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Review

Biological properties of mesenchymal stem cells derived from adipose tissue, umbilical cord tissue and bone marrow

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Abstract: Regenerative medicine employs stem cells to repair or to restore the function of damaged tissues. Major sources of stem cells are embryonic as well as adult tissues; however, adult stem cells are preferred for cell based regenerative therapies. Mesenchymal stem cells (MSCs) are a type of adult stem cells and they hold great promise for regenerative therapeutics. Beside other sources adipose tissue, bone marrow and cord tissue are common sources of MSCs. Significant biological differences may exist in MSCs derived from different sources due to which cells from some sources may be favoured over others for clinical use. MSC origin may be an important consideration to determine biological activity and potential use in regenerative medicine. Therefore, it is important to consider the biological characteristics of MSCs isolated from these sources. The current study briefly discusses essential characteristics (such as isolation procedures, identification, proliferative capacity and differentiation potential) of MSCs derived from umbilical cord tissue, adipose tissue and bone marrow.

Keywords: mesenchymal stem cells; adipose tissue; bone marrow; cord tissue; proliferation; differentiation

1. Introduction

The majority of damaged human tissues do not regenerate spontaneously and therefore require alternative regenerative options for a complete recovery. Regenerative medicine, a new field of medicine, can replace the damaged tissues of the body with intact tissues thereby restoring their function [1]. As conventional medicine can only treat a disease or delay its progression without restoring or replacing the spoilt tissues or organs, regenerative medicine has succeeded to achieve considerable attention. The principle of regenerative medicine is simple and involves collection of cells (stem cells), culturing of these cells, and transplantation of cells into body with or without modification of their biological properties. Stem cells are undifferentiated cells with self-renewal ability and potential to differentiate into specific lineage if an appropriate environment is provided [2].

Stem cells can be harvested from embryonic as well as adult sources (such as adipose tissue, bone marrow, cord tissue etc). The source of embryonic stem cells (ESCs) is inner cell mass of embryonic blastocysts. These cells can differentiate into any type of cell in the body and are therefore pluripotent [3,4]. Due to their diverse differentiation potential, they have superior regenerative potential. However, the controversy due to their origin and potential immunogenicity and tumorigenicty limit their use for clinical purposes [5]. In addition, current hallmark studies have successfully converted somatic cells into pluripotent stem cells [6–8]. These cells are called as induced pluripotent stem cells (iPSCs) and their characteristics closely resemble ESCs [8,9]. Although these cells are patient specific and may have great clinical potential, their current utility is limited to drug screening and disease modelling [10]. The problems related with iPSCs include use of retroviral or lentiviral vectors for gene insertion and neoplastic development due to induced genes [11]. Due to such problems with ESCs and iPSCs use, adult stem cells are preferred for use in regenerative medicine. Adult stem cells unlike ESCs or iPSCs are not controversial and therefore they are considered possible candidates for stem cell based regenerative therapies [12,13].

One of the best documented populations of adult stem cells is mesenchymal stem cells (MSCs) that have pervasive use in research as well as in clinics [14]. The scientific and medical communities have played more with these cells as compared to other cell types and thus their isolation methods, characterization, potential uses and outcomes are more established. MSCs have unique regenerative properties such as multi-lineage differentiation potential, high proliferative ability, and potent immunomodulatory and anti-inflammatory properties [15]. Recent success of pre-clinical studies has opened new avenues for their clinical use for various diseases and disorders (www.clinicaltrials.org). As a result numerous clinical trials have been registered using MSCs, aiming for regeneration of tissues (such as bone, cartilage etc.) or treatment of disorders such as cardiovascular [16], neurodegenerative [17], kidney disease [18], erectile dysfunction [19], liver diseases [20], graft-vs-host disease [21] etc.

MSCs were first isolated from bone marrow; however, latter studies confirmed their presence in other tissues such as adipose tissue, umbilical cord blood, peripheral blood, amniotic membrane, gut, liver, blood vessels, skeletal muscles, hair follicles, skin, tonsils etc [22–32]. Currently, protocols are available to isolate MSCs from all of these tissues. Adipose tissue, bone marrow and cord tissue have been commonly used in research and pre-clinical studies due to their easy isolation, characteristics and importantly their potential medical uses [26–32].

The niche of cells may affect their biological characteristics and therefore MSC origin may be an important consideration to determine their biological activity and potential use in regenerative

medicine. Significant differences may exist in biological activities of MSCs derived from different sources [32–33]. The cells isolated from different sources may also exhibit different potential for clinical applications [30,34]. Therefore, it is enviable to consider the regenerative characteristics of MSCs isolated from different sources. The current study briefly discusses various characteristics of MSCs derived from different adult sources such as cord tissue, adipose tissue and bone marrow.

