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# Isolation of mesenchymal stem cells from bone marrow aspirate

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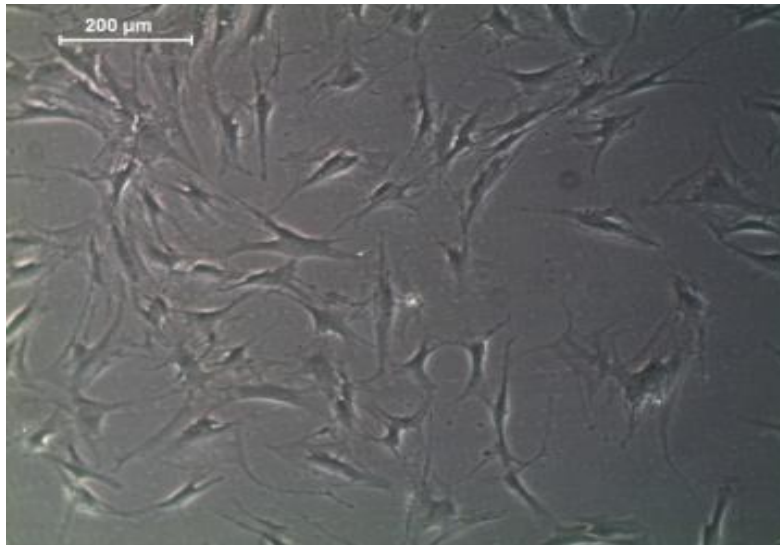
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## The cellular composition of bone marrow

Bone marrow is a spongy tissue present within the central cavity of many large bones of the body that has been shown to perform many important regenerative functions. There are two types of bone marrow: red marrow and yellow marrow. Hematopoiesis, the process of generating blood cells takes place in the hematopoietic tissue of the red marrow, and so, unsurprisingly, all mature blood cell types, with the exception of lymphocytes, are found to be present in this fraction, along with the numerous blood progenitors, including hematopoietic stem cells. The stroma of the bone marrow contains the yellow marrow, and consists of all tissues that are not related to hematopoiesis. This includes blood vessels called sinusoids, as well as adipocytes, osteoblasts and connective fibroblasts.

Bone marrow is generally considered to contain two distinct stem cell systems: CD45-positive hematopoietic stem cells, HSC, and CD45-negative mesenchymal stem cells, MSC; of which HSCs are the more abundant and the better understood cell type [1]. HSCs are a multipotent population of cells that express markers including the hematopoietic marker CD45 as well as CD34, CD133 and CD117. MSCs are a more complex cell type that reside in the stroma of the bone marrow, as well as in other tissues and organs such as adipose tissue [2] and notably umbilical cord blood [3]. In mice they have even been located in more obscure areas such as the liver, kidneys and lungs [4]. They express markers such as Stro-1, CD90 and CD106 but are CD34-, CD45- and CD133-negative [5], although no consensus over a complete and unique set of identifying markers currently exists [1], largely because of conflicting reports over their potency, but are also characterised by their fibroblast-like morphology (see Figure 1). Initially MSCs were believed to be purely osteogenic [6] but were later shown to be a multipotent cell type capable of differentiating into connective tissue cells such as osteoblasts, adipocytes and chondrocytes [7,8]. However, more recent findings have led research groups to hypothesise that they could be very “plastic”; capable of differentiating into a greater number of different cell types including cardiomyocytes [9], neural cells [10,11] and

endothelial cells [8]. It is thought that MSCs and HSCs are interrelated because secreted cytokines from one group have been shown to affect the differentiation pathway taken by the other [12].



