Potential Use of Mesenchymal Stem Cells in Regenerative Medicines: A Review

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

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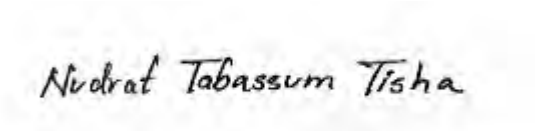

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Approval

The thesis/project titled “A review on the Potential Use of Mesenchymal Stem Cells in Regenerative Medicines: Prospects and Challenges” submitted by Nudrat Tabassum Tisha (18146009) of Summer, 2021 has been accepted as satisfactory by the School of Pharmacy, Brac University in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on August 3, 2022.

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Ethics Statement

This study does not involve any kind of animal or human trial

Abstract

The utilization of Mesenchymal Stem Cells (MSCs) in biomedicine has expanded dramatically over the past 50 years. MSCs are a diverse group of cells, extracted from fat, synovium and bone marrow with the ability to self-renew and differentiate into several types and thus can be utilized for various biomedical applications. As MSCs do not express major histocompatibility complexes or immune-stimulating molecules, they are not detected by the immune surveillance system within a host body which prevents graft rejection after transplantation, thus making MSCs an ideal candidate to be used as regenerative medicine. Unlike therapeutic approaches that deliver a single agent at a fixed dose, MSCs produce bioactive chemicals and impulses that are site controlled, adding to their advantage to be used for tissue engineering. More than 950 clinical trials are currently examining the potential medical benefits of MSCs for various biomedical purposes, including regenerative medicine. Development of new approaches to comprehend and unleash the potential of MSCs, as well as new delivery modalities and patient selection, should lead to significant breakthroughs in the future years and more therapeutic opportunities for MSCs in the field of regenerative medicine. The present review aims to provide a comprehensive overview of the preclinical and clinical applications of MSCs in regenerative medicine and further discuss the challenges associated with developing MSCs for regenerative medicine with future prospects.

Keywords: mesenchymal stem cells; regenerative medicine; differentiation; clinical applications, tissue reconstruction

Dedication

Dedicated to my Beloved Parents, Friends & Respected Faculty Members who have always been there to support me

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To begin, I am grateful to Almighty Allah (SWT) for the good health and wellbeing during the project which was vital to complete the project work on time.

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Chapter 1

Introduction

1.1 Background

Mesenchymal stem cells (MSCs) are a diverse group of cells that can self-renew and differentiate into several types of cells (Caplan, 2007). Recent research have has shown that adipose tissue (Ibrahim et al., 2020), dental pulp (Suchaneka et al., 2009), the endometrium (Schwab et al., 2008), peripheral blood (Tondreau et al., 2005), the periodontal ligament (Park et al., 2011), placenta (Guimarães-Camboa et al., 2017), the synovial membrane (de Bari et al., 2001), and umbilical cord blood (Guimarães-Camboa et al., 2017) along with bone marrow (Friedenstein et al., 1968) as sources of mesenchymal stem cells. MSCs possess numerous benefits such as multiple proliferation capability, multipotency, immunomodulation, and paracrine action. They are an ideal choice for cell therapy, regenerative medicine, immunological regulation, and tissue engineering due to their unique characteristics, including the absence of ethical issues pertaining to embryonic stem cells. As a result, they have been widely employed in clinical practice during the last decade to treat a variety of traumatic and degenerative illnesses. While there is no agreement on a single surface molecule to identify MSCs, the International Society for Cellular Therapy (ISCT) identified three cardinal stem cell properties that must be met for a cell to be called an MSC (Dominici et al., 2006). They are:

(a) *in vitro* differentiation ability that can transform into osteocytes, adipocytes or chondrocytes;

(b) adhesive to polymer on standard culturing; and

(c) expression of cell surface markers namely CD73, CD90, and CD105, but not CD11b or CD14, CD34, CD45, CD19, or CD79a, or HLA-DR. MSCs additionally exhibit surface markers such as CD10, CD13, CD29, and CD44 (Crisan et al., 2008).

Despite the various sources of MSCs, only a small amount can be obtained when aspirated from a particular source. To be therapeutically useful, MSCs must be grown *in vitro* across numerous population doublings (PDs) to produce number of sufficient cells pre–implantation (Sheng, 2015). The chronological age of a donor has a major impact on the quality and lifetime of MSCs - MSCs from older donors function worse than younger counterparts due to lower proliferation ability and overall differentiation potential. Furthermore, beyond the age of 30, their regeneration capacity reduces (A. T. Wang et al., 2019).

