A Systemic Review of Adult Mesenchymal Stem Cell Sources and their Multilineage Differentiation Potential Relevant to Musculoskeletal Tissue Repair and Regeneration

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Abstract

Adult mesenchymal stem cells (MSCs) were first isolated from bone marrow by Friedenstein in 1976. These cells were clonogenic, non-haematopoietic, and able to replicate extensively in vitro. The fields of regenerative medicine and tissue engineering have grown dramatically since their inception. In the decades since, MSCs have been identified from mesoderm-, endoderm- and ectoderm-derived tissues. In light of our ageing population, the need for effective cell-based therapies for tissue repair and regeneration is ever-expanding.

Online published articles were searched for using the PubMed/MEDLINE and Ovid databases, and relevant articles fulfilling the pre-defined eligibility criteria were analysed. To date, MSCs have been isolated from a number of adult tissues, including trabecular bone, adipose tissue, bone marrow, synovium, dermis, periodontal ligament, dental pulp, bursa and the umbilical cord. Bone marrow MSCs are currently considered the gold standard, with which newly discovered sources are compared on the basis of their renewal capabilities and multipotency. Furthermore, MSCs have been successful in the regeneration of osteonecrosis, osteoarthritis, bony defects, fracture remodelling and so on.

Unfortunately, significant hurdles still remain and will need to be overcome before tissue engineering using MSCs becomes routine in clinical practice. Thus, further research and understanding is required into the safe and effective sourcing and application of mesenchymal stem cells in musculoskeletal applications.

Keywords

Mesenchymal stem cells, MSC, MSC applications, musculoskeletal applications, stem cells, tissue engineering.

Introduction

Adult mesenchymal stem cells (MSCs) were first isolated from bone marrow, described as mononuclear cells, similar to fibroblasts. These cells were clonogenic, non-haematopoietic and they adhered to plastic in culture, replicating extensively in vitro [1, 2, 3]. Evidence of their differentiation capacity was demonstrated by Friedenstein et al., [2] in seminal studies revealing the ability of MSCs to generate cartilage, bone, myelosupportive stroma, adipocytes, and fibrous connective tissue. The fields of regenerative medicine and tissue engineering have grown dramatically since their early inception in the 1960s [4]. In the decades since, MSCs have been identified from mesoderm-, endoderm, and ectoderm-derived tissues. These have been known by many different names other than the original 'mesenchymal stem cells' coined by Arnold Caplan [5], including; mesenchymal stream cells [6], bone marrow stromal cells [7] and marrow-isolated adult multipotent inducible cells [8].

Considering our ageing population, the need for effective cell-based therapies for tissue repair and regeneration will continue to increase. 1 in 2 adults older than 18 years (107.7 million persons) reported experiencing a musculoskeletal condition lasting 3 months or longer [9]. Indeed, age-related musculoskeletal disorders represent a major cause of morbidity globally and result in enormous costs for health and social care systems [10]. The ability of MSCs to differentiate in vitro into chondrocytes, osteocytes and myocytes holds great promise for the future of tissue regeneration and repair in musculoskeletal diseases [11].

The purpose of this systematic review is to summarise evidence from the most recent studies outlining different sources of adult MSCs and their suitability in musculoskeletal applications. Studies were identified using Pubmed/MEDLINE database. Despite the existence of other comparable reviews, this area of medicine is growing at a significant pace, and therefore a more current review is called for.

Stem cells are an undifferentiated population, capable of endless self-renewal and differentiation down one or more lineages to produce specialised cell types [12]. They can be categorised into two classes 1) embryonic stem cells (ESCs) and 2) adult stem cells. The earliest stem cell in the human body, the fertilised egg, is totipotent and has the

capacity to differentiate into any cell derivative of the ectoderm, endoderm and mesoderm, essentially into all cell types of the human body [13, 14]. However, their application in tissue engineering is greatly hindered by the risk of tumour formation, immune concerns and political and ethical considerations. In contrast, post-natal adult stem cells (somatic cells) are multipotent, immunocompatible, with no ethical concerns related to their use [15]. Located in many tissues of the human body, they are required to restore normal function via repair and regeneration of tissues in vivo. They exist in a quiescent state until activated by mediators of injury or disease [16]. MSCs are non-haematopoietic cells of mesodermal derivation that are present in a number of post-natal organs, and connective tissues and are multipotent [17]. Hence, the use of MCSs in clinical applications is being investigated further as they are not burdened by the same ethical concerns and restrictions associated with embryonic stem cells [18].

