a thin connective tissue layer that covers the bone extremities of the joint [4]. The AC presents a notable matrix structure organization [6], a limited number of

chondrocytes [7], and a rich water content [8]. The most important biomechanical functions of the AC include weight bearing and a smooth distribution of forces to the adjacent subchondral bone, providing nonfrictional motion of joints [4, 8]. The AC is divided into three layers. The most superficial one is thin, with a smooth surface. In this layer, the collagen fibers are aligned parallel to the tissue surface. In the middle, the articular cartilage is constituted by larger collagen fibers, with a nonparallel organization structure. The deep zone has a parallel alignment of the collagen fibers, vertically to the tissue surface [8]. The unique matrix structure, rich in collagen fibers, proteoglycans, and interstitial fiber, provides a viscoelastic environment that allows the AC to support its biomechanical functions [8]. It has been well established that the AC has limited self-healing capacities [3–5, 7–15] due to its intrinsic characteristics, namely its avascular nature, limited number of resident stem cells, and unique matrix organization [4]. Partial defects on mature cartilage do not heal spontaneously. On the other hand, complete defects are associated with the formation of fibrocartilage, which presents inferior mechanical characteristics [4]. Injuries affecting both, AC and subchondral bone, named osteochondral lesions, often evolve to secondary osteoarthritis (OA) [8, 9]. OA is a syndrome, characterized by AC degeneration, matrix loss, fissure formation, culminating with defects on the cartilage surface, and impacting on surrounding articular tissues, such as the subchondral bone, joint ligaments, the synovial membrane (SM), and periarticular muscle tissue [16], culminating with joint dysfunction and severe pain [9]. OA is one of the most frequent diseases affecting individuals worldwide, thus representing a major impact on the society's health [17]. Many other diseases culminate in OA, if not diagnosed early and treated, such as osteochondritis dissecans, affecting specially teenagers and young adults [18].

Chondrocytes are highly specialized cells, responsible for the production and maintenance of healthy cartilage matrix [19, 20]. However, these cells are particularly differentiated, with poor migration and proliferation abilities; thus, treatment represents a problematic challenge [17]. Several surgical treatment approaches have been developed in the past years. However, they all have inherent problems, impacting on patients' long-term healing process [21]. Surgical procedures that stimulate the bone marrow (BM), such as abrasion, distraction, drilling, and microfractures, are said to promote chondrogenesis phenomena, by inducing the BM mesenchymal stem cells (MSCs) from the subchondral bone. However, in most cases, these techniques lead to the formation of fibrocartilaginous tissue, instead of hyaline cartilage, probably due to an overloading of the BM and a small number of MSCs available, and the repaired cartilage often degenerates in the long term [3–5, 11]. Alternative regenerative approaches, regarding cartilage tissue engineering, are being developed, in order to overcome these disadvantages. Mosaicplasty is characterized by the transplantation of various small autologous osteochondral grafts to the injured joint site [13]. This procedure, however, is not suitable for OA patients or suffering from rheumatoid arthritis (RA), as chondrocytes in these patients have different biological properties [14]. This procedure promotes a short-term relief on patient's symptoms but fails to repair the damaged tissue and hyaline cartilage [4]. RA is a systemic autoimmune disease, characterized by a continuous inflammation phenomenon, a result of an intrinsic imbalance, culminating in a major synovial hyperplasia, bone, and cartilage damage [15]. Treatments involving artificial prosthesis are quite invasive and lifetime limited [9], as well as the mosaicplasty treatment technique is invasive and causes damages to the donor site [13] and fails to restore functional, as well as, phenotypically stable hyaline cartilage [4].

