3 Adipose-Derived Stem/Stromal Cells, Stromal Vascular Fraction, and Microfragmented Adipose Tissue

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3.1 Introduction

Adipose tissue, for a long time, has been considered merely a storage of excess energy, but more recent evidence has helped shed some light on its role [1], comprising energy balance storage [2], as well as regulating bone metabolism, hematopoiesis, and the inflammatory response [3].

Adipose is a highly vascularized structure, composed of a heterogeneous mixture of cell populations, primarily derived from interlobular and perivascular connective tissues, consisting of mature adipocytes, preadipocytes, fibroblasts, vascular smooth muscle cells, endothelial cells, resident monocytes/macrophages, and lymphocytes, as well as progenitor cells and mesenchymal stem/stromal cells (MSCs). The presence of MSCs within this tissue (ASCs, adipose-derived stem/stromal cells) has recently drawn significant clinical attention due to their purported paracrine effects and multipotent differentiation capacity [4]. To date, its use as source of pro-regenerative cells has been successfully reported in a variety of preclinical and clinical applications, including musculoskeletal conditions, cardiac diseases, ischemia, amyotrophic lateral sclerosis, diabetes, and Alzheimer's and Parkinson's diseases [5]. Considering the promising results achieved so far, a wide array of lab-driven technologies is actively studied to undergo the process of a more efficient translation into the clinical setting.

Adipose tissue either can be used to isolate ASCs or can be processed at the point of care to obtain adipose-derived products. In the former case, ASCs are efficiently isolated by tissue enzymatic digestion and then culture expanded as adherent monolayers. In this setting, ASCs are generally consistent with the International Society for Cellular Therapy (ISCT) accepted attributes mesenchymal stromal cell populations (MSCs). Differently, adipose tissue can be processed at the point of care into cell suspensions or microfragments that have been commonly referred to as stromal vascular fraction (SVF) or microfragmented adipose tissue (microfat), respectively [6].

Both these strategies for the use of adipose-derived therapeutic cellular products have advantages and pitfalls. The approach based on cultured ASCs provides a standardized cell population of stem/stromal cells, compared to the use of SVF or microfat, in which different cell types (i.e.,

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endothelial cells, progenitor cells, and leukocytes) are represented together with mesenchymal stem/stromal cells [7]. On the other hand, the use of microfat or SVF has the theoretical and practical advantages of providing a point of care therapy that does not imply the cost and risk of in vitro culture expansion. Moreover, preparation of SVF and microfat may preserve the tissue native niche, which is composed by different cell types including stem and progenitor cells.

Still many controversial points animate the debate on the most effective procedure. To shed some light, in the next paragraphs, a more in-depth description of both cells and techniques as well as applications will be discussed, with a final focus on orthopedic-related tissues and diseases.

3.2 Adipose-Derived Stem/Stromal Cells and Adipose-Derived Products: Two Sides of the Same Moon

3.2.1 SVF and Microfat

Although they have some similarities, including being prepared at the point of care and the characteristic of preserving the tissue niche, SVF and microfat also present some substantial differences.

The adipose tissue SVF is defined as a heterogeneous population of freshly isolated cells comprising all the different types of cells residing in the tissue such as fibroblasts, preadipocytes, vascular smooth muscle cells, endothelial cells, resident monocytes/macrophages, and lymphocytes, except mature adipocytes. The process to obtain SVF may exceed the definition of "minimal manipulation" as it is frequently based on enzymatic tissue digestion. However mechanical dissociation, albeit less efficient in terms of cell recovery, is currently favored mainly for regulatory reasons. In contrast, microfat, obtained by mechanical processing only, is composed of clusters of blood- and lipids-free adipose tissue ranging from tens to few hundred micrometers in diameter, containing all the adipose tissue cells, including adipocytes, within their native niche [8, 9]. Moreover, microfat, beyond preserving the cell composition, also preserves the tissue microarchitecture [10]. Borrowing the concept from the world of bone marrow and bone marrow concentrate (BMAC), it is quite common to refer to these adipose-derived products as "cell concentrates." Actually, this is improper since, especially for microfat, the production process is not designed to concentrate any population type, but rather to eliminate blood and lipid residuals known to be pro-inflammatory agents [10]. Both the SVF and microfat have similar nucleated cell number per gram of product, as well as similar proliferation abilities and the expression of the typical MSC marker CD90; nevertheless, the proportion of cells positive for CD34 and CD45 appears to be higher in SVF compared to microfat [11, 12], underlying the higher blood contamination in SVF.

