INTRODUCTION

Cell-based therapy and regenerative medicine offer a new hope for the treatment of various diseases in which there is loss of substance, volume, tissue or organ damage. Recently, scientists have been interested in mesenchymal stem cells (MSCs) due to their capacity to differentiate into many cell lineages.[1]

A stem cell is characterized by its ability to undergo self-renewal, multi-lineage differentiation and form terminally differentiated cells.[2, 3]

Ideally, a stem cell that could be used in regenerative medicine applications should meet the following set of criteria: [2] (i) It should be found in abundant quantities (millions to billions of cells), (ii) It can be collected and harvested by a minimally invasive procedure, (iii) It can be differentiated along multiple cell lineage pathways in a reproducible manner and (iv) finally, It can be safely and effectively transplanted to either an autologous or allogenic host.

Stem cells can be classified into embryonic and adult stem cells. Embryonic stem cells are totipotent, while adult stem cells are pluripotent. A totipotent cell is a cell that is able to divide and produce; foetal membranes and pluripotent cells. Pluripotent cells are cells that can differentiate into either **endoderm** e.g. interior stomach lining, gastrointestinal tract and lungs or **mesoderm** e.g. muscle, bone, blood and urogenital or **ectoderm** e.g. epidermal tissues and nervous system.[4] Although embryonic stem cells posses enormous potentials, their use is limited due to ethical issues.[5]

Adult stem cells can be isolated from postnatal tissues. These cells were initially thought to have differentiation capacity limited to their tissue of origin, however recent studies have demonstrated that they have the capacity to differentiate into cells of mesodermal, endodermal and ectodermal origins. [4]

Historical perspective:

More than a century ago, the presence of progenitor cells in the bone marrow with the capability of differentiating to bone were identified. A series of landmark observations by ***Friedenstein et al***; founder of mesenchymal stem cell (MSC) concept, led to identification of the clonogenic potential of fibroblast-like cells residing in bone marrow.[6]

By culturing of bone marrow on plastic culture dishes, Friedenstein et al were able to discard non-adherent hematopoietic stem cells and identify plastic adherent cells. These plastic adherent cells were spindle shaped and formed foci of two to four cells, which remained inactive for 2–4 days and then multiplied rapidly. After passaging several times in culture, they became more homogeneously fibroblast-like in appearance. They also were found to have the ability to make small colonies that resemble cartilage or bone.[6] The term “mesenchymal stem cells” (MSCs) appeared in the early 1980s and was largely popularized by ***Caplan***; the father of human mesenchymal stem cells (hMSCs)[7]

Mesenchymal stem cells (MSCs):

MSCs are a group of multi-potent somatic stem cells that have the potential for mesodermal and non-mesodermal differentiation, i.e. can be guided in vivo or in vitro to terminally differentiate into osteoblasts, adipocytes, chondrocytes, skeletal myocytes, tenocytes, cells of visceral mesoderm, neurons, hepatocytes and endothelium.[8, 9] *(figure 1)*

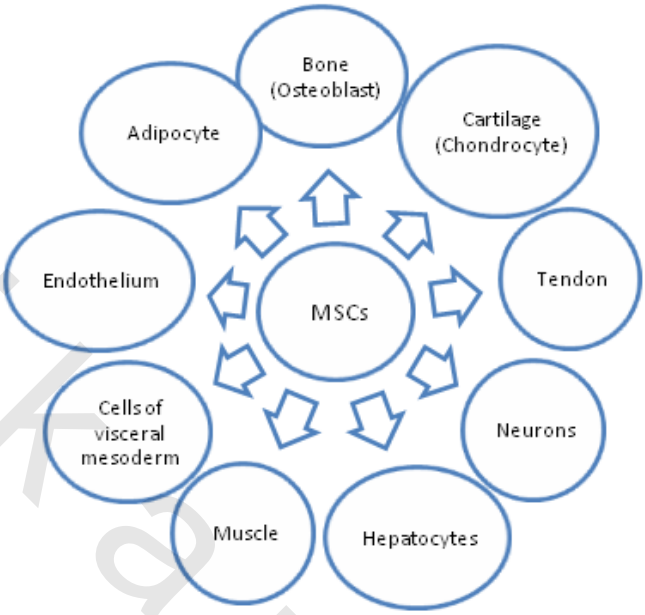

Figure (1): MSCs have the ability to terminally differentiate in vivo or in vitro into osteoblasts, adipocytes, chondrocytes, skeletal myocytes, tenocytes, cells of visceral mesoderm, neurons, hepatocytes and endothelium.