The term ‘mesenchymal stem cell’ has been applied to these cultured cells as they have a high capacity for self-renewal and the ability to differentiate into a number of different tissues of mesenchymal origin [19]. Today, they are described as ‘fibroblast-like’ cells that can mature along multiple different pathways according to their trophic activity [20]. MSCs are defined by the International Society of Cellular Therapy as multipotent stromal cells on the basis of three agreed-upon characteristics [21]. Firstly, their adhesion and ability to form colonies when initially plated on tissue culture plastic; secondly, they must be capable of extended *in vitro* expansion, while maintaining the potential to differentiate along osteoblastic, adipocytic and chondrocytic pathways; thirdly, they must not exhibit CD14, CD34 and CD45 and human leukocyte antigen-DR (HLA-DR) which are characteristic epitope markers of haematopoietic stem cells (HSCs), and they must express the following set membrane molecules; CD73, CD90 and CD105 [21, 22, 23].

Between all these tissues, bone marrow (BM) was the first source reported to contain hMSCs and is considered the main source of these stem cells for clinical and experimental applications. However, the percentage of hMSCs obtained from BM is very low, approximately 0.01–0.001% of total mononuclear cells [5, 24]. Furthermore, bone marrow aspirates seldom yield more than $5x10^4$ cells per 20 mL of aspirate, which further diminishes as patients age [25]. This is a major limitation, as the volume of bone marrow that can be harvested is minuscule when compared with the number of cells necessary to regenerate the injured tissues [26]. What is more, the harvesting procedure is painful and associated with significant morbidity [27]. Consequently, new techniques of MSC isolation, expansion, and differentiation have been added to the therapeutic repertoire for

musculoskeletal regeneration, thus overcoming these issues [28]. Other sources of MSCs therefore hold great promise for regenerative therapies in the musculoskeletal system.

An awareness of the dual role exhibited by MSCs in tissue repair has increased. It is now known that MSCs also release trophic factors that alter the local environment, facilitating replacement by local progenitors [29]. Furthermore, MSCs secrete numerous bioactive factors that influence resident precursor cells (with similar stromal origins) to undergo differentiation [30]. These factors mediate key aspects of tissue repair, including angiogenesis and secretion of neuroregulatory peptides and cytokines that have a crucial role in inflammation [30]. This *in vivo* role of MSCs in tissue repair is presumed to be initially dependent on signalling cascades initiated at sites of injury. Yet, what ensues thereafter is compelling. As platelets aggregate, they release densely packed alpha granules laden with cytokines. An influx of macrophages and neutrophils follows, hyper stimulating the inflammatory responses. Blood vessel permeability increases, setting the stage for MSC homing [31]. Homing is the mechanism by which MSCs migrate and aggregate to injured tissues [31]. Moreover, they produce essential cytokines such as transforming growth factor B, vascular endothelial growth factor and epidermal growth factor and secrete an array of bioactive molecules that stimulate local tissue repair [32]. It is suggested that MSCs secrete paracrine factors that elicit regenerative responses to enhance angiogenesis [33], promote skin wound healing [34], stimulate fracture healing [35], repair nervous degeneration [36], and treat cardiovascular disease [37]. These novel abilities exhibited by MSCs illustrate why this area of medicine has grown dramatically in the last decade.

A fascinating feature of MCSs are their ability to modulate immune and inflammatory responses and their ability to release active molecules that affect cell migration, proliferation and survival at the site of the lesion [20]. Interest in MSCs have further expanded in recognition of their ability to release growth factors and to adjust immune responses [38]. MSCs are easily isolated from patients and can be used autologously and allogenically, allowing for re-implantation in human subjects without triggering an immune response [39]. Indeed, MSCs have been found to suppress inflammatory T-cell proliferation, and found to inhibit the maturation of monocytes and myeloid dendritic cells resulting in an immunomodulatory and anti-inflammatory effect **[20]**. Research highlighting the pro-inflammatory cytokines involved in the destruction of hyaline cartilage and development of degenerative osteoarthritis has identified the potential of MSCs as disease modifying agents [40]. Therefore it seems that MSCs have scope beyond

their multi-lineage potential such as treating graft-versus-host, cardiovascular, neurological and autoimmune disease [41].

