Enhancing the Potency of Mesenchymal Stem Cells for Tissue Regeneration

Sylvia Müller, Kenny Dalgarno, Anne Dickinson, Xiao-nong Wang and Lindsay Nicholson

Abstract

Mesenchymal stem cells (MSCs) are adult stromal cells with multi-lineage differentiation potential and immunomodulatory properties. They can be isolated relatively easily from several tissues, including bone marrow and adipose tissue and can be expanded ex vivo to yield the large numbers required for a therapeutic dose. As such, MSCs are considered promising cell source that can be used in cellular therapy for a wide range of diseases, including bone and cartilage defects. However, despite the huge number of clinical trials utilising MSCs, their clinical application has yielded variable results, often attributable to different donor sources and expansion protocols. The objective of this concise review is to summarize recent developments regarding tissue source of MSCs, use of phenotypic markers for MSC selection, as well as potential in vitro modifications that may be stratified and applied to enhance the potency of MSCs for clinical use in regenerative medicine.

Keywords

Mesenchymal stem cell, CD271, Bone marrow, Clinical trials, Regenerative medicine

Introduction

Mesenchymal stem cells (MSCs) are adult stem cells with fibroblast-like morphology. In the late 60’s and 70’s, transplantation studies showed that a small sub-population of cells present in the bone marrow could aid osteogenic regeneration [1]. Studies by Friedenstein, Chailakhjan and Lalykina [2] named this population ‘colony forming unit fibroblasts’, due to their colony forming ability. This was changed in 1987 to ‘Osteogenic stem cell’ [3]. The nomenclature used to describe this cell type is contentious. The term ‘Mesenchymal stem cell’ came into use in the 1990’s, being coined by Caplan [4] and this remained as the term for these cells until an International Society of Cellular Therapy (ISCT) position paper by Horwitz et al., [5] proposed that the official term be changed to “multipotent mesenchymal stromal cells”. The paper stated that as plastic adherent populations isolated from the bone marrow shared certain properties they should be classed together as one type of cell. However, the word ‘stem’ was removed as not all plastic adherent cells derived from the bone marrow exhibit the stem cell properties. The word stromal was used as its replacement owing to the stromal location MSCs are derived from in the original tissue.

The current classification of MSCs was set by the International Society of Cellular Therapy (ISCT) in the position paper by Dominici et al. [6]. This listed the three minimal criteria that must be met to define cells as MSCs: plastic adherence, tri-lineage differentiation potential (osteogenic, chondrogenic and adipogenic) and expression of a specific surface phenotype. In addition to the minimal criteria, MSCs have also been described as hypo-immunogenic [7] (though current research disputes this and have described them as immune-evasive [8]) and immunomodulatory [9-15] making an allogeneic “off-the-shelf” therapy an attractive possibility. Unsurprisingly, given MSCs capabilities they show great potential for a wide range of clinical uses, most notably in treating graft versus host disease (GvHD) [16-21] and defects in bone and cartilage [22-29]. Indeed, the number of indicated uses for these cells continues to gain pace; illustrated by the fact that as of October 2015 there were 554 clinical studies involving mesenchymal stem cells listed on clinicaltrials.gov. However, to date, MSCs used clinically have yielded heterogeneous results, though no adverse effects have ever been reported. This heterogeneity can be largely attributed to differences in donor source and ex vivo expansion methods. This review summarises the current MSC selection criteria, as well as potential in vitro modifications that may be utilised to enhance the potency of MSCs for clinical use, with an emphasis on regenerative medicine.

Donor Source

MSCs have been isolated from a number of tissues including the bone marrow (BM) [30], adipose tissue (AT) [31], umbilical cord blood (UCB) [32], Wharton’s jelly [33] and dental pulp [34]. MSCs from different sources have been extensively compared and have shown to have a unified phenotype and similar morphology. The main variation in cells from different sources lies in their differentiation potential, proliferation rates and the potency of their immunosuppressive actions.