Bone marrow harbors multiple types of stem/progenitor cells including; hematopoietic stem cells (HSCs), endothelial progenitor cells (EPCs) and bone marrow-derived mesenchymal stem cells (BM-MSCs).[10, 11] BM-MSCs typically exist at a very low frequency within the bone marrow mono-nucleated cell population (0.01%-0.1% of total bone marrow cells).[12]

Sources of mesenchymal stem cells (MSCs):

MSCs can be isolated form bone marrow (BM), umbilical cord (UC) and adipose tissue (AT). It should be noted that MSCs have recently been also isolated from periosteum and fetal tissues.[5, 13]

Bone marrow represents the main source for the isolation of multi-potent MSCs, however there are some features that could restrict or make the use of BM-MSCs unacceptable, such as; highly invasive harvesting procedure and the significant decline in number, differentiation potential and maximal life span of these cells with increasing age.[14] Therefore, it became essential to search for other sources of MSCs.

MSCs were isolated from bone marrow by Bochev et al[15] as follows ; 5-10 ml aspirate obtained from subjects were centrifuged at 1200 rpm for 10 minutes and the supernatant plasma was discarded. Bone marrow nuclear cells were obtained after erythrocyte removal by lysis buffer, then washed twice in a sterile phosphate buffer saline

(PBS) and cultured in 6-well plates at a concentration of 1.0 x10^5 nucleated cells/ cm^2 in 2 ml low glucose Dulbecco's modified Eagle's medium (LG-DMEM) containing 10% foetal bovine serum (FBS) then incubated in a humidified atmosphere containing 5% CO_2 at 37^{o}C.

An alternative source of MSCs is the ***umbilical cord***.[14] MSCs are isolated from different compartments of the umbilical cord, including ; umbilical vein sub-endothelial zone, umbilical cord blood and specifically Wharton's jelly.[16, 17] Wharton's jelly is the parenchyma within the umbilical cord, a mucoid connective tissue surrounding umbilical cord arteries and vein.[16] Wharton's jelly can be further divided into three anatomical regions ; where MSCs can be derived from the perivascular zone, the intervascular zone and the subamnion.[17] Umbilical cord as a source of MSCs is favored than bone marrow as it is a less invasive method and can be obtained without harm for the mother or the infant. [18] However, controversy still exists whether umbilical cord could serve as a source for multipotent MSCs as some scientists didn't succeed in isolating MSCs from it. [19]

MSCs from umbilical cord blood (UCB) were isolated by kern et al [14] as follows ; prior to isolation, the anti-coagulated cord blood was diluted 1:1 with 2 mM EDTA-PBS. The mononuclear fraction was initially seeded at a density of 1x 10^6 mononuclear cells/ cm^2 into FBS -pre-coated culture plates. Non-adherent cells were removed 12-18 hours after initial plating. Cells were then cultured in low glucose Dulbecco's modified Eagle's medium (LG-DMEM) containing 10% mesenchymal stem cells growth supplements (MSCGS) and incubated in a humidified atmosphere containing 5% CO_2 AT 37^{0}C.

Adipose tissue appear to be a very encouraging alternative source of MSCs and regenerative cell therapy, as it could serve as a more convenient rich source of MSCs. Adipose tissue can be obtained in a less traumatic manner and in larger quantities than bone marrow. In addition, adipose-derived mesenchymal stem cells (AT-MSCs) show lower morbidity during the harvesting procedures [5, 15]

Criteria of mesenchymal stem cells (MSCs):

At sites of injury, it has been established that most, if not all, MSCs are derived from perivascular mesenchymal stem cells, pericytes, that are on the tissue side of blood vessels and sinusoids.[20, 21] This location has been identified in MSCs derived from bone marrow, umbilical cord [22] and adipose tissue. [23-25] In vivo, MSCs exert their influence by the secretion of massive amounts of growth factors and cytokines. MSCs secrete bioactive molecules that ; inhibit apoptosis, limit the field of damage or injury, inhibit fibrosis, stimulate angiogenesis, stimulate the mitosis of tissue-specific and tissue-intrinsic progenitors, such as cardiac or neural stem cells.[26]

It is important to note that there is no universally agreed set or specific single marker to identify these cells. [27] To address this problem, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) proposed a set of standards to define human mesenchymal stem cells (hMSCs) for both laboratory-based scientific investigations and for pre-clinical studies.[27]