Autologous chondrocyte transplantation (ACI) is a cell-based technique that consists of harvesting chondrocytes from a nonweight bearing joint, first reported by Brittberg et al. [9]. Chondrocytes are expanded *in vitro* in a monolayer culture and then implanted in the lesion site. Despite the small amounts of donor cartilage used, it is necessary to minimize the invasiveness of the technique [13]. During the *in vitro* expansion period, many chondrocytes dedifferentiate and become unsuited to produce stable hyaline cartilage, thus impacting the final clinical outcome [4, 18]. Further, an uneven distribution of the transplanted chondrocytes at the lesion site is very common, as well as the diffusion of the cells from the cartilage defect [8]. To overcome these difficulties, transplantation of tissue-engineered cartilage was developed, evolving *ex-vivo* techniques, however with short-term successful results [18], in part due to intrinsic characteristics of the AC, as its antiadherent properties, which do not facilitate the integration of repaired tissue into the adjacent cartilage tissue [8].

2.2. Skeletal muscle injuries and repair mechanisms

Musculoskeletal disorders also impair the life and well-being of millions of individuals. They are usually characterized by long and incomplete healing processes that culminate into permanent musculoskeletal lesions [1].

Regarding the muscular tissue, in specific, the skeletal muscle, its constitution includes syncytial fibers that are characterized by the presence of a peripheral, postmitotic myonuclei [22]. Under experimental conditions, the skeletal muscle presents notable regeneration ability. Concerning clinical disorders, injuries or ischemia results in considerable tissue loss, that is, generally, not replaced [23]. In an adult, the intrinsic healing capacity of the skeletal muscle tissue relies on the presence of a resident, mononuclear, undifferentiated cell population, known as satellite cells (SCs) [22]. These cells are located between the sarcolemma of myofibers and the basal lamina [1, 24] and have the ability to migrate considerable distances, within the muscle tissue [23]. In a mature, healthy musculoskeletal tissue, SCs are predominantly on a mitotically quiescent state and respond to environmental signaling [22]. It is well established that microenvironmental signals are responsible for gene reprograming and cell phenotype changes [25]. Those signals, resulting from biophysical phenomena, such as growth, injuries, or weight bearing, induce existing SC to proliferate, differentiate, and fuse to existing muscle fibers, thus, mediating postnatal muscle regeneration [22, 26]. These environmental signals comprehend the release of growth factors from the impaired muscle fibers [1, 23], more accurately, myogenic regulatory factors (MRF), which are MyoD, Myo5, myogenin, and MRF4 [22]. The first two growth factors have a more active role during the embryonic development of the skeletal muscle lineage. After division, the SCs become myoblasts, which undergo a terminal differentiation process and fusion to the preexisting muscle fibers. Myogenin is responsible for promoting the terminal differentiation process and fusion [22]. MyoD also plays an important role by promoting the beginning of the proliferation phase of the SC. The absence of MyoD implies a cycle where SCs suffer several division rounds but return to a quiescent state [27]. On the other hand, lack of myogenin causes a severe deficit in the muscle tissue differentiation, resulting in the formation of unfunctional muscle fibers [28]. Thus, satellite cells recapitulate the MRF expression from the embryonic stage, during muscle repair processes. But, when in a quiescent state, SCs do not express detectable levels of MRF [1].

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Primary myopathies are characterized by a progressive atrophy of skeletal muscle fibers, thus resulting in deterioration, and compromising movements [2]. As the intrinsic repair ability of the mature skeletal muscle is limited, and pharmacology suppression of the inflammatory and immune response only provides a mild and finite effect, alternative cellular therapies have been developed, aiming at promoting the healing process [1, 2, 22]. Myoblasts would be an obvious choice, due to their role in the muscle repair mechanism. However, they are poorly expandable *in vitro* and undergo senescence quite easily [2, 22]. It is reported that about 90% of the transplanted myoblast cells die within the first hours [25]. Most genetic muscular disorder defects lie in the protein binding between the extracellular matrix and the cytoskeleton of the muscle cell, thus resulting in mechanical stress and continuous contraction movements, leading to muscle degeneration, and consequent tissue loss [2]. The Duchenne muscular dystrophy (DMD) is one of the most common genetic disorders in children. It is characterized by the lack of dystrophin at the muscle fiber sarcolemma. This disorder results in progressive and irreversible muscle degeneration and consequent death [1, 22]. Regarding these genetic disorders, myoblasts exist in small number and are not easily recovered in muscle biopsies [22]. Moreover, in the earlier stages of the disease, SCs divide to form myoblasts that fuse to the existing muscle fibers. However, those SCs transport the exact same genetic defect as the other muscle cells they are replacing. Thus, they will eventually die too [2].

