Adipose Derived Stem Cells – Where Are We Now?

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Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are multipotent stromal cells that can differentiate into a variety of lineages, including osteocytes, adipocytes and chondrocytes. This differentiation capacity, in addition to their release of trophic factors and immunomodulatory properties, holds great promise for cell therapies and tissue engineering.

In the 1960s Alexander Friedenstein described a population of plastic-adherent cells that emerged from long-term cultures of bone marrow that have colony forming capacity and osteogenic differentiation characteristics *in vitro* as well as *in vivo* upon re-transplantation[1]. In light of their capacity to differentiate into bone, fat, cartilage and muscle in culture and an emerging link to the embryonic development of mesenchymal tissues, the term "mesenchymal stem cell" was coined in 1991 by Arnold Caplan[2]. Observation of these cells in culture led to a definition of MSCs by the International Society of Cell Therapy (ISCT) that included a propensity to adhere to laboratory culture plastic and the capacity to differentiate into at least bone, cartilage and fat[3]. MSCs were subsequently found to have a characteristic, although not specific, set of surface markers, with additional functions including the secretion of immunomodulatory factors[4].

It is now known that MSCs exist in almost every tissue, including subcutaneous fat, and adipose tissue isolated from the knee fat pad[5]. While these cells share common characteristics, they are distinctive, and reflect aspects of their tissue of origin. For example, MSCs from bone marrow exposed to transforming growth factor (TGF)-β form chondrocytes and make cartilage extracellular matrix, while adipose-derived MSCs (ADP-MSC) from fat require both TGF-β and bone morphogenetic protein (BMP)-6 to make cartilage[6]. In contrast to MSCs derived from bone marrow, there is no reduction in the yield of MSCs from fat as age increases[7]. Furthermore, the yield and viability of MSCs from fat are minimally affected by gender, BMI or storage time of tissues[8].

Adipose-derived MSCs

A range of adipose-derived MSC preparations are available which can be broadly categorized based on whether the contained MSCs have been enriched through culture or machine purified (Table 1).

Table 1. Summary of the principal MSC preparations from adipose tissue

Preparation	Harvest/preparation technique	Preparation time	Typical yield	Purity	Likely FDA pathway
Culture-derived ADP-MSC	1. Lipoaspirate 2. Digestion 3. Centrifugation 4. Cell culture 5. Delivery	2-4 weeks	Unlimited through culture expansion	Culture enriched	351
SVF (containing ADP-MSC)	1. Lipoaspirate 2. Digestion 3. Centrifugation 4. Delivery	90 mins	31 x10^6 per 200mls lipoaspirate	Hetero-genous	351
FACS purified ADP-MSC	1. Lipoaspirate 2. Digestion 3. Centrifugation 4. FACS purification 5. Delivery	120 mins	31 x10^6 per 200mls lipoaspirate	Purified	351

SVF, stromal vascular fraction; ADP-MSC, Adipose-derived MSC; FACS, fluorescent activated cell

1. Culture-derived ADP-MSCs

Traditionally, MSCs have been isolated in laboratory culture, being selected from total cell suspensions based on their ability to adhere and proliferate for several weeks of primary culture[9]. Since MSCs make up a small fraction of mononuclear cells from adipose tissue, the fat tissue is typically digested and centrifuged to separate layers of blood, fat and mononuclear cells (the so-called stromal vascular fraction or SVF) according to their density. The mononuclear cell layer, containing MSCs, is subsequently plated onto plastic and the number of cells is allowed to increase over time. During the culture, MSCs predominate and become the most abundant cell type. Despite the process of enrichment through plastic adherence, it is inevitable that even cultured preparations will be contaminated by non-MSC populations, and the contribution of each contained population to the repair process cannot be definitively established. Despite demonstrating excellent potential as sources of cells for tissue engineering, there are clear challenges that restrict widespread clinical application[10]. At present MSCs must be expanded in number over several weeks prior to implantation and this process adversely affects the function of the cells and their therapeutic potency[11]. As these types of cell have traditionally been identified through a process that requires several weeks of culture in a laboratory, their preparation is time consuming and precludes autologous use in emergency situations. There is increasing evidence that the culture of MSCs introduces potential risks of immunogenicity[12], infection[12], genetic instability[13] and tumorogenicity[14].

2. Stromal Vascular Fraction (SVF)

The SVF of adipose tissue have been used directly in therapeutic applications with the aim of harnessing the potential of the contained stem cells, while avoiding the risks associated with laboratory culture. This type of therapy is heavily studied by many biotech companies developing benchtop systems that produce SVF at the bedside, which makes the cell mixture conveniently available shortly after harvesting adipose tissue. However, SVF is highly heterogeneous and includes multiple non-MSC types, such as inflammatory cells, hematopoietic cells, endothelial cells, and non-viable cells among others. Available studies using SVF show poor and unreliable tissue formation, or lower tissue regeneration efficacy relative to cultured MSCs[15]. In fact, recent studies have suggested that the presence of endothelial cells has inhibiting effects on bone differentiation, among other lineages[16]. Furthermore, variability in cell composition presents clear barriers to approval from regulatory bodies such as the Food and Drug Administration (FDA) of future stem cell-based therapeutics, potentially including reduced safety, purity, identity, potency and efficacy[17].