Both products have shown anti-inflammatory and immunomodulatory potential, and reparative effects in vivo [13], and safety in a growing number of clinical trials [14–16], including musculoskeletal diseases. Moreover, the undisputable practical advantages associated to the use of SVF and microfat over culture-expanded ASCs have made them very popular among the orthopedic community, as revealed by the increasing number of publications reporting the results of their application [17].

3.2.2 Culture-Expanded Adipose-Derived Stem Cells (ASCs)

Within the SVF, not all the cells are likely to have a therapeutic effect [18]. Among them ASCs have a role of paramount importance in regenerative medicine, and therefore many therapeutic approaches are based on the use of these cells only. A small fraction of the adipose tissue is in fact represented by ASCs that can be isolated and induced to proliferate in culture to generate expanded populations. The process starts with the enzymatic isolation of the SVF, and then it further proceeds with in vitro expansion in appropriate culture media leading to the loss of the native adipose structure and the achieve-

ment of a homogeneous population of expanded cells that can be rigorously characterized in terms of cell markers, morphology, and secretory profiles. Interestingly, adipose tissue contains up to 3% of MSCs, whereas in bone marrow it is reported between 0.002% and 0.02% [19]. The identification of the heterogeneous stem/stromal cell types and native phenotypes in their environment is still a matter of debate [20]. There is growing evidence supporting the hypothesis that these cells and more in general MSCs reside in a perivascular location. Consistently, the ability of MSCs to stabilize blood vessels and contribute to tissue homeostasis in both physiological and injury conditions has also led some authors to propose that MSCs are a subpopulation of pericytes [21].

Culture-expanded ASCs match the criteria reported in the ISCT guidelines aimed to standardize the concept and metrics used for culture-expanded products and the appropriate use of the term MSCs. The definition and required attributes for MSC included the adherence to plastic support, the capacity for tri-lineage differentiation (adipocyte, chondroblast, osteoblast) in vitro, the expression of cell surface markers (CD73, CD90, and CD105), but the lack of cell surface markers associated with hematopoietic stem cells and progenitors (CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR) [22]. More recently, other potentially useful markers have been proposed, like positivity for CD13, CD29, and CD44 and absence of CD31 and CD235a [23]. Further, cell size and granularity, telomere length, senescence status, trophic factor secretion, and immunomodulation ability [24, 25] can also be evaluated. The opportunity to characterize ASCs, in theory, should lead to more reproducible product assessment and outcomes [26]. However, since the techniques of expansion can affect the relative proportion and features of the expanded cell populations [27, 28], individual batches of ASCs can vary significantly with respect to these metrics. All the aforementioned are attributes that must be considered as predictive of the potency of any culture-expanded cell population that may be used in regenerative medicine [29]. Therefore, being able to optimize the population attributes, including the secretion of soluble factors, might allow the development of tailored cell-based protocols to achieve the desired result.

However, this strategy requires a GMP facility and a minimum of two procedures (harvest and administration) to complete the treatment, increasing the cost for both patients and NHS or other payors.

3.3 Influence of Patient-Specific Factors on Adipose-Derived Cells and Products

The tissue source selection, processing methods, injection techniques, cell composition, and cell dose have been extensively studied for years, and the efforts of researchers are still aimed at their standardization. Nevertheless, the variability in terms of outcome suggests the presence of patient-specific factors such as age, body mass index (BMI), gender, and harvest sites as confounding variables in the evaluation.

Studies have shown a slight decrease in the overall yield of nucleated cells with increasing age [30], as well as a significant decrease in the proliferative and differentiation capacities of culture-expanded ASCs [31]. This result is in keeping with studies on bone marrow-derived expanded MSCs where age is negatively correlated with cell viability and overall potential [32]. Nevertheless, despite a lower yield of pro-regenerative cells per gram of tissue, the autologous transplantation of ASCs seems to be still a feasible option for elderly patients [33].