1.2 Rationale and Objectives of the Review

Due to ethical concerns using embryonic stem cells, MSCs holds promise to be used for tissue reconstruction and in regeneration treatments due to their capacity to self-renew and differentiate into tissue-specific cells such as osteoblasts, chondrocytes, and adipocytes. They are useful for musculoskeletal regeneration treatments and also to treat age-related orthopedic degenerative disorders with other clinical problems as they orchestrate tissue formation, maintenance, and repair. Significantly, MSCs secrete substances that aid in tissue healing and promote both engraftment and trophic activities such as autocrine and paracrine effect. However, the establishment of consistent methods for MSC production and characterisation, as well as defined functionality testing for assessing their biological potential, are key variables in their therapeutic relevance.

Therefore, the present review aims to

- point out the potential sources of MSCs
- provide a comprehensive overview of the preclinical and clinical applications of MSCs in regenerative medicine
- discuss the challenges associated with developing MSCs for regenerative medicine

Chapter 2

Mesenchymal Stem Cells: An Overview

2.1 A Concise Summary of Stem Cells:

The first step of understanding how MSCs work is to have knowledge of stem cells and its functionalities. Stem cells are specialized cells that are yet to differentiate. They give rise to specialized cells that forms the foundation for organs or tissues, whether they are embryonic, fetal, or adult (Keyes and Fuchs, 2018). In the postnatal and adult stages of development, stem cells that are unique to certain tissues can arise in organs that are differentiated. Owing to this unique property, they play a key role in organ regeneration after injury (Sagaradze et al., 2020).

All embryonic stem cells (ESCs) have their genesis in the zygote and blastocyst, which are the building blocks of human body development. Organs are then formed from the germ layers. There are progenitor cells that have not yet been fully differentiated, aid in generation of the bones, marrow, blood, liver, muscles, brain, adipose tissue, gastrointestinal tract, and skin. Tissue stem cells, also known as progenitor cells, give rise to the fully formed and specialized cells of a tissue or organ. These cells may stay inactive throughout injury and recovery, but they activate and proliferate when required (Kitadate et al., 2019). Proliferation properties of these cells are different in each tissue. Specifically, stem cells in bone marrow continually grow to feed cells during normal turnover or damage and proliferate for wounded cells in the pancreas, heart, or nervous system following injury.

2.1.1 Classification of Stem Cells

Stem cells varies in terms of their ability to differentiate depending on where and how they were formed (Kolios & Moodley, 2012). Depending on their capacity to develop, there are five distinct stem cell types: totipotent (also known as "omnipotent") cells, pluripotent cells,

numerous cell types, oligopotent cells, and unipotent cells (Figure 1) (Kolios & Moodley, 2012). They may also be categorized into four groups based on their origin. Adult, fetal, ESCs and induced pluripotent stem cells (iPSC's) all fall under the umbrella term "stem cell." Table 1 highlights the characteristics of the different stem cell types.