2. Isolation of Mesenchymal Stem Cells

MSCs can be isolated from a variety of mesenchyme tissues with already established protocols. The isolation and culture technique may vary according to the species and tissue type. Following protocols can be used for the isolation of MSCs from bone marrow, adipose tissue and cord tissue. Bone marrow MSCs (BM-MSCs) are obtained from bone marrow aspirate [35–37]. The bone marrow aspiration is an invasive and painful procedure, and it may poses risk of infection [37]. MSC isolation procedure involves several post aspiration steps that reduce contamination with other cell types. Briefly, BM-aspiration is followed by mechanical disruption with repeated pipetting to create a mixture of stromal and hematopoietic cells. The erythrocytes can be removed using Ficol or Percoll in a density gradient centrifugation [37,38]. The centrifugation procedure separates the cells into various layers. The layer containing mononuclear cells (under plasma layer) is taken in a new centrifuge tube, washed with PBS, and cells are seeded in tissue culture flasks at 37 ℃, 5% CO_2 in humid environment. MSCs rapidly adhere to plastic surface while hematopoietic cells remain in suspension [39,40]. To obtain pure MSC population, stem cell culture medium can be replaced with fresh medium within 12–24 hours. This step is important as it eliminates the contamination of other types of cells from MSCs. In the initial passages the resultant BM-MSC population is still highly heterogeneous and requires positive or negative selection of MSCs with specific markers using FACS (flourescnce activated cell sorting) or MACS (magnetic cell sorting).

The isolation methods of MSCs from adipose tissue although are diverse but follow certain standard procedures such as washing, enzymatic or non-enzymatic disrucption of adipose tissue, centrifugation and plating. The differences in different protocols are attributed mainly to the type of enzyme used and its concentration, centrifugation speed, number of washing steps, stainer size, erythrocyte lyses step and also culture conditions [41–49]. MSCs from adipose tissue were isolated for the first time by Zuk et al., in 2001 [41]. Adipose tissue-MSCs (AT-MSCs) can be isolated from the biological materials generated during lipoplasty, lipectomy and more commonly during liposuction [50]. Small amounts of adipose tissue (50 ml–100 ml) can be obtained under local anesthesia. For isolation of MSCs from fat, the lipoaspirates are washed 3 to 5 times with phosphate buffered saline (PBS), followed by enzymatic digestion with collagenase usually collagenase type I or type IV [22,51], dispase or trypsin [51]. The digested tissue solution is filtered through cell strainers (70 um–100 um) and centrifuged at 1000 rpm. The centrifugation steps separate the stromal vascular fraction (SVF) as pellet from mature adipocytes. These mature adipocytes at the top are discarded while the cell pellet (SVF) is taken in tissue culture flasks and incubated at standard culture conditions (37 ℃, 5% CO_2, under humid environment). SVF is a heterogeneous mixture of several cell populations such as endothelial cells, fibroblasts, pre-adipocytes, blood cells and MSCs [53]. Spindle shaped MSCs adhere to plastic surfaces of culture dishes while other cell types remains in suspension. Like BM-MSC cultures, removal of non-adherent cells within 12–24 hours is important to avoid MSC contamination with other types of cells. In addition to enzymatic digestion

methods, recently non-enzymatic digestion methods to isolate MSCs from adipose tissue have been developed [54,55]. In non-enzymatic isolation methods different techniques to disrupt the adipose tissue have been used. For example Shah et al., simply shaked a mixture of lipoaspirate and PBS in a tube for 1–2 minutes and the supernantant was taken a new tube and centrifuged at 1200 rpm for 5 minutes to obtain SVF as pellet [56]. Similarly, blender mixer and sonicators have been used to obtain SVF non-enzymatically [57]. Besides these initial steps, further processing of culturing SVF and to obtain AT-MSCs is similar to enzymatic methods.