UCB-MSCs have faster growth rates than BM- and AT-MSCs [35,36] at the expense of a weaker differentiation potential [37]. UCB-MSCs can be problematic to isolate as the procedure requires a large volume of blood processed immediately after collection [38]. Bone marrow collection is an invasive and unpleasant process which may only yield a small number of stromal cells. Adipose tissue and liposapirate can be collected as biological waste from cosmetic
procedures [39], and contains more cells per gram of tissue compared to bone marrow [40,41]. As well as their longevity in in vitro culture, AT-MSCs produce higher levels of cytokines and have stronger immunosuppressive potential [42]. However the differentiation potential of BM-MSCs has already been primed for cell doubling and long term culture [45]. This suggests that those cells are faster proliferators.

Phenotype

A single marker that can identify all MSCs in a population has yet to be found, instead, a mixed panel of positive and negative markers has been put forward by the ISCT. The phenotype for a population of MSCs is 95% positive expression of CD73, CD90 and CD105 and less than 2% positivity for CD14, CD19, CD34, CD45 and HLA-DR [6]. The three positive markers are consistently found on MSCs, independent of passage or source and not found on other cell types in this combination. Non expression of the negative markers ensures the absence of contaminating cell types of haematopoietic lineage.

Though this panel is currently used as the standard for MSC classification, it is likely to change as more research is conducted into the properties of MSCs. The disadvantage of the current phenotypic classification of MSCs is that the CD73/CD90/CD105 population is highly heterogeneous in its functional capacity [13, 44, 45]. Clonal studies have found that during in vitro culture, less than half of BM-MSCs clones possessed the tri-lineage differentiation potential [44]. This heterogeneity becomes more pronounced as with continual cell doubling and long term culture [45]. This suggests that those cells in the conventionally isolated MSCs have already been primed for specific lineage. To obtain a purer sample of MSCs, various single and combination markers have been examined as potential targets for specific lineage. To obtain a purer sample of MSCs, various single and combination markers have been examined as potential targets for
table 1, with the surface marker, CD271, either used alone or in combination with other markers leading the way as the frontrunner for MSC selection, as discussed below.

CD271

CD271, also known as low-affinity nerve growth factor receptor (LNGFR) or p75 NTR (neurotrophin receptor), is one putative marker that potentially identifies a pure MSC population [46]. In the 1990s, studies using immunoelectron microscopy and immunohistochemistry found that fibroblast-like cells throughout the bone marrow expressed CD271 [47,48]. The majority of research using CD271+ MSCs has used bone marrow as the source tissue, including the pivotal work by Quirici et al., [49] which detailed the first successful isolation and characterisation of CD271+ cells. Work has also suggested that CD271+ MSCs are found in the greatest concentration in the trabecular bone surrounding the bone marrow [50,51]. Despite this however, some studies have successfully isolated CD271+ MSCs from adipose tissue [52] and dental pulp [53] though, to date, they have not been found in the umbilical cord blood or Wharton’s jelly [54,55]. The characteristics of the CD271+ population of bone marrow-derived cells are in line with the standard requirements for MSC classification; however, they are coupled with more potent functional properties. This includes their osteochondral differentiation ability, colony forming potential and immunomodulatory characteristics.

The immunomodulatory properties of CD271+ MSCs were assessed by Kuçi et al. [56,57]. Even at low concentrations, the ability of CD271+ cells to inhibit mononuclear cell proliferation was greater than that of the CD271− MSC population. Additionally, Kuçi et al., [58] showed that co-transplantation of CD271+ MSCs and T cells can lead to an increased level of T cell repopulation. Jones et al. [59] proposed that the CD271+ cells are MSC precursor cells. Churchman et al. [60] published a transcriptional study of CD271+ MSCs and standard cultured MSCs found that the CD271+ population expressed greater levels of genes involved in bone production, suggestive that the CD271+ MSC cells are primed to undergo osteogenesis. In line with this, differentiation studies have found that human MSCs enriched for CD271+ cells have a greater osteogenic potential compared to the non-enriched fraction. Despite a possible bias for osteogenic expression, CD271+ MSCs have also been shown to undergo greater levels of chondrogenic differentiation in vivo and in vitro [61,62].