**Figure 1 - A light microscope image showing the typical size and morphology of human MSCs.**

Other stem/progenitor cell types have also been identified in bone marrow which may be linked to HSCs and MSCs or may explain their perceived capacity for multilineage differentiation. Examples include endothelial progenitor cells, EPC, also known as angioblasts, which are another resident bone marrow stem cell type that has been shown to circulate in peripheral blood and contribute to regeneration; in this case particularly of vascular tissue in damaged organs such as the pancreas, lung and heart [13]. Multipotent adult progenitor cells, MAPC, are another cell type, reported by several groups to be capable of differentiating into the three germ layers: endoderm, mesoderm and ectoderm [14,15,16], but are unable to differentiate into hematopoietic cells. This is in contrast to the so-called “pluripotent stem cells” (PSC) which are able to do so, despite lacking the CD45 hematopoietic marker. CXCR4-positive “very small embryonic/epiblast-like”, VSEL, stem cells have since been identified as another pluripotent stem cell-type in murine bone marrow [17] and human umbilical cord blood [18]. These were initially thought to be CXCR4-positive “tissue committed stem cells”, TCSC [5], and it is hypothesised that these are a dormant sub-population of PSCs which may participate in hematopoiesis and the turnover of monopotent progenitors [19]. TCSCs, or VSEL stem cells, may also play a role in regenerating damaged tissues or organs as they were shown to respond to the presence of SDF-1 [20], HGF and LIF [21]; all of which have been shown to be released by damaged tissue, and SDF-1 in particular is secreted by bone marrow fibroblasts. It is also considered that VSEL cells may be more likely to turn cancerous if exposed to mutagenic substances [19]. VSEL stem cells have also been shown to be more abundant in the bone marrow of younger donors, and have consequently been considered to play some part in the ageing process, along with telomere shortening which is known to result from stem cell division [22]. It is thought that the actions of

these cells may previously have been mistakenly attributed to the perceived plasticity of HSCs or MSCs [5,22].

## **Why isolate MSC populations?**

Ultimately, the goal of stem cell research is to produce therapeutic treatments which can restore or establish a normal function to damaged tissues or organs [23]. These treatments will be produced through the expansion and differentiation of human stem cells, and can be either allogeneic or autologous in nature. Allogeneic treatments involve the use of cells obtained from a donor, which is beneficial to the patient as, ideally, only one medical procedure would be required to implant the therapeutic product. However, in many cases this type of therapy would also require the use of immuno-suppressive drugs in order to combat the possibility of immune rejection of the transplanted cells. This complication might not always occur with stem cell therapies, however, because evidence suggests that some populations, including MSCs [24], and embryonic stem cells [25], may be immune-privileged, although this has been strongly disputed more recently [26,27]. Autologous therapies, where the patients themselves are the donors, do not carry the risk of immune rejection, but do require an additional procedure to surgically extract the required cells for the treatment. Another problem with this method is that the quality of the extracted stem cell samples from bone marrow is, to some degree, related to the age of the patient [22], leading to variability in the outcomes of the treatments.

Due largely to their due to the multipotent differentiation capacity, MSCs derived from bone marrow are thought to be highly promising, not only for use in stem cell therapies, but also as tools in research, either with the goal of discovering a novel stem cell-based therapeutic treatment or for generating a source of clinically relevant cells which can act as a model for small-scale, *in vitro* drug testing. The requirements of instruments and techniques used for separating MSCs from bone marrow for research purposes will naturally be quite different from those used for producing a stem cell therapy. For example, in therapeutic production it is usually essential for separation systems to be capable of high throughput and a high yield. Following the isolation of MSCs from bone marrow, an expansion protocol would require a certain number of cells to satisfy a minimum seeding density so as to ensure the cells are able to survive and proliferate in a healthy state. It is estimated that between  $10^9$  and  $10^{10}$  of these cells would be required for many cell therapies [28], and it is usually desirable to create a bank of frozen cells for each patient, particularly in the case of autologous therapies, so that the treatment can be re-applied if necessary. Hence, being able to extract the maximum yield of MSCs from a given bone marrow sample could dramatically reduce the waiting time for the patient and significantly improve the chances of a positive outcome from the treatment.

Conversely, instruments for research purposes are not likely to be required to handle large volumes, and maximising the yield of MSCs, although desirable, might not be so vital for the often relatively small scale expansion processes. Similarly, while maintaining sterility is clearly very important in both cases, if a contamination occurs in research, the impact in terms of time and costs is usually minimal because the work generally takes place at a lower scale. The worst case scenario is perhaps that erroneous results are obtained, if the infection is not detected. On the other hand, in the case of therapeutic production, the results of an undetected infection can of course be disastrous, highlighting the need for stringent quality controls. This should never occur, however, because any separation technique for therapeutic production must conform to Good Manufacturing Practice standards, which should ensure that the instruments used are demonstrably capable of reproducible quality and sterility in the final product.