To isolate CT-MSCs, cord tissue pieces are obtained aseptically after cesarean sections. Depending on the requirement 1–3 inches of cord tissues are sufficient to start a CT-MSC culture. CT-MSCs are obtained either by enzymatic digestion [58,59] or by explant-culture technique [22,23,61–64]. In the explant tissue culture method of MSC isolation, cord tissue is minced into small pieces (1–2 mm^3) which are seeded in tissue culture treated flasks or dishes. When the tissue fragments adhere to plastic surface of tissue culture flasks, culture medium (MEM supplemented with FBS (5%–10%) and non-essential amino acids (1%)) is slowly added to avoid detachment of pieces. The outgrowth of cells from the tissue pieces can be observed within a week (3–7 days) after culturing. When sufficient number of cells is obtained, tissue pieces are removed and fresh medium is added to allow these cells to proliferate for few more days. Most of the research laboratories prefer explant culture method for MSC isolation from cord tissue as it is inexpensive and gives pure MSC populations [60,63,65,66]. However, cord tissue pieces often detach and float in the medium resulting in low number of cells as floating pieces do not give MSCs. The second method is the digestion of cord tissue using enzymes [58,59]. The enzymes used for this digestion are either collagenases (such as collagenase type 4), dispase, hyaluronidase or a mixture of collagenase and trypsin is used [58,61,62,67]. In this method, cord tissue is first cut into small pieces and digested using collagenase (or collagenase and trypsin) at 37 ℃ for 30–60 minutes. The solution is then filtered through cell strainer (70 um or 100 um), centrifuged at 1000 rpm to obtain cells as pellet. The cell pellet is cultured in tissue culture flasks at 37 ℃, 5% CO_2 in humid environment. Stem cell medium is replaced within 24 hours with fresh medium to remove cells that were not plastic adherent.

Overall, it is less invasive and harmless to obtain either adipose tissue or cord tissue as compared to bone marrow tissue. Bone marrow aspiration is painful and over-harvesting and may be a risk for human health.

3. Morphology and Phenotypic Characteristics

MSCs isolated from bone marrow, adipose tissue and cord tissue are morphologically and phenotypically similar [22,68,69]. Irrespective of the tissue source, MSCs display long spindle shaped fibroblastic morphology when cultured in suitable culture media [22,41,58,69]. Primary cultures of MSCs; however, may exhibit heterogeneous morphology with various shapes that become homogenous in successive cell passages. In the initial passages no differences in MSC morphology have been detected [22,70]. However, morphological changes may appear during in vitro expansion. Such morphological changes may appear when the cells become senescent. For example MSCs exhibit expanded morphology instead of typical spindle shape in long term in vitro cultures which is a hallmark of senescent cells. Expanded (senescent) morphology of cells may appear earlier or later during expansion and it may depend on the cell source. For example, Dmitrieva RI et al., [70] found that in terms of morphology, MSCs from adipose tissue and bone

marrow were very similar in the initial passages; however, during in vitro expansion of these cells, senescence was detected earlier in BM-MSCs as compared to AT-MSCs. Similar results were obtained in another study in which onset of senescence was compared among MSCs isolated from adipose tissue, bone marrow and cord tissue [71]. The results indicated that BM-MSCs senesce much earlier than AT-MSCs and CT-MSCs [71]. In this study BM-MSCs became senescent after 30 population doublings while AT-MSCs and CT-MSCs exhibited senescence after 70 population doublings [71].

Although MSCs are initially separated from heterogeneous mixture due to their ability to adhere plastic surfaces of culture dishes, they can be further purified on the basis of their surface markers (phenotypes). Most common methods of MSC phenotype determination are phenotype detection using FACS or MACS. Both of these techniques use a combination of a set of markers to define MSC population [22,69]. MSCs have been shown to express different classes of cell surface marker proteins including cellular adhesion molecules, integrins, selectins, chemokine receptors and membrane-bound receptors but lack hematopoietic lineage markers. Overall, it can be anticipated from the results of various studies that MSCs show a highly variable cell surface marker profile [69,72] and a lot of controversy exists in literature regarding "true MSC marker" [32,33,73]. Despite this controversy, ISCT (International Society for Cellular Therapy) proposed the minimal criteria for human MSCs [74]. According to ISCT proposal, for a cell population to be MSC, >95% cells must exhibit positive expression of CD73, CD90 and CD105 and must lack CD34, CD45, CD14 or CD11b, CD79-α or CD19, HLA-DR (<2%). Keeping in view these minimal criteria, several publications have analyzed the surface antigens of MSCs isolated from various tissues. These studies showed reproducible expression of CD44, CD73, CD90 and CD105, and absence of CD14, CD34 and CD45 in MSCs isolated from adipose tissue, cord tissue and bone marrow [69,73,75,76]. The analysis of MSCs using flow cytometry showed the expression of certain mesenchymal and haematopoietic markers with no significant differences among the three kinds of MSCs [22,69,75]. All three kinds of MSCs were negative for the hematopoietic stem cells markers CD34 and CD45, but positive for the typical MSC markers CD29, CD73, CD90 and CD105 [22,69,75].