Conversely, work by Mikami et al. [53] showed that prolonged overexpression of CD271 in murine MSCs leads to the inhibition of lineage commitment. This disparity between commitment versus inhibition to lineage differentiation may be ascribed to species variation. Aomatsu et al. [63] found that signalling from the protein SCRG1 maintains expression of CD271 on MSCs in culture, with the production of SCRG1 decreasing during osteogenesis. This would suggest that CD271 is a MSC marker of stemness. The possible

<table>
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<tr>
<th>Marker (AKA)</th>
<th>Comments</th>
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<tr>
<td>CD44</td>
<td>CD44 is a receptor for the ECM component Hyaluronan and may mediate the migration of MSC.</td>
<td>[169]</td>
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<tr>
<td>CD73</td>
<td>Classic MSC characterisation marker in combination with CD90 and CD105. May be a mediating factor in MSC differentiation.</td>
<td>[6, 170]</td>
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<tr>
<td>CD105 (Endoglin)</td>
<td>Though expressed by numerous cell types including endothelial cells and fibroblasts, enrichment of CD105+ cells from bone marrow has been seen to result in large numbers after short term culture. Classic MSC characterisation marker in combination with CD73 and CD90</td>
<td>[6]</td>
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<td>CD106 (V-CAM-1)</td>
<td>Highly expressed on placental MSCs and weakly expressed on AD and BM MSCs. CD106+ MSCs have a weaker clonogenic potential but greater immuno-suppressive capabilities.</td>
<td>[171]</td>
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<td>CD146 (MCAM)</td>
<td>CD146 is expressed by pericytes and perivascular MSCs. High CD146 expression in MSCs suggests commitment to the vascular smooth muscle cell line.</td>
<td>[172-174]</td>
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<td>CD271 (LNGFR)</td>
<td>Potential marker for precursor MSCs located in the trabecular bone. Isolation or enrichment for CD271 may result in a more homogeneous population of cells with a greater growth, differentiation and immuno-suppressive characteristics compared to PA-MSCs.</td>
<td>[60, 56, 60]</td>
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<td>STRO-1</td>
<td>Isolation of STRO-1+ cells from the bone marrow stroma yields colony forming cells with osteogenic potential. STRO-1+ MSCs have a greater migratory capacity then STRO-1− cells; however it is also a marker expressed by CD34+ cells.</td>
<td>[175-179]</td>
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<td>SSEA-4</td>
<td>SSEA-4 is classically considered to be a marker for embryonic stem cells, however it has also been shown to be expressed by BM-MSCs.</td>
<td>[180]</td>
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<td>CD271+CD90+CD106+</td>
<td>CD271+CD90+ population contains a higher proportion of clonogenic cells. In this fraction, the CD106+ cells were the faster proliferators.</td>
<td>[181]</td>
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<tr>
<td>Stro-1+CD106+</td>
<td>Stro-1+CD106+ samples sorted directly for bone marrow were reported to be homogeneous due to consistent expression of type 1 collagen, however the rate of proliferation was still variable and strongly inhibited in some colonies.</td>
<td>[182]</td>
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<td>MSCA-1+CD56+</td>
<td>Sorted MSCA-1+CD56+ populations displayed a homogeneous phenotype, as well as a strong chondrogenic potential.</td>
<td>[183]</td>
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<tr>
<td>CD271+CD140b+</td>
<td>From sorted BM samples the CD271+CD140b population contained strongly clonogenic MSCs. CD140b was only seen on these cells.</td>
<td>[184]</td>
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<tr>
<td>CD13+CD105+CD45−</td>
<td>Isolated through cell sorting, this bone marrow-derived cell population expressed all classic MSC characteristics. Authors note that post expansion the population had an altered phenotype with decreased HLA-DR expression and increased CD146 expression.</td>
<td>[185]</td>
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stemness properties of CD271+ cells have also been found in research on cancer cells. A population of CD271+ cells have been found in squamous cell carcinomas of the head and neck [64], melanoma [65], osteosarcoma [66], and osseous squamous cell carcinoma [67]. These studies have found that the CD271+ cells proliferate faster [68], are more likely to contain colony forming cells, have greater expression of stemness genes [66,67] and have a higher level of self-renewal [69]. Additionally, these CD271+ populations are more likely to metastasise [65] and are more chemo-resistant than CD271+ cancer cells [67,70]. It is not to suggest that CD271 is a marker of tumorigenicity in MSCs, rather it exemplifies the fact that CD271 is a marker of greater growth potential and resilience.

