Chapter -I

General Introduction and Objectives
Abstract
This thesis focuses on the manufacturing of human bone-marrow derived mesenchymal stromal cells (BM-MSCs) in serum and serum free culture conditions for therapeutic applications. This introduction chapter provides the readers with an overview of classification and importance of stem cells with an emphasis on mesenchymal stromal cells. A brief update on currently available cell therapy products has been provided. Finally, the objectives and outline of the thesis are described.
1.1 Introduction
The term “stem cell” was first proposed by the Russian histologist Alexander Maksimov in 1908 at congress of hematologic society. But the term stem cells was adopted after using stammzella form Ernst Haekel, Theodor Boveri early around 1900 stating the potential of stem cells for self-renewal and differentiation [1]. Later the concept of the stem cell was described by Pappenheim as immortal with self-renewal properties and without cellular senescence [2]. Stem cells are the cells with the ability to divide for indefinite periods in culture and to give rise to specialized cells. The stromal cells are non-blood cells of bone marrow or fetal liver, which are capable of supporting growth of blood cells in vitro.

These stem cells have the unique potential to develop into many different cell types in the body during early life. The stem cells are the part of internal repair systems to repair or replenish damaged or aged cells. Stem cells are unspecialized cells capable of renewing through cell division and in some physiological conditions, become specific cells to perform organ specific functions. Because of their unique regenerative potential, stem cells offer a hope of new treatment for various disease indications such as osteoarthritis (OA), diabetes and other degenerative diseases. The modality to use these stem cells for cell based therapies to treat diseases is referred to as regenerative medicine.

1.2 Classification of stem cells
1.2.1 Can A Classification
Can A (2008) described the conceptual approach to classifying the stem cells beginning from the early morula stage totipotent embryonic stem cells to the unipotent tissue resident adult stem cells [3]. Two major types of stem cells are embryonic stem cells (ES) and adult stem cells. Other types such as pluripotent stem cells (iPSCs) are produced by reprogramming adult cells to regain ES like characteristics.

1.2.2 General classification stem cells
Stem cells can be classified into five types based on their ability to differentiate or their potency. The stem cell types are as follows:
1.2.2.1 Totipotent stem cells
These stem cells have the ability to differentiate into any type of cells in the body. A fertilized egg is totipotent and has total potential for about four days. Totipotent cells can form all cells in a body plus the extra embryonic or placental cells. The zygote is totipotent because its cells can become any type of cell and they have unlimited replication abilities. As the zygote continues to divide and mature, its cells develop into more specialized cells called pluripotent stem cells.

1.2.2.2 Pluripotent stem cells
These stem cells have the ability to differentiate into several different types of cells that make up the body. Like totipotent stem cells, pluripotent stem cells can self-renew and give rise to trillions of cells in the body. Specialization in pluripotent stem cells is minimal and therefore they can develop into almost any type of cells. Embryonic stem cells (ESCs) and stem cells from aborted fetuses are two types of pluripotent cells.

- **Induced pluripotent stem cells** (iPS cells) are genetically altered adult stem cells that are induced or prompted in a laboratory to take on the characteristics similar to embryonic stem cells. Although iPS cells behave like and express some of the same genes that are expressed normally in embryonic stem cells, they are not identical to embryonic stem cells.

1.2.2.3 Multipotent stem cells
These stem cells have the ability to differentiate into a limited number of specialized cell types. Multipotent stem cells typically develop into any cell of a particular group or type.

- **For example**, bone marrow stem cells can produce any type of blood cells. However, bone marrow cells don’t produce heart cells. Adult stem cells and umbilical cord stem cells are examples of multipotent cells.

- **Mesenchymal stem cells** are multipotent cells of bone marrow that have the ability to differentiate into several types of specialized cells related to, but not including blood cells. These stem cells give rise to cells that forms specialized connective tissues, as well as that support the formation of blood.
1.2.2.4 Oligopotent stem cells
These progenitor stem cells have the ability to differentiate into a few cell types. The progenitor cells have the ability to differentiate into a few cell types.

- Lymphoid or myeloid stem cells specifically can give rise to various blood cells such as B and T cells and not to different blood cell types like RBC.
- Progenitor cells of vascular stem cells have the capacity to become either endothelial or smooth muscle cells.