## **Separation Techniques**

### **Initial purification of bone marrow aspirate**

Human bone marrow is most commonly extracted from the posterior iliac crest under local anaesthetic. The initial sample should be around 0.3mL in volume, as attempts to draw out more marrow will cause the sample to be diluted with peripheral blood. The syringe used to perform the biopsy will have been prefilled with a sodium heparin anticoagulant solution [29] which will dilute the sample significantly in any case. This technique is clearly very prone to operator variability which is evident by the fact it is recommended that the samples should be checked during the biopsy to ensure the presence of marrow particles, called “spicules” [29]. Therefore, in addition to the natural, largely age-related, variability that will occur with bone marrow samples [22], the quality of the sampling technique will cause further inconsistencies, and the MSC population will differ in size as a result.

MSCs are thought to account for only 1 in  $10^5$ - $10^6$  bone marrow cells [30], but will be a far greater proportion of the adherent population. Therefore the first step when attempting to isolate them is often to exploit their adherent properties by suspending the bone marrow aspirate in a tissue culture-treated plastic flask [6,31]. This technique filters out the non-adherent cells, such as the hematopoietic cells, which do make up a relatively large portion of the bone marrow, and, depending on the bone marrow sample size, may be necessary to increase the quantity of desired cells in the sample, as additional purification steps can leave only  $10^3$ - $10^4$  cells in some cases [30]. This can then be followed by additional purification steps because even the adherent fraction of cells from bone marrow constitutes a highly heterogeneous population, as mentioned earlier [5].

An alternative approach is to first centrifuge the bone marrow sample while employing a density gradient [31]. Percoll and Ficoll are commonly used density gradient solvents which can be used to clearly separate the mononuclear cells from the anuclear red blood cells. Following centrifugation, the red blood cells will have collected at the bottom of the tube with the mononuclear cells, including the desired stem cells separated from them by a band of solvent. The procedure can be further enhanced through use of an antibody mixture, RosetteSep(R), which binds red blood cells to unwanted cell types causing them to be filtered to the bottom of the tube during centrifugation, thus further concentrating the desired cells in the sample. A potential problem with using density gradient centrifugation is that the VSEL stem cells, mentioned previously, can be lost from the mixture due to their small size [17].

### Cell surface markers for bone marrow populations

Stem cells usually exist in a very low concentration within a given tissue. In order to distinguish them from other cell populations, it is possible to exploit the cell surface markers which are unique for each cell type. These are proteins molecules, also known as “receptors” that coat the surface of all cells, and are able to bind to other cells, surfaces or proteins. They are used *in vivo* to signal other cells and to induce functionally significant cellular reactions, for example to stimulate production of a particular protein. As mentioned earlier, CD (cluster of differentiation) markers are commonly used to identify stem cell types in bone marrow, but additionally a number of antibody-binding receptors, antigens, can also be used. A selection of surface markers that have been reported for the isolation of bone marrow cells are shown in Table 1. It must also be considered that many different cell types may have a number of markers in common, so in order to isolate a specific stem cell populations contained within the highly heterogeneous bone marrow, or to further separate the sub-populations of the adherent fraction, a combination of different markers must be used. Therefore it is important not only to know the markers for the cell type that you wish to isolate, but also to be aware of the markers of the other cells contained in the bone marrow.

Cell type	Identifying markers		References
<b>Mesenchymal stem cell (MSC)</b>	+ve	CD13, CD29, CD44, CD54, CD55, CD59, CD73, CD90, CD105, CD106, CD166, CCR2 (CD192), SB-10 (CD166), STRO-1, SB-10	[32,33,34,35,36]
	-ve	CD11b, CD14, CD19, CD31, CD34, CD45, CD79a, CD80, CD86, HLA-DR, vWF <sup>-</sup>	
<b>Multipotent adult</b>	+ve	CD13, CD90 <sup>low</sup> , Flk-1 <sup>low</sup> , Oct3/4, SSEA-1, VCAM <sup>low</sup> , <b>CD44<sup>low</sup></b> , <b>MHC I<sup>low</sup></b>	[37,38,39]