It is important to note that MSCs lose the surface expression of CD271 in culture [71]. If CD271 is a marker of precursor MSCs with no lineage commitment, then the isolation of CD271+ cells could produce a population of cells with greater osteogenic potential as none (or very few) of the cells have committed to either the adipogenic or chondrogenic lineage. Taken together, this suggests that the CD271+ MSC populations are less potent in comparison to standard MSC populations and CD271 enrichment could yield a better product for clinical applications. With the advantages of CD271+ populations containing a higher concentration of colony forming cells [57] and having stronger differentiation and immunomodulatory potential, fewer cells would need to be isolated to be as efficacious as larger populations of standard MSCs. Furthermore, the requirement for a smaller cell number would reduce the total cost of production and decrease the risk of potential contamination during culture by using either uncultured cells or shorter expansion times. Indeed, a recent decrease the risk of potential contamination during culture by using fewer cells would need to be isolated to be as efficacious as larger populations and CD271 enrichment could yield a better product for clinical applications.

In vitro Culture Modifications

As well as selecting specific cells from a defined tissue source, alterations to the standard method of cell expansion can have a positive impact on the potency of the resulting cell product. MSCs are highly responsive to chemical and physical signals from their surrounding environment and can be both negatively and positively impacted by alterations in their culture conditions [73]. Different modifications to the traditional culture system can have different impacts on the functional properties of MSCs, as discussed below.

Serum

Possibly one of the most common alterations to standard MSC culture conditions is the replacement of foetal calf serum (FCS), also known as foetal bovine serum. In standard culture conditions, MSCs are grown in media containing 10% FCS, providing the cells with the growth factors needed for expansion. Although the use of FCS for clinical-grade MSC expansion is currently permissible, it is associated with several disadvantages and implementation of a standard xeno-free culture condition is most likely required for future clinical production. For example, cells cultured in FCS can retain bovine proteins on their cell surface and these can cause an allergic reaction in the receiving patients [74-76]. Additionally, there is a risk of transmitting prion-based diseases from cows to humans. Though recent studies have shown that these proteins can be filtered out of contaminated media [77], sources are still restricted to certified prion-free countries such as Australia and New Zealand. These, coupled with the obvious ethical problems attached to the use of FCS in cell culture [78] make alternatives actively sought, whilst maintaining or enhancing, MSC function.

The current competitors to replace FCS in cell culture are human serum and platelet lysate. These are both derived from human sources, which would remove the use of xenogenic material in culture [79]. Human serum for cell culture can be collected from either an autologous or allogeneic source. The use of autologous serum would reduce the chance of a negative reaction occurring from the serum-cultured cells, however the quality of the serum may vary from different patients. The use of allogeneic serum would eradicate this problem by pooling sera collected from numerous donors to produce a more standardised product. Cultures supplemented with 10% autologous serum were seen to have higher rates of proliferation and greater osteogenic potential compared to cultures with the standard 10% FCS [80,81]. Nimura et al. [82] suggested that the increased growth rate of cells in human autologous serum may be due to the increased level of platelet-derived growth factor (PDGF) compared to FCS. Studies have also seen that MSCs grown in autologous human serum are more genetically stable compared to those grown in FCS [83,84]. The use of allogeneic human serum for MSC expansion yielded comparable growth rates to FBS and MSCs of a similar quality and functionality [85,86]. In contrast, a study by Shahdadfar et al. [83] found that MSCs cultured with allogeneic serum were more likely to be affected by growth arrest.