A higher BMI has been associated with a reduced number of viable mature adipocytes per gram of tissue, a lower differentiation capacity of the culture-expanded ASCs, reduced capacity of cell migration, and angiogenic and proliferative abilities [30], probably due to the low oxygen condition and inflammatory conditions observed in adipose tissue of obese patients. Interestingly, the effect of BMI on cell performance can be reverted. Bariatric surgery and diet-induced long-term calorie restriction could improve cultured ASCs profile, with reduced DNA damage,

improved viability, and extended replicative life span [34]. This evidence is in line with studies reporting a positive connection between weight loss and reduced inflammation [35].

The role of gender and donor site is still controversial. Some studies on human ASCs isolation failed to show any difference in adipose tissue native stem/stromal cell concentration, prevalence, or yield by gender. However, another study suggested that men might have a higher yield compared to women [36]. Likewise, the ideal donor site for fat harvest is yet to be defined.

Some studies [37–39] showed that fat from the lower abdomen and medial thighs has higher yield compared to the upper abdomen, trochanteric region, knees, and flanks but similar differentiation potential. However, previous studies suggest that the choice of donor site has little effect on fat graft outcomes and the choice should be based on ease and safety of access to the tissue [30]. Other parameters, such as diet, lifestyle, drug consumption, and smoke and alcohol habit, should be also investigated to identify a possible influence on the pre-regenerative properties of adipose-derived cells or products.

3.4 The Rationale for Using Injections of Culture-Expanded ASCs or Adipose-Derived Products

Both ASCs and adipose-derived products can be delivered mainly with two approaches, which imply different mechanisms of action. The first one relies on the seeding of cells/SVF or microfat on scaffolds to generate tissue and organs, and it is typically used in association with surgery, such as repair of focal chondral lesion or tendon rupture, as well as treatment of critical bone defects. Cells/SVF or microfat are seeded on a support (scaffold) and can exert their function by both paracrine regulations of the microenvironment and direct differentiation into tissue-specific cells, albeit not complete. The second approach relies on the direct delivery of cells/SVF or microfat to damaged sites, typically by injections or infusions. In this case, many findings suggest that, despite still being a valid model in different applications [40], the direct cell trans-differentiation mechanism would not be the main responsible for the benefits observed after MSCs transplantation, but rather the therapeutic effect is related to the secretion of soluble factors able to regulate the cross-talk with resident cells [41]. However, in the absence of adequate support for attachment, cells alone after injection on the site are generally stressed, sometimes leading to a rapid death [42]. In this view the delivery of cells within their niche, as it happens with SVF and even more with microfat, could protect them from this phenomenon. Nevertheless, the initiation of the restoration process is guaranteed by the initial cross-talk between stem/stromal cells and resident cells, and therefore their long term-survival at the site of injection is not a strict requirement for their functioning. The low engraftment rate documented in lung injury models or cardiac infarcts after MSCs infusion [43, 44], and studies demonstrating similar or even improved organ function upon infusion of MSC-derived conditioned medium (MSC-CM) with respect to whole MSCs [45], are all supporting a paracrine role of MSCs. Therefore, the research interest is also shifting on the characterization of secreted factors, collectively termed as the "secretome."

3.4.1 Paracrine Potential (Soluble Mediators and Exosomes/Microvesicles)

The term secretome refers to the wide array of secreted factors, such as cytokines and chemokines or lipids with trophic and immunomodulatory activities [46]. Since Caplan's description of MSCs as "drugstores," i.e., elements that recognize injury signals and became activated in order to release bioactive molecules able to modulate local immune response and to establish a regenerative microenvironment [47], a number of elements, such as trophic (anti-scarring, anti-apoptotic, mitogenic, angiogenic), immunomodulatory, and also antimicrobic factors, were identified in MSCs secretome [48]. Therefore, the traditional paradigm of MSCs as a "cell replacement tool" has been now enriched by a new vision of MSCs as "sensing cells" that inter-

