REVIEW ARTICLE

Cell therapy for bone fracture repair: A comparative preclinical review of mesenchymal stromal cells from bone marrow and from adipose tissue

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Abstract: Over the last decade, there has been an increasing interest among researchers for human mesenchymal stromal cells (MSC). Their regenerative properties, multilineage differentiation capacity and immunomodulatory properties make them promising candidates for treatment in various conditions. Emerging biotechnology companies specialized in cellular and regenerative therapies have been focusing their interest on MSC-based therapies, and their use in clinical trials has steadily increased. Notably, MSC are currently tested in clinical trials addressing unmet medical needs in the field of bone fracture repair and more specifically in non-union and delayed union fractures where the bone repair process is impaired. Although MSC can be isolated from various tissues, the most commonly studied sources are bone marrow (BM) and adipose tissue (Ad). In this article, we reviewed the literature directly comparing BM- and Ad-MSC for their *in vitro* characteristics and *in vivo* osteogenic potential to determine which source of MSC would be more appropriate for bone fracture repair. As considerable variations in experimental settings between studies were found, our review was based on studies meeting specific sets of criteria, notably regarding donors' age and gender. This review of side-by-side comparisons suggests that while BM-and Ad-MSC share common general characteristics, BM-MSC have a higher intrinsic osteogenic capacity *in vitro* and bone repair potential *in vivo*.

Keywords: adipose tissue-derived mesenchymal stromal cells, bone marrow-derived mesenchymal stromal cells, MSC, proliferation, yield, immunophenotype, osteogenic differentiation, *in vivo*, preclinical, regenerative medicine, cell therapy, human, review

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1. Introduction

Regenerative medicine is a promising emerging therapeutic field, notably for bone repair. With their unique biological properties, human mesenchymal stromal cells (MSC) may be particularly interesting for this field. MSC are non-hematopoietic multipotent stromal cells that were first isolated and characterized from bone marrow (BM) in 1968 by Friedenstein *et al*.[1]. Using their plastic adherence property, the authors were able to isolate fibroblast-like clonogenic cells that they called colony forming unit-fibroblasts (CFU-F)[1,2]. These cells have been shown to exhibit a strong replication capacity *in*

vitro and to differentiate into different cell lineages including osteoblasts, chondrocytes and adipocytes[3–5]. In addition to this tri-lineage differentiation capacity, MSC may also be able to give rise to other mesodermal lineage cells such as skeletal muscle and tendon cells[6–11]. Since then, MSC have been isolated from other sources (adipose tissue, synovial membrane, placental tissues, umbilical cord blood, blood, dental pulp, dermal tissue or trabecular bone) than bone marrow[10, 12–18].

Recently, the heterogeneity in isolation procedures and culture protocols prompted the Cell Committee of the International Society for Cellular Therapy (ISCT) to harmonize the definition of MSC[19]. MSC were defined according to the following 3 minimum criteria:

(i) MSC must be purified based on their plastic adherence when maintained in standard culture conditions;

(ii) MSC must express the mesenchymal markers CD105, CD73 and CD90 and must not express the hematopoietic markers CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR;

(iii) MSC must be able to differentiate in appropriate *in vitro* conditions into osteoblasts, adipocytes and chondrocytes.

MSC from bone marrow were the first to be investigated twenty years ago in a clinical trial by Lazarus *et al*., in patients with non-Hodgkin's lymphoma[20]. Since then, the number of clinical trials using MSC has increased steadily. To date, according to data reported by the US National Institutes of Health[21], more than 500 clinical trials using human MSC are either completed or ongoing. In these clinical trials, autologous or allogeneic MSC are used for the treatment of various diseases, including haematological diseases, autoimmune diseases, liver, kidney and lung diseases, neurological diseases and cartilage and bone diseases (e.g., osteonecrosis, spinal fusion, delayed and non-union fractures).

2. Rationale of Cell Therapy for Fracture Repair

Bone being naturally regenerative, fractures are currently well-managed in the majority of patients. However, there are traumatic situations in which bone fails to regenerate itself leading to non-union[22]. While there is no universally accepted definition, non-union refers to a fracture that has not united within 6 to 9 months after fracture onset and that requires additional surgical or non-surgical intervention. An overall rate of 5 to 10% non-union is reported in the literature[3]. Standard treatment options (i.e., bone auto- or allograft) typically involve highly invasive surgeries, which have a significant morbidity associated to considerable risk of complications (in 20 % of patients), such as infections, and may require months of rehabilitation[24,25]. Although the exact aetiology remains unknown, the impaired bone repair capacity in non-union fractures was reported to be associated with excessive mechanical instability of the fracture, reduced bone vascularity, reduced number of progenitor cells (at fracture site as well as in iliac crest bone marrow), along with a decreased proliferation capacity of said progenitors[26–28]. Therefore, cell-based therapy, by providing additional functional MSC, could offer an appealing therapeutic alternative to treat bone fractures.

BM-MSC have historically been the most widely investigated in clinical trials[29–31]. But supported by interesting preclinical results, the number of clinical trials involving Ad-MSC has significantly increased over the past years[29,32,33]. In the US National Institutes of Health database[21], 11 delayed and non-union fracture repair trials using BM-MSC or Ad-MSC are reported, among which 9 with BM-MSC and 2 with Ad-MSC for non-union applications (Table 1). These trials are all either “starting” or “recruiting” and one can expect the results to be available in the near future.