Generally, MSCs are fibroblast-like cells in morphology that posses the following ***three*** criteria; [27] MSCs have the ability to adhere to plastic surface in standard culture conditions. ***Biologically***, they possess multi-potent differentiation potential , i.e. in vitro

differentiation into osteoblasts, chondrocytes and adipocytes. ***Finally***, MSCs have specific surface antigen (Ag) expression; by immunophenotyping, they are **Positive** for ; *CD90* (known as Thy-1), *CD73* (known as ecto 5' nucleotidase) and *CD105* (known as endoglin), and **negative** for ; *CD45* ; is a pan-leukocyte marker, *CD34*; marks primitive haematopoietic progenitors and endothelial cells, *CD14 or CD11b*; are prominently expressed on monocytes and macrophages, the most likely haematopoietic cells found in MSCs' culture, *CD79a or CD19*; are markers of B-cells that may also be found in MSCs' culture and remain vital through stromal interactions and *HLA-DR* ; major histo-compatability complex class II (MHC Class II).[27]

The biological property that most uniquely identifies MSCs is; their ability to differentiate into osteoblasts, adipocytes and chondrocytes using standard in vitro tissue culture-differentiating conditions [27] Differentiation to osteoblasts can be demonstrated by staining with Alizarin Red or von Kosa staining, while differentiation to adipocytes can be demonstrated by staining with Oil Red O and differentiation for chondroblasts can be demonstrated by staining with Alician blue or immune-histochemical staining for collagen type II. [27]

On molecular basis, MSCs were shown to have a transcriptional co-activator with PDZ-binding motif tafazzin gene (TAZ) that was identified as an early "molecular rheostat" modulating mesenchymal stem cells' differentiation. [28, 29] TAZ has been shown specifically only in bone marrow-derived mesenchymal stem cells (BM-MSCs). [1] However, a recent article by Cho et al [30] suggests that TAZ is also expressed in AT-MSCs.

In case of adipogenic differentiation, although several transcriptional key events regulating the differentiation of pre-adipocytes into mature adipocytes have been identified in the last decade, master genes committing the multi-potent MSCs to adipocytes are still awaiting discovery.[1] Adipogenic differentiation is mainly promoted by peroxisome proliferator-activated receptor gamma (PPAR-γ).[1] There is evidence that the ability of AT-MSCs to grow and differentiate varies among fat depots and changes with age. [1] For example, PPAR-γ is expressed more by the adipose tissue samples taken from the arm.[31] Younger patients have increased PPAR-γ-2 expression in all depots, whereas older patients have consistent elevated expression only in the arm and thigh depots.[31]

Bone morphogenetic proteins (BMPs) have been postulated to play a role in the selective differentiation of MSCs into either the osteoblastic or adipogenic lineage ; the temporal expression or loss of the BMP receptors may play a key role in determining the lineage commitment of the mesenchymal stem cells MSCs into osteoblasts or adipocytes.[32] Osteogenesis is most evident in AT-MSCs from the flank and thigh, as compared with those from the arm and abdomen. This may be due to elevations in bone morphogenetic protein 4 (BMP 4) and bone morphogenetic protein receptor 1B (BMPR 1B).[33]

The main difference between BM-MSCs and AT-MSCs at the early stages of their in vitro culture is their ***growth rate***. Newly isolated AT-MSCs expand faster and reach full confluence earlier than do BM-MSCs. Studies shows higher expansion potential of AT-MSCs compared to BM-MSCs through 4-6th passage.[14]

Analysis of ***proliferation capacities*** of MSCs derived from bone marrow, umbilical cord and adipose tissue. BM-MSCs were shown to possess the lowest population doubling numbers through 4-6th passages, followed by AT-MSCs after 3rd passage. However, UC-MSCs displayed the highest doubling numbers in all passages.(14)

During in vitro culture, mesenchymal MSCs posses ***limited life span and finally undergo replicative senescence*** indicated by loss of proliferation and altered morphology. UC-MSCs has the highest ratio of cells undergoing senescence within early passages, followed by BM-MSCs. The lowest senescence ratio within early passages displayed by AT-MSCs. (14) Despite many colonies reaching senescence at early stages, UC-MSCs can be cultured longest, followed by AT-MSCs whereas BM-MSCs showed the shortest culture period.(14)