Methods

Online published articles were searched for using the PubMed/MEDLINE and Ovid databases.

With a PubMed/MEDLINE database search, the following MeSH-terms (Medical Subject Headings) and controlled vocabulary thesaurus was used for indexing articles: ‘mesenchymal stem cells’ [MeSH], OR ‘MSC [MeSH], OR ‘stem cells’ [MeSH], OR ‘MSC applications’ [MeSH], OR ‘musculoskeletal applications’ OR ‘tissue engineering’ [MeSH].

Limits were set for: ‘English language’, ‘full text’, ‘adults 19+’ and a publication date in the last 5 years. Furthermore, the reference lists of relevant articles found were searched for additional studies deemed relevant.

A total of 543 articles were identified through the PubMed/MEDLINE database search and were assessed for eligibility following the inclusion and exclusion criteria.

With regard to the Ovid database, a search was conducted using the same key words with the addition of: bone marrow, adipose tissue, muscle, periosteum, synovial membrane, osteonecrosis, osteoarthritis, osteogenesis imperfecta and muscular dystrophy. A total of 630 articles were identified and assessed for their relevance based on the eligibility criteria.

Eligibility Criteria

The following inclusion criteria was used to select appropriate studies.

- Identification of sources of MSCs in human adult tissues

Sources of Mesenchymal Stem Cells

- Information about the suitability and applicability of MSCs in musculoskeletal conditions in adults

Articles were excluded on the basis that they:

- Did not include information about MSCs
- Involved stem cells not found in adults, e.g. embryonic stem cells
- Did not refer to potential uses of MSCs in musculoskeletal diseases.

Figure 1 in the results section shows the stepwise selection procedure for this systematic review.

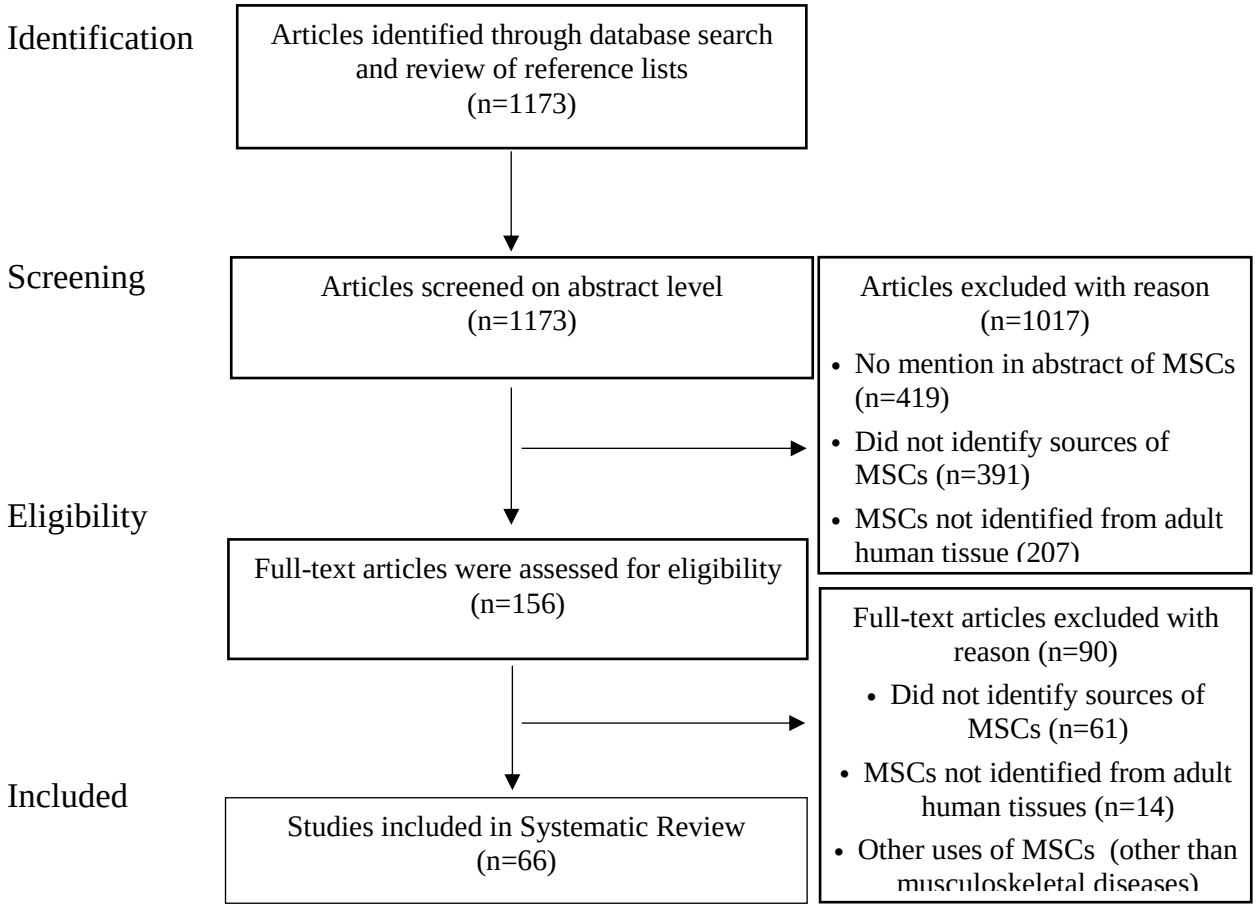
Of the 543 articles identified on PubMed/MEDLINE, 27 met the predefined inclusion criteria and were analysed.