<b>progenitor cell (MAPC)</b>	-ve	CD34, CD45, CD117 (c-kit), MHC II, NANOG, <b>CD44, MHC I (conflicting reports)</b>	
<b>Multipotent adult stem cell (MASC)</b>	+ve	CD13, CD29, CD44, CD49a, CD49b, CD73, CD90, CD105, CCR2 (CD192), MHC I, CCR10, FGFR1, FGFR2, IL6ST, PDGFRA, PDGFRB, TGFBR1, TGFBR2	[40,36]
	-ve	CD14, CD34, CD38, CD45, CD133, CD117 (c-kit), HLA-DR	
<b>Very small embryonic-like (VSEL) stem cell</b>	+ve	CD34, AC133, c-Met, CXCR4AP, LIF-R, SSEA-1 (mouse), SSEA-4 (human),	[17]
	-ve	CD29, CD45, CD90, CD105, Lin, HLA-DR, MHC I	

Table 1 - Characteristic markers for several non-hematopoietic stem cell types reported to have been found in bone marrow. Adapted from Ratajczak *et al*, 2008.

Cell receptors must be “tagged” with the specific signalling molecules that they bind with *in vivo* in order to separate stem cell populations from within a heterogeneous tissue such as bone marrow. Further, these signalling molecules must be modified or attached to another particle or molecule, which can then be applied using some technique to either separate or merely distinguish the tagged cells.

Most techniques for separating samples of stem cells from heterogeneous populations involve the use of surface markers in the attachment of either: 1) magnetic particles, allowing the user to separate the tagged population through the application of magnetic forces; or 2) fluorescent proteins or molecules so that cells can be isolated based on their light-scattering or fluorescence properties. Other separation techniques exist that fall outside these 2 main categories, and some will also be addressed in this review. Some of these techniques are shown in Figure 2.