Preclinical studies have been and are still being conducted to understand the characteristics of BM- and Ad-MSC and to assess their regenerative potential in bone repair models. Notably, a growing number of preclinical studies have been undertaken to directly compare the two types of MSC with the aim to determine their commonalities and specificities. However, the heterogeneity in experimental settings used by different teams makes the comparison between studies difficult and conclusions hard to draw. This article aims to provide a critical review of the preclinical studies investigating *in vitro* and *in vivo* the characteristics and the bone formation potential of BM- and Ad-MSC.

3. Methodology

Only a few specific studies which directly compared human BM-MSC and Ad-MSC were selected for this review. The analysis of this literature underlines considerable variations between results obtained from different research teams that may be linked to the heterogeneity in (i) donors and donors’ characteristics (e.g., health status, gender, age), (ii) *in vitro*/*ex vivo* protocols (e.g., sample isolation sites and methods,

Table 1. List of clinical trials using BM-MSC and Ad-MSC in delayed and non-union fractures

Study Title	Status	Identifier	Phase	Product	Cell source	Sponsor
BM-MSC						
Treatment of Atrophic Non-union Fractures by Autologous Mesenchymal Stem Cell Percutaneous Grafting	Not yet Recruiting (Nov 2017)	NCT01429012	II	BM-MSC	Autologous	University Hospital of Liege
Phase 2b/3 Study on Autologous Osteoblastic Cells Implantation in Hypotrophic Non-Union Fractures	Recruiting (Jun 2017)	NCT01756326	II/III	Differentiated BM-MSC (PREOB®)	Autologous	Bone Therapeutics S.A, Belgium
Phase 1/2a Study on Allogeneic Osteoblastic Cells Implantation in Delayed-Union Fractures	Recruiting (Jul 2016)	NCT02020590	I/II	Differentiated BM-MSC (ALLOB®)	Allogeneic	Bone Therapeutics S.A, Belgium
Autologous BM-MSC Transplantation in Combination With Platelet Lysate (PL) for Nonunion Treatment	Recruiting (Aug 2017)	NCT02448849	II/III	BM-MSC + Platelet Lysate	Autologous	Royan Institute, Iran
Mesenchymal Stem Cells; Donor and Role in Management and Reconstruction of Nonunion Fracture	Recruiting (Jan 2016)	NCT01626625	I	BM-MSC + HA	-	Indonesia University, Indonesia
Mesenchymal Stromal Cells for the Treatment of Non-union Fractures of Long Bones	Recruiting (Dec 2017)	NCT02230514	I/II	BM-MSC (XCEL-MT-OSTEO-ALPHA) + allograft	Autologous	Banc de Sang i Teixits + Hospital ASEPEYO Sant Cugat, Spain
Mononucleotide Autologous Stem Cells and Demineralized Bone Matrix in the Treatment of Nonunion/Delayed Fractures	Not yet recruiting	NCT01435434	-	BM-MSC + DBM (Ignite®)	Autologous	Hadassah Medical Organization
Evaluation of Efficacy and Safety of Autologous MSCs Combined to Biomaterials to Enhance Bone Healing	Active, not Recruiting (Nov 2015)	NCT01842477	I/II	BM-MSC + BCP granules	Autologous	Institut National de la Santé Et de la Recherche Médicale, France - REBORNE project
Evaluation the Treatment of Nonunion of Long Bone Fracture of Lower Extremities Using Mononuclear Stem Cells From the Iliac Wing Within a 3D Tissue Engineered Scaffold	Unknown (last update Nov 2013)	NCT01958502	II	BM-MSC (not expanded) + collagen matrix	Autologous	Emdadi Kamyab Hospital, Iran
Ad-MSC						
A Clinical Trial to Assess the Effect of HC-SVT-1001 in the Surgical Treatment of Atrophic Pseudarthrosis of Long Bones (BONECURE)	Recruiting (Apr 2017)	NCT02483364	II	Ad-MSC (HC-SVT-1001) + TCP matrix	Autologous	Laboratorios Salvat, Spain
Allogenic Mesenchymal Stem Cell for Bone Defect or Nonunion Fracture (AMSC)	Recruiting	NCT02307435	0	BM/Ad/UC-MSC (3 treatment groups)	Autologous	Indonesia University

culture conditions) and (iii) readouts, outcomes and methods of analysis. In addition other undefined factors can also influence MSC properties.

From 54 studies which directly compared BM-MSC and Ad-MSC, only a few studies that compared BM-MSC and Ad-MSC from donors matched for age and/or gender were retained for analysis. Eighteen studies reporting data about yield and proliferation, immunophenotype, differentiation potential and/or bone formation/repair potential *in vivo* were considered. Five studies compared BM-MSC and Ad-MSC isolated from the same donors[33–38], which represents the most robust comparison as they are not biased by inter-individual variabilities, in particular age, gender and health status. Nine studies comparing age-matched donors were also retained, which include 1 study on female donors only[39], 1 study on 50% male and 50% female donors[40], 2 studies on a close but not identical number of females and males[41,42], and 5 studies on donors matched for age but without specifying the gender[43–47]. Finally, 4 studies compared the two types of MSC isolated from healthy donors, but with unknown age and gender[48–51].

For bone formation/repair potential, there were limited *in vivo* studies which compared both types of MSC. Therefore, 2 studies comparing BM-MSC and Ad-MSC with unknown health condition, age and gender of donors[52,53] and 5 studies using the two types of MSC isolated from other species[54–58] were considered for this review.