Contrary to this, potential differences in surface markers have also been detected in MSCs isolated from different tissue sources. For example, when BM-MSCs and AT-MSCs were compared, differences in the expression of CD49 and CD106 were obvious between the two cell types [77]. In this study CD49 was expressed in AT-MSCs while BM-MSC lacked it [77]. Contrary to this, BM-MSC exhibited expression of CD106 while it was absent in AT-MSC [77]. Interestingly some AT-MSCs may exhibit expression of CD34 (which is a hematopoietic lineage marker) in early in vitro culture stages, whereas BM-MSCs did not express this marker [32,33,78]. Similarly, CD146+ cells were more enriched in BM-MSCs as compared to AT-MSCs and CT-MSCs; however, CD146+ cells declined more rapidly in subsequent passages [70,79]. Furthermore, BM-MSCs can be distinguished from culture-expanded AT-MSCs using CD36 and CD106. Culture expanded AT-MSCs lacked the expression of CD106 while displayed expression of CD36 [53]. The above mentioned information raises the possibility that MSC-phenotypic markers may vary depending on the MSC source. To date, there exists no single marker for the identification of MSCs [32,33,73]; however, CD146 may be the most appropriate markers for the characterization and purification of MSCs isolated from various sources [79]. Similarly, CD271 is also considered specific marker for purification of BM-MSCs [80,81] and AT-MSCs [71,78]. However, this marker seems inadequate

for the purification of MSCs isolated from other tissues such as Wharton's jelly of cord tissue [82] and umbilical cord blood [83].

It is interesting to know that although MSCs reside in different tissues throughout the body, there may be a common tissue pool (such as bone marrow) to house these cells. From this pool MSCs home to other tissues in response to specific biological needs. Furthermore, the discrepancies in the expression of surface markers on MSCs isolated from different tissues are due to the fact that immunophenotypes may be dynamic. Biological age of donor, isolation methods, culture conditions, in vitro expansion may have potential influence on MSC phenotype and therefore contribute to the inconsistent reports [32,33,79]. In addition, the expression of some antigens may be artificially induced by in vitro culturing (such as SSEA-4), by certain growth factors and cytokines, and by disease conditions [79].

4. Proliferative Potential and Senescence Characteristics

The proliferation power of MSCs is of immense significance that determines their potential clinical use. The long term proliferative potential of MSCs is determined by the time and number of population doublings. For this long term proliferation analysis, MSCs are serially cultured in regular expansion medium, and initial and final number of cells is noted to find out the number of population doublings and the time taken per population doubling [84]. Overall, MSCs have high proliferative potential but differences may exist depending on the origin of these cells. Most studies indicate that, in comparison to BM-MSCs and AT-MSCs, UC-MSCs exhibit a higher proliferation capacity [22,68,85]. Choudhery et al., [22] have shown that the population doublings of CT-MSCs are significantly higher as compared to AT-MSCs. Similarly the doubling time of CT-MSCs is significantly lower as compared to doubling time of AT-MSC [22,85]. However, CT-MSCs may show earlier morphological changes and a more rapid decline in proliferation ability. Another reason reported for higher proliferative potential of CT-MSCs is that their proliferation is not inhibited by direct cell-cell contact [22,75] while other cells stop dividing at confluence. Population doubling analysis of adipose tissue MSCs and bone marrow MSCs indicate that AT-MSCs are more proliferative as compared to BM-MSCs [69]. When different donor sites of adipose tissue MSCs in the same individual were compared, cells from different depots showed different characteristics. For example, subcutaneous adipose tissue derived MSCs showed faster proliferated (doubling time, 4 ± 1 days) than those isolated from the omental region (doubling time, 5 ± 1 days) [86]. Besides cell origin, there are other factors that can contribute in altering the proliferation potential of MSCs. Such contributing factors are numerous such as the cultivation conditions and medium supplements. A variation in doubling time was also observed at different passages and use of different culture medium [68].

Traditionally, the ability of MSCs to proliferate in *in vitro* cultures can also be tested using colony-forming-units (CFs) assay. In this assay MSCs are counted and seeded in low numbers (such as 20 cells/cm^2) in regular medium for two weeks [22,64,76,84]. During this period the cultures are usually not fed with fresh medium. After two weeks cells are fixed and the cultures are stained with a dye (such as 0.1% crystal violet). The colonies with more than 30 cells are counted under a phase contrast microscope [22]. Each colony represents the clones from a single cell and its size represents the proliferative capability of cell. It has been shown that MSCs derived from adipose tissue, bone marrow and cord tissue are all clonogenic; however, with differences. For example, in one study