act with tissue progenitor cells through a paracrine action, which stimulates the innate potential of the tissue in the repair and modulation of inflammatory and immune reactions. These features have defined the rationale behind the use of MSCs as therapeutic tool in treating joint diseases like osteoarthritis. Accordingly, MSCs were shown to modulate the function of the immune system typically dysregulated during joint inflammation, by suppressing B cells and inhibiting T cells proliferation, together with attracting regulatory T cells and promoting the release of anti-inflammatory factors [49]. Even more importantly, MSCs were reported to promote in macrophages the transition from pro-inflammatory M1 to anti-inflammatory M2 polarization, inhibiting the release of pro-inflammatory cytokines (TNF-α and IL-1β), and augmenting the secretion of anti-inflammatory molecules (IL-10) [50]. As a consequence, polarization switch may reduce the cartilage degeneration mediated by inflammatory macrophages [51]. Consistently, the effectiveness of native or culture-expanded ASCs (and related products) paracrine action was demonstrated on chondrocytes and tenocytes exposed to pathological conditions, with results suggesting a restoration of tissue homeostasis [52, 53]. Then, a significant amount of research explored the possibility of modulating these factors through the adoption of different culturing conditions, paving the way for the development of acellular therapeutic interventions for autoimmune, inflammatory, and malignant diseases and tissue regeneration from cellular secretions derived from MSCs (Fig. 3.1).

Among all the components of the secretome, extracellular vesicles (EVs) were also identified as active entities [54]. EVs embed different type of molecules (DNA, mRNAs, miRNAs, pre-miRNAs, ncRNAs, and proteins), can be found in different biological fluids, and are secreted by a wide range of cell types including MSCs [55]. The recent advent of omics techniques allowed a better characterization of these vesicles and fostered research on their involvement in the regulation of different biological processes [56]. Consistently, EVs from MSCs showed an immunosuppressive role on many types of immune cells [57]. In specific, treatment of T cells in vitro resulted in a marked decrease in proliferation and downregulation of IFN-γ and TNF-α secretion [58], with inflammation efficiently suppressed in vivo [59]. Moreover, EVs from cultured ASCs had positive effects in skin regeneration and cardiac, liver, and neuroprotection [60] with strong attractive properties as potential therapeutic candidates also in the orthopedic settings since the reported attenuation of the inflammatory response and the degeneration after both tendon or cartilage injury [61, 62].

Overall, although further studies involving the safety and duration of EVs therapeutic effect are needed, MSC-derived EVs are the most promising candidates for a rational design of next-generation cell-free MSC-based therapeutics mainly derived from adipose tissue. In fact, the use of EVs avoid potential safety concerns typical of cell-based approaches (i.e., tumorigenicity and undesired spontaneous differentiation). Considering their natural biogenesis process, EVs are generated with high biocompatibility, enhanced stability, and limited immunogenicity, which provide multiple advantages as drug delivery systems over traditional synthetic methods. In this context, EVs can penetrate the tissues and be bioengineered to enhance the targetability, avoiding off-target effects. In comparison with cell-based approaches, their manufacturing is also more competitive in terms of cost-effectiveness. In this perspective, few clinical trials of Phase I, II, and III have been opened in the last years, covering diseases such as macular holes (NCT03437759) or diabetes mellitus type 1 (NCT02138331) or ischemic stroke (NCT03384433) [63]. Rational and potential of extracellular vesicles—exosomes are reported more in detail in Chap. 11.

3.5 In Vitro and Preclinical Findings

As already mentioned, the interest in the use of ASCs and adipose-derived products such as SVF and microfat in musculoskeletal applications is dramatically increasing over the last years. In the following paragraph, we will comment on the most relevant findings of in vitro preclinical