AT-MSCs and BM-MSCs can be directed in vitro cultures using suitable induction media towards ***osteogenic, adipogenic and chondrogenic differentiation***, while UC-MSCs can only be directed towards ***osteogenic and chondrogenic differentiation with non-detectable adipogenic differentiation capacity***.(14)

Mesenchymal stem cells (MSCs) as an 'injury drugstore':

MSCs have been well characterized with their ability to produce a range of growth factors and cytokines, which inspired the designation of these cells as a kind of 'injury drug store'.(25) An interesting subset of this factory of cytokines is the factors that have been shown to have a profound effect on modulating the immune system.(34)

MSCs have been used as a therapeutic tool based on their capacity to differentiate directly into end-stages phenotypes. In addition to this, MSCs have also been shown to secrete trophic factors that contribute to repair via promotion of vascularization, inhibition of cell death and modulation of the immune response.(25) Of particular relevance to the therapeutic applications of MSCs is their post-implantation fate and their route of delivery to the site of injury/inflammation.(25)

There are two principal methods to introduce cells into the body ; local delivery into the tissue and systemic delivery. Local delivery can be further classified either cells embedded in a scaffold or local injection e.g. intra-peritoneal (IP), intra-cardiac or intra-muscular. Systemic delivery can be further defined by the vascular route ; intra-venous (IV) or intra-arterial (IA). The optimal method of delivery of MSCs will depend on their mechanism of action at the site of injury/inflammation.(25) If MSCs can exert their effect distally, for example, by secreting cytokines into the circulation,(34) then, it may not be necessary for the cells to be located at the site of injury, for example, when MSCs were injected intra-peritoneally, they were able to prevent the damage caused by collagen-induced arthritis despite the lack of detectable presence in the arthritic joints.(35) However, if MSCs need to be present at the site of injury, for example, by differentiating into replacement cells (36) or by the local production of anti-apoptotic or angiogenic factors (37), then the delivery system must place cells at, or allow MSCs to migrate to the site of injury.(25)

Clinical applications of mesenchymal stem cells (MSCs):

Autologous AT-MSCs were used for the regenerative treatment of **widespread traumatic calvarial bone defects**.[38] A 7-years-old girl with post-traumatic calvarial defects was treated with autologous cancellous iliac bone combined with autologous AT-MSCs, fibrin glue and a biodegradable scaffold. Postoperative computed tomography showed new bone formation and almost complete calvarial continuity was obtained.[1]

The transfer of AT-MSCs combined with free fat has been reported to play an important role in **maintaining the volume of the injected fat tissue**.[39] Injection of free fat together with AT-MSCs isolated from the equivalent liposuction aspirates, termed cell-assisted lipotransfer, could become an alternative to soft tissue augmentation surgery, including cosmetic breast augmentation.[40] A new aim could be the use of fresh AT-MSCs isolated from half of the fat tissue mixed with platelet-rich plasma and combined with the other half.[1]

Autologous AT-MSCs therapy could also be used to treat **fistulas in patients with Crohn's disease**. In a study of five patients with Crohn's disease, the external openings in six of eight fistulas were closed by inoculation of the fistulas with autologous AT-MSCs.[41] Since that report was published, AT-MSCs have been also used to repair trachea-mediastinal fistulas caused by cancer ablation.[42]

The therapeutic potential of AT-MSCs for wound healing can be anticipated for the treatment of **chronic ulcers caused by radiation therapy**.[38] Twenty patients being treated for the side effects of radiotherapy, and with severe symptoms or irreversible functional damage, received autologous AT-MSCs delivered via repeated hypoinvasive computer-assisted injections. The clinical outcome was systematic improvement or remission of symptoms in all patients evaluated.[1]

Cultured MSCs can be used in treatment of **Osteogenesis imperfecta (OI)**.[13] Osteogenesis imperfecta (OI) is a disease in which osteoblasts produce defective type I collagen, which leads to osteopenia, multiple fractures, bone deformities, and shortened stature. Mesenchymal progenitors in transplanted marrow were shown to migrate to bone in children with OI and then give rise to osteoblasts whose presence correlates with an improvement in bone structure and function.[13] Culture-expanded human mesenchymal stem cells (hMSCs) were infused into children who had previously undergone conventional bone marrow transplantation (BMT), it was found that some cells engrafted in defective bone and differentiated to osteoblasts capable of extending the clinical benefits of BMT. [43] Thus, allogenic MSCs can be safely transplanted to children with OI without provoking an immune response, and some cells home to the bone marrow.[13]