Platelet lysate is obtained through repeated freeze-thaw cycles of platelets isolated from peripheral blood [87]. Human platelet lysate has been found to be applicable for the in vitro expansion of epithelial cells, fibroblasts, and MSCs from multiple sources [88-92]. Doucet et al., [93] demonstrated that platelet lysate is rich in growth factors which enhance the proliferation of MSCs. Further studies have found the phenotypic and morphological properties of MSCs grown in platelet lysate are strongly comparable to those grown in FCS. Expansion of MSCs has continuously been shown to be more efficient in platelet lysate compared to in FCS, as the platelet lysate-MSCs have faster growth rates [94-97]. Studies that have assessed the genetic stability of MSCs cultured in platelet lysate have reported a high level of chromosomal stability [97]. Lange et al. [98] suggested that culture of MSCs in platelet lysate could maintain their stemness properties due to an increased expression of cell cycle-related genes and a decrease in genes specific for lineage commitment. Additionally, studies have shown that in in vitro conditions the immunomodulatory properties of platelet lysate-expanded MSCs are either equal to, or stronger than, FCS-expanded MSCs [99]. However, a clinical trial by von Bonin et al., [100] using platelet lysate-expanded MSCs to treat acute GvHD, found that only 2 of the 13 patients responded positively to the initial treatment. Despite the low response rate in this trial, it did show that there was no negative reaction from using platelet lysate expanded MSCs, thus highlighting the clinical feasibility of using platelet lysate-expanded MSCs.

Some disadvantages to using platelet lysate for cell expansion have also been reported. Firstly, it has been suggested that MSCs cultured in platelet lysate are less plastic adhesive due to a decreased expression of adhesion-related genes [101]. This could make the initial isolation of freshly harvested cells problematic as many isolation protocols rely on the plastic adhesion of MSCs. Secondly, there is a level of lot-to-lot variability in different batches of platelet lysate which can result in problems with standardising for use in culture [102], however, the use of pooled platelet lysate batches can reduce this variability.

Growth Factor Supplements

The addition of recombinant growth factors in serum-supplemented media can enhance the growth potential of MSCs, making shorter culture times as effective as longer periods. Additionally, media containing recombinant growth factors could entirely replace serum supplementation in culture media. Matrin et al. [103] reported that the addition of 1 ng/ml fibroblast growth factor 2 (FGF-2, also known as basic fibroblast growth factor, bFGF) to media containing 10% FCS resulted in the formation of larger colonies and faster growth rates compared to the MSC cultured in control media. The addition of FGF-2 to standard osteogenic induction media also enhanced the level of matrix deposition from MSC samples. Other work has also found that the addition of FGF-2 can enhance the growth of MSCs in culture, as well as the eventual differentiation
potential of the cells [104-106]. However, addition of FGF-2 can cause low level surface expression of HLA class I and II [107], which could potentially stimulate an immune response.

Platelet-derived growth factor (PDGF) is another growth factor often used to supplement MSC culture medium, sometimes with mixed results. Cassidy et al. [108] found that 5 ng/ml PDGF added to the culture media enhanced the proliferation of MSCs, though the osteochondral differentiation potential of the cells was not consistent in vitro and in vivo. The same paper showed addition of transforming growth factor beta (TGFβ) had a similar effect as PDGF on the growth of MSCs. Gharibi et al. [109] found that stimulation of the PDGF beta receptor (PDGFRβ) can induce the expression of cell cycle-associated proteins and increase the rate of proliferation, and Tokunaga et al. [110] suggest that PDGFRβ may also inhibit osteogenic differentiation.

Some work has found that a combination of different growth factors can be as effective as serum supplementation in culture media. Ng et al. [111] and Chase et al. [112] detail a combination of TGFβ, PDGF and FGF2 added to serum-free media being as effective as serum-containing media in stimulating proliferation without altering the gene expression profile of the cells.