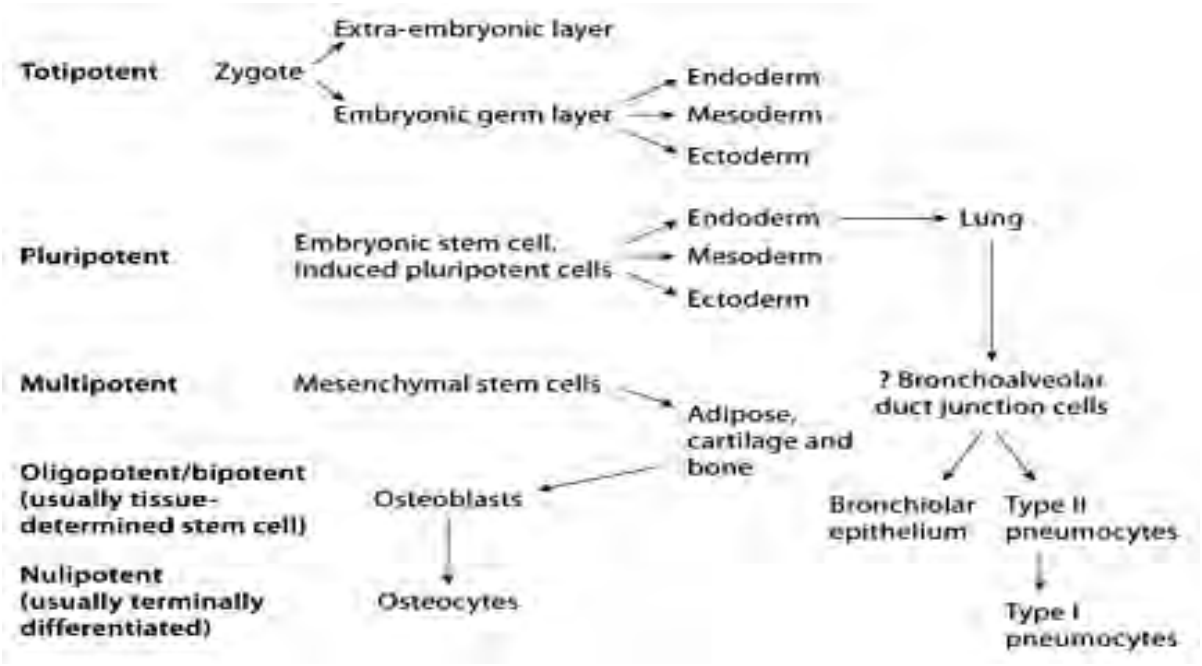

Figure 1: *Taxonomy of stem cells. Totipotent cells are seen in both embryonic and extraembryonic tissues. Multipotent cells can only produce cells from a single germ layer, but pluripotent cells can develop cells from all three germ layers. Type II pneumocytes are oligopotent and develop into type I alveolar pneumocytes, while bronchoalveolar duct junction cells in the lung may be multipotent. (adapted from Kolios & Moodley, 2012)*

Table 1: *Classification of Stem Cells and their Characteristics (adapted from Kolios & Moodley, 2012)*

Differentiation Potential	**General Characteristics**	**Origin**	**Specific Characteristics**
Totipotent or omnipotent	In the beginning of growth, you can see the cells that are most similar to each other. The totipotent cells of a fertilized egg and the cells from the first two divisions can become both embryonic and extra-embryonic tissues. The embryo and the placenta are made in this way.	Zygote	A zygote forms when an egg and sperm unite. Fertilized egg is another term. After 4 days, pluripotent cells specialize. Don't form a whole organism
Pluripotent	Three germ layers produce all tissues and organs (ectodermal, endodermal, and mesenchymal). Embryonic stem cells (ESCs) originated from the blastocyst's inner cell mass. Takahashi and Yamanaka reprogrammed somatic cells to become pluripotent. iPSCs are like ESCs in many	ESCs	It comes from the blastocyst, a 5–6-day-old embryo stage. Cells can shift into the three major germ layers or be cultured without transforming. Transcription factors like Nanog and Oct4 maintain stem cells undifferentiated so they can make more.

	aspects.	iPSCs	iPSCs are created from reprogrammed adult somatic cells. These cells are identical to human ESCs in form, proliferation, surface antigens, gene expression, epigenetic state of pluripotent cell-specific genes, and telomerase activity.
Multipotent	Germ cells comprise a single layer in most tissues. Mesenchymal stem cells (MSCs) are versatile. They come from bone marrow, fat, bone, Wharton's jelly, umbilical cord blood, and other tissues. MSCs adhere to cell culture plates and have surface markers. Mesoderm cells generate fat, bone, cartilage, and muscle. MSCs were used to make ectoderm-derived neural tissue.	Fetal stem cells	Live in a stem cell niche. A stem cell niche determines how these cells divide and become distinct kinds. Most tissue-resident stem cells are latent but wake up when injured or repaired.

Oligopotent	Oligopotent stem cells may self-renew and create several lineages. Hematopoietic stem cells can differentiate into myeloid and lymphoid lineages.	Adult or somatic stem cells	Adult-derived. MSCs and placental stem cells (himan amnion epithelial cells) are examples. These cells minimize inflammation and speed animal damage healing. Different layers of germ cells can be transformed into tissue in a lab, but only to a limited extent.
Unipotent	Can only divide into one cell type and generate one lineage. Muscle stem cells can only mature.	Muscle Stem Cells	Transforms into one sort of cell. One-way changeability.

2.1.2 Stem Cells in Clinical Practice and Regenerative Medicine

Using stem cells in medical research and in the development of new treatment options in clinical practice is crucial to medical advancements. Researchers in the fields of biology and medicine can take advantage of their unique features (Daley, 2010). Owing to their usefulness in the study of human development and organogenesis, ESCs are an essential component in the field of regenerative medicine (Chien, 2008). Stem cells like iPSCs will be used to produce new and safe treatments. Stem cells may be able to restore damaged tissue or even regenerate whole organs. Improving our understanding of disease mechanisms and developing cell treatments for degenerative disorders can be achieved by using iPSC-derived human disease models.

Cell treatment has been studied for all degenerative disorders. Stem cell immunomodulatory effects have showed promise in inflammatory illnesses such as diabetes (Trivedi et al., 2008),

cirrhosis (Kuo et al., 2008), Crohn's disease (Cassinotti et al., 2008), chronic myeloid leukemia (Gratwohl & Heim, 2009), and nervous system disorders (Burt et al., 2009). Stem cells' immune-modulating activities are useful in many instances, say researchers.