4. Comparison of BM-MSC and Ad-MSC Characteristics

It is noteworthy to mention that BM-MSC and Ad-MSC share the general minimum criteria defined by Dominici *et al.* in 2006[19]. However, preclinical studies identified the differences regarding their yield, proliferation, phenotype and differentiation properties. This section aims to discuss the commonalities and differences reported for BM-MSC and Ad-MSC (Table 2), with the focus on studies which involves direct comparison between the BM-MSC and Ad-MSC isolated from donors sharing similar characteristics as described above.

4.1 Collection, Isolation, Yield and Proliferation

The use of MSC for clinical applications requires a large number of cells, and although MSC can be extensively expanded *in vitro*, this expansion is not indefinite due to replicative senescence[59,60]. With the availability of different MSC sources, comparative studies have been conducted to determine whether, and to what extent, the source affects *in vitro* the expansion capacity and yield of MSC.

BM-MSC are isolated from bone marrow aspirates usually from the iliac crests. Alternative harvesting sites are femoral heads (from total hip arthroplasty of an elderly patient population), sternum and less frequently vertebrae. The commonly applied preparation method for the generation of BM-MSC is density gradient centrifugation, but other methods rely on their properties to adhere to plastic[1,61–66].

Ad-MSC are isolated from lipoaspirates obtained during liposuction, lipoplasty or lipectomy procedures. The commonly applied preparation method implies enzymatic digestion with collagenase followed by centrifugation, washing and plating onto plastic surfaces[67]. Ad-MSC can be isolated from any types of white adipose tissue, including subcutaneous and omental fat[61,63,67–70].

While the MSC isolation rate from Ad and BM sources is comparable, the yield and expansion capacity are reported higher for Ad than for BM preparations[6,63,71–73]. Indeed, across literature (without taking into account donors' characteristics), studies and reviews generally report that Ad-MSC have a higher expansion capacity than BM-MSC both in terms of proliferation rate and cell doubling number[31,61,63]. In addition, Ad-MSC would have longer proliferation capacity through cell culture passages and a lower senescence rate compared to BM-MSC[31,61,63].

The analysis of selected comparative studies (10) reporting proliferation and yield data gives a contrasted picture with 5 studies favouring Ad-MSC, 3 favouring BM-MSC and 2 supporting equivalence.

Zhu *et al.*[41] and Bochev *et al.*[33] showed that Ad-MSC reach confluence faster than BM-MSC (but with marked differences between studies: 6 vs 9 days (to reach ~8×10^4 cells from an initial seeding of 5×10^3 cells/well) and 15 vs 20–22 days). Zhu *et al.*[41] and Chen *et al.*[37] found that Ad-MSC have a shorter doubling time (28 hours vs 39 hours[41]).

Further, Chen *et al.*[37] showed that with young donors (36.4 ± 11.8 years old), 5/8 BM-MSC cultures reached passage 9 and with elderly donors (71.4 ± 3.6 years old), 2/4 BM-MSC cultures reached passage 7[37]. On the contrary, the aging effect for Ad-MSC was not significant up to passage 9 for both the young and elderly donor groups. Ad-MSC achieved a cumulated cell number of 10^8 within 4 passages and 10^{13} within 7 passages whatever the donors' age (young or elderly groups), while BM-MSC from both age groups reached a cumulated cell number of 10^8 after 5 passages but only BM-MSC from young adult donors reached 10^{13} at passage 8; the cumulated cell number of BM-MSC from the elderly group did not go over 10^9[37]. These results suggest that contrarily to Ad-MSC, BM-MSC proliferation capacity decreases with passage numbers and age. Dmitrieva *et al.*[38] observed a similar proliferation rate between both types of MSC at early passages: the population doubling time was about 1–1.5 day for both types of MSC. However BM-MSC population doubling time increased by passage 3 (by ~5 fold) and CFU-F frequency declined by passage 4 (by ~4 fold) while population doubling time and CFU-F were maintained through passages in Ad-MSC cultures. Also, signs of senescence appeared earlier in BM-MSC cultures than in Ad-MSC (passage 4 vs 6)[38]. Najar *et al.*[48] showed that after the primoculture, Ad-MSC yield a 6 fold higher CFU-F number compared to BM-MSC (number of CFU-F obtained from 10^6 cells initially seeded: 5080 ± 553 for Ad-MSC vs. 852 ± 135 for BM-MSC).

In contrast, Vishnubalaji *et al.*[44] obtained a higher number of CFU-F for BM-MSC than for Ad-MSC at passage 1 (25.9 ± 2.9 *vs.* 17.9 ± 2.8), but CFU-F frequency of BM-MSC decreased with passage numbers while it was maintained in Ad-MSC cultures (CFU-F number: 8.5 ± 3.8 *vs.* 24.2 ± 2.8 at passage 4). Jin *et al.*[46] found that 2.5 times more CFU-F were formed

from BM-MSC than from Ad-MSC (16.5 ± 4.4 *vs.* 6.4 ± 1.6 at passage 3) and population doubling of BM-MSC was higher than that of Ad-MSC (by ~3 fold at passage 2 and ~2 fold at passage 3), although cell growth of both BM-MSC and Ad-MSC arrests at passages 11–12. In addition, one study reports that the expansion potential of BM-MSC was retained at later passages than that of Ad-MSC (passage 10 vs passage 7)[36].

Finally, 2 studies[34,40] did not show differences in yield, proliferation or senescence between BM-MSC and Ad-MSC.