Hypoxia

Certain culture modifications aim to replicate the internal environment that MSCs are subjected to within the body. Currently, the standard culture environment for cells is in an incubator at 37°C with 5% CO₂ and 20% O₂; however the oxygen content within our the standard culture environment for cells is in an incubator at 37°C environment that MSCs are subjected to within the body. Currently, some work has found that a combination of different growth factors can be as effective as serum supplementation in culture media. Ng et al. [111] and Chase et al. [112] detail a combination of TGFβ, PDGF and FGF2 added to serum-free media being as effective as serum-containing media in stimulating proliferation without altering the gene expression profile of the cells.

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Whether hypoxic conditions also enhance the differentiation potential of MSCs is still unclear with some reports showing greater differentiation from cells cultured in 2-5% O₂ [122-123] and others suggesting that low oxygen inhibits differentiation [113,118,122]. The main consensus is that a hypoxic environment can maintain stemness in a cell population, with studies showing an increased or prolonged expression of genes such as Oct-4 and NANOG [116,120,124]. One such explanation for this improvement under low oxygen concentration is the maintenance of hypoxia-inducible factor I-alpha (Hif-1α). This is a transcription factor that regulates the expression of hypoxia-dependant genes. Under normoxic conditions the protein is susceptible to degradation by the proteasome [125], therefore is only stable in low oxygen environments. Active Hif-1α can inhibit the activation of the E2A-p21 complex which leads to cellular senescence and apoptosis [126]. A second hypothesis is that the normoxic environment can lead to a production of reactive oxygen species (ROS) within the cell, causing oxidative stress [119] and compromising the stability of DNA [127].

Mechanical Stimulation

Similar to hypoxia, the addition of mechanical stimulation to culture is also based on physiological conditions. As the body moves and the blood pumps, cells are subject to a degree of shear stress. This can be replicated in vitro with the use of bioreactors in which cells can be cultured [128]. These can be ‘spinner flasks’ containing a propeller which continuously stir the cell-containing media in one direction or flow perfusion bioreactors, which pump media across the cell sample. A third option is to provide cells with mechanical stimulation by simulating mechanical loading.

The mechanical stimulation provided by the movement of fluid within these devices has been shown to lead to an increased activation of mitogen-activated protein kinase (MAPK)-linked signalling pathways [129-132]. As the MAPK signalling pathways are key regulators of differentiation and proliferation, the functional benefit of mechanical stimulation is an enhanced level of osteogenesis. Certain studies have sought to discover whether mechanical stimulation could induce dexamethasone-independent osteogenic differentiation in MSCs. Holtorf et al. [133] tested MSCs cultured on a titanium fiber scaffold with or without flow perfusion-derived mechanical stimulation in the absence or presence of dexamethasone. Analysis found high levels of alkaline phosphatase activity, calcium deposition and osteopontin secretion, key markers of osteogenesis, in samples subjected to flow perfusion only in those that were preconditioned with dexamethasone-treatment. Mechanical stimulation is not limited to osteogenesis; Mauck et al. [134] demonstrated that mechanical loading of MSCs in a hydrogel can improve chondrogenic differentiation in MSCs. This study showed an increased level of aggrecan gene transcription and glycosaminoglycan production, signifying the enhanced production of cartilage.

Osteogenesis can also be augmented through mechanical loading and strain. This is believed to be due to an increased activation of the ERK/MAPK signalling pathway which regulates differentiation through inhibiting adipogenesis in favour of osteogenesis [135-137]. Other studies have failed to show conclusive evidence that mechanical stimulation (whether by flow perfusion or mechanical loading) can provide osteogenic induction in the absolute absence of dexamethasone. However, it does appear that if cells are subjected to mechanical stimulation during osteogenic induction, they will produce higher levels of bone deposition compared to the cells differentiated without mechanical stimulation [138,139].