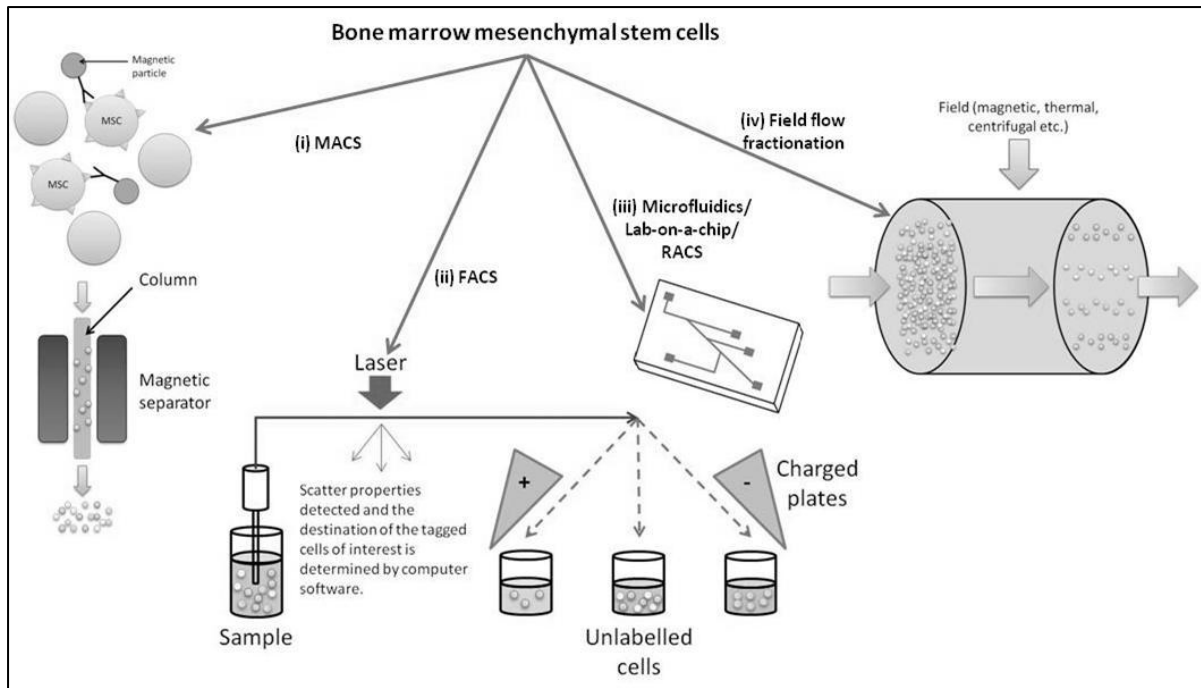


Figure 2 – An overview of the separation techniques described in this chapter: (i) Magnetic-activated cell sorting (MACS®): in this case the desired cells (MSCs) expressing a specific antigen attach to an antibody-bound magnetic particle before being run through a magnetic separator column. The MSCs are shown to be retained on the column; (ii) Fluorescence-activated cells sorting (FACS): the sample, containing MSCs which have previously been tagged with a fluorescent marker, is injected into the system where the cells are hit by a laser. The resulting fluorescence and scatter data are detected by a computer, which then determines which are the cells of interest and causes a charge to be induced, allowing them to be finally separated using charged plates; (iii) Microfluidics/Lab-on-a-chip/Raman-activated cell sorting: a number of methods for separation or analyses of cell populations currently exist using this technology. One separation system involves placing the sample on the chip, and using different flow rates, cells could be isolated based on their ability to pass through channels of different sizes [41]; (iv) Field flow fractionation: a perpendicular field is applied to a flowing sample of the mixed population, which causes separation of the different cell types based on characteristic properties such as their size, shape and flexibility [42].

## Magnetic separation systems

Magnetic-activated cell sorting, MACS®, is the most commonly used method of sorting cells by magnetic forces, and is a registered trademark of Miltenyi Biotec GmbH (Bergisch Gladbach, Germany). Using this separation technique, the cells of interest are labelled with 50nm diameter, superparamagnetic beads and sorted using a packed column. Separation can be achieved by first coating the magnetic beads with an antibody, which is known to selectively bind to the desired cell type, and incubating them with the sample [43]. Once the cells have bound to the particles, the mixture is passed through a small column under the influence of a strong magnetic force. This induces a high gradient magnetic field in the column matrix, causing the particle-bound cells to be retained while the untagged cells pass through (see Figure 2). The column is washed with buffer to ensure no unwanted cells remain within the matrix, before the magnetic force is removed and the tagged cells can be eluted from the column. The magnetic beads can then be removed from the cells using enzymes. This separation system is quite flexible, and can be very quick depending on the



method of tagging the cells that is required. When only one labelling step is required to bind an antibody to the magnetic particle, i.e. if the cells can be directly attached via the antibody, then the entire separation may take as little as 30 minutes. However, it may not be possible to bind the cells and the beads directly, and an intermediary antibody, either biotinylated or fluorochrome-bound, for example, might be required instead.

Above is a description of a technique for *positively* selecting the cells of interest. As discussed earlier, this is not always possible because there is significant overlap in the surface receptors expressed by different cells types. Therefore it may be necessary to employ different strategies using MACS in isolating the desired cells. For example, it may be preferable to bind magnetic particles to a significant population of unwanted cells, allowing the cells of interest to pass straight through the column, while many of the impurities remain bound. The desired cells can then be positively selected from the remaining mixture as before. There have been reports of MACS being used to isolate MSC populations from bone marrow [44,45,46], umbilical cord blood [45] and lipoaspirate samples [47]. Gronthos and Zannettino reported the use of the MACS system to isolate “bone marrow stromal stem cells” (BMSSC), a population of cells that display similar characteristics to MSCs. The STRO-1 antigen was the only marker used in the initial isolation with magnetic sorting, but the population was then further enriched using FACS. The CD106 marker was used to separate “STRO-1<sup>bright</sup>/CD106<sup>+</sup>” BMSSCs from the nucleated red cells and lymphocytes present in the STRO-1-positive population [48].

Another magnetism-based separation system, the magnetic particle concentrator (MPC<sup>®</sup>; Dynal Biototech.), has been used to isolate mesenchymal stem cells from murine bone marrow [30]. Three immunodepletion separations were performed using markers: CD11b, CD34 and CD45, which were bound with superparamagnetic “Dynabeads<sup>®</sup>” (Dynal Biototech.).