3. MSC sources applicable for musculoskeletal regeneration

Regenerative medicine approaches regarding stem cell therapies have been developed in the past decades as a promising strategy, focusing primarily on immune/anti-inflammatory modulation [15] and cancer treatment [29]. Furthermore, their potential has been employed in cartilage [30] and skeletal muscle repair [1], the latter in a more immature state of development.

MSCs represent a fair candidate to innovative therapies because of their intrinsic unique abilities. MSCs were first harvested from the bone marrow by Friedenstein in 1976 [29, 31, 32], but now their presence is well established in virtually all postnatal tissues [5, 31–34], being involved in the tissue growth and homeostasis [31]. They have since been isolated from different adult tissues [30–32, 35, 36], such as fat, bone marrow, bone, cartilage [6], periosteum [32], nervous tissues, tendon, ligament, epithelium, SM, lung, peripheral blood, skeletal muscle, and nonadult tissues, such as amniotic fluid, placenta, and umbilical cord blood and stroma [29].

MSCs are plastic-adherent cell, fibroblast-like [29, 36], able to self-renew [32, 37]. They are characterized by an extensive proliferation ability in culture and have the potential to differentiate *in vitro* into different lineages [5, 32, 33, 36], including adipogenic, chondrogenic, osteogenic [6, 29, 31, 35], myogenic [6, 31, 35, 37], and neurogenic [6, 37].

MSCs' unique characteristics explain the interest of application on the development of regenerative cell therapies: ease of isolation, high expansion rates *in vitro*, low immunogenicity, and multipotency [4]. However, MSCs' definition and characterization still represent a challenge in the actual days.

According to the International Society of Cellular Therapy (ISCT), MSCs are characterized based on three important criteria: cell adherence, when cultured in standard conditions [38, 39], expression of specific cell surface markers (cluster of differentiation (CD)73, CD90, and CD105) and negative to others (CD45, CD34, CD11b, CD79a, and HLA-DR), and differentiation potential *in vitro* into multiple lineages: osteogenic, adipogenic, and chondrogenic, in defined culture conditions [38, 39]. They are furthermore characterized with respect to colony-forming unit fibroblast (CFU-F) [38, 40]. General consensus has not yet been established regarding specific cell surface markers [4]. It is not possible to characterize these cells only by the use of one specific cell marker [41]. Different protocols are applied to compare MSCs from different sources, always respecting the minimal criteria proposed by the ISCT. Nevertheless, there is no uniformity among the different characterization protocols [4]. The parallel expression or exclusion of several cell surface markers, associated with other ISCT criteria, is a frequent approach to MSC identification [41]. They share nonhematopoietic cell surface markers, such as CD29, CD44, CD73, CD90, and CD105 and human lymphocyte antigen (HLA) [29]; CD9, CD44, CD54, CD90, CD166 [41]; and CD44, CD79, CD90, CD105 [39]. They are usually negative for hematopoietic markers, such as CD34 e CD45 [39].

3.1. Synovial membrane-derived MSCs

MSCs from different sources present unlike characteristics, such as phenotype, proliferation capacity, and differentiation ability [29, 38], thus affecting the cell biological properties and therapeutic potential [4]. These intrinsic differences are influenced by the tissue of origin environmental factors [12, 29]. There has been an increasing interest in developing cell-based therapies, with the use of MSCs, with or without scaffolds. However promising for regeneration therapies, the most suitable source of MSCs for cartilage and for muscle repair still remains controversial [4].