There are a number of problems with regenerative medicine and cell therapy. Immune-rejection is one of the most important and hard-to-solve problems. No one knows yet how stable stem cells are in terms of their genes. Tumors have been linked to genomes that are not stable. The use of ESCs or iPSCs in therapeutic cell transplantation can lead to spontaneous teratomas and other tumor-related diseases, even though it's possible that stem cells' ability to keep dividing on their own can cause cancer to grow in the tissue they are transplanted into (Cunningham et al., 2012). There have also been worries about using stem cells from embryos (ESC). Concerns about the destruction of an embryo during the making of ESCs are one of them. With the help of induced pluripotent stem cells, this can no longer happen.

2.2 Mesenchymal Stem Cells and their Sources

Human Mesenchymal Stem Cells (hMSCs) may self-renew and grow into a range of tissues in culture (Caplan, 2007; da Silva Meirelles et al., 2006). They also have the ability to express cell-specific signals, according to emerging studies (CD10, CD13, CD44, CD73, CD90, CD105, and CD146).MSCs are perivascular cells with cell-specific markers (CD10, CD13, CD44, CD73, CD90, CD105, and CD146) (Crisan et al., 2008). hMSCs utilized in treatment arise from many sources. MSCs can be found in adult bone marrow, fat, blood, and tooth pulp. Newborns have placenta, amnion, and umbilical cord cells (Klingemann et al., 2008). MSCs from birth-related tissues may exhibit astonishing biological qualities, such as a high ability to proliferate, a long life expectancy, and the ability to differentiate (Hass et al., 2011). There are various clinical sources of hMSCs. Adult-derived MSCs can be detected in bone marrow, adipose tissue, blood,

and tooth pulp. In most human MSC studies, bone marrow and stromal vascular adipose tissue are the most common adult donor tissues (Ibrahim et al., 2020). Umbilical cord and placenta tissue are also "mature" despite being young. Self-renewal, high proliferative power, life duration, and differentiation potential are unique biological properties of these tissues (Guimares-Camboa et al., 2017).

hMSCs are being researched in clinical trials to see if they can regenerate and modify the immune system, notably in host vs. graft responses (Rodrguez-Fuentes et al., 2021). Still, these cells' immunophenotypes vary by origin, which explains their diverse reactions. Mature MSCs express CD44, CD90, CD105 (SH2), and CD166, as well as CD14, CD34, and CD45 (Carvalho et al., 2019). Dental pulp MSCs are more likely to become neurons in the lab than other MSCs (Suchaneka et al., 2009). Placental MSCs have less-developed surface markers. Amnion- or cord-derived MSCs cannot develop into adipocytes *in vitro*. Both placenta- and umbilical cord-derived MSCs carry hematopoietic growth factor genes, which helps hematopoietic stem cells to proliferate (la Rocca et al., 2009; Sivasubramaniyan et al., 2012).

MSCs can differentiate *in vitro* and emit bioactive trophic chemicals. MSCs can heal cartilage and subchondral bone, according to various preclinical investigations on animal models of knee osteoarthritis (Wang et al., 2019). Animal studies demonstrate MSCs reduce ischemic stroke (Vu et al., 2014). Similar lab tests were done on rheumatoid arthritis animals for heart, lung, and metabolic problems (Rodríguez-Fuentes et al., 2021). Given their flexibility, MSCs appear to be able to produce therapeutic agents in a variety of human tissue injury types.

Chapter 3

Isolation of MSCs from Different Sources

MSc can be isolated from a numerous sources of tissues, namely adipose tissue, bone marrow, human umbilical cord blood and synovium that are all sources of MSCs, among which bone marrow is one of the most important source. Multilineage cells from human umbilical cord blood were the first to be described in early 2000 as a source of MSCs other than bone marrow. MSCs were discovered in adipose tissue and synovium in 2001, respectively, and synovium MSCs (SMSCs) were extracted (de Bari et al., 2001). Figure 2 and Table 2 provide techniques for the extraction, identification, and culture of MSCs from various tissues or organs (Han et al., 2019). Cells are isolated from diverse tissues, digested, and subsequently cultured for three to five days, after which non-adherent cells are discarded and adherent cell cultures are maintained until the desired passage is reached. MSCs are typically cultured in low glucose Dulbecco's modified eagle medium (LG-DMEM) with 1% (w/v) antibiotic/antimycotic and 10% (v/v) fetal bovine

serum (FBS) as the major culture medium. Table 2 also includes a list of markers that are expressed on the surface of MSCs. It must be noted that rabbits are a commonly utilized animal model for cartilage and bone tissue regeneration investigations and further studies are required about this animal model for MSC identification process. Furthermore, the surface markers of MSCs generated from rabbit tissue need additional testing (Han et al., 2019).