Allogenic MSCs infusion can be used for treatment of **metachromatic leukodystrophy (MLD)**; an autosomal recessive disease due to the deficiency of aryl-sulfatase A enzyme, that results in accumulation of sulfatides causing demyelination of central and peripheral nervous system and **Hurler's syndrome (MPS-IH)**; an autosomal recessive disease due to the deficiency of alpha-L-iduronidase enzyme that results in accumulation of heparan sulfate and dermatan sulfate in lysosomes [44] Transplantation of allogenic hematopoietic stem cells (HSCs) can significantly halt disease progression and improve survival of patients with MLD and Hurler's syndrome.[45, 46] This effect is believed to be due to tissue infiltration of macrophages that express normal arylsulfatase-A and

alpha-L-iduronidase that are missing in MLD and MPS-IH, respectively [45] and transfer of normal enzyme into defective cells by endocytosis. However, nervous system and skeletal deformities persist and often progress. [47] Some of the residual defects could be corrected by use of supplemental cell therapy by infusion of donor bone marrow-derived allogenic multipotent mesenchymal stem cells (MSCs) as they express high amounts of alpha-L-iduronidase and aryl-sulfatase-A, deficient in MPS-1H and MLD respectively.[44]

MSCs can be used in treatment of some **orthopaedic diseases** as ; degenerative arthritis, osteoarthritis, aggravated rheumatoid arthritis, lumbar inter-vertebral disc degeneration and osteoporotic fractures.[48]

Immunomodulatory properties of mesenchymal stem cells (MSCs):

Transplanted allogenic MSCs can be detected in recipients for extended periods of time, indicating that these cells weren't recognized by immune system and thus, they weren't cleared. Also, it has recently been reported that; BM- MSCs play a role in reducing the incidence and severity of graft-versus-host disease (GVHD) during allogenic bone marrow transplantation. These findings suggest that MSCs posses immune-regulatory properties, but the mechanisms remain to be investigated.[49] *(figure 2)*

These immune-modulatory properties may be attributed to the fact that ; MSCs express a large number of receptors that help them to interact with T-cells; MHC-Class I and a variety of adhesion molecules including; vascular cell adhesion molecule (CD106; VCAM), intracellular adhesion molecule 1 (ICAM-1), activated leukocyte cell adhesion molecule (CD166; ALCAM), lymphocyte functional antigen-3 (CD58; LFA3) and also other antigens that can find their counterpart ligand on T-cells. [13] However, They lack expression of MHC-Class II on cell surface,[50, 51] but Western blotting assays on cell lysates has shown that they contain intracellular deposits of MHC-Class II allo-antigens. [52, 53]