Scaffolds

Standard MSC culture utilises a two-dimensional plastic surface on to which the cells adhere. This may be of use to in vitro research; however, it does not replicate the natural environment in which cells have evolved in and grown. Though hypoxia and mechanical stimulation may provide some of the features of their in vivo environment, changing to a three-dimensional scaffold may also be required to obtain the best possible potency from a cell population. Scaffolds are often used for tissue regeneration and can be utilised to ensure direct application of cells into an area of damage. Depending on the specific requirements, scaffolds can be made from a range of different materials including ceramics, Bioglass®, synthetic polymers, hydroxyapatite, and biological materials such as hyaluronic acid or collagen. Shared characteristics of these different materials are that they are non- immunogenic and can support the growth of the surrounding cells [140].

The stiffness of a scaffold material can impact on the cellular functions by dictating the pathway of lineage commitment in the interacting cell. Fu et al. [141] highlighted that the stiffness of a material can affect MSC differentiation. The authors produced a scaffold made of elastic, ‘polymer polydimethylsiloxane’, the surface of which was covered in small protrusions. The rigidity of these was dependant on their length, with the shorter protrusions having the greater rigidity than the longer, more flexible protrusions. They found that the cells cultured on the rigid scaffold were more spread out (in their morphology) and displayed highly organised cytoskeletons. These cells were also seen to be more likely to undergo osteogenesis. In contrast, those cells grown on a more pliable structure were more rounded in shape with a disordered actin structure and predisposed to undergo adipogenic differentiation. Therefore, scaffolds made from stiff materials are more likely to be used for bone regenerative uses; this includes ceramics and bio-glasses, which can also bond quickly to bone [142]. Conversely, materials that are more pliable can be used for cartilage and soft tissue regeneration [143-145], as well as in the production of hydrogels for wound healing [146].

As well as directing the fate of cells through their basic structure and topography, scaffold materials can impact on the functions of cells through the presentation and release of proteins, molecules, and ions. The presentation of proteins and molecules will often be linked to the structure and function of the extracellular matrix (ECM) which provides structure and signals for cell growth and function. By
Genetic Modification

Numerous studies in rodent and murine models have found that insertion of new genes into the genome of the MSC can prolong their survival, improve their functional characteristics, and enhance their production of therapeutic proteins (comprehensively reviewed in Park [157]). For example, the induced expression of CXCR4 in MSCs has been shown to enhance their homing potential and led to improved repair of cardiac [158], liver [159], and bone tissues [160–161]. The overexpression of BCL-2 has been shown to protect cells from apoptosis and improve their differentiation potential [162]. Genetically modified MSCs can also be applied to cancer therapies, with studies showing that cells modified to express IFN-α [163], IFN-β [164], TNFαF10 [165], IL-12 [166] or CCL5 [167] can increase survival in murine models of haematological malignancies and solid tumours such as multiple myeloma [165] and pancreatic carcinoma [167], respectively.

Good Manufacturing Practice (GMP)

Irrespective of the donor source, the growth conditions or the status of genetic modification, the production of MSCs for any form of cellular therapy must be ensured to be compliant with Good Manufacturing Practices (GMPs), guaranteeing the safety, efficacy and reproducibility of MSC production. Any product designed for clinical application must follow strict GMP guidelines; thus any culture modifications used to enhance the potency of MSCs for clinical use must be GMP-transferrable. These are the rules and regulations that govern the production of medical products and therapies with the aim to safeguard the health of a patient by ensuring that all steps taken during production have been verified and that the product is regularly tested for contamination [168]. GMP compliance requires every process to be well documented so that any adverse event can be easily traced back to source, whilst also standardising the production process to guarantee a level of quality in each batch of product (Table 1 and Figure 1).

Conclusion

There is an increasing demand for MSCs in clinical applications, however, to date the research successes have yet to be fully translated from the “bench to the bedside”. Only when the optimal donor source and selection criteria, in combination with the addition of bioactive materials and factors, are coupled with GMP-requirements can their full potential be exploited.
Acknowledgement

This work was supported by a grant from Arthritis Research UK Award 19429 and CellEurope project, FP7-people-2012-ITN, No. 315983.

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