One of the key disadvantages of both FACS and MACS technology is that they require samples to be in single cell suspension considering, as mentioned previously, the initial purification of MSCs from bone marrow aspirate often involves allowing the MSCs, amongst other cells, to adhere to tissue culture plastic. Given that enzymatic dissociation of adherent cells with, for example, trypsin, can lead to proteolytic damage of cell surface proteins, it is important that the method of cell harvesting is carefully considered when FACS or MACS is used for adherent cell purification. Although MSCs can be harvested using an enzyme-free dissociation buffer, viability is lower than if trypsin is used [49], highlighting the need for purification methods which work *in situ*. One such method is laser-mediated cell purification. Cytellect (California, USA) have generated a laser-enabled analysis and processing (LEAP<sup>™</sup>) platform which combines imaging capability with laser technology to purify cell

populations *in situ* in tissue culture well plates by eliminating unwanted cells by necrosis, apoptosis or cell lysis [50]. For instance, labelled HeLa cells were effectively removed from a monolayer of unlabelled HeLa cells, resulting in approximately 100% purity [51]. If MSCs could be distinguished from other bone marrow cell populations by brightfield imaging or by fluorescently marking the unwanted cells (ie negative selection), this technology could be used to damage and lift off unwanted cells which could then be washed away. Potential issues might include the processing time for a tissue culture flask and yield of purified cells as Szaniszló and colleagues showed a loss of 10-20% of untargeted cells in their HeLa experiments, following laser treatment. Some optimisation of the system for use with MSCs may also be required because the need for lower cell densities at the time of treatment if the unwanted cells are present at higher than 5% of the population has also been reported [50,51], and this could be problematic with MSCs due to their low abundance, even within the adherent population.

## **Optical separation systems**

### **Flow Cytometry**

The term cytometry describes the process by which the characteristics of single cells (or particles of a similar size) are measured [52]. There are many different forms of cytometry, each with their own unique features; however the most popular is undoubtedly flow cytometry, FC [53]. FC is an extremely powerful, high-throughput, diagnostic technique which can measure the physical and/or chemical characteristics of single cells as they pass individually through a laser beam [52,54,55]. Exposure to the laser beam causes light scattering in two planes, forward angle light scattering and right angle light scattering, which provide information about the size and refractive properties of the cell [54]. More specific cell characteristics can then be determined through the use of fluorescent probes and multiparametric analysis can be performed. Flow cytometers also have the added ability to sort cells within a heterogeneous mixture (also known as a cell or flow sorter) based on the light scattering and fluorescence characteristics of the cells [52]. Such flow cytometers are referred to as fluorescence activated cell sorting (FACS) devices. Exposure to the laser beam causes light scattering in two planes, forward angle light scattering (FALS) and right angle light scattering (RALS), which provide information about the size of the cell and refractive properties of the cell (Hewitt CJ, 2006; see Figure 3). In addition to this, FC allows for multiparametric analysis which can be used to quantify cell constituent relationships [56].