1.2.2.5 Unipotent stem cells
These stem cells have unlimited reproductive capabilities, but can only differentiate into a single type of cell or tissue. Unipotent stem cells are derived from multipotent stem cells and form an adult tissue.

- Skin cells are one of the most prolific examples of unipotent stem cells. These cells most readily undergo cell division to replace damaged cells.

1.3 Mesenchymal stromal cells/stem cells
Human mesenchymal stromal cells (hMSCs) have therapeutic potential in the treatment of Osteoarthritis (OA), Critical limb ischemia (CLI), Liver cirrhosis (LC), Chronic obstructive pulmonary disease (COPD), Diabetes mellitus (DM), Acute myocardial infarction (AMI), Cerebral stroke (CS), Dilated cardio-myopathy (DCM), Parkinson disease (PD), spinal cord injury etc. MSCs are multipotent non-hematopoietic clonogenic cells capable of providing safe and reliable way to repair, restore, maintain or replace the damaged cells and help in retaining functional properties. They are also capable of self-renewal and differentiation into multiple tissues [4]. MSCs are heterogeneous subset of stromal stem cell population that can be isolated from various sources of adult tissues. In vivo administration of MSCs has peripheral tolerance and migrates to injured site, where they can inhibit the release of pro-inflammatory cytokines and enhance the survival of damaged cells/tissue. Overall, the hMSCs are the promise of regenerative medicine in the 21st century as they are not only involved in the regeneration of tissue, but also in prognostics and diagnostics use.
1.4 Classification of MSCs

MSCs can be broadly classified into different types based on source, immune response and cell shape/size. The following section deals with the classification and major properties of each class of stem cells.

1.4.1 Based on source MSCs can be derived from various different sources and can be classified based on the source. These MSCs can be found in nearly all tissues and are mostly located in perivascular niches. Based on the source, MSCs were classified as below:

1.4.1.1 Bone marrow derived MSCs

Bone marrow derived stem cells first described by Friedenstein et al. (1976) are still the most widely studied cell type and often designated as the gold standard [5]. Bone marrow aspiration (BMA) is an invasive and painful procedure for healthy volunteer donors. The commonly used procedure for the generation of MNCs from bone marrow is density gradient centrifugation [6] and then MSCs are isolated from MSCs during culture process. The morphological characteristics of MSCs may slightly differ based on the source, for example BM-MSCs are morphologically larger than AT-MSCs [7]. The mean population doubling time (PDT) of BM-MSCs is about 49 h up to passage 7 and it increased thereafter [8]. Peng et al. (2008) described the PDT of 61.2 h in BM-MSCs and 45.2 in AT-MSCs based on logarithmic growth phase [7]. Kernal et al. (2006) found the sign of cell senescence in BM-MSCs at passage 7, whereas in ASC showed the same at passage 8 onwards [8]. There have been extensive preclinical studies using MSCs wherein most of these are performed in mice, rats and monkeys models. In the human system, BM-MSCs are most frequently used in multiple clinical trials for various disease indications.

1.4.1.2 Adipose derived MSCs

AT-MSCs are also called as adipose-derived stem cells (ASCs) are usually isolated from the stromal vascular fraction (SVF) of homogenized adipose tissue material generated during liposuction, lipoplasty, or lipectomy procedures by enzymatic digestion with collagenase followed by centrifugation and washing [9]. ASCs may in fact be vascular stem cells (VSC), as these cells differentiate into smooth muscle and endothelial cells that are assembled into newly formed blood vessels during angiogenesis and neo-vasculogenesis. Schaffler et al. (2007) defined the surface marker
set for AT-MSCs (ASCs) as positive for CD9, CD29, CD44, CD54, CD73 (SH3), CD90, CD105 (SH2), CD106, CD146, CD166 and HLA-I expression, and negative for CD14, CD31, CD34, CD45, CD133, CD144, HLA-DR, STRO-1 and HLA-II expression [10]. AT-MSCs have shown higher proliferation capacity than BM-MSCs [8]. AT-MSCs have PDT in the range of 45-61 h depending on the origin of ASCs from various region of the body [7, 8]; Chondrogenic differentiation of AT-MSCs (ASCs) has been observed at enhanced levels under hypoxic conditions where osteogenesis is inhibited. In contrast, enhanced osteogenic differentiation of AT-MSCs (ASCs) can be induced under normoxia [11, 12].