In summary, a review of data from comparative studies does not give consistent results about the yield and proliferation capacity of BM-MSC and Ad-MSC and definitive conclusions seem difficult to draw. Further, the lack of quantitative data (e.g., size of CFU, absolute cell count seeded or collected…) makes detailed analyses difficult. Although BM and Ad tissue donors' characteristics were similar within studies, they were different from one study to another. Also, besides donors' characteristics, MSC clonogenic ability and proliferation rate can also depend on the harvesting site and culture conditions, which could explain the different outcomes obtained between studies. The development of standardized procedures, protocols and methods is therefore necessary to ensure reliable and comparable results between studies and research teams.

4.2 Cell Morphology and Identity

All the studies which focused on MSC morphology (whatever the donors' characteristics) agreed that there are no or few observable differences between BM-MSC and Ad-MSC as both exhibit a similar fibroblast-like, elongated spindle shape, morphology[33,47,61,74,75].

When analysing the immunophenotypic data reported by comparative studies (15), only a few subtle differences in surface marker expression between BM-MSC and Ad-MSC could be identified (48 markers reported in total, either tested extensively such as CD73 or CD45 or occasionally like CD49c or CD133)[33,35–48] (Table 3).

BM-MSC and Ad-MSC both express the stromal surface markers CD13, CD73, CD90, CD105 and CD166, and STRO-1 to a lesser extent. Both do not express the hematopoietic and immune markers CD3, CD4, CD11a,b,c, CD14, CD16, CD19, CD24, CD33, CD34, CD38, CD45, CD56, CD58, CD62b, CD79a and HLA-DR nor the endothelial/platelet markers CD31, CD36, CD133 or CD117. They have both been also shown to express the integrin CD29 (β-integrin) and CD44 (hyaluronan receptor).

Our review has identified only a few markers that are differentially expressed between BM-MSC and Ad-MSC: these markers however have not been the most extensively studied. CD49d (integrin α4) is reported to be expressed by Ad-MSC (although inconstantly) but not or only weakly by BM-MSC, whereas CD106 (vascular cell adhesion protein 1) and PODXL (podocalyxin-like protein 1) are moderately to strongly expressed by BM-MSC but not expressed by Ad-MSC[35,36,39,41].

Through extensive literature review, it is found that most of the studies agree that both types of MSC have similar profiles of surface antigen expression[7,31,62,68]. Several studies have reported that Ad-MSC express the hematopoietic progenitor cell antigen CD34 immediately after isolation and during the first stages of cell culture[31,76–80] while it is not expressed by BM-MSC[31,62,80,81]. However, no difference in CD34 expression is reported in side-by-side comparisons between BM-MSC and Ad-MSC (Table 3). These divergences may be explained by variations in used protocols (e.g., different culture media, culture duration, proliferation stage, passage number, donors' characteristics, antibody sources).

4.3 BM-MSC and Ad-MSC Gene Signature

Understanding at the molecular level the pathways that distinguish BM-MSC from Ad-MSC in their differentiation toward the osteoblastic lineage may help designing cell therapies for bone repair. Based on mRNA analysis such as qRT-PCR, microarray or RNAseq, studies evidenced differences between BM- and Ad-MSC that are implicated in various signalling pathways. This could suggest functional differences between both MSC types.

According to the selection criteria of this review (i.e., age and gender matching donors), 6 articles have been selected[39,42–44,47,81]. From these 6 publications however, 2 were discarded because the number of cell passages was either too large or not clearly specified[43] (using P2 to P6 cells without additional clarification), which can cause artefactual background, or because the comparison was not performed with non-induced BM-MSC and Ad-MSC (comparing only differentiated cells)[44]. Therefore, only 4 papers were kept for this review. In these articles, BM- and Ad-MSC were expanded with a known and limited number of passages (P1 to P3), were grown in minimal conditions

without induction towards lineage and were directly compared[39,42,47,81].

In Brocher *et al.*[42], the expression of genes of the osteogenic, angiogenic and adipogenic pathways from BM-MSC and Ad-MSC was compared. The comparison of expression level of *BMP2* and *BMP4* coding for 2 osteogenic growth factors showed that BM-MSC expressed significantly higher mRNA levels of these factors compared to Ad-MSC (fold change between BM-MSC and Ad-MSC was 5 for *BMP2* and 3 for *BMP4*). The same was observed for mRNA coding for the pro-angiogenic molecules VEGF and ANGPT1 with a fold increase of 2 and 5, respectively[42]. VEGF is notably known to stimulate bone formation by increasing angiogenesis, to maintain MSC survival and to promote osteoblast differentiation. Finally, genes involved in adipogenesis, such as those coding for the visfatin and adiponectin proteins, were downregulated in BM- compared to Ad-MSC. Similarly, Noël *et al.*[81] showed by microarray from Passage 1 (P1) BM-MSC and Ad-MSC that genes involved in chondro/osteogenesis, such as *HES1*, *DLX5*, *TWIST1*, *BGLAP*, *OSX*, *SOX9*, *WNT5A*, *TGFB1* and *VEGF* were upregulated in BM-MSC. These results were confirmed recently in Reinisch *et al.*[47], which showed that genes related to chondro/osteogenesis such as *RUNX2*, *RUNX3*, *BGLAP*, *MMP13*, *ITGA10*, *DLX5*, *DLX6*, *SPP1*, *BGLAP*, *ALPL*, *SPP1*, *COL2A*, *COL10A*, and *ACAN* were upregulated in BM-MSC compared to Ad-MSC. The same researchers also confirmed that hemato/angiogenic genes such as *INHBA*, *FZD1*, *TLR4*, *DGFC*, *ANGPT1*, *VEGFA*, *CTGF*, and *VEGFC* were also upregulated in BM-MSC, confirming Brocher's results[42,47].