Of the 630 articles identified on the Ovid database, 39 met the standards of the eligibility criteria and were analysed.

In total, 66 articles met the standards of the eligibility criteria and were analysed in this systematic review.

Results

Figure 1.



Tables 1 and **Table 2** summarise the most significant findings taken from the articles included in the review.

Table 1 identifies the most recent research outlining the different sources of adult MSCs and their multilineage differentiation potential. MSCs have been identified and isolated from a multitude of adult tissues, albeit in small numbers. Based on their clonogenic and multipotent differentiation activities, MSCs have, to date, been isolated from bone

marrow [42],[43], adipose tissue [44], synovium [66], dermis [45], periodontal ligament [46], dental pulp [47], gingival tissues [48], bursa [49], ligaments [50], peripheral blood [51] as well as prenatal and perinatal sources such as the umbilical cord [52, 53]. Bone marrow MSCs are currently considered the gold standard, by which newly discovered sources of MSCs are compared on the basis of renewal and multipotency [43]. Bone marrow MSCs are typically isolated from the iliac crest, but they have also been found in bone marrow cavities such as the vertebrae bodies [54]. Interestingly, Cavallo *et al* found that multipotent MSCs could be harvested from the iliac crest bone marrow of cadaveric donors 24 hours after death [43]. These cells demonstrated similar or better musculoskeletal differentiation potential, but a lower frequency of colony forming units when compared to MSCs derived from adipose and term placenta tissues [43].

Nevertheless, MSCs derived from adipose tissue (aMSCs) are fast becoming a more attractive source and have been isolated from various locations in the body. The easiest source for aMSCs is the abdomen following liposuction plastic surgeries, due to the large quantities removed and otherwise discarded fatty tissue [43]. Adipose-derived MSCs are ubiquitously available and have been shown to be up to 500 times more prevalent than bone marrow MSCs when comparing an equivalent volume of tissue (lipo-aspirate vs bone marrow aspirate) [55]. Similarly, MSCs isolated from trabecular bone and synovium during total joint replacement procedures e.g. knee joint reconstruction, are easy to obtain and have comparable proliferative capabilities [56].

It has been reported that cells isolated and cultured from the dermis exhibit characteristics of MSCs [42]. These cells were shown to be capable of undergoing in vitro differentiation into adipocytes and neurones [45]. Furthermore, a study by Vaculik and colleagues demonstrated the differentiation ability of dermal MSCs into adipogenic, osteogenic and chondrogenic lineage [57]. However, reports are still limited and further research is required to fully characterise and determine their capacity [58].