Bone marrow-derived MSCs (BM-MSCs) were the first stem cells applied in cartilage injury therapy studies. Although their chondrogenic potential has been established, the improper differentiation of BM-MSCs during cartilage regeneration has been repeatedly reported [4], leading to the need to study alternative MSC sources.

3.1.1. Characterization of synovia-derived MSCs

Synovia membrane-derived MSCs (SM-MSCs) were first identified in 2001 [31] and are a promising source of MSCs regarding musculoskeletal therapies, due to their intrinsic characteristics. They present a high self-renewal ability [31, 40], superior potential for chondrogenic differentiation [3, 4, 10, 12, 30, 32, 37], and a high proliferative capacity [3, 4, 10, 12, 29, 32, 37, 40], both compared to nonjoint (bone marrow, adipose tissue, and umbilical cord) and joint tissues (AC and synovial fluid) [4]. SM-MSCs have been successfully differentiated into osteogenesis, chondrogenesis, and adipogenesis [31, 35], as well as toward myogenesis, but at a minor extent. They maintain their intrinsic differentiation characteristics, regardless of the donor, age [4, 6, 31], cell passage number, or cryopreservation [31]. SM-MSCs can produce hyaline-like cartilage tissue, under specific conditions, becoming a promising approach to cartilage injury therapies [4].

SM-MSCs are easily expandable in vitro, maintaining a stable profile, and retaining their multidifferentiation ability, even over 10 passages [4, 12, 31, 32]. The SM is easy to harvest [40] and can be collected from any joint, without impairing the AC tissue [3]. It can be obtained arthroscopically, with a minor degree of invasiveness [10] through a small biopsy [31] and with minor complications at the donor site [12]. It is a quite accessible source, as the SM is routinely removed from OA patients for knee replacement or other arthroscopic interventions [15]. It can be cryopreserved and stored for future use, as it is not negatively influenced by cryopreservation methods [31].

3.1.2. The synovial membrane as a niche to SM-MSCs

The SM is composed of two different layers: the synovial lining, rich in fibroblast-like synoviocytes and macrophage-like synoviocytes, and the subintimal layer, constituted by fibrous tissue, blood vessels, and immune cells. The origin of SM-MSCs has been speculated to be from the synovial lining, as they are biologically similar to the fibroblast-like synoviocytes [4]. SM-MSCs are more closely related, developmentally, to chondrocytes, in comparison to other MSC sources [17] revealing proximity of the gene expression profiles of SM-MSCs and chondrocytes when compared, for example, to BM-MSCs, adipose tissue-MSCs and umbilical cord-MSCs [4, 42], which further support the SM-MSCs' superiority in chondrogenesis differentiation. SM-MSCs have been found in healthy joints, confined to the subintimal layer, but also in OA individuals, in a more diffuse distribution through the joint tissues [4]. As there has not yet been established a specific marker to identify MSCs, it is not possible to address the topographic distributions of MSCs in the joint [31, 43]. Various theories have been proposed for their origin. They can be derived from precursor cells to enter the joint from the circulation and/or they can have been recruited from the BM through vascular channels [31].

The SM is a very responsive tissue upon cartilage injury. It responds to full thickness defects with proliferation and chondrogenic differentiation [44], but the most common source of reparative cells in these cases are the BM-MSCs, as they can infiltrate directly from the subchondral part of the defect into the joint space [4]. In partial defects, a positive response from the SM can be observed, by the formation of a continuous layer of SM-MSCs extending from the SM across the normal AC into the injury site [45]. The recruited SM-MSCs migrate toward the lesion site under chemotactic signals and undergo chondrogenesis, stimulated by the transforming growth factor β (TGF-β) [46].

The synovial fluid (SF) is a viscous and clear, rich in hyaluronic acid, liquid in intimate contact with the AC and the SM. It represents a route for exogenous cells to access the cartilage injury site [4] and 'free-floating' MSCs have been identified and isolated. Different theories have been proposed to address the origin of the MSCs present in the SF. They can have their origin in the disrupted cartilage, bone, SM, periosteum, and also in the BM itself. Regarding the SM, cells can shed into the lumen or through reported vascular pericytes of the SM [43].