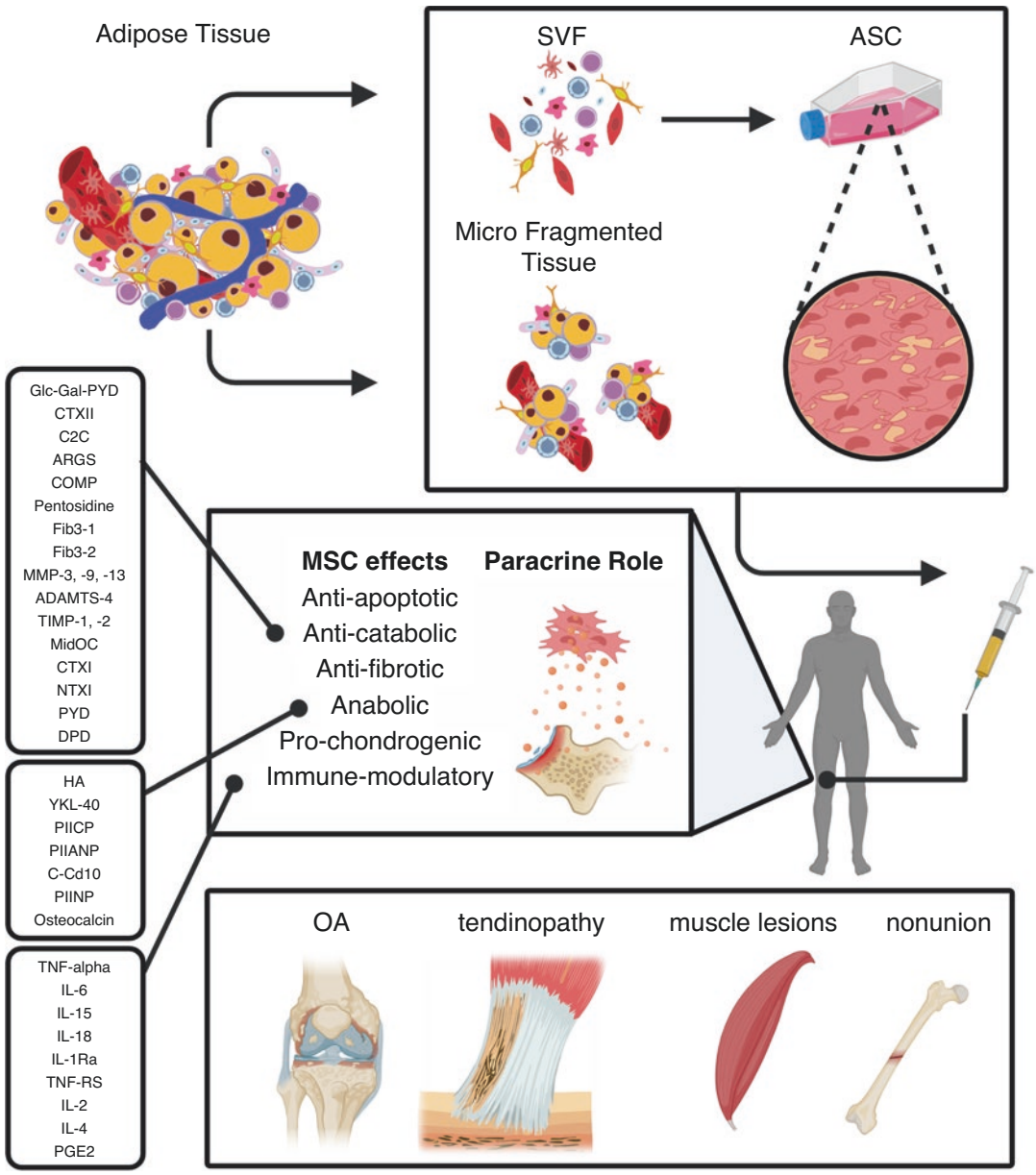

Fig. 3.1 Adipose-derived products: applications and properties

studies published so far for the treatment of joint lesions/degeneration, tendon and bone repair, as well as muscle lesions, to give the readers significant insights about their mechanisms of action. Given the preclinical settings, most of the studies show the results of the use of culture-expanded ASCs, although some results are about the unprocessed products. Up-to-date reviews and meta-analysis can also provide the readers with the most recent papers about the clinical applications of both ASCs and SVF and microfat [17, 64–66].

3.5.1 Focus on Culture-Expanded ASCs and SVF/Microfat in Joint Degeneration

Articular cartilage degeneration eventually gives rise to osteoarthritis (OA), the main cause of disability in developed countries [67]. The current conservative options may relieve symptoms but are ineffective in the restoration of the damaged tissues. Recently, innovative therapies for cartilage regeneration showed efficacy [68, 69], with particular regard to MSCs thanks to their immunomodulatory and pro-regenerative potential [70].