Figure 2: *Mesenchymal stem cells isolated from the mouse adipose tissue using a standard extraction method (adapted from Han et al., 2019).*

Table 2: *Differentiation and cultivation of MSCs from diverse tissue sources (adapted from Han et al., 2019)*

Types of MSCs	**Culture Medium Used**	**Source for Extraction**	**Markers**
Bone Marrow Stromal Cells	LG-DMEM with 1% (*W*/*V*) antibiotic/antimycotic,		Rabbit: $CD29^+$, $CD44^+$, $CD73^+$, $CD81^+$, $CD90^+$, $CD166^+$, $CD14^-$, $CD34^-$, $CD45^-$, $CD117^-$, and $HLD\text{-}DR^-$
			Mouse: $CD29^+$, $CD44^+$, $CD73^+$, $CD90^+$, $CD105^+$, $Sca\text{-}1^+$, $CD14^-$, $CD34^-$, $CD45^-$, $CD11b^-$, $CD31^-$,

	10% (*V*/*V*) FBS	Rabbit, mouse and rat: tubular bones, e.g., femurs and	Vcam-1$^-$, C-Kit$^-$, CD135$^-$, CD11b$^-$, Ia$^-$, and CD86$^-$
			Rat: CD29$^+$, CD44$^+$, CD54$^+$, CD73$^+$, CD90$^+$, CD105$^+$, CD106$^+$, Sca-1$^+$, CD14$^-$, CD34$^-$, CD45$^-$, and CD11b$^-$
		Human: tubular bones and iliac crest bone marrow	CD29$^+$, CD44$^+$, CD73$^+$, CD90$^+$, CD105$^+$, Sca-1$^+$, CD14$^-$, CD34$^-$, CD45$^-$, CD19$^-$, CD11b$^-$, CD31$^-$, CD86$^-$, Ia$^-$, and HLA-DR$^-$
Human Umbilical Cord Blood-derived Mesenchymal Stem Cells	LG-DMEM, 1% P/S, 250 ng mL^{-1} amphotericin B, and 10% (*V*/*V*) FBS	Blood of human umbilical cord	CD29$^+$, CD44$^+$, CD73$^+$, CD90$^+$, CD105$^+$, CD166$^+$, CD14$^-$, CD31$^-$, CD34$^-$, CD45$^-$, CD106$^-$, and HLA-DR$^-$
Mesenchymal Stem Cells from Adipose Tissue	DMEM with 1% (*W*/*V*) P/S, 10% (*V*/*V*) FBS	Rabbit, mouse and rat: subcutaneous adipose	Rabbit: CD44$^+$, CD105$^+$, NG2$^+$, CD34$^-$, and CD45$^-$
			Mouse: CD34$^+$, CD44$^+$, CD45$^+$, CD90$^+$, MHC-I$^+$, MHC-II$^+$, and CD117$^-$.
		Human: subcutaneous adipose in abdomen, buttocks, and abdominal zone	Rat: CD44$^+$, CD73$^+$, CD90$^+$, MHC-I$^+$, CD31$^-$, and CD45$^-$
			Human: CD29$^+$, CD44$^+$, CD73$^+$, CD90$^+$, CD105$^+$, CD146$^+$, CD166$^+$, MHC-I$^+$, CD31$^-$, CD45$^-$, and HLA-DR$^-$
Synovial Mesenchymal	DMEM or αMEM	Synovium,	Rabbit: CD44$^+$, CD90$^+$, and CD105$^+$
			Mouse: CD29$^+$, CD44$^+$, CD90$^+$, CD34$^-$, CD45$^-$, and CD107$^-$

Stem Cells	with 1% (*W*/*V*) P/S, 250 ng mL^{-1} amphotericin B, and 10% (*V*/*V*) FBS	especially in knee joints, of human, mouse, rat, rabbit, pig, etc.	Rat: $CD90^{+}$, $CD11b^{-}$, and $CD45^{-}$
			Human: $CD10^{+}$, $CD13^{+}$, $CD49^{+}$, $CD44^{+}$, $CD73^{+}$, $CD90^{+}$, $CD105^{+}$, $CD147^{+}$, $CD166^{+}$, $CD14^{-}$, $CD20^{-}$, $CD31^{-}$, $CD34^{-}$, $CD45^{-}$, $CD62^{-}$, $CD68^{-}$, $CD113^{-}$, $CD117^{-}$, $HLA\text{-}DR^{-}$, and ALP^{-}

Chapter 4

Regenerative Medicine Using MSCs

Since 1995, clinical trials have used MSCs to treat numerous disorders (Lazarus et al., 2005). Preclinical and clinical studies show its usefulness in regenerative medicine and tissue engineering. Figure 3 shows how research focuses on reconstructing fragile tissues such the musculoskeletal and neurological systems, heart and liver, cornea and trachea (Han et al., 2019).