3.1.3. Harvesting and isolation methods for SM-MSCs

For regenerative medicine purposes, it is helpful to harvest the greatest cell number, with greatest potential, from the smallest amount of tissue possible, minimizing the harvesting impact on

the tissue source. Isolation methods have not been exhaustively characterized. For example, Sugita et al. proposed an isolation method without filtration to be more effective in collecting more cells from a smaller sample [37], in contrast to the common ones, which undergo filtration.

SM-MSCs harvested from OA joints have the same osteogenic and chondrogenic differentiation potential [41, 47], although they present superior proliferation abilities, in comparison to SM-MSCs from healthy joints [47].

3.1.4. SM-MSCs in vitro culture

Fickert et al. reported that after the harvested cells adhered in monolayer cultivation, the subtype of cells expressing CD markers enriched remarkably [41]. During *in vitro* culture, SM-MSCs become homogeneous populations after the first passages. During expansion, hematopoietic and endothelial markers almost disappear and they present a stable molecular profile between passage 3 and 10 [31].

Enriched subpopulations of SM-MSCs present more efficient chondrogenic differentiation abilities [4]. A possibility of cell selection to get more homogeneous populations [40] involves the use of growth factors.

Ashton et al. first reported chondrogenesis of MSCs [48]. A specific medium for *in vitro* chondrogenesis was described containing transforming growth factor beta (TGF-β), dexamethasone [49], and bone morphogenetic proteins [50]. Evaluation of MSCs' chondrogenesis potential is currently performed with a micromass technique [21].

Another group suggested the combination of growth factors: insulin-like growth factor I (IGF-I), basic fibroblast growth factor (FGF), and TGF-β_1, applied early in culture, with a posterior addition of TGF-β_1, and reported an enhanced proliferation of SM-MSCs with chondrogenic potential. It has been well established that growth factors induce important effects on MSCs' differentiation potential. TGF-β is known to promote collagen II and proteoglycan expression; furthermore, it enhances cell recruitment into the prearticular tissue, regulating MSCs' condensation during cartilage formation; TGF is also involved in the expression of Sox-9, a gene responsible for a major regulation of the chondrogenesis differentiation. FGF promotes proteoglycan synthesis and IGF-I plays a role in chondrogenesis regulation, augmenting the expression of chondrogenic markers, thus, impacting on skeletal growth [51].

Although there has been some concern about the chondrogenic stability of SM-MSCs *in vitro* [52], a recent study reported SM-MSCs to present a significantly higher expression of chondrogenic markers and a stable chondrogenic phenotype [47].

3.1.5. SM-MSCs' therapeutic applications

Therapeutic strategies with resource to SM-MSCs have been developed in the past years, mostly for osteoarticular tissue regeneration. **Table 1** summarizes the most relevant studies applying SM-MSCs in *in vivo* models, which will be extensively addressed in this section.

Intra-articular (IA) administration of SM-MSCs has been widely reported. Nakamura et al. reported intra-articular transplantation of SM-MSCs in a pig model, inducing repair of

SM-MSC donor species	Lesion model		Delivery mode	Refs.
	Host	Lesion type/site		
Rat SM-MSCs	Rat	Patellar groove osteochondral defect	Intra-articular magnet	[3]
Porcine SM-MSCs	Pig	Femoral condyle full-thickness osteochondral defect	IA administration	[30]
		Chondral defect in the knee femoral condyle	Scaffold-free TEC	[6, 8, 33, 58]
Rabbit SM-MSCs	Rabbit	Femoral trochlear groove full-thickness osteochondral defect	Local adherent technique	[5]
			Cell aggregates	[54]
			Collagen gel + periosteum coverage	[40]
		Femoral trochlear groove osteochondral defect	PRP gel	[10]
		Femoral trochlear groove partial-thickness osteochondral defect	IA administration after chondroitinase ABC treatment	[53]
		Femoral condyle full-thickness osteochondral defect	Tissue construct: fibrin glue + polyglycolic acid netting	[59]
Human SM-MSCs	Rabbit	Femoral trochlear groove full-thickness osteochondral defect	Cell aggregates	[54]
	Mouse	Tibialis anterior muscle injury	Injection	[22]