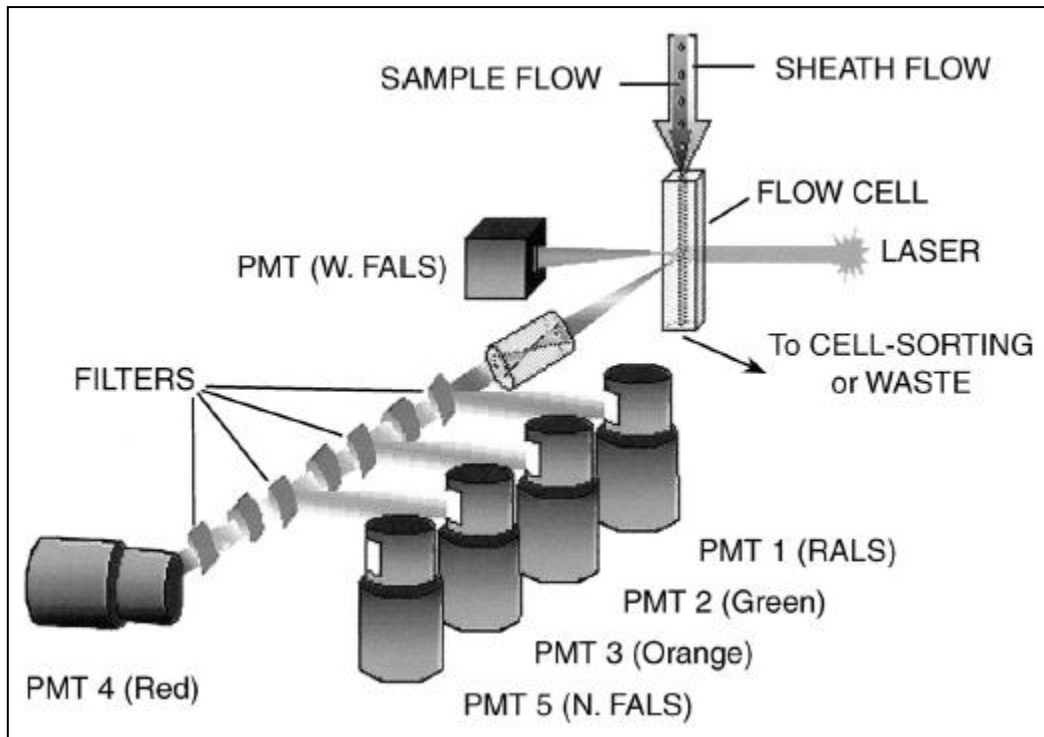


Figure 3 - Schematic of a typical flow cytometry instrument. A laser passes through a flowing sample of cells causing light to scatter, which is detected in two directions: 1) Forward angle light scatter (FALS), measured at  $180^\circ$  to the beam; and 2) Right angle light scatter (RALS), measured at  $90^\circ$  to the laser. Photo multiplier tubes (PMT) are used to measure light emitted due to fluorescent tags on cells of interest, also at  $90^\circ$  from the angle of the laser. From Hewitt & Von-Caron, 2004.

FACS technology has been used to purify MSCs from heterogeneous cell populations based on the positive identification of cell surface markers expressed by MSCs [35]. For example FACS has been used to identify and isolate MSC subsets from bone marrow [57] and  $CD9^+$ ,  $CD90^+$  and  $CD166^+$  mesenchymal progenitors from synovial membranes of osteoarthritic patients [58]. Initially sorting cells by FACS was quite time consuming, however, high speed sorters are now available and with continuing advances in instrumentation and software (recently reviewed in Preffer & Dombkowski, 2009), the use of FACS in the MSC field is likely to grow.

One of the main disadvantages associated with using FACS in the stem cell field, however, is our current lack of knowledge of specific or unique cell markers for cell types such as MSCs and so a complex regime of positive and negative selection may need to be used to isolate the cells of interest. Other disadvantages are thought to include an altered cell viability and/or function as a result of the probes used (through both their physical interaction and in the washing protocols used which may result in the loss of cells), physical stresses exerted on the cells by flowing through the nozzle, laser damage and osmotic stress and potential contamination of cells. However, since cells are measured and sorted on an individual basis using filtered ( $0.2\mu\text{m}$ ) sheath fluid the latter is probably unlikely. Despite these perceived drawbacks, there are several reports in which stem cells or stem cell-derived cells survived and have been cultured successfully *in vitro* without

contamination for up to 6 weeks or have even been transplanted into an animal model [59,60,61]. New models of FACS machines also exist which can be operated inside a biological safety cabinet thereby lowering the risk of contamination. The iCyt Reflection Cell Sorter (iCyt, Illinois, USA) system is one example, and is capable of not only preventing contamination of the cells but also reducing the biohazard associated with using cells in an open system. Generally speaking, these new FACS instruments remain expensive, are currently only used for analytical, lab-scale purposes [62] and are unlikely to be suitable for large scale separations.

## **Other Techniques**

### **Field-flow fractionation**

This family of techniques is based on separation occurring by differential retention of analytes (ranging from proteins to whole cells) in a fluid stream flowing through a very thin, empty channel with a field applied in a perpendicular direction, but there are many variants [42]. It has the ability to sort cells based on biophysical properties, such as cell size, shape, flexibility, membrane roughness, and has previously been used to sort mammalian cells such as red blood cells [42]. More recently it was reported that this technique could even detect distinct fractionation profiles of MSCs from different tissue sources and it was able to accurately separate MSCs from epithelial cells [63]. The advantages of this system include that it could be used to isolate cells which are not well characterised in terms of cell surface markers, it is biocompatible, relatively low cost and could be scaled up, making it an attractive method to pursue in the stem cell field.