CFU frequency was estimated 0.0029 ± 0.0008% and 0.12 ± 0.096% in BM-MSCs and AT-MSCs, respectively [71]. Choudhery et al., [22] compared the clonogenic potential of AT-MSCs and CT-MSCs and found significant differences in CFU frequency. In this study CT-MSCs cultures produced fewer colonies as compared to AT-MSCs cultures. Interestingly, the same study showed more population doubling and less doubling time for CT-MSCs as compared to AT-MSCs. The possible explanation for this might be the primitive nature of cells that requires more time to mature and form CFUs. CT-MSCs even do not form colonies when cultured at very low numbers such as 100 cells per culture flasks (unpublished data). It is worth noting that there were tenfold more CFU-F units following an AT-MSCs harvest as compared to BM-MSCs [87] but controversies exist in this regard [73].

Overall the proliferation rate decreases in the order CT-MSCs > AT-MSCs > BM-MSCs, while the colony-forming ability decreases in the order of AT-MSCs > BM-MSCs > CT-MSCs.

5. Tri-lineage Differentiation Capacity

Besides plastic adherence capacity and surface marker expression, multi-lineage differentiation capacity towards osteoblasts, adipocytes, and chondrocytes have also been proposed as minimal criteria to characterize MSCs by the International Society for Cellular Therapy [74]. This tri-lineage potential is one of the important characteristic features of MSCs. MSCs show this differentiation potential (tri-lineage differentiation) independent of the origin of tissues, however, level of differentiation may vary depending on the cell source. Several studies have successfully differentiated these cells into adipose, bone and cartilage [22,32,33,51,68]. Other studies have also demonstrated their differentiation into muscles, cardiomyocytes, neuronal cells and endothelial cells [22,88–91].

In a comparative study by Sakaguchi et al., [92], human AT-MSCs showed superior adipogenic potential as compared to BM-MSCs and CT-MSCs. The results of several other studies have indicated the same results in which AT-MSCs showed superior adipogenic differentiation as compared to CT-MSCs [69,93]. Choudhery et al., [22], compared differentiation potential of AT-MSCs and CT-MSCs. In this study cells were differentiated into adipose, bone, cartilage and neurons. The results indicated that cell from both of these sources differentiated into adipose, bone, cartilage and neurons but with differences. For example, adipogenic, neurogenic and osteogenic potential of AT-MSCs was superior as compared to CT-MSCs while in terms of chondrogenic differentiation potential MSCs from adipose and cord tissue were equivalent [22]. In another study, when AT-MSCs and CT-MSCs were compared, no difference was found in osteogenic differentiation potential, but prominent adipogenic differentiation was observed in AT-MSCs as compared to CT-MSCs [93]. Li CY et al., [69] induced AT-MSCs and BM-MSCs to differentiate into bone, cartilage and adipose. The comparison indicated that osteogenic and chondrogenic differentiation potentials of BM-MSCs were greater as compared to AT-MSCs [69]. Contrary to this the fatty vacuole deposits were significantly higher in AT-MSCs as compared to BM-MSCs [69]. In another study osteogenic differentiation was found in BM-MSCs and AT-MSCs but not in other of MSCs [73]. Overall, MSCs isolated from all of these sources can show differentiation into multiple lineages but differences may exist.

Clinical Trials

NIH database (http://clinicaltrials.gov/) keeps record of human clinical trials. A search using the term "mesenchymal stem cells" of this website yielded 360 entries of clinical trials that utilized MSCs. Out of 360 clinical results, 151 were recruiting, 55 yet not recruiting and 154 studies have been completed. 61 studies were using allogenic MSCs from different sources. In allogenic studies mostly BM-MSCs and CT-MSCs were used. Major conditions treated with MSCs were cardiovascular diseases, osteoarthritis, neurodegenerative diseases (such as ALS, Alzhiemr's, Parkinsons), Kidney disease, liver diseases, multiple sclerosis, spinal cord injury etc. BM-MSCs were utilized in 151, AT-MSCs in 40 and CT-MSCs in 18 clinical trials.

6. Conclusion

It can be said without doubt that stem cell based regenerative therapies are very promising for the regeneration of damaged human organs and tissues. After the success of preclinical studies, several research groups have provided initial clinical reports for the use of stem cells in a broad spectrum of human diseases. For stem cell based therapies different sources of MSCs are available that may differ in their biological activities. Although MSCs are present in almost all tissue of the body, bone marrow, adipose- and cord tissues are major MSC sources. MSCs isolated from all of the sources display the basic characteristics of being MSCs, but with significant differences. Therefore the specific characteristics should be kept in mind when designing a therapy for a specific medical condition.

Conflicts of interest

There is no conflict of interest regarding this paper.

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