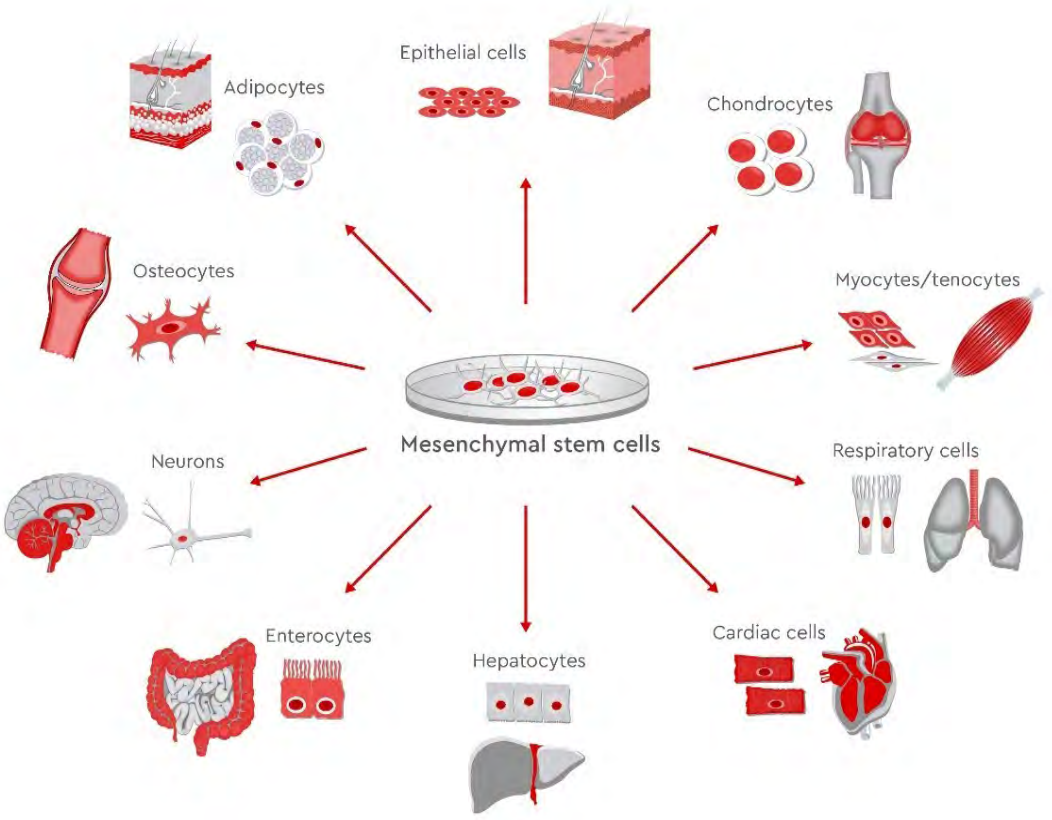

Figure 3: *Using mesenchymal stem cells for multiple differentiation in tissue repair (adapted from Han et al., 2019)*

4.1 What is Regenerative medicine?

Regenerative medicine replaces or regenerates human cells, tissues, or organs to restore normal function (Mason & Dunnill, 2008). Even though building artificial organs is an old notion, it may be a promising next step in clinical research. RM can be achieved by employing cell-filled scaffolds, natural or man-made structures to facilitate healing and cell proliferation, or MSCs in cell-based therapies (Mao & Mooney, 2015).

Data from preclinical and clinical studies thus far show that regenerative medicine can be used to treat both chronic diseases and acute injuries, as well as a wide range of illnesses and conditions, which include wounds, cardiovascular disease, cancer treatments, and more. Organ and tissue transplantation now relies on a very limited donor supply and often significant immunological problems, but regenerative medicine technologies may be able to overcome these hurdles in the near future. Regeneration can also be aided by the body's natural healing response, however mature humans have a limited ability for regeneration compared to lower animals (Kami & Gojo, 2014). By the incorporation of MSCs, regenerative medicine has a potential of treating numerous medical conditions, some of which are discussed below.

4.2 Cardiovascular Therapeutics Using MSCs

Cardiovascular disease, especially heart failure, is a leading cause of mortality and illness worldwide. Therefore, new cardiovascular therapies are needed. MSC-based therapy techniques should be used to treat acute and chronic heart failure. MSCs can inhibit cardiomyocyte neovascularization and ischemic cell death. Human adipose-derived MSCs may cure cardiovascular disorders (Siciliano et al., 2015).