Pivotal in vitro studies reported the ability of culture-expanded ASCs to induce chondrocyte proliferation and extracellular matrix production, through their paracrine activity with anti-inflammatory, anti-apoptotic, and chondrogenic properties [71].

Also, the potential of autologous ASCs infusion for osteochondral defects treatment has been assessed in numerous animal models [72, 73]. Interestingly, the successful regeneration of cartilage has also been reported with an allogeneic transplant of ASCs in a sheep OA model [74]. Similar results have been observed in a rabbit model, where ASCs infusion promoted histological healing [75]. Single intra-articular injections of ASCs have been tested in dogs with hip OA. ASCs-treated animals were reported to have improved their condition [76] with improved limb function within 3 months from the procedure [77]. Conversely, the intravenous injection of ASCs in dogs with elbow OA failed to significantly improve the animals' conditions [78].

For what concern adipose-derived products, in a model of goat osteochondral defect, the application of SVF showed higher regeneration compared to the controls. SVF-treated animals exhibited more extensive collagen type II, hyaline-like cartilage, and more tissue native-like content of glycosaminoglycan in the cartilaginous layer. Moreover, in the defect regions, it has been observed more intense collagen type I staining [79]. Similar results have been obtained in a rat model of full-thickness cartilage defect treated with native stem/stromal cell-enriched microfat where it was able to effectively restore cartilage tissue [80]. A very interesting paper reports a direct comparison of cultured ASCs, SVF, and microfat for the treatment of OA in a rabbit model of bilateral transection of the anterior cruciate ligament. The rabbits were either left untreated or injected with culture-expanded ASCs or SVF or 300μl of microfat. The analysis conducted at 2- and 4-month follow-ups showed no macroscopic differences among the groups. However, at both experimental times, microfat showed the most promising results with a more uniform cartilage staining and a smoother cartilage surface than the untreated group [81].

3.5.2 Focus on Culture-Expanded ASCs and SVF/Microfat in Tendon Repair

Tendon tissue has poor healing potential, given by the limited cellular content and vascularization. Thus, the response to treatment is generally low, and prolonged recovery is needed [82]. In addition, spontaneous tendon repair often fails in adequately restoring the structural and molecular composition of the tissue, often resulting in scar tissue rich in collagen type III, more vulnerable to injuries and relapses [83]. Surgical repair also showed frequent relapses. Conservative treatments were able to improve symptoms, but none of them provided a long-term solution [84], and therefore, the application of ASCs or adipose tissue-derived products has been explored for tendon regeneration.

In vitro models demonstrated that the co-culture of primary tenocytes and ASCs could drive the differentiation of the latter into tenocytes in vitro [85, 86]. In vivo, in a mice tendon repair model, the local administration of ASCs has been reported to accelerate the tendon healing process through differentiation of ASCs into tenocytes, and by increasing the expression of angiogenic growth factors [87]. Similar results were obtained on a rabbit calcaneal tendon injury model, which showed that the application of ASCs associated with platelet-rich plasma

increased the resistance of tendons as well as the amount of collagen type I, VEGF, and FGF [88]. More recently, using a rat tendinopathy model, the application of ASCs significantly improved the pathological picture [89]. ASCs have also been used on racehorses suffering from superficial flexor digitorum longus tendon (SFDLT) lesions. The injection of ASCs significantly improved healing, with treated horses showing shorter periods of lameness and better organization of collagen fibers in the injured tendon [90]. Similarly, in a horse model of collagenase-induced SFDLT lesions, the administration of ASCs resulted in a better organization of collagen fibers and a reduction of the inflammatory infiltrate. Besides, the ultrasound evaluation showed a lack of lesion progression compared to the control group [91].

Analyzing the effect of uncultured adipose tissue products, some authors reported that in vitro microfat significantly increased the proliferation rate of tendon progenitor cells as well as the expression of VEGF, which is crucial for the neovascularization of the tissue during the healing process [92]. In a similar experimental model, it was also demonstrated that microfat was effectively able to counteract the detrimental effect of experimentally induced inflammation in co-cultures with autologous tenocytes [53]. Likewise, in a rotator cuff tear model in rabbits, the application of native stem/stromal cell-enriched SVF caused a significant improvement in few physiological parameters, and it accelerated the transformation of collagen fibers from type III to type I, the crucial step of repaired tissue maturation [93].