The same trend was shown for *BGLAP, SPARC* and *RANKL* by Pachón-Peña *et al.*[39]. Finally, as in Brocher *et al.*, adipogenesis-related mRNA levels in non-induced Ad-MSC largely exceeded (although without reaching statistical significance) those of BM-MSC, for *PPARG* (0.83 ± 1.73 *vs.* 0.03 ± 0.09), *CEBPA* (0.02 ± 0.02 *vs.* 0.01 ± 0) and *FABP4* (1.75 ± 4.09 *vs.* 0.02 ± 0.02)[39].

Altogether, these 4 studies evidence that the expression of genes which are related to the chondro/osteogenic and angiogenic pathways are largely more expressed in BM-MSC compared to Ad-MSC. Whereas, an opposite observation for the adipogenic pathway suggests that both MSC exhibit a gene signature related to their source (Table 2).

4.4 *In Vitro* Multilineages Differentiation Potential

In vitro analysis of MSC differentiation potential under appropriate culture conditions revealed that BM-MSC and Ad-MSC displayed the capacity of differentiation into the adipogenic, chondrogenic and osteogenic lineages. Yet, the important question which raised in the context of bone tissue engineering is whether MSC from both sources have similar osteogenic potential.

To answer this question, 14 articles were selected based on the selection criteria used for this review. From these 14 articles, 6 have investigated the trilineages differentiation potential[34,36,40,46,47,81], 5 the osteogenic and the adipogenic potential[33,38,39,43,44], 1 the osteogenic and chondrogenic potential[45] and 2 the osteogenic potential only[37,42].

MSC can be induced to differentiate along the three lineages using culture medium supplemented with different factors. For osteogenic differentiation the medium is generally supplemented with dexamethasone, β-glycerophosphate and ascorbic acid. This medium induces osteoblastic differentiation that can thereafter be revealed using staining protocols for ALP activity, calcium deposition or matrix mineralization (e.g., Alizarin Red, Von Kossa and Giemsa staining) or by mRNA analysis measuring the expression levels of osteoblastic gene.

Im *et al.*[45] showed that after 2 weeks of culture in osteogenic medium, 85% of BM-MSC were ALP positive against 28% for the Ad-MSC, reaching 92% and 45% respectively after 3 weeks of culture. The number of mineralization nodules and the calcium content were also quantitatively higher in BM-MSC than in Ad-MSC[45]. Five other studies (using ALP activity, Von Kossa, Alizarin Red staining or qPCR) confirmed the conclusions made by Im *et al.*[45] that under osteogenic induction, BM-MSC display higher osteogenic capacity than Ad-MSC[33,36,37,44,81]. It was also shown after 3 weeks of osteogenic induction that the number of mineralizing colonies (number of Alizarin Red positive colonies vs the total number of colonies) was higher for BM-MSC (~43%) than for Ad-MSC (~22%)[36]. At early timepoint (10 days of induction) too, BM-MSC were shown to have significantly higher osteogenic potential than Ad-MSC (2 fold increase in fluorescence-based calcium level quantification, *p=0.02*)[44]. Further, three key bone genes, *OSX*, *ALPL* and *BGLAP* were shown to be more strongly up-regulated in differentiated BM-MSC than in differentiated Ad-MSC[37,81].

Seven studies found that BM- and Ad-MSC displayed the same osteogenic differentiation capacity after osteogenic induction[34,38,39,42,43,46,47]. Jin *et al*., Reinisch *et al.*, and Wagner *et al.* showed that BM- and Ad-MSC can successfully differentiate into osteogenic lineage based on ALP[46,47] or Von Kossa staining[43]. The comparisons are however not quantitative and therefore difficult to be interpreted. Four studies showed that there is no significant quantitative difference between induced BM- and Ad-MSC by quantification of ALP activity[34,42] or colonies positive for Alizarin Red staining[38,39].

Finally, only one study suggests that after 3 weeks of osteogenic induction, Ad-MSC showed higher calcium deposition compared to BM-MSC based on Alizarin red staining, but this was not quantitatively assessed[40].

Among these 14 studies, 6 concluded on the superiority of BM-MSC[33,36,37,44,45,81], 7 observed equivalence[34,38,39,42,43,46,47] and a single one superiority of Ad-MSC[39]. Taking into account only studies (10 out of the 14) providing quantitative osteogenic assessments, the superiority of BM-MSC was demonstrated in 6 studies[33,36,37,44,45,81] and the equivalence in 4[34,38,39,42].

Similar trends were observed for the adipogenic and chondrogenic potentials. Indeed among the 11 studies investigating the adipogenic capacity of BM- and Ad-MSC, 3 reported a higher potential for Ad-MSC[36,39,44] and 8 reported equivalence[33,34,38,40,43,46,47,81]. Finally, among the 7 studies investigating the chondrogenic capacity of BM- and Ad-MSC, 4 reported a higher potential for BM-MSC[36,40,45,47], 1 a higher potential for Ad-MSC[35] and 2 equivalence[44,81].

Although the results are not completely consistent, comparative data tend to support a more pronounced osteogenic and chondrogenic potential of BM-MSC as compared to Ad-MSC (6 studies against 4 for osteogenic potential; 4 against 3 for chondrogenic potential) (Table 2).