The discovery of MSC-like cells deriving from induced pluripotent stem cells (iPSCs) is intriguing. This combines the advantage of the unlimited proliferative capacity of iPSCs with the well-known properties of bone marrow MSCs which could lead to the ability to generate large amounts of highly uniform batches of MSCs [41]. Furthermore, the possibility of reproducing patient specific multipotent human MSC-like cell preparations is a promising enterprise in the field of regenerative medicine.

Peripheral blood MSCs are being increasingly studied, sharing similar biological characteristics with MSCs derived from bone marrow or adipose tissue [51]. They offer an autologous low-cost source of stem cells which may be easily harvested from patients' blood via non-invasive procedures [51].

Table 1. Sources of Adult MSCs and their Multilineage Differentiation.

Adult MSCs Sources	Multilineage Differentiation Potential	Reference
Dermis	Osteocyte, Adipocyte, Chondrocyte, Neuronal, Glial, Myofibroblast, Melanocyte, Schwann cell, Myocyte	Vapniarsky *et al*., 2015 [45]
Adipose tissue	Osteocyte, Adipocyte, Chondrocyte	Yang *et al*., 2014 [44] Iwen *et al*., 2014 [59]
Dental pulp, periodontal ligament, gingival tissues(dental tissues)	Osteocyte Adipocyte, Chondrocyte Osteoblasts	Di Benedetto *et al*., 2014 [47] Lei *et al*., 2014 [46] Rodriguez-Lozano *et al*., 2012 [48]
Inflamed gingival tissues	Osteocyte, Adipocyte, Chondrocyte	Ge *et al*., 2012 [60]
Temporomandibular Joint (TMJ) Synovium	Osteocyte, Chondrocyte, Adipocyte, Neurogenetic lineages	Koyama *et al*., 2011 [61]
Bone Marrow	Osteocyte	Gao *et al*., 2014 [42]
Bone Marrow (Iliac Crest of cadaveric donors)	Osteocyte, Chondrocyte	Cavallo *et al*., 2011 [43]
Muscle	Osteocyte	Gao *et al*., 2014 [42]
Induced pluripotent stem cells (iPSCs)	Osteoblast, Adipocyte, Chondrocytes	Hynes *et al*., 2013 [62] Tang *et al*., 2014 [63]
Urine	Smooth muscle cells, skeletal muscle cells	Bharadwaj *et al*., 2013 [64]
Synovium	Chondrocytes	Chang *et al*., 2014 [65]
Bursa	Tenocytes, Osteoblasts, Adipocytes, Chondrocytes	Song *et al*., 2013 [49]
Perivascular stem cells	Osteoblasts	Askarinam *et al*., 2013 [66]
Infrapatellar fat pad (IFP)	Chondrocytes	Liu *et al*., 2012 [67]
Human intervertebral disc cartilage endplate (CEP) — degenerated human CEP	Osteoblasts, Adipocytes, Chondrocytes	Liu *et al*., 2011 [68]
Synovial membrane	Osteoblasts, Adipocytes, Chondrocytes	Kim *et al*., 2011 [69]
Facet joints & interspinous ligaments	Osteoblasts, Adipocytes, Chondrocytes	Kristjansson *et al*., 2016 [50]

Sources of Mesenchymal Stem Cells

Adult MSCs Sources	Multilineage Differentiation Potential	Reference
Peripheral blood	Osteocyte	Wu *et al*., 2015 [51]
Total Knee Replacement (TKR) tissues (adipose; synovial tissue; subchondral trabecular bone, osteoarthritic cartilage)	Osteocyte, Adipocyte, Chondrocyte	Labusca *et al*., 2013 [56]

With regard to the musculoskeletal applications of MSCs, **Table 2** summarises the more recent available evidence. Skeletal defects are emerging as key targets for treatment using MSCs due to the high responsiveness of bone to interventions in animal models [38]. Musculoskeletal applications are of utmost importance as pathological or traumatic orthopaedic events are becoming one of the most recurrent causes of disability in the world [25]. Despite limited evidence-based trials supporting the use of MSCs in treating musculoskeletal disease, their clinical importance has been highlighted and a growing body of research is now emerging.