3.5.3 Focus on Culture-Expanded ASCs and SVF/Microfat in Bone Repair

Bone fractures and segmental bone defects are a significant source of patient morbidity and place a substantial economic burden on the healthcare system. Generally, after damage, bone can regenerate itself, but in the case of significant loss of tissue, surgery with bone grafts or bone substitutes is required. These approaches may be characterized by long immobilization periods, donor site morbidity (in case of autologous graft), muscular atrophy, and potential complications such as infection, pain, or hemorrhage [94, 95] that may lead to incorrect graft integration, resorption, and eventually relapses [96]. Therefore, potential applications of ASCs in this context have then been explored [95, 97, 98].

In vitro studies have reported, under specific stimuli, the ability of ASCs to differentiate into osteocytes, unequivocally showing markers of the mature tissue [99, 100]. Interestingly, it has been reported that osteogenic induction might not be mandatory as the primary function of adhesion, migration, proliferation, and differentiation can also be achieved using native ASCs [101, 102]. Animal models mainly relied on the use of scaffolds populated by ASCs, with few applications involving ASCs injection. Some studies explored the use of ASCs and osteocyte-induced ASCs in the context of distraction osteogenesis (DO) [94]. In a rabbit model of tibial defect, the authors reported a shorter consolidation period using osteo-differentiated or undifferentiated stem/stromal cells compared to the control, but osteo-differentiated ASCs seem to perform better in terms of tissue density and quality [103]. Similarly, in a rat model of DO, the authors demonstrated that the injection of ASCs resulted in a significantly higher density and fracture strength after 6 weeks, supported by molecular evidence as ASCs' derived tissue expressed osteogenic markers [104].

For what concern the uncultured adipose tissue product, mechanical generated-SVF (mSVF) and enzymatic generated-SVF (eSVF) were compared to test whether the mechanical approach influences the biological features and functions of SVF. Albeit less efficient in terms of cell recovery and CFU-F than eSVF (five times less), mSVF preserved the functions of cell populations within the adipose tissue, with similar osteo-differentiation commitment and similar release of VEGF, HGF, IGF-1, and PDGF-bb, involved in pathways mediating osteochondral repair and cell migration, and of the anti-inflammatory cytokine IL-10 [105].

3.5.4 Focus on Culture-Expanded ASCs and SVF/Microfat in Muscle Repair

Among musculoskeletal tissues, the muscle is more prone to regenerate after injury, thanks to the presence of satellite cells, a subpopulation with stem cell-like properties [106, 107]. Although these cells are able to regenerate muscle tissue after strains, tears, or lacerations, they fail to resolve conditions of greater damage with significant muscle tissue loss, indicated as volumetric muscle loss injuries [108].

As per the other tissues, the use of ASCs for muscle regeneration and repair may rely on direct differentiation or on the release of paracrine effectors. Indeed, ASCs are able to differentiate in vitro into skeletal myoblasts and myotubes, and they maintain myogenic potential also after expansion [109], but if properly stimulated using dedicated scaffold, they may also differentiate in vivo [110].

ASCs with specific myogenic properties, and able of homing to the injured muscle tissues, have been obtained [111] and used in a mice model of Duchenne muscular dystrophy, with promising results [112].

The potential of ASCs to regenerate the skeletal muscle showed to be comparable to muscle-derived progenitor cells in a volumetric muscle loss injury murine model employing tissue-engineered muscle repair (TEMR) construct [113].

Cultured homologous ASCs injected into injured soleus muscles showed an acceleration of skeletal muscle repair in rat [114].

Similar results were obtained when human ASCs were implanted in a model of murine hind limb ischemia: an improvement in the functionality of the damaged limb occurred faster than in the control mice. In this work, the authors hypothesize a paracrine action of IL-6 released from ASCs, leading to stimulation of M2 macrophages and inducing muscle repair through neovascularization [115].