Table 2. General comparison of BM-MSC and Ad-MSC characteristics

Characteristics	BM-MSC	Ad-MSC	References
***In vitro* proliferation capacity**	Limited	Limited ***Greater in 5/10 selected studies***	[59–60] [33,36–38,40–42,44,46,48]
	Greater in 3/10 selected studies		[33,36–38,40–42,44,46,48]
	Equivalent in 2/10 selected studies		[33,36–38,40–42,44,46,48]
Cell morphology	Fibroblast-like morphology	Fibroblast-like morphology	[33,61,74,75]
Immunophenotype	Express stromal surface markers Do not express most haematopoietic lineage and immune markers Do not express endothelial/platelet markers CD31, CD36, CD133 and CD117 Express CD29 and CD44 ***Do not express or weekly express CD49d*** ***Moderately to strongly express CD106 and PODXL*** ***Do not express CD34***	Express stromal surface markers Do not express most haematopoietic lineage and immune markers Do not express endothelial/platelet markers CD31, CD36, CD133 and CD117 Express CD29 and CD44 ***Express CD49d (but not constantly)*** ***Do not express CD106 and PODXL*** ***Do not express or express CD34 at early stages***	[7,31,33,35–46,48,63,68,76–78,80] *See Table 3 for detailed comparison of the results from the selected studies*
Gene signature	***Chondro/osteogenic genes:*** *BMP2, BMP4, OSX, BGLAP, ALPL, VEGF, HES1, DLX5, TWIST1, BGLAP, OSX, SOX9, WNT5A, TGFB1, DLX6, COL2A, COL10A and ACAN* ***Upregulated*** ***hemato/angiogenic genes:*** *INHBA, FZD1, TLR4, DGFC, ANGPT1, VEGFA, CTGF, and VEGFC* ***upregulated***	***Adipogenic genes,*** *FABP4, NAMPT, ADIPOQ, PPARG, CEBPA* ***upregulated***	[39, 42, 47, 81]
Multilineage differentiation capacity			
Osteogenic potential	***> 6/14 studies***	***> 1/14 studies***	[33,34,36–40,42–47,81]
	Equivalent in 7/14 selected studies		
Adipogenic potential		***>3/11 studies***	[33,34,36,38–40,43,44,46, 47,81]
	Equivalent in 8/11 selected studies		
Chondrogenic potential	***>4/7 studies***	***>1/7 studies***	[34, 36, 40, 45–47, 81]
	Equivalent in 2/7 selected studies		

Table 3. Immunophenotypic comparison of BM-MSC and Ad-MSC

		BM-MSC	Ad-MSC
Hematopoietic and immune markers			
CD3	Lymphocyte	–(3)	–(3)
CD4	Lymphocyte	–(1)	–(1)
CD10	Hematopoietic progenitor	–(2)+(1)	–(1)+(2)
CD11a	Integrin α L-Leukocyte	–(1)	–(1)
CD11b	Integrin αM-Leukocyte/NK	–(1)	–(1)
CD11c	Integrin αX-Leukocyte	–(1)	–(1)
CD14	Macrophage	–(10)	–(10)
CD16	NK/macrophage	–(1)	–(1)
CD19	Lymphocyte B	–(5)	–(5)
CD24	B cells	–(1)	–(1)
CD33	Monocyte	–(1)	–(1)
CD34	Early hematopoietic	–(13)	–(12)+(1 low)
CD38	Plasma/B Cell	–(2)	–(2)
CD45	Hematopoietic	–(13)	–(13)
CD56	NK/macrophage	–(2)	–(2)
CD58	Leukocyte/macrophage	+(1)	+(1)
CD59	Immune	+(1)	+(1)
CD62b	Adhesion-leukocyte	–(1)	–(1)
CD79a	B cells	–(3)	–(2)+(1)
CD184	CXCR4	–(2)	–(2)
HLA-DR	Immune	–(8)	–(8)
HLA-ABC	Immune	+(4)	+(4)
Mesenchymal markers			
CD13	Mesenchymal/myeloid marker	+(4)	+(4)
CD73	Mesenchymal	+(12)	+(12)
CD90	Mesenchymal	+(12)	+(12)
CD105	Mesenchymal	+(12)	+(12)
CD166	Mesenchymal	+(9)	+(8/1 moderate)
CD271	Mesenchymal	–(2)	–(2)
STRO-1	Mesenchymal progenitor	+(4)	+(3/1 low)
SSEA4	Mesenchymal	–(1)	–(1)
Integrin, cadherin and adhesion markers			
CD29	Integrin β1	+(9)	+(9)
CD44	Hyaluronan receptor	+(8)	+(8)
1. CD49d	*Integrin α4*	*–(2)+(1 low)*	*–(1)+(2)*
CD49c	Integrin α3	+(1)	+(1)
CD49f	Integrin α6	+(2)	+(2)
CD54	ICAM1	+(3 low)	+(2 low/high)–(1)
CD104	Integrin β4	–(1)	–(1)
2. CD106	*VCAM1*	*+(2/2 low)*	*–(4)*
CD144	V cadherin - Endothelial	–(1)	–(1)
CD146	M CAM	+(4)	+(2 low)–(2)
Platelet and endothelial markers			
CD31	Platelet/Endothelial	–(7)	–(7)
CD36	Platelet/Endothelial	–(2)	–(1)+(1)
CD117	Stem cell	–(3)	–(3)
CD133	Endothelial	–(1)	–(1)
FLK1	VEGF receptor	–(1)	–(1)
VEGFR2	VEGF receptor	–(1)	–(1)
Others			
CD147	Neurothelin	+(1)	+(1)
PODXL	Podocalyxin-like protein 1	+(1)	–(1)

The immunophenotype data reported by 15 studies directly comparing BM-MSC and Ad-MSC isolated from donors with similar characteristics were compared[33,35–48].