To date, bone marrow-derived MSCs have been used as a regenerative therapy for cartilage regeneration in osteoarthritic knees [70], in the healing of upper limb fractures and in bone non-union [71], and osteonecrosis of the femoral head [72] amongst other applications. Its potential for human tendon tissue engineering [73], bone defect and fracture regeneration and remodelling has also been demonstrated [71, 74]. Furthermore, injection of adipose-derived MSCs into the retro-patellar joints of three patients presents a new promising, safe and effective non-surgical method of treating patients with chondromalacia patellae [75]. The study revealed a continuous anterior knee pain improvement of 80-90% after 3 months [75]. Freitag *et al*., 2015 [76], are currently conducting a pilot randomised controlled trial with 40 participants investigating isolated femoral condyle chondral defects. The study aims to compare arthroscopic microfracture alone versus in combination with postoperative autologous adipose derived MSC injections. Intra-articular chondral defects remain a huge challenge, particularly where inadequate healing predisposes a patient to the development of osteoarthritis. Moreover, preclinical trials have indicted the capacity of MSCs to influence chondral repair, a major development in the management of chondral defects [76].

Another notable discovery is the isolation of MSC's from TMJ synovium, which has been shown to aid the repair of osteoarthritis and osteonecrosis of the TMJ in immunodeficient mice [77]. Furthermore, MSC's derived from umbilical cords were found to alleviate,

effective and safely, the symptoms of Juvenile Idiopathic Arthritis [78]. Finally, a level II randomised controlled trial was conducted evaluating articular cartilage regeneration in patients with chondral lesions: these were treated with arthroscopic subchondral drilling followed by postoperative intra-articular injections of hyaluronic acid, with and without peripheral blood MSCs [79]. It was revealed that post-operative intra-articular injections of autologous MSCs from the peripheral blood in combination with hyaluronic acid resulted in an improvement of the quality of articular cartilage repair over the control group, shown by histology and MRI [79].

Table 2. Sources of MSCs and their suitability in Musculoskeletal Applications.

Adult MSCs Sources	Musculoskeletal Application	Reference
Adipose Tissue	Intra-articular injection of 1.0 x 10^8 AD MSCs into the osteoarthritic knee improved function and pain of the knee joint without causing adverse events, reducing cartilage defects by regeneration of hyaline-like articular cartilage	Jo *et al.*, 2014 [70]
Adipose Tissue (derived from osteoporotic patients) (opASCs)	Osteogenic potency of opASCs to offer new possibilities for osteoporosis-related bone tissue engineering in male and female patients	Jiang *et al.*, 2014 [80]
Adipose Tissue	Potential for human flexor tendon tissue engineering — following reseeding on human tendon scaffolds *in vivo* and aid in graft integration	Schmitt *et al.*, 2013 [73]
Adipose Tissue	Creation of vascularised tendon equivalent in vitro, which could easily be detached from the bioreactor, thus facilitating implantation at the lesion site	Vindigni *et al.*, 2013 [81]
Adipose Tissue	Chondrocytes from MSCs of adipose tissue grown in nodules were able to express lubricin, and collagen type I and II, indicative of hyaline cartilage formation. Chondrocyte nodules producing lubricin could be a novel biotherapeutic approach for the treatment of cartilage abnormalities.	Musumeci *et al.*, 2011 [74]
Adipose Tissue	Restoration of damaged tissue (softened cartilages) in patients with chondromalacia patellae (who have continuous anterior knee pain)	Pak *et al.*, 2013 [75]
Adipose Tissue	Case Series — Bone formation in osteonecrosis of femoral head & cartilage regeneration in knee osteoarthritis	Pak *et al.*, 2011 [72]