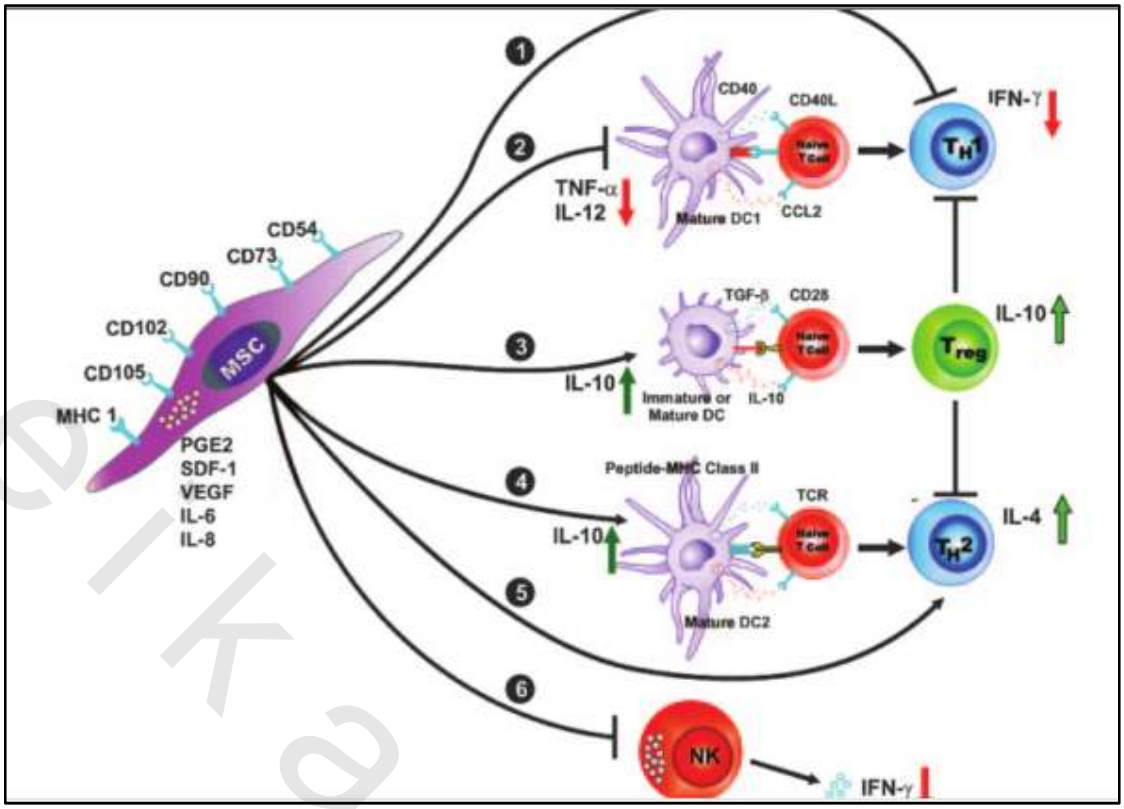

Figure (2): MSCs mediate their immune-modulatory effects by interacting with cells from both the innate (DC, pathways 2-4; NK cell, pathway 6) and adaptive immune systems (T cell, pathways 1 and 5). MSCs act through inhibition of TNFα secretion and promotion of IL-10 secretion which may affect dendrtic cell (DC) maturation state and their functional properties, resulting in shifting the immune response toward an anti-inflammatory/tolerant phenotype. Alternatively, when MSCs are present in an inflammatory microenvironment, they inhibit IFN γ secretion from TH1 and NK cells and increase IL-4 secretion from TH2 cells. It is likely that MSCs also mediate their immune-modulatory actions by direct cell-cell contact as well as by secreted factors. ***CCL*** indicates a chemokine ligand, ***TCR*** indicates T-cell receptor. *In the courtesy of Aggarwal et al.* [49]

MSCs can be induced to express MHC Class II and Fas ligand (CD95L) by interferon-gamma (IFN γ) treatment.[54] Also, they don't express co-stimulatory molecules B7-1 (CD80), B7-2 (CD86), CD40, and CD40 ligand (CD154) and therefore, they don't activate allo-reactive T-cells. [55] Thus, MSCs have historically been regarded as hypo-immunogenic cells. [56]

MSCs interact with cells of the immune system and are capable of altering the outcome of the immune response by inhibiting two of the most important pro-inflammatory cytokines; tumour necrosis factor-alpha and interferon-gamma (i.e. TNFα and IFN γ) and by increasing expression of suppressive cytokines including ; IL-10.[49]

IL-10 is an anti-inflammatory cytokine which inhibits the secretion of pro-inflammatory cytokines by the macrophages and dendritic cells. It inhibits the activities of type 17 T-helper cells (Th17) [57] and the differentiation of blood monocytes into dendritic cells.[58] One of IL-10 most significant immune-regulatory effects is the induction of tolerance in dendritic cells by inhibition of molecules which are crucial for antigenic presentation such as the B7 complex. Moreover, IL-10 directly inhibits the CD28 expression on T-helper surface, which is the specific ligand for the B7 complex.[58]

MSCs can alter the cytokine secretion profile of dendritic cells. MSCs up-regulate regulatory cytokines such as IL-10 and down-regulate inflammatory cytokines such as; IFN γ, IL-12 and TNFα. The down-regulation of TNFα by dendritic cells leads to; inhibit dendritic cells maturation, migration to lymph nodes and hence the ability to stimulate allo-T cells by altering the expression of several receptors and co-receptors necessary for antigen capture and processing.[59-61]. Thus, inducing a more anti-inflammatory or tolerant dendritic cell phenotype.[62, 63]

MSCs can suppress proliferation of natural killer cells (NK cells) and decrease the secretion of IFN γ by stimulated NK cells. It was reported that this is due to; combination effects of cell-to-cell contact and soluble factors, including ; transforming growth factor-beta1 (TGF-β1) and prostaglandin E2 (PGE2). [64] They can also inhibit B-cell proliferation and immunoglobulin production. [65]