Following catastrophic myocardial infarction or the cardiac condition, myocardium's potential for rejuvenation is not sufficiengt to heal damaged cardiomyocytes. As Toma and colleagues

reported in 2002 (Toma et al., 2002), whenever Bone Marrow Stromal Cells (BMSCs) got transplanted into the mouse heart, it appeared to grow into cardiomyocytes, a conclusion that was supported in subsequent studies. Adipose-derived Stem Cells (ADSCs) were found to be the best source of MSCs capable of transforming into cardiomyocytes in subsequent experiments (Lei et al., 2013). ADSCs were successfully transferred into an infarcted pig heart model by spraying, which was shown to be more cost-effective and less intrusive (Mori et al., 2018). Cardiomyocyte recovery is facilitated by MSCs' capacity to supply vast quantities of angiogenesis, anti-apoptosis as well as mitogenic chemicals, as well as their competence in decreasing cardiac fibrosis (Figure 4). (Gubert et al., 2021). The immunomodulatory impacts of MSC therapy worked on patients with non-ischemic cardiomyopathy by enhancing the left ventricular ejection fraction, as per Butler and colleagues (Butler et al., 2017). Heart tissue that has undergone a myocardial infarction is more resistant to oxidative stress when MSCs and cardiomyocytes are cultured together. MSC development into cardiomyocytes can be aided by 5-azaacytidine. After myocardial infarction in rats (Jung et al., 2018), IGF-1-transfected MSCs lowered infarct size and prevented fibrosis and cardiomyocyte death. MSCs fused onto cardiomyocytes have been shown to boost cardiac function because of the capacity of Interleukin-7 (IL-7) in rising cell proliferation also aiding in the restoration of injured myocardium, according to researchers (Haneef et al., 2018). The secretion of vascular endothelial growth factor (VEGF) by MSCs in a severe ischemia of limb situation led to myocardial healing as well (Fujita & Kawamoto, 2017).

MSC-based therapy has been tested on dogs (Sun et al., 2009), rats, mice, rabbits, and pigs (Cai et al., 2016) in a number of preclinical studies, which showed that MSCs can be used to treat heart disease. Preclinical tests have shown that treatments based on MSCs are safe and show promise.

The mechanism of MSCs-based cell therapy must also be elucidated. In the past, the primary method for MSCs mending injured tissue was based on an *in vitro* research on cardiomyogenic differentiation (Makino et al., 1999). Many investigations have shown that *in vivo* cardiomyogenic differentiation does not take place to a considerable extent (Noiseux et al., 2006). Now, the "Paracrine effect" (Martire et al., 2016) is the deemed mechanism. Instead of contributing directly to the production of new tissue, some donor cells stimulate the patient's own cells to mend injured tissue. The above occurs as a result of the paracrine effect, in which the donor cells produce substances that tell the patient's cells to modify their behavior. Experts have been inspired to move this application from preclinical to clinical testing because of the promising findings they have observed. In the ClinicalTrials.gov database (https://clinicaltrials.gov/), there are records of more than 60 clinical trials of MSC-based treatments for heart failure. Several clinical studies have shown that this method is safe and works well. On the other hand, the effectiveness of clinical trials varies. When MSCs block TNF signaling, inflammation in the heart and lungs goes down. But there are a number of important problems that need to be fixed before this treatment can be used to help people with heart failure (Perin et al., 2011).

Preclinical and clinical evidence suggests that employing MSCs to relieve cardiac disease is a realistic alternative. Aside from cells and pulmonary hypertension, MSC immunotherapy has also been widely researched as a therapeutic option. Slightly swollen cardiomyopathies, myocardial infarctions, and peripheral ischemic vascular diseases are a few of the more common causes of sudden cardiac death (Murphy et al., 2013).