Sources of Mesenchymal Stem Cells

Adult MSCs Sources	Musculoskeletal Application	Reference
Adipose Tissue, Bone Marrow	Potential cell-based intervertebral disc (IVD) regeneration when combined with GDF6 growth factor.	Clarke *et al.*, 2014 [82]
Bone Marrow	Highly autologous treatment can be effective and safe in long-term healing of bone non-unions. This tissue engineering approach resulted in successful clinical and functional outcomes for all patients.	Giannotti *et al*., 2013 [71]
Bone Marrow	Allogenic bone scaffold loaded with MSC in the reconstruction of mandibular continuity defects (e.g. after tumour resection, maxillofacial injury, osteomyelitis)	Zamiri *et al*., 2013 [83]
Bone Marrow	Radiographic healing of 8 different types of upper limb fractures and pseudo-arthritis and delayed consolidation. No adverse events were highlighted. This is encouraging but not conclusive and further investigation needed.	Giannotti *et al.*, 2013 [84]
Bone Marrow	Case Report — Adequate bone regeneration of alveolar bone atrophy in 58 year old patient — potential novel options in dental implant treatment with severe alveolar bone atrophy.	Yamada *et al*., 2013 [85]
Bone Marrow	Meta Analysis — Implantation of autologous MSCs into the core decompression track, particularly when employed at early (pre-collapse) stages of osteonecrosis of the femoral head (ONFH), would improve the survivorship of femoral heads and reduce the need for hip arthroplasty	Papakostidis *et al.*, 2015 [86]
Bone Marrow	Preliminary Report — Osteoarthritis of the knee improved in terms of walking time to pain, number of stairs, pain visual analogue scale, crepitus & range of movement.	Davatchi *et al.*, 2011 [87]
Periodontal Ligament, Gingival Tissues	Suitable stem cell sources for tendon engineering	Moshaverinia *et al*., 2014 [88]
TMJ Synovium	Potential use for TMJ repair and regeneration e.g. osteoarthritis, osteonecrosis	Wu *et al.*, 2014 [77]
Induced pluripotent stem cells (iPSCs)	iPSCs derived from adult marrow CD4+ cells — potential to promote bone regeneration in dental, craniofacial, and orthopaedic repairs	Tang *et al.*, 2014 [63]
Bursa	Potential for application in tendon repair — formed tendon-like tissue *in vivo.*	Song *et al.*, 2013 [49]

Sources of Mesenchymal Stem Cells

Adult MSCs Sources	Musculoskeletal Application	Reference
Umbilical Cord	UC-MSCs have the potential to reduce inflammatory cytokines, improve immune network effects, adjust immune tolerance, and effectively alleviate the symptoms and they might also provide a safe and novel approach for Juvenile Idiopathic Arthritis (JIA)	Wang *et al.*, 2015 [78]
Peripheral Blood	Intra-Articular injections of peripheral blood MSCs following arthroscopic subchondral drilling of knee chondral defects resulted in an improvement of the quality of articular cartilage repair over the same treatment without peripheral blood MSCs.	Saw *et al*., 2013 [79]

Discussion

A review of the current evidence regarding the sources of MSCs as well as their suitability in musculoskeletal applications has kindled the debate as to whether bMSCs or aMSCs is the most valuable in terms of multilineage potential, proliferation ability and use in regenerative stem cell therapy.

An ideal MSC source has yet to be identified where an MSC population could be easily harvested in abundance, with minimal morbidity and with high purity [89]. The most common source of MSCs to date has been bone marrow. Yet, aspirating bone marrow is an invasive and painful procedure, sometimes requiring general or spinal anaesthesia [90]. These limitations led to the exploration of alternative tissue sources and the possibility of niches containing undiscovered MSCs with similar characteristics to bone marrow derived MSCs.

The discovery of adipose-derived stem cells in rodents by Rodbell in 1964 was a significant advancement in the field [91]. Multipotent stem cells within adipose tissue are one of the most promising MSC populations identified, since human adipose tissue is ubiquitous and can be easily obtained in large quantities with very little donor site morbidity or patient discomfort [92]. Furthermore, there is a greater yield from adipose tissue than from other stem cell reservoirs, a significant factor for use in regenerative medicine. As many as 1×10^7 aMSCs can be isolated from 300 ML of lipoaspirate [92]. To strengthen the debate for aMSCs, bMSCS have been shown to lose potency with age with certain disease states like osteoporosis, whereas aMSCs are thought to be a more

efficient regenerative cell course given their protection from physiological stress [93]. Indeed, aMSCs have been shown to have a greater potential for proliferation, higher rates of colony formation, and greater tolerance to serum deprivation-induced apoptosis than their bone marrow counterparts [94].