NK: Natural Killer, -: absence of expression, +: expression, low: low expression, moderate: moderate expression, high: high expression. The number in brackets corresponds to the number of studies reporting the corresponding result.

5. *In Vivo* Osteogenic Potential of BM-MSC and Ad-MSC

In order to evaluate the osteogenic potential of BM-MSC and Ad-MSC *in vivo*, the same approach used for assessing the *in vitro* MSC characteristics was applied. The *in vivo* literature is however limited. From the 11 articles directly comparing BM-MSC and Ad-MSC in bone formation/bone repair model, only 2 meet the criteria defined for this review[41,47]. From the others, 4 articles do not specify the BM and/or Ad donors' characteristics[50–53] and 5 compared BM-MSC and Ad-MSC isolated from others species such as rats[54], rabbits[55], dogs[56], pigs[57] and sheep[58]. Our analysis was therefore based on the 6 articles using human BM-MSC and Ad-MSC and when relevant, confronted to the results obtained with other species.

To address the *in vivo* osteogenic potential, two aspects will be discussed in this section. First, the ectopic bone formation capacity investigated in 5 out of the 11 selected articles[42,47,50,52,54], and second, the orthotopic bone formation capacity evaluated in 6 out of the 11 selected papers[51,53,55–58].

5.1 Ectopic Bone Formation Capacity of BM-MSC and Ad-MSC

The direct comparison of ectopic bone formation capacity of BM-MSC and Ad-MSC has been studied in 5 articles: 4 using human MSC (with 2 meeting the selection criteria for this review[42,47]) and 1 using rat

MSC.

Ectopic bone formation commonly occurs with BM-MSC in presence of calcium phosphate ceramics such as β-tricalcium phosphate (β-TCP) and/or hydroxyapatite (HA) after subcutaneous implantation in immunocompromised mice[82–86]. This ectopic bone formation model is useful to assess the intrinsic capacity of cells to produce mineralized matrix outside a bone environment, even if this model is partly biased by the use of osteoconductive scaffolds.

In an experiment comparing BM-MSC and Ad-MSC from 14 different donors (7 per source and matched for age), Brocher *et al.*[42] have shown that BM-MSC seeded on β-TCP scaffolds induced new bone deposition onto the scaffold (6/7 donors and 11/14 scaffolds) whereas no bone deposition was observed with Ad-MSC (0/7 donors and 0/22 scaffolds) 8 weeks after subcutaneous implantation. The Ad-MSC results were moderately improved (1/3 donors and 2/6 scaffolds) by prolonging the observation period to 12 weeks[42]. In Reinisch *et al.*[47], similarly, after subcutaneous implantation in mice, Ad-MSC seeded onto Matrigel™ were not able to induce bone formation contrarily to BM-MSC. This study is interesting because the superiority of BM-MSC over Ad-MSC was demonstrated using non-bioactive scaffold. Similar results were reported in rats (r) where the bone volume measured by µ-Computed Tomography (µCT) within the pores of scaffold was over 100 fold higher with rBM-MSC (6.85mm³ ± 1.89) than with rAd-MSC (0.05 mm³ ± 0.05) 6 weeks after implantation; this was further confirmed by histology[54].

The Ad-MSC literature however shows that a pre-induction or a pre-differentiation step would be required for Ad-MSC to promote bone formation[87–90]. This would therefore suggest a lower intrinsic bone forming capacity of Ad-MSC compared to BM-MSC.

This is supported by Brocher *et al.* and Hattori *et al.* who showed that pre-induction increases the rate of ectopic bone formation by Ad-MSC to the levels of non-induced and induced BM-MSC[42,52], but not by Reinisch *et al.* who confirmed the superiority of BM-MSC over Ad-MSC even under an induction protocol (β-TCP/HA, osteogenic stimulation and PTH injection)[47].

However using µ-CT, pre-differentiated BM-MSC have been shown to display higher bone deposition onto the scaffolds (9.1 mm³ ± 1.1) than pre-differentiated Ad-MSC (1.3 mm³ ± 0.1)[50]. This was also shown by Brocher *et al.* with 7% and 3% of bone deposition for BM-MSC and Ad-MSC respectively, although here the difference did not reach statistical significance. Interestingly too, this last study also demonstrated that pre-induction of BM-MSC is sufficient for new bone formation without scaffold (3/3 donors and 6/6 scaffolds) whereas Ad-MSC (0/5 donors and 0/10) still require the osteoconductive microenvironment provided by the scaffold[42].

In summary, BM-MSC have higher intrinsic bone formation capacity than Ad-MSC. Under pre-induction, the conclusion is more controversial but the results suggest that ectopic bone formation capacity of BM-MSC is quantitatively better than that of Ad-MSC and does not require osteoconductive scaffold.

5.2 Bone Repair Capacity of BM-MSC and Ad-MSC

From the large body of literature investigating the bone repair potential of BM-MSC and Ad-MSC on calvarial and long bones defects, direct comparisons between BM-MSC and Ad-MSC have been performed in only 6 articles: 3 (only 1 with human MSC) in critical size calvarial defect and 3 (only 1 with human MSC) in critical size long bone defect.