Table 1. Animal studies applying SM-MSCs for musculoskeletal repair.

cartilage defects. Cell adherence to the injury site was observed through fluorescent labeling [30]. Recently, SM-MSCs from OA individuals have been reported to suppress T-cell proliferation and to suppress T-reg populations *in vitro*, when cocultured with allogenic lymphocytes. Thus, indicating their ability to suppress the immune response and prevent OA development [15, 35]. Yan et al. reported SM-MSCs' ability to prevent autoimmune disease and recover self-tolerance after repeated IA administrations of SM-MSCs from OA individuals to a collagen-induced arthritis murine model. They observed a superior histological and clinical scores in treated individuals, with inferior tumor necrosis factor α (TNF-α), interferon gamma (IFN-γ), and interleukin (IL) 17A, and increased IL-10 levels [15]. Another group also reported their ability to display indoleamine 2,3-dioxygenase (IDO) activity, after stimulation with IFN-γ and/or TNF-α that has been recently correlated to the T-cell suppressive mechanism in humans [35].

To address the problem of dispersion of injected cells inside the articular joint capsule, Hori et al. proposed the use of an intra-articular magnet, to conduct the IA administered cells to the injured site, where an intra-articular magnet is placed. They successfully reported the mobilization of the injected cells to the lesion site [3].

Nevertheless, IA administration has also been reported to be insufficient, as it results in an increased number of T-cell recruitment, relating to the development of synovitis [53]. A possible explanation for this reaction, in comparison to other therapeutic techniques, can be the

number of SM-MSCs injected to the joint, which is considerably higher when performing IA injection and that most of them adhere to surrounding tissues [5].

Koga et al. compared the effectiveness of IA injection of SM-MSCs with a local adherent technique using a rabbit model. They reported an *ex-vivo* 60% attachment to the defect in the local adherent technique, 10 min after the beginning of the procedure. *In vivo*, they registered a 24 h attachment that showed improved histological scores for 24 weeks, in comparison to the IA administration technique. This technique was scaffold free, with no periosteal coverage of the inserted cells. They also performed the *ex-vivo* technique in humans, with similar results [5].

Another study applied SM-MSCs in a full-thickness defect, with collagen gel covered with periosteum, in a rabbit model. They showed that SM-MSCs undergo differentiation and evolve into chondrocytes, responding to environmental cues, and remain active for at least 24 weeks. They also demonstrated an abundant cartilage matrix production. However, the cartilage became thinner after the 24 weeks, suggesting long-term incomplete healing process [40].

Later, the same group investigated the possibility to transplant aggregations of SM-MSCs for cartilage regeneration. The aggregates were produced easily by the hanging drop technique. They reported an improved cartilage matrix synthesis from SM-MSC aggregates, compared to SM-MSCs cultured in monolayer. They adhere to the defect by surface tension. Successful cartilage repair was achieved with transplantation of a low-density aggregate. These findings suggest a way to improve cartilage repair techniques, with minor loss of SM-MSCs. However, they propose the use of fibrin glue to improve results in a future study [54].

Lee et al. reported the application of SM-MSCs into the cartilage defect of rabbits, embedded in platelet-rich plasma gel (PRP), as it was previously studied with chondrocytes. PRP is defined as a volume of plasma fraction of autologous blood that is composed of a higher platelet concentration. It is described to be an important source of growth factors, enhancing chondrogenesis and proliferation of MSCs. They concluded that SM-MSCs in association with PRP showed improved results, in comparison to PRP alone. However, the applicability of this technique may not be suited for all osteochondral defects, and the clinical benefits of PRP are still controversial [10]. Chiang et al. also applied a PRP hydrogel with SF-MSCs in a porcine model, with satisfactory results [55].