3. Purified ADP-MSCs

Traditional methods of MSC isolation have depended on a period of cell culture for two reasons. Firstly, as the exact location or phenotype of MSCs in tissues was not known, it was not possible to target specifically these cells for purification. Instead researchers had to wait for these cells to emerge from cell culture where they would overgrow other cell types. Recent insights into the perivascular origin of MSCs combined with advances in multicolor flow cytometry have enabled prospective purification of innate MSCs to homogeneity on the basis of established perivascular markers[18]. All vascularized tissues are now possible immediate sources of purified MSCs, which can now be isolated using fluorescence activated cell sorting (FACS). The second reason for historic dependence on culture has been the need to expand cell populations to clinically relevant numbers[19]. Low stem cell yield and donor site morbidity limit the use of fresh autologous bone marrow, periosteum and the majority of other MSC sources[10]. Relative to the lower yield, limited donor sites, and morbidity associated with bone marrow or periosteal harvest, adipose tissue is now a well-documented, easily accessible, abundant and dispensable source of perivascular MSCs[20]. Approximately 15 million perivascular MSCs can be purified per 100 ml of lipoaspirate - a sufficient number of cells to treat a broad range of musculoskeletal disorders without culture expansion[21]. Using this technology, MSCs can now be isolated without the need for cell culture to treat a range of orthopaedic applications.

Adipose-derived MSCs in the clinical orthopaedic literature

Enthusiasm for the use of adipose-derived MSCs is illustrated by the prodigious number of clinical studies registered at www.clinicaltrials.gov. A systematic search of the published clinical orthopaedic literature from 2012 – present identified 51 clinical studies evaluating MSC-based preparations for musculoskeletal applications of which 14 (27%) evaluated the use of MSCs derived from fat (Figure 1a). The cells were being used in the treatment of osteoarthritis and osteochondral defects (Figure 1b) and produced a range of contrasting results that will be summarized in this presentation.

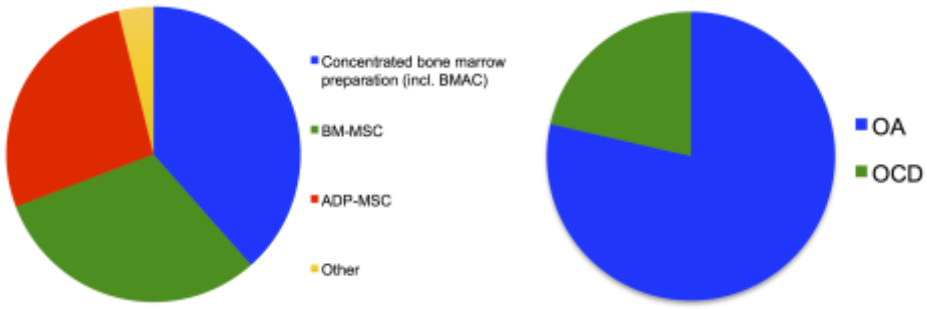

Figure 1. (a) Mesenchymal stem cells preparations used in the orthopaedic clinical literature. (BMAC, bone marrow aspirate concentrate; BM-MSC, bone marrow derived mesenchymal stem cells; ADP-MSC, adipose derived mesenchymal stem cells) (b) Indications for adipose derived MSC - based therapies in the published clinical orthopaedic literature.

Key challenges and future work

1. Identifying the mechanism of therapeutic effect

It has generally been hypothesized that MSCs would achieve a therapeutic effect by means of tissue engraftment. However, few studies have documented substantial beneficial effects of engraftment, although some benefit is likely to be achieved through the trophic and immunomodulatory properties of MSCs. Further research is required to fully characterize the mechanism by which MSCs exert beneficial effects. If engraftment is not central to the mechanism of action, the need for actual delivery of cells could be questioned. Considerable clinical hurdles (such as rejection and tumorogenicity) and regulatory challenges could be bypassed if the responsible therapeutic cytokine released by cells were identified.

2. Developing stringent assays of efficacy

Stringent assays to demonstrate the efficacy of MSCs that are indication specific are lacking. Most researchers have examined potentially heterogeneous MSC populations derived from cultures with high plating density that do not accurately reflect cell behavior at the clonal level. More stringent studies using clonally derived MSC populations may enable the identification of subpopulations of cells better able to maintain their stem cell–like properties. These potency assays could help increase the efficacy and safety of cell therapies using large numbers of culture-expanded cells. Similarly, the identification of subsets of MSCs isolated through FACS may facilitate purification of populations most suited to specific indications.