In rat critical size calvarial defect model, Wen *et al.* showed using X-Ray and histomorphometry that human BM-MSC and Ad-MSC have similar bone healing rate 8 weeks after implantation[53]. Autologous transplantation of BM-MSC and Ad-MSC in rabbit and pig calvarial model supports these observations. Indeed, mineralization rate and new bone formation volume determined by µ-CT were not significantly different between the two sources of MSC 12 weeks after implantation[55,57]. Nonetheless, more adipose tissue was observed in bone defect areas with Ad-MSC than BM-MSC indicating that Ad-MSC undergo adipogenic differentiation even in a bone healing environment.

In critical size long bone defect, whereas one study in dogs showed comparable healing rate between canine BM- and Ad-MSC[56], two studies demonstrated that BM-MSC display higher bone healing capacity than Ad-MSC[51,58]. In rat femoral critical defect, new bone volume fraction values (BV/TV) quantified by µ-CT were significantly higher in human BM-MSC compared to human Ad-MSC 12 weeks after implantation, but the difference was lost with pre-induction[51]. Similarly, in a model of a critical size defect of sheep tibia, radiographic evaluations — confirmed histologically — revealed higher levels of newly formed bone

in ovine BM-MSC than in ovine Ad-MSC[58].

Taken together, these 6 studies suggest that the bone repair potential of BM-MSC and Ad-MSC is site dependent. Whereas similar bone repair was observed in calvarial defect model, BM-MSC show better bone repair potential in the long bone defect model. A key element that might explain this difference is the type of ossification occurring during bone repair. Whereas flat bones such as calvaria undergo intramembranous ossification, long bones are repaired through endochondral ossification. During long bone fracture repair, endochondral ossification occurs following four overlapping phases. After injury, disruption of vessels leads to hematoma formation followed by an intense angiogenesis and the formation of a soft callus. This angiogenesis is critical to the formation of the callus and therefore for bone healing and VEGF is a key player in this process (for review[91,92]). During bone fracture repair, VEGF is highly expressed and secreted by osteoblasts. Literature has showed that BM-MSC express more VEGF than Ad-MSC (see BM- and Ad-MSC gene signature section). BM-MSC could therefore promote better angiogenesis than Ad-MSC which may explain the higher osteogenic potential of BM-MSC in long bone defect compared to Ad-MSC.

In summary, the *in vivo* data reported here support that BM-MSC display inherent bone formation capacity superior to that of Ad-MSC. Indeed, the studies discussed in this review showed that BM-MSC have a higher bone formation capacity in non-induced as well as in induced conditions. Further studies have shown that Ad-MSC require pre-induction to promote bone formation. Yet, even with pre-induction, Ad-MSC fall short of BM-MSC quantitatively in terms of amount of newly formed bone. This inherent superior bone formation capacity of BM-MSC is further confirmed by the results from long bone defect studies. However, as BM-MSC and Ad-MSC have similar osteogenic potential in the calvarial critical size defect, one hypothesis is that Ad-MSC osteogenic potential could depend on the bone healing environment that is delayed when an endochondral ossification step occurred.

6. Discussion and Conclusion

The interest of MSC for regenerative medicine and their use as therapeutics in clinical trials has considerably increased over the last decade. This interest has been fed by the discovery of various sources of MSC, which has triggered their preclinical comparison. However, despite the large number of studies performed and articles published, an analysis of the current state of literature underlines a strong heterogeneity between studies, making the comparison difficult.

In the present article, we reviewed, in the context of bone repair, the general characteristics and the osteogenic capacity of MSC from bone marrow and adipose tissue, which are the two main sources of MSC currently studied preclinically and clinically. In this review, literature was screened for side-by-side comparison of BM and Ad-MSC and only articles that compared MSC from the same donors or from age-and/or gender matched donors were retained.

With respect to yield and proliferation, the analysis of comparative data gives inconsistent results and the superiority of one source over the other could not be concluded (5 studies favoured Ad-MSC, 3 favoured BM-MSC and 2 supported equivalence). This could be explained by the fact that many factors other than donors' age or gender (e.g., harvesting site, isolation procedures, time of cell processing, culture medium and supplements, initial seeding density, passage numbers and so on) could affect the proliferation rate and culture yield of MSC.

The analysis of comparative data yields only few differences in the immunophenotype of BM-MSC and Ad-MSC (such as for CD49d, CD106 and PODXL), both MSC populations displaying the general profile of surface antigen expression defined for MSC[19]. However, at the molecular level, gene expression profiling revealed that BM and Ad-MSC have different genetic signatures suggesting a commitment toward the cell fate of their origin (i.e., osteo/chondroblastic for BM-MSC and adipocytic for Ad-MSC).

All *in vitro* studies investigating the differentiation potentials of BM-MSC and Ad-MSC when directed toward osteogenic, chondrogenic and adipogenic lineages under appropriate culture conditions agree that both have the capacity to differentiate into the three lineages. However, while some studies report that BM-MSC have a greater osteogenic differentiation capacity and Ad-MSC a better adipogenic capacity, others claim that BM-MSC and Ad-MSC display similar osteogenic, chondrogenic and adipogenic differentiation potentials. Discrepancies may be explained by difference in cell culture conditions (e.g., cell culture medium, cell passages, induction reagents). Finally, *in vivo* comparative studies support a greater osteogenic potential of BM-MSC both qualitatively and quantitatively.

In view of the results of this critical literature review, the development of standardized protocols and

methods, the establishment of additional cell specifications and the application of rigorous quality controls are required to ensure reliable and consistent results in preclinical studies involving MSC.

Conflict of Interest and Funding

All authors are employees of Bone Therapeutics S.A. or Skeletal Cell therapy Support, an affiliate of Bone Therapeutics S.A. This review has no direct link on the company's products and the authors declare that no conflict of interest exists.

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