1.4.1.3 Cord blood derived MSCs

The umbilical cord blood (UCB) collected immediately after the birth of baby can be used as a rich source of stem cells. UCB derived stem cells are rejected less often by the host tissue, possibly because they do not possess cell-surface molecules that can be recognized and attacked by the host's immune system. Cord blood derived MSCs (CB-MSCs) were first described by Erices et al. in 2000 [13] and were isolated using Ficoll gradient centrifugation method [14]. An average of 40-45% of the processed cord blood samples have colony forming efficiency of 1-11% and visible morphological features appear within 7-21 days [14]. These CB-MSCs were characterized by higher proliferation and differentiation potential with longer telomeres. There is no significant variation in phenotypic marker expression while compared to BM-MSCs. CB-MSCs do not elicit tumorigenicity after subcutaneous injection in immune-deficient nude mice, but CB-MSCs genetic expression profile showed POU5F1-gene, OCT4, Nanog, SOX2 of embryonic stem cell markers [15].

1.4.1.4 Wharton’s Jelly MSCs

Wharton’s jelly MSCs (WJ-MSCs) can be described as an amenable, plentiful, and inexpensive source of multipotent MSCs with promising potential for use in regenerative medicine applications. Fresh human umbilical cords can be obtained after full-term births and proper standardization process has to be followed for processing the cord [16]. The clean and non-contaminated tissues should be used for processing of umbilical cords [17]. Explants culture and enzymatic digestion are two basic methods to isolate MSCs from the human umbilical cord. In explants culture, based on the
mesenchymal migratory capabilities which is also called “plate and wait” where the cord segments are simply plated in the medium and the MSCs are obtained by waiting for them to migrate out [18]. The explants culture protocol is simple, reliable and less labor-intensive, leading to pure and viable cultures, when dealing with a large number of samples received simultaneously [19]. In enzymatic digestion, the cord tissue pieces are digested in an enzyme solution to release the cells. With this method, the number of cells isolated from the umbilical cord is fixed and known while compared with explants culture. But over digestion of tissue may result in diminished cellular viability and altered cellular function [19-23]. WJ is a gelatinous tissue within the umbilical cord that contains MSCs. WJ-MSCs’ stemness and immune properties appear to be more robustly expressed and are more comparable with fetal than adult-derived MSCs. Other advantageous features include ease of sourcing, in vitro expandability up to 80 or more population doublings, differentiation abilities, immune-evasion, and immune-regulation capacities [24, 25]. WJ-MSCs possess the typical extra-embryonic perinatal MSCs properties. Generally WJ-MSCs are very similar to the properties of placenta-derived MSCs (PD-MSCs) and cord blood-derived MSCs (UCB-MSCs) [25]. Clinical application of WJ-MSCs includes improvement in cardiac function, when co-cultured with fetal MSCs [26].

1.4.1.5 Placental derived MSCs

The human PD-MSCs can be isolated from the cotyledons present towards the maternal side of the placenta. The unique properties of multipotent PD-MSCs can be described as an amenable, widely available without ethical concerns and without compromising cell cycle pattern, apoptosis pattern and pluripotency associated endogenous gene expression pattern even after culturing up to 25-30 passages [27]. PD-MSCs exhibit low or no immunogenicity as the placenta acts as an immunological barrier between the fetus and the maternal immune system [28, 29]. Further, placenta synthesizes various hormones, enzymes, neurotransmitters, and cytokines [30, 31]; PD-MSCs may secrete active factors that facilitate the repair of bone defects.