Lee and his group also proposed a treatment with chondroitinase ABC in a rabbit *ex-vivo* partial defect model, to promote the adhesion of transplanted SM-MSCs. The proteoglycan antiadherent properties impact on the cell adhesion ability, to the cartilage surface [4, 53]. Chondroitinase ABC is an enzyme that depolymerizes the glycosaminoglycan side chain, thus, exposing to the underlying fibronectin, which presents cell adhesive properties. As such, this approach enhanced cell adhesion. However, the repaired tissue showed lack of hyaline-like cartilage content [53].

It is generally accepted that a three-dimensional (3D) environment enhances cell proliferation and differentiation abilities. Artificial scaffolds, composed of synthetic polymers or biomaterials, are often used. However, they are related to various issues with regard to long-term safety, such as degradation *in situ*, retention, and transmission of infectious agents. Scaffold-free tissue-engineered constructs (TECs) were developed in order to overcome the previous

drawbacks. MSCs are cultured in monolayer with addition of ascorbic acid and are then submitted to shear stress, resulting in their detachment and spontaneous contraction to form the 3D structure, similar to what is observed with collagen gels [56].

TECs based on allogenic SM-MSCs have been applied on cartilage defects from varied species. Shimomura et al. used a TEC based on SM-MSCs derived from immature and mature pigs, in order to address the age dependency in chondrogenic and proliferation abilities of SM-MSCs. No differences were reported between the groups, suggesting no age dependency [6]. Ando et al. similarly used a porcine allograft model, with a basic TEC composed of collagen I, III, vitronectin, and fibronectin. The TEC showed to have stable adhesion to a porcine cartilage matrix, in an explant culture, possibly due to the adhesion properties of fibronectin. When cultured in chondrogenic medium, enhanced expression of glycosaminoglycans and chondrogenic matrix genes, as collagen II and aggrecan, was observed [33, 56], suggesting that SM-MSCs in the TEC retain their chondrogenic potential [56]. They also proposed a xeno-free system for the development of this technique, as the TEC is produced without an exogenous scaffold, with autologous serum and MSCs. A chondrogenic-like tissue was formed in the defect, *in vivo*, with similar mechanical properties to a normal cartilage and progression of OA phenomena was prevented, compared to untreated defects [33].

In terms of human SM-MSCs, investigators proved human SM-MSC-derived TECs to be rich in fibronectin and vitronectin. This group demonstrated cells' ability to adhere to human chondral fragments [56], as it was previously demonstrated in the pig model. They also applied a xeno-free technique, by using human serum [5, 56]. Autologous human serum is reported to be more effective in promoting SM-MSCs' proliferation, in comparison to other MSC sources [5, 10, 57].

Later, Fujie et al. developed a similar TEC in a porcine model and reported a mechanical vulnerability at the repaired tissue boundary, indicating commitment of long-term durability from the repaired tissue, regardless of the apparent secure tissue continuity and histological quality [8]. Ando et al. developed a new TEC in 2012, showing to have histological defects at the superficial layer of repaired cartilage that presented a stiffness surface and lower water-retaining capacity. Thus, improvement is still needed regarding TEC strategies for cartilage defects' long-term repair [58].

Pei et al. reported the use of an allogenic SM-MSC-based premature tissue construct in a full-thickness osteochondral defect. They combined SM-MSCs from a rabbit with fibrin glue and seeded into polyglycolic acid netting. Incubation in a bioreactor lasted 1 month, with growth factor enrichments. After 6 months, the defects were covered by a hyaline-like tissue, well adhered to the surrounding healthy cartilage, presenting collagen II and glycosaminoglycans. However, contamination with macrophages was an issue in the *in vitro* assays [59].