MSCs inhibit T-cell proliferation via direct contact through engagement of inhibitory molecule programmed death 1 (PD-1) to its ligands; programmed death-ligand 1 (PD-L1) and programmed death-ligand 2 (PD-L2) leading to modulate the expression of different cytokine receptors and transduction molecules for cytokine signaling. [66]

T-cell receptor has the ability to recognize antigens. T-cell activation requires co-stimulatory signals involving specific molecules on the surface of both T-cells and dendritic cells. Given the absence of surface expression of these key co-stimulatory molecules by MSCs, it has been proposed that MSCs could also render T-cells anergic. [67]

MSCs have been shown to increase the number of immune-regulatory T-cells (T-regs) [68]; either CD4+ CD25+ cells or CD4+ CD25+ FoxP3+ T-cells in different models and assays. [69] CD4+ CD25+ FoxP3+ cells produce the immunosuppressive cytokine IL-10, and thus MSCs also exert indirect immunosuppressive effect.[69-72]

MSCs can also cause type 1 T-helper cells (Th1 cells) to decrease IFN γ and the type 2 T- helper cells (Th2 cells) to increase secretion of IL-4 leading to a shift from the prominence of pro-inflammatory Th1 cells towards an increase in anti-inflammatory Th2 cells.[73]

They can mediate suppression of T-cell proliferation by the secretion of immunosuppressive cytokines ; TGF-ß and/or IL-10.[55, 56] Also, MSCs can modify T-cell functions by soluble factors including; IL-6, IL-8, PGE2 and vascular endothelial growth factor (VEGF).[49]

MSCs secrete high levels of IL-6, and this leads to; inhibition of the differentiation of dendritic cells, inhibition of the secretion of pro-inflammatory cytokines.[74] It also causes the secretion of other immunosuppressive factors such as PGE2 by MSCs acting by an

autocrine mechanism. [75] IL-6 also directly causes an increase of the numbers of immune-regulatory T-cells CD8+FoxP3+.[76, 77]

Studies also showed elevated levels of PGE2 by MSCs in co-cultures. Inhibitors of PGE2 synthesis masked the overall suppressive effects, suggesting that it may be responsible for much of the MSCs- mediated immune-modulatory effects in vitro. [49]

In presence of IFN γ, MSCs express tryptophan catabolizing enzyme indoleamine 2,3 di-oxygenase (IDO) activity that in turn degrades essential tryptophan in the media and hence, may lead to inhibition of T-cell proliferation, suggesting that IDO plays a role in MSCs-mediated immune-suppression.[78]

The observation that human mesenchymal stem cells (hMSCs) can be isolated from decidua [79], amniotic fluid[80], fetal blood [81] and umbilical cord blood [82, 83] may indicate a role for MSCs in fetal tolerance. Fetal immune responses to paternal antigens are suppressed by a phenomenon called "immune privilege." [67] The emerging data on the mechanisms contributing to immune privilege in the pregnant uterus show striking similarity to the immune-suppressive effects of MSCs, including the production of indoleamine 2,3 di-oxygenase (IDO) [78] and support the hypothesis that MSCs are involved in fetal tolerance.[84]

Pros and cons of Clinical trials using MSCs in humans:

Clinical studies have demonstrated promising results in treating patients with cancer, in reducing the incidence of graft-versus-host-disease (GVHD) after allogenic bone marrow transplantation, in promoting heart tissue recovery from massive myocardial infarction, in improving the recovery of patients after amyotrophic lateral sclerosis (ALS) and in treating fatal disorders such as metachromatic leukodystrophy (MLD) and Hurler syndrome (MPS-IH).[44, 85-87]

Graft-versus-host disease (GVHD) is the major complication associated with allogenic hematopoietic stem-cell transplantation (HSCT), which significantly impacts on non-relapse mortality. Based on the timeframe and type of organ involvement, GVHD can be characterized as; acute or chronic. Prevention strategies have almost exclusively been directed at reducing acute GVHD which is the most important risk factor for chronic graft-versus-host disease GVHD.[88] It involves a pathophysiology that includes host tissue damage, increased secretion of pro-inflammatory cytokines (TNFα, IFN γ, IL-1, IL-2, IL-12), and the activation of dendritic cells, macrophages, natural killer cells and cytotoxic T-cells.[89] MSCs were first shown to accelerate the recovery in a patient with a severe case of GVHD [50, 73] Early feasibility studies showed safety with no evidence of adverse events associated with infusion of autologous human mesenchymal stem cells (hMSCs). A recent study assessing the role of in vitro expanded, third party, MSCs co-infused with transplantation in high-risk settings, such as mismatched unrelated donor HSCT, suggests that MSCs may reduce life-threatening GVHD. The results from these HSCT trials have shown that MSCs' infusions are well-tolerated, but long-term safety data have yet to be established along with the production of guidelines for how to culture and expand MSCs.[88]