Peripheral blood MSCs are of increasing interest as they share similar characteristics with MSCs derived from bone marrow and adipose tissue. They offer an autologous low-cost source of cells which can be easily harvested from patients' blood via a relatively non-invasive procedure [51]. A recent trial, assessed the efficacy of intra-articular injections of peripheral blood MSCs following arthroscopic subchondral drilling of knee chondral defects. The trial found a statistically significant improvement in cartilage quality after histological and MRI analysis at 18 months, when compared to subchondral drilling without MSCs [79].

Interestingly, a recent study revealed that the age and BMI of patients has a strong influence on the differentiation pattern of MSCs. aMSCs proliferation isolated from individuals aged <30 years was greater than those individuals aged >50 years old [44]. Moreover, BMI correlated with osteogenic differentiation; an increased BMI seemed to enhance osteogenesis. Finally, bMSCs were strongly induced to differentiate along both osteogenic and adipogenic lineages, whereas aMSCs predominantly differentiated into the chondrogenic lineage. Thus, it seems that the type of regeneration required must be carefully considered when selecting MSCs for use in clinical tissue engineering [44].

Another important finding is the MSCs capacity for immunomodulation. It has been demonstrated that these properties can be employed to alleviate inflammatory conditions [20]. Pre-clinical research has uncovered that MSCs derived from bone marrow, adipose, synovial and umbilical-cord are able to suppress the functions of different immune cells, thus highlighting their potential for therapeutic use in autoimmune disease such as rheumatoid arthritis [95].

Another question of significance is their response to ageing. In the UK, our ageing population means that the prevalence of age-related musculoskeletal disorders are increasing [16]. It has already been established that MSCs are promising cell sources for regenerative therapies, but their application is likely to be complicated by patient factors such as age and specific illnesses [96]. Considering the fact that MSCs are being currently investigated for the treatment of disorders such as osteoarthritis, osteoporosis and osteonecrosis of the femoral head, it is paramount that the effects of ageing on MSCs are studied [96]. It has been demonstrated that bMSCs proliferation rate and differentiating potential decrease with donor age [97]. Despite the adverse affects of ageing on bMSCs, leading to impaired proliferation, senescence, and chondrogenic response, it seems

muscle- and adipose-derived MSCs exhibit no negative effects [96]. Not only that, age reduces the overall cell yield and adipogenic potential of all MSC populations, whilst osteogenesis and clonogenicity remain unchanged [96].

It has been established that high donor-to-donor variability exists between the pre-, peri- and post-menopausal age groupings. Interestingly, cell lines derived from postmenopausal donors demonstrate a relatively high propensity for osteogenic differentiation and a relatively low proclivity for adipogenic differentiation [98]. Indeed, it can be concluded that MSCs undergo a decline in their expansion capacity with physiological ageing, meaning that age-related change may be detrimental for their successful use in tissue regeneration therapies [99].

Despite the promise of MSCs, it must be acknowledged that they possess characteristics that limit their usage. Firstly, MSCs exhibit significant heterogeneity between different sources, as well as amongst a single isolation of cells [100]. This heterogeneity can cause different behaviour with regard to their proliferation and differentiation patterns [101]. Despite little published evidence supporting the clinical application of MSCs in treatment therapies, regenerative medicine clinics throughout the US are already offering ultrasound and fluoroscopy-guided, minimally invasive treatments using MSCs harvested via bone marrow and adipose tissue aspirations [102, 103]. The sudden proliferation of these treatments, at a stage where evidence-based studies are lacking, raises significant concerns. Indeed, major uncertainty still surround MSC-based therapies, notably abnormal growth and potential cancer evolution [83].

Conclusion

Mesenchymal stem cells provide promising therapeutic possibilities for tissue engineering. Their ability for repair and regeneration of a variety of tissues such as bone, cartilage and muscle is exciting for the field of tissue engineering in the context of the rising global burden of musculoskeletal disorders. Unfortunately, significant hurdles still remain before tissue engineering using MSCs becomes routine in clinical practice. The future of this rapidly growing field depends not only on technological advancements but more importantly on the continued proliferation of organised clinical trials to investigate in more detail their efficacy and safety**.** The process of transferring tissues from the laboratory into human recipients is still in its infancy. New scaffold materials and conditions are necessary to demonstrate successful clinical outcomes**.** Nevertheless,

research into the clinical application of mesenchymal stem cells will continue until safe and effective means of tissue engineering are fully understood.

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None

Conflict of Interest

None declared.

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