1.4.1.6 Amniotic fluid derived MSCs

The human Amniotic fluid derived MSCs (AF-MSCs) were first reported in 2003 by Prusa et al. (2003) as OCT-4 positive cells, a pluripotent markers for identification
General introduction and objectives

Subsequently there were several reports on the use of AF-MSCs for further characterization [33-36]. Both amniotic membrane and AF contains sub-population of MSCs and these AF are usually obtained amniocentesis, a pre-natal diagnostic procedure to evaluate the health status of fetus during pregnancy. AF-MSCs express all the MSC characteristics that were determined by ISCT. Second trimester AF were considered to be rich source of multipotent MSCs. These AF-MSCs were reported to possess increased in vitro proliferation potential of about 250 population doublings with a doubling time of 1.6 days [37]. Besides, it shows increased multipotency with long telomeric lengths and these cells can be placed in between ESCs and postnatal cell sourcing material like WJ, UB etc. Miranda S et al. (2011) reported accumulated cell number of about $1.0 \times 10^{22}$ and $9.7 \times 10^{23}$ with no visible alterations in chromosome and retain their tri-lineage differentiation capacity during their life span [38]. These MSCs exhibit embryonic intra cellular markers like Nanog and Sox2 throughout their life span. Recently, AF-MSCs are considered the most valuable tools to induce liver repair and liver function by cell transplantation as they can release many factors in curing liver related diseases [39]. CD44+/CD105 sorted MSCs from AF are tested in mouse model for pre-mature ovarian failure and has shown long term in vivo survival and proliferation [40].

MSCs isolated from fetal parts like placenta, umbilical cord and UCB have an advantage of wide availability, no ethical concern, low/no oncogenicity, high expandability and low risk of bacterial/viral contamination. ESCs have highest proliferation and potency and then it decreases gradually from prenatal cell source like AF-MSCs and post natal cell source like WJ-MSCs, adult tissue like adipose, and then BM and lastly the peripheral blood derived MSCs.

1.4.1.7 Synovial MSCs

Synovium also has considerable regeneration capability seen during its reappearances following surgical synovectomy [41]. Synovial Fluid-MSCs (SF-MSCs) from intra-articular ligament injury patients have 100 times more MSCs than healthy donors. SF-MSCs were more in OA than other arthropathies and suggesting possible role of SF-MSCs in arthritis pathophysiology [42]. Hence knowledge and characteristics of SF-MSCs in diseased OA patients will help to understand and develop strategy for OA treatment. Although there is potential role of SF-MSCs in ligament regeneration and
these SF-MSCs were consistently shown higher differentiation potential during in vitro chondrogenesis compared to variable chondrogenesis shown by BM-MSCs [43]. Further, strategies developed to pre-condition the MSCs with 10 ng/ml of TGF-β1 resulted in higher proteoglycan expression. This is of high importance in tissue engineering application of MSCs in chondral defects [44].

1.4.1.8 Dental pulp MSCs
The dental pulp is the centre part of the tooth consisting of small mass of living connective tissue, blood vessels, nerves and stem cells. The search for easily accessible MSCs other than BM has originated the investigations in dental tissues cells. The first type of MSC like cells were isolated from human dental pulp and termed as post natal dental pulp stem cells (DPSCs) [45]. Recently, MSC characteristics were identified in cells derived from dental pulp, stem cells from human exfoliated deciduous teeth (SHEDs), periodontal ligament stem cells, dental follicle precursor cells and apical papilla of dental tissue [46]. Besides, DPSCs possess all mesenchymal characteristics like differentiation, phenotypic marker expression, immune-privilege properties and have characteristics of ectomesenchyme (due to its interaction with neural crest), express a few embryonic markers and possess higher neurogenic potential [47]. DPSCs are highly proliferative as compared to BM-MSCs and ASCs and transcriptome profiling of DPSCs revealed an inherent propensity towards the neuro-ectoderm lineage [48]. DPSCs are a source of neurotrophic factors like nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), glial derived neurotrphic factor (GDNF), CXCL12 etc [49, 50]. The neuroprotective effect of DPSCs can be used to cure the central nervous disorders such as spinal cord injury, Alzheimer’ disease and Parkinson disease, as it promote the in vivo survival of trigeminal and sympathetic ganglia [50, 51].

1.4.1.9 Peripheral blood derived MSCs (PB-MSCs)
PB-MSCs are one of the easily accessible sources and form the alternative to painful cell sourcing of BM-MSCs. MSCs isolated from PB shows all basic MSCs characteristics, besides isolation and characterization of PB-MSCs can be compared with their BM-MSCs. Two ml of PB and BM were showed 5.4 and 5.5 million of cells at passage 2, demonstrated similar tri-lineage differentiation, and similar phenotypic
marker expression. Moreover, qualitative and quantitative test of chondrogenic potential showed similar s-GAG levels in both cultures [52]. Comparison of UC-MSCs and PB-MSCs exhibit similar morphology, proliferation capacity and multilineage differentiation potential; differences were only in the clonogenic efficiency and the immune-phenotype [53]. These results also demonstrate that the PB-MSCs are good alternative source for cellular therapies.