There are several obstacles that face the use of MSCs in clinical trials including ; MSCs characteristics and phenotype vary according to the passage cycle, culture conditions and their source. Also, the use of FBS to maintain MSCs' culture represents a safety issue, as FBS has been associated with transmission of diseases or undesirable immune responses to it. Trials using autologous platelet-rich-plasma (PRP) as a source of growth factors and cytokines instead of FBS have recently been made.(50)

It is essential also to define an effective cell dose (ECD) equivalent for cell therapy; which is the minimum cell dose required to exert a therapeutic effect, protocol of administration that results in optimal efficacy and to develop refined immunologic and molecular assays capable of monitoring therapeutic efficacy.(25)

The ***protocol of administration*** depends on the question whether MSCs perform best when present at the site of injury/inflammation or not ***(as mentioned earlier in introduction, page 6)***. Advantages of local injection include; delivery of MSCs to the site of lesion, for example, MSCs were found to migrate towards an ischemic lesion of the brain when injected near the location of the ischemia and appeared morphologically, to differentiate into microglia in animal models.(90) The disadvantage of local injection is that it can lead to further tissue damage from injection bolus (91), also MSCs can't migrate out of local tissues into the circulatory system, which is problematic if MSCs need to be present in multiple body compartments or if the injury is systemic.(25)

Intravascular injection has the advantage of being minimally invasive, and it allows wide distribution of cells throughout the body. The most common method for delivery of MSCs is intravenous injection. However, cells delivered intravenously have to first pass through the lungs before they can distribute throughout the body.(25) This presents a major problem which has been termed ***pulmonary "first pass" effect***, which results in significant entrapment of cells.(92) This effect may be attributed to; MSCs deformability and endothelial cell adhesion molecules. While arterial delivery of MSCs allows cells to bypass the lungs and thus avoiding pulmonary first-pass effect. (25)

The techniques of assessing bio-distribution of MSCs can be categorized into ***in vivo*** and ***in vitro*** methods.(25) Examples of ***in vivo*** methods include; ***bioluminescence***: in which cells are transduced to express luciferase and can then be imaged through their metabolism of luciferin resulting in light emission(93), ***fluorescence***: in which cells are either loaded with a fluorescent dye or transduced to express a fluorescent reporter which can then be imaged, ***radionuclide labeling***: where cells are loaded with radionuclides and localized with scintigraphy(94), ***positron emission tomography (PET)*** or ***single photon emission computed tomography (SPECT)*** and ***magnetic resonance imaging (MRI)***: in which cells are loaded with paramagnetic compounds (e.g., iron oxide nanoparticles) are traced with a magnetic resonance imaging (MRI) scanner. ***In vitro*** methods include; quantitative PCR, flow cytometery. (25) With these new tracking methods, it is now easier to know long-term fate of transplanted MSCs.

Malignant transformation of MSCs may represent also a problem. It may occur as a result of expansion in vitro for extended periods of time due to a risk of chromosomal instability.(95)

To date the identification of MSCs has also been difficult and challenging. It is interesting that since the first description of MSCs by Friedenstein et al (6), his method for

isolation of MSCs from bone marrow has largely remained the ***"standard of practice"*** ; depending on the ability of fibroblast-like cells to adhere to the plastic substrate of a cell culture plate, together with a concurrent lack of adherence of marrow-derived hematopoietic cells.(96)

Although many scientists have successfully isolated MSCs from adipose tissue, there is still no ***standard method*** for isolating and culturing highly homogenous population of MSCs. An essential requirement for the effectiveness of regenerative strategies is the good yield of stem cells, which is mainly depending on source and processing method. In addition, it is desirable that cells retain over time the capacity to proliferate and maintain their multi-potency. (97)

The development of a standardized and reproducible method for isolating and culturing AT-MSCs is a basic requirement to validate their use in experimental protocols, since the methodological differences may influence the properties of isolated cells and influence the results.(48)

The ***aim*** of this study is to develop an efficient method for isolation and identification of MSCs.