### **Antibody-column**

A method of separating cell populations has been described by Mahara & Yamoaka, whereby CD34-positive cells were bound to a cell rolling column containing immobilized anti-CD34-antibodies [64]. The column surface was activated using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (WSC) and filled with a solution of the mouse anti-human CD34 antibody. A cell suspension containing CD34-positive KG-1a, and CD34-negative HL-60 cells was injected by syringe at a constant rate of 50 $\mu$ L/min, with the column tilted at an angle. Phosphate-buffered saline (PBS) was also washed through the column to promote cell rolling. The authors of the study claim the system to be superior to MACS in that it is capable of separating cells based on their surface marker *density*, due to the additional cell rolling [64]. They also indicated their belief that this system would prove quicker and less damaging than other separation techniques, as well as producing a highly pure population of cells. The system was considered a potential application for the separation of bone marrow MSCs in this study.

## **Microfluidic Technology**

Microfluidic (MF) or lab-on-a-chip (LOC) techniques fall under the area of nanotechnology, however in recent years, both MF and LOC techniques have been developed and utilised for the investigation of biological phenomena, with the cell being at the crux of the investigations [65]. MF or LOC devices allow for not only the analysis of single cells, but also provide a platform for cell culture and can integrate and automate cell manipulation with detection techniques [65]. Although MF and LOC have not been employed for the separation of MSCs from bone marrow, MF has been applied to amniotic fluid MSCs. The cells were isolated based on their size and the sample flow rate, and as a result, their ability to pass through differently-sized microfluidic channels. An initial separation efficiency of MSCs of 82.8% was achieved, but with repeated cycling, it was increased to 97.1% [41].

## **Raman activated cell sorting (RACS)**

Microfluidic technologies are being extended for use in novel cell sorting techniques, an example of which being the recently developed integrated optofluidic Raman activated cell sorting platform, which was created in California, USA [66]. This system is based on laser tweezers Raman spectroscopy which uses a laser beam to both optically trap individual cells and as an excitation source to generate a Raman spectrum or fingerprint. By integrating this with a multichannel microfluidics device it allows for automated delivery of cells to the laser trap and sorting of the cells based on their fingerprint. Whilst still very much in its infancy, more proof-of-principle work has shown that using Raman microspectroscopy with hESCs, human foetal left ventricular cardiomyocytes and hESC-derived cardiomyocytes each have a Raman fingerprint and that therefore RACS could potentially be employed as a non-destructive, label-free sorting method in stem cell science [67]. At the moment the systems' throughput and efficiency are low compared to an established system like FACS but it may prove useful in instances where unique cell markers cannot be identified or when investigating cells which may become altered or activated upon antibody binding to its surface markers.

## **Conclusions**

The ultimate goal of separating the constituent cell populations in bone marrow is the identification of the multiple phenotypes present within the mixed marrow community, potentially for use in clinical therapies. These cell populations are small in number and show diversity in their origins and differentiation capability. This presents a major challenge for the methodologies that have been developed for isolation and separation. In addition, the identification of a 'true' pluripotent stem cell, which is capable of continuous division and differentiation into the three germ layers, is a

difficult task due to the very small populations present in the marrow and the fact that the markers that truly indicate these traits are yet to be fully understood.

This review outlines the markers which have been identified for the isolation of different fractions from within a bone marrow sample, including MSCs. Routine approaches for large scale identification have been put into practice for isolating stromal and haematopoietic populations, while other markers for many of the sub-populations are still being defined. Technologies which have been developed for the isolation of stem cell populations within bone marrow range from magnetic to optical to microfluidic techniques. Current research includes developing single cell technologies for isolating and defining low numbers of 'stem' cell populations which can form cloned stock populations for allogeneic therapies. These technologies form an important basis for ultimately bringing cell therapies to the clinic for treatment of a variety of diseases.

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