1.4.1.10 MSCs derived from ESCs

Search for alternative source that provides consistency, robustness and enable large scale manufacturing to produce downstream progenitor cells by MSCs has led to ESCs as a source. Potential of manufacturing single batch of cell therapy products sufficient to repeat treatments in multiple patients eliminating the variability and improving the cell quality can be achieved by MSCs derived from ESCs. The use of ESC derived MSCs enables cost-effective production system similar to that of other biotech processes. Several researchers have developed methods for differentiating MSCs from ESCs [54-56]. These hESC-MSCs are very similar to morphological, functional, biological and even in their gene expression profile [57] to BM-MSCs. Recently, MSCs were even derived from human induced pluripotent stem cells (hiPSCs) and confirmed the MSCs characteristics in vitro and shown in vivo bone formation in mice [58].

MSCs were even derived from the lung [59], and the heart [60] and search in all other organs is on.

1.4.2 Classification of MSCs based on immune response

Waterman et al. (2010) classified two homogenously acting phenotypes as MSC1 and MSC2 based on MSC polarization of toll like receptor (TLR) signaling [61]. MSC1 are TLR4-primed hMSCs elaborate pro-inflammatory mediators whereas MSC2 are TLR3 primed hMSCs have immunosuppressive properties. Further studies done by the same group in 2012, showed that MSC1 does not support in vitro tumor cell growth whereas MSC2 favor tumor cell growth [62]. A clear clinical benefit of cell based therapy prevails even if 0.1 to 1% of infused cells reach target sites. These benefits are due to local immune modulation by these cells rather than differentiation or replacement of damaged target tissue [63, 64].
1.4.3 Classification of MSCs based on shape and size

MSCs derived from various cell sources have heterologous MSC population and within the same source MSCs tends to generate various sub-populations of MSCs. Mets and Verdonk (1981) classified MSCs based on cell shape into rapidly proliferating smaller spindle-shaped cells as type-I MSCs and very slow proliferating, large and flat cells as type-II MSCs [65]. Similarly, Colter et al. (2000) have identified, defined and classified MSC subpopulations [66] based on rapidly proliferating small cyclic self-renewal cells as recycling stem (RS) cells. The RS cells that grow very rapidly at low seeding densities of 1.5 or 3.0 cells/cm² to produce single cell derived colonies and reached stationary phase in culture. These include major proportion of large and moderately granular cells and a minor population of small and agranular cells. These major populations of large cells at stationary phase are called mature MSCs or mMSCs and minor population of small granular cells as called as RS-1. Reseeding these cultures at low seeding density area new population of small and granular cells called RS-2 cells. Colter et al. (2001) extend his previous observation to demonstrate a third type of cells as extremely very small rapidly self-renewing round cells as RS cells [67]. RS enriched MSCs have a higher potential and express a distinct surface epitopes which can be used to distinguish the small cells from the large cells. Sakiya et al. (2002), further classified the RS based MSCs into thin spindle shaped cells as RS-1A, little wider shipped shaped cells as RS-1B and still wider spindle shaped cells as RS-1C [68]. A time dependent transition of early progenitors of RS1A to RS1B and RS1C was observed after seeding cells at 1 to 1000 cells/cm².

It may not be appropriate to use single sourced MSCs to treat various target diseases. MSCs from various sources and types have a distinct process and unique gene and molecular signatures which propels them to specific lineage propensity, besides qualifying basic MSCs characteristics. Therefore careful attention is needed in choosing appropriate MSC source for specific disease indications.