A recent report was the first to investigate OA therapies resorting to exosomes, which result in the paracrine secretion of trophic factors by MSCs. They compared the therapeutic abilities of SM-MSC exosomes and the ones produced from human induced pluripotent stem cells in OA, using a mouse model. Stronger effects were observed by human induced pluripotent stem cell exosomes, representing a possible alternative to MSCs for OA treatment [17].

Regarding human *in vivo* research, Sekiya et al. reported a promising study involving 10 individuals with articular defects. SM-MSCs were successfully applied locally and rested for 10 min for adherence, as the same investigators reported before in pigs and rabbits. The therapy efficacy *in vivo* was evaluated, according to MRI, histological, and clinical scores. Only one patient presented fibrous cartilage in the deep-zone, although, in general, the results were satisfactory and promising [60].

De Bari and his group investigated the potential use of SM-MSCs for muscle repair in mdx mouse model for DMD. They demonstrated that human SM-MSCs had the capacity to contribute for the formation of myofibers and long-term persisting SC. The cells were injected into the blood stream, engrafting in several tissues. However, they only acquired muscle phenotype within the skeletal muscle tissue, verifying their sensitiveness to environmental cues. They observed that the administration of SM-MSCs restored the sacrolemmal expression of dystrophin and rescued the expression of mouse mechano-growth factor (MGF). MGF is involved in muscle repair and maintenance but is undetectable in dystrophic mdx mouse, even after mechanical stimulation. They also reported that a subpopulation of the injected cell remained for several months as SC. These findings suggest the significant role of SM-MSCs in restoring pathophysiologic features of the dystrophic muscle in the animal model [22].

4. Conclusion

In summary, MSCs play an important role in embryonic development, postnatal growth, repair, and regeneration mechanisms, as well as in maintaining tissue homeostasis, and synovial membrane mesenchymal stem cells are a promising, easily available source. Despite relevant recent advances, challenges still remain on the use of MSCs as standard therapeutic options for clinical applications.

Although BM-MSCs remain the most studied source of MSCs, as they were the first to be characterized, SM-MSCs are an easily available source with proven enhanced chondrogenic, osteogenic, and myogenic differentiation ability. Nonetheless, their characterization, as established by the expression of specific cell surface markers, may be affected by interindividual heterogeneity and major differences in cellular marker expression profiles may be found in nonhuman species.

The most effective administration route for SM-MSC application *in vivo* remains to be defined and the genetic stability of the cells must be assured both *in vitro* and *in vivo*. Also, the biomechanics and secretory profile of these cells must be further studied, in order to comprehend the mechanism of regenerative capacity of these cells and secretion profile in signaling factors, growth factors, cytokines, and other bioactive molecules, and their role on chondrogenic as well as osteogenic and myogenic differentiation.

SM-MSCs present themselves as a promising source of MSCs that are becoming the targets of several research groups worldwide. Their application *in vivo* in preclinical and clinical trials is envisioned for the therapeutics of musculoskeletal disorders.

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Conflict of interest

The authors declare no conflicts of interest.

Abbreviations

AC	articular cartilage
ACI	autologous chondrocyte transplantation
CD	cluster of differentiation
CFU-F	colony-forming unit fibroblast
BM	bone marrow
DMD	Duchenne muscular dystrophy
FGF	fibroblast growth factor
HLA	human lymphocyte antigen
IA	intra-articular
IDO	indoleamine 2,3-dioxygenase
IFN-γ	interferon gamma

IGF-I	insulin-like growth factor I
IL	interleukin
ISCT	International Society of Cellular Therapy
MGF	mechano-growth factor
MRF	myogenic regulatory factors
MSCs	mesenchymal stem cells
OA	osteoarthritis
PRP	platelet-rich plasma gel
SC	satellite cells
SF	synovial fluid
SM	synovial membrane
TEC	tissue-engineered construct
TGF-β	transforming growth factor β
TNF-α	tumor necrosis factor α

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