The paracrine activity of ASCs for muscle regeneration has been investigated specifically in different animal models. The conditioned media of ASCs have been suggested to improve muscle tissue healing in a rabbit model of critical limb ischemia [116]. The effects of ASC whole secretome or isolated extracellular vesicle fraction were evaluated in an in vivo cardiotoxin-induced skeletal muscle injury model, and this study demonstrated that both extracellular vesicles and soluble molecules released in the ASC secretome promote muscle regeneration acting in synergistic manner [117].

Interestingly, the rat ASCs paracrine activity for muscle regeneration can be improved by pretreatment of stem/stromal cells with IL-4 and SDF-1. Indeed, ASCs treated with these factors were able to improve muscle structure and function and decrease fibrosis in a rat model of skeletal muscle injury [118].

In an attempt to determine the importance of the direct use of ASCs, ASCs and ASC-conditioned medium were used in type I collagen hydrogel, and the action of these constructs were directly compared in volumetric muscle loss rat model. The results indicated that hydrogels bearing ASCs or conditioned medium only were able to induce similar increase of angiogenesis and myogenesis, as well as M2 stimulation, suggesting that both elements retain an immunomodulatory role on macrophages transition. A decrease of inflammation and collagen deposition was also observed, resulting in improved muscle repair [119], confirming once more the pivotal ASCs paracrine role.

3.6 Conclusions

The rationale for the use of adipose-derived stem/stromal cells and adipose-derived products such as SVF and microfat, as well as their safety profile, for the treatment of several musculoskeletal conditions is strong and well documented in both in vitro and preclinical studies. The possibility of local survival and differentiation of tissue-derived cells and the formation of new tissues is theoretically appealing but as yet unproven. Moreover, this effect could be mainly observed when the adipose-derived cells or products are associated with surgery and delivered locally at the injury/defect site. Paracrine action mediated by soluble

factors as well as by exosomes and microvesicles may play a key role in ASCs-based therapies by modulating the microenvironment, especially in a setting of injury or degeneration. In some cases, ASCs or the adipose tissue-derived products may act not only on symptoms relief but also as disease-modifying agents, possibly reverting the pathological progression. The current efforts of the scientific community are aimed to improve the knowledge of the most effective strategies to improve the therapeutic effects of these approaches. In particular, cell priming, that is the modulation of the secretory ability of cells through the use of cytokines and growth factors, hypoxia, pharmacological drugs, biomaterials, or different culture conditions, has been indicated as one of the most promising ones. In fact, an appropriate priming can modulate the cell secretory profile so that the molecule cargo is able to exert a specific therapeutic effect for each different pathology. Regardless of the mechanism of action, the optimization of dose and delivery strategies to achieve both predictable and durable positive effects needs to be further evaluated in high-quality clinical studies. While ASCs have the undisputable advantage of being homogeneous and therefore more controlled, SVF and microfat are easier to use and do not have to follow strict regulatory pathways. Overall, both are associated with pros and cons, and only further research studies will allow to identify the best approach for the different musculoskeletal pathologies and the different type of patient.

Take-Home Messages

- The presence of ASCs within the adipose tissue has drawn significant clinical attention due to their purported paracrine effects and multipotent differentiation capacity. Practical methods to exploit the properties of adipose tissue at the point of care, such as SVF and microfat, have been developed to promote an efficient use into the clinical setting.
- ASCs, SVF, or microfat can be delivered in association with surgery for the treatment of local defects or through injection to damaged sites to treat wider areas of degeneration.
- The main therapeutic effect of ASCs and adipose-derived products is mainly mediated by the release of soluble factors as well as by exosomes that interact with the resident cells creating a pro-regenerative microenvironment.
- The efficacy of ASCs, SVF, and microfat for the treatment of several musculoskeletal conditions, as well as their safety profile, is well documented in both in vitro and preclinical studies. Therefore, there is a high potential of the individual fat component to be used in regenerative medicine.
- While ASCs have the undisputable advantage of being homogeneous and therefore more controlled, SVF and microfat are easier to use and do not have to follow strict regulatory pathways. Further research studies will allow to identify the best approach for the different musculoskeletal pathologies and type of patient.

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