1.5 Overview of cell therapy products

At this point, there are a significant number of cell based clinical trials approved by many national and international regulatory bodies are on-going and many of them are registered in clinicaltrials.gov. The fast growing cell therapy industry is integrated with
many public companies and private partnerships for product launch and services. These primarily depend on the level of investments in these sectors and the growth may slow slightly over the next five years to 2018, but it may still outpace the majority of the economy largely revolving pharmaceuticals, biotechnology and medical device manufacturing companies. Many of the cell therapy industry already are aiming to create new blockbuster treatments and some cell therapy companies have already launched the product (Table 1.1) in the market and creating a way and market edge in cell therapy space. These translational researches towards cell therapy products create clarity on guidelines, review process and streamline the product approval requirement to market. The outcome of the cell therapy products bring us critical factors in evaluating the process and improves the regulatory timely approval process for clinical trials.

**Table 1.1** Approved cell therapy product

<table>
<thead>
<tr>
<th>Year</th>
<th>Product Name</th>
<th>Company</th>
<th>Source</th>
<th>Indication</th>
<th>R.Agency</th>
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<tbody>
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<td>Carticel</td>
<td>Genzyme</td>
<td>Auto-Chon</td>
<td>Articular cartilage</td>
<td>USFDA</td>
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<tr>
<td>1998</td>
<td>Apligraf</td>
<td>Oganogenisis</td>
<td>Allo-Keratino + Scaffold</td>
<td>Diab.foot ulcers</td>
<td>USFDA</td>
</tr>
<tr>
<td>2009</td>
<td>ChondroCelect</td>
<td>TiGenix</td>
<td>Auto-Chondrocytes</td>
<td>Cartilage defects</td>
<td>EMEA</td>
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<td>2010</td>
<td>Provenge</td>
<td>Dendreon</td>
<td>Auto-Dendritic cells</td>
<td>Prostate cancer</td>
<td>USFDA</td>
</tr>
<tr>
<td>2010</td>
<td>Gintuit</td>
<td>Oganogenisis</td>
<td>Auto-Dendritic cells</td>
<td>Prostate cancer</td>
<td>USFDA</td>
</tr>
<tr>
<td>2011</td>
<td>Hearti-Cellgram</td>
<td>FCB-PharmiCell</td>
<td>Auto- BM-MSCs</td>
<td>Post-myocardial infarctions</td>
<td>KFDA</td>
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<tr>
<td>2012</td>
<td>Prochymal</td>
<td>Osiris</td>
<td>Allo-BM-MSCs</td>
<td>GVHD</td>
<td>Canada</td>
</tr>
<tr>
<td>2012</td>
<td>La Viv</td>
<td>Fibrocell Science inc</td>
<td>Auto-fibroblast</td>
<td>Severe nasolabial fold wrinkles</td>
<td>USFDA</td>
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<td>2012</td>
<td>Cartistem</td>
<td>Medipost</td>
<td>Allo-MSCs from UCB</td>
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<tr>
<td>2012</td>
<td>Cupistem</td>
<td>Anterogen</td>
<td>Auto-fat derived stem cells</td>
<td>Anal fistula in Crohns Disease</td>
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<td>Duke university</td>
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<td>GVHD</td>
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<td>2013</td>
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Abbreviations: Allo- Allogeneic; Auto- Autologous; BM-MSCs- Bone marrow mesenchymal stromal cells; Chon- Chondrocytes; Diab - Diabetic ; EMEA – European medical agency; GVHD-Graft versus host disease; HPC- hematopoietic progenitor cell; Keratino – keratinocytes; OA- Osteoarthritis (OA); R-agency – Regulatory agency; UCB- Umbilical cord blood; KFDA – Korean FDA; USFDA – United state federal drug administration.
Overview of cell based clinical trial was presented to understand the opportunities and challenges for successful therapeutic applications. Moreover, the outcomes of a large number of cell clinical-trial outcomes for treatments are not documented in peer-reviewed journals. Unfortunately rationale for the clinical application has lagged behind laboratory observations [69]. Well-designed clinical trials will be critical for determining whether MSCs can be effective in treating disease indications. Currently there are various clinical trials on regenerative medicine with various approaches to succeed in clinical trial end points. Table 1.2 presents the major on-going clinical trials in regenerative medicine. In these studies, several initiatives were implemented to accelerate our understanding to bring safe and efficacious development of regenerative medicine product.