3. Establishing proof of safety

Methods of establishing proof of the safety of stem cell therapies are needed. These methods must include identification of off-target effects of stem cells delivered locally and the ability to definitively establish the risk (currently considered minimal) of malignant transformation.

4. Widespread adoption of minimum reporting standards

The reporting of MSC preparation protocols and its composition is inadequate and inconsistent. Poor reporting of the variables which may critically influence outcome precludes interpretation, prevents others from reproducing experimental conditions and makes comparisons across studies impossible. We encourage the widespread adoption and compliance of emerging minimum reporting standards for clinical studies evaluating the use of MSCs in orthopaedics[22] (www.mibo-statement.org).

References

1. Friedenstein AJ, Piatetzky S, II, Petrakova KV. Osteogenesis in transplants of bone marrow cells. J Embryol Exp Morphol 1966;**16**(3):381-90.
2. Caplan AI. Mesenchymal stem cells. J Orthop Res 1991;**9**(5):641-50.
3. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;**8**(4):315-7.
4. Nauta AJ, Fibbe WE. Immunomodulatory properties of mesenchymal stromal cells. Blood 2007;**110**(10):3499-506.
5. Crisan M, Yap S, Casteilla L, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 2008;**3**(3):301-13.
6. Hennig T, Lorenz H, Thiel A, et al. Reduced chondrogenic potential of adipose tissue derived stromal cells correlates with an altered TGFbeta receptor and BMP profile and is overcome by BMP-6. J Cell Physiol 2007;**211**(3):682-91.
7. Harris LJ, Zhang P, Abdollahi H, et al. Availability of adipose-derived stem cells in patients undergoing vascular surgical procedures. J Surg Res 2010;**163**(2):e105-12.
8. West CC, Hardy WR, Murray IR, et al. Prospective purification of perivascular presumptive mesenchymal stem cells from human adipose tissue: process optimization and cell population metrics across a large cohort of diverse demographics. Stem cell research & therapy 2016;**7**:47.
9. Murray IR, Peault B. Q&A: Mesenchymal stem cells - where do they come from and is it important? BMC biology 2015;**13**(1):99.
10. Murray IR, West CC, Hardy WR, et al. Natural history of mesenchymal stem cells, from vessel walls to culture vessels. Cell Mol Life Sci 2013.
11. Ma T. Mesenchymal stem cells: From bench to bedside. World J Stem Cells 2010;**2**:13-7.
12. Gad S. *Pharmaceutical Manufacturing Handbook: Regulations and Quality*. Hoboken, NJ: John Wiley and Sons., 2008.
13. Dahl JA, Duggal S, Coulston N, et al. Genetic and epigenetic instability of human bone marrow mesenchymal stem cells expanded in autologous serum or fetal bovine serum. Int J Dev Biol 2008;**52**(8):1033-42.
14. Ren Z, Wang J, Zhu W, et al. Spontaneous transformation of adult mesenchymal stem cells from cynomolgus macaques in vitro. Exp Cell Res 2011;**317**(20):2950-7.
15. Cheung WK, Working DM, Galuppo LD, et al. Osteogenic comparison of expanded and uncultured adipose stromal cells. Cytotherapy 2010;**12**(4):554-62.
16. Rajashekhar G, Traktuev DO, Roell WC, et al. IFATS collection: Adipose stromal cell differentiation is reduced by endothelial cell contact and paracrine communication: role of canonical Wnt signaling. Stem Cells 2008;**26**(10):2674-81.
17. Anz AW, Hackel JG, Nilssen EC, et al. Application of biologics in the treatment of the rotator cuff, meniscus, cartilage, and osteoarthritis. The Journal of the American Academy of Orthopaedic Surgeons 2014;**22**(2):68-79.
18. Corselli M, Crisan M, Murray IR, et al. Identification of perivascular mesenchymal stromal/stem cells by flow cytometry. Cytometry A 2013;**83**(8):714-20.
19. Murray IR, Corselli M, Petrigliano FA, et al. Recent insights into the identity of mesenchymal stem cells: Implications for orthopaedic applications. Bone Joint J 2014;**96-B**(3):291-8.
20. James AW, Zara JN, Corselli M, et al. Use of human perivascular stem cells for bone regeneration. J Vis Exp 2012(63):e2952.
21. James AW, Zara JN, Corselli M, et al. An abundant perivascular source of stem cells for bone tissue engineering. Stem Cells Transl Med 2012;**1**(9):673-84.
22. Murray IR, Geeslin AG, Goudie EB, et al. Minimum Information for Studies Evaluating Biologics in Orthopaedics (MIBO): Platelet-Rich Plasma and Mesenchymal Stem Cells. Journal of Bone and Joint Surgery (America) 2017;**In Press**.