Table 1.2 Clinical trial update on Investigational New drugs in cell therapy

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Product Name</th>
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<td>2</td>
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<td>Osiris</td>
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<td>Shire</td>
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<td>4</td>
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<tr>
<td>5</td>
<td>MPC</td>
<td>Mesoblast</td>
<td>Phase-III</td>
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<tr>
<td>6</td>
<td>StemEx</td>
<td>Gamida Cell</td>
<td>Phase-III</td>
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<tr>
<td>7</td>
<td>Liftegrast</td>
<td>Shire</td>
<td>Phase-III</td>
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<tr>
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<td>PLX Cell</td>
<td>Pluristem</td>
<td>Phase-II</td>
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<td>Bluebirdbio</td>
<td>Phase-II</td>
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<td>Multistem</td>
<td>Athersys</td>
<td>Phase-II</td>
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1.6 Objectives of the research work

Title: A novel approach to produce bone marrow derived mesenchymal stromal cells for therapeutics applications

1.6.1 Media optimization of human BM-MSCs in HPL, SFM and FBS.

1.6.2 Characterization of pre-isolated human BM-MSCs cultured in large-scale serum free conditions.
1.6.3 Isolation, expansion and characterization of BM-MSCs in serum free conditions.

1.6.4 Molecular analysis and comparison of BM-MSCs cultured in serum containing and serum free media.

1.6.5 *In vitro* efficacy induction to particular disease indication.

1.7 Outline of thesis

The regenerative potential of multipotent MSCs has generated tremendous interest in using these cells for curing various debilitating diseases. Many different culture conditions have been used for large scale expansion of MSCs *in vitro* however, majority of the studies relied heavily on using media containing FBS for optimal growth of these cells. Human platelet lysate (HPL) was evaluated as an alternative to FBS in large-scale culturing of BM-MSCs for therapeutic applications. Dulbecco’s modified Eagle Medium (DMEM) of low glucose (LG) and Knock out (KO) were used with HPL as LG-HPL and KO-HPL and with FBS as LG-FBS and KO-FBS to culture the BM-MSCs. Optimization studies reveals that 10% HPL supported BM-MSCs growth and subsequent isolation efficiency generated >90 X 10^6 MSCs in LG-HPL. We have investigated the ability of HPL to support growth and expansion of BM-MSCs and compared the results with the cells grown in standard FBS containing medium. Later we have screened 5 different commercially available SFM for their ability to support growth and expansion of pre-isolated undifferentiated BM-MSCs and compared the results with the cells grown in standard FBS containing medium as control. In addition, based on initial screening results, BD-SFM was evaluated in large scale cultures for the performance and culture characteristics of BM-MSCs. Out of the 5 different serum free media, the BD-SFM enhanced BM-MSCs growth and expansion in Cell STACK (CS), but the cell yield per CS-10 was less when compared to the control medium. Our results suggest that BD-SFM enables large scale expansion of BM-MSCs for therapeutic use, even if the donor banks of MSCs were already grown in FBS containing media.

Complete characterization of BM-MSCs from isolation onwards includes isolation kinetics, growth kinetics, immunosuppressive and immunogenicity assays. The identification of suitable commercially available SFM or XFM for their suitability
for the isolation and ex vivo expansion of MSCs were investigated with the cells grown in FBS containing media as control. Isolation kinetics, proliferation, morphology, surface marker expression, MTT assay, CFU-F assay, immunogenicity, immunosuppressive assay, interferon-gamma induction studies, RT-PCR of IDO analysis, and exogenous IDO assay were employed for investigating the effect of SFM on MSCs. BD-SFM and MSX supported isolation, sequential passage, differentiation potential and acceptable surface marker expression profile of BM-MSCs. However cells cultured in control media has significantly higher proliferation rate than BD-SFM or MSX. BM-MSCs cultured in BD-SFM or MSX media supported the isolation and expansion of the cells, maintain MSC characteristics, and are preferred by regulators as it is not containing bovine serum. While BM-MSCs cultured in BD-SFM and MSX media adhered to all MSC characteristics, but in a few parameters, the performances of cells cultured in BD-SFM was superior to that of MSX media. Finally, this study explores the feasibility of erythropoietin (EPO) based induction of MSCs to secrete higher secretome having propensity towards angiogenesis to target cell therapy for certain specific disease indications.

1.8 References
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