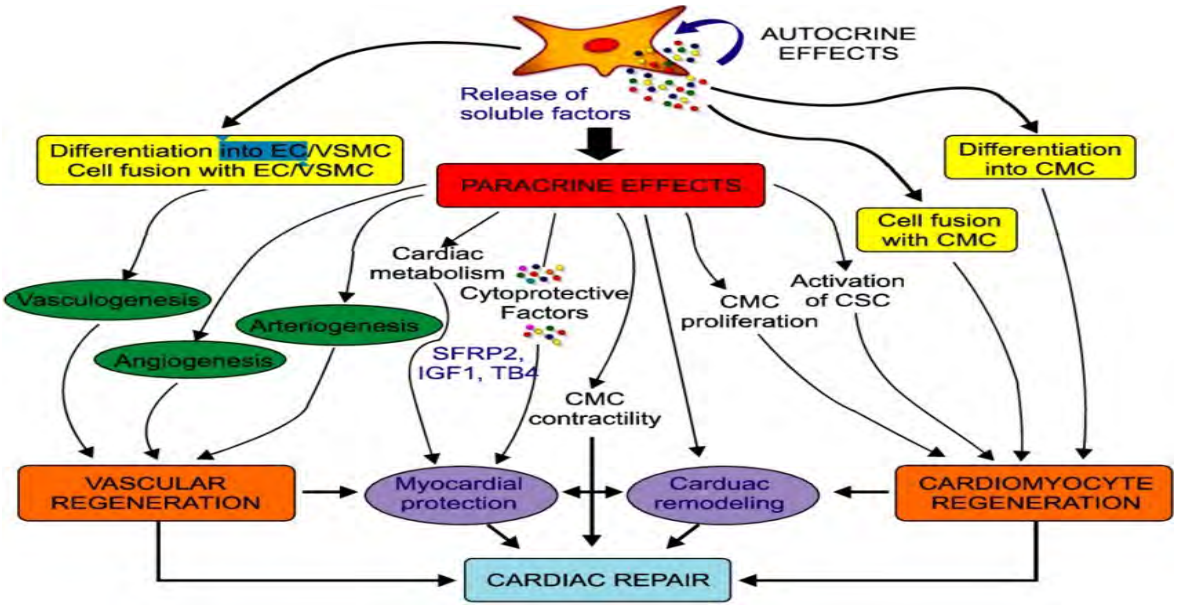

Figure 4: *This* diagram *shows how MSCs aid in heart regeneration. It is conceivable to heal the heart with MSCs because of their ability to generate angiogenesis, vascularization and cardiomyocyte differentiation. A variety of regenerative and anti-inflammatory agents, and several other benefits, are provided by MSCs' paracrine actions (adapted from Gubert et al., 2021)*

4.3 Treating Diseases Affecting Bones

Configurable MSCs can cure bone disorders. Proteins include bone morphogenetic proteins-2 (Youssef et al., 2017), insulin-like growth factors (IGF-1) (Reible et al., 2018), TGF-1 (Qu et al., 2018), and growth differentiation factor 5 (Youssef et al., 2018) can transform MSCs into cartilage, bone, or tendon (Qiu et al., 2018).

In order to function properly, skeletal muscle is required. However, volumetric muscle loss (VML) as a result of a severe traumatic injury or a tumor removal is not treatable in any way. Hence skeletal muscle phenotypic differentiation of MSCs *in vivo* has been observed.

Using MSCs and a decellularized ECM scaffold, Pumberger et al. (2016; Qiu et al., 2016) and Qiu et al. (2016) have shown that VML damage can be healed with this technique. Pumberger et al. (De Bari et al., 2003) discovered that MSCs may employ paracrine signaling to heal muscles. PLGA/MSCs increased cartilage growth and repair. Toosi et al. (Sackstein et al., 2008) studied Lb-MISCs. Cryopreservation did not affect how Lb-MISCs developed and altered. This study suggests Lb-MSCs can cure bone disorders.

Osteoblasts can be generated from MSCs, but they cannot move to bone on their own. Bone regeneration can be achieved using modified MSCs. For developing MSCs, enzymatic modification is among the other ways (Yang et al., 2017). An enzymatic alteration by Sackstein and his colleagues (Chen et al., 2015) produced stereospecific carbohydrate substitution that did not compromise cell survival or undesirable phenotypic consequences. HCELL (hematopoietic cell E-selectin/L-selectinIt-gianc) has been demonstrated to be a powerful E-selectin ligand on the cell surface of MSCs. In an immunocompromised mouse model, intravenous injected modified HCELL expressing human MSCs penetrated the bone marrow within hours. Using a new collagen scaffold, Chen et al. found that MSCs were better able to find their way into osteochondrocytes. Collagen scaffolds were made and the migration of MSCs within the scaffolds was observed *in vitro*. MSCs' migration was aided by the collagen scaffold and SDF-1, both of which were included. Scaffolds and SDF-1 were used to improve cartilage regeneration in a rabbit model of osteochondral defects (OCD). On the other hand, a different study has employed gene transfer to boost the ectopic expression of CD49d (a4 integrin) on MSCs (Aggarwal and Pittenger, 2005). In a mouse model, bone homing was enhanced by raising CD49d levels. While maintaining stem cell properties, the transplanted MSCs were also shown to be capable of producing new bone cells in the growth plates of the mice's leg bone. It is

possible that this study's genetically modified approach of achieving MSCs bone homing will be useful in the development of targeted therapeutics for osteopenic bone deficiencies (Murphy et al., 2013).

4.4 Musculoskeletal Tissues Regeneration

Recent research has utilized MSCs to examine musculoskeletal tissues, such as ligaments, tendons, and the meniscus, that are not composed of bone or cartilage. More focus is being paid to meniscus regeneration. Using MSCs intra-articularly to aid meniscal regeneration was a first step, and the results were encouraging (Shapiro et al., 2017). MSC-loaded hydrogels and scaffolds were employed to rebuild the meniscus in a similar way to how they have been used for cartilage regeneration. The meniscus-derived decellularized matrix has stronger histocompatibility than natural or synthetic polymer materials and is far more effective at promoting MSC growth. When repairing meniscal damage, tissue-engineered constructs that do not require scaffolds are an option (Toratani et al., 2017). Tarafder et al. (Tarafder et al., 2018) used connective tissue TGF and TGF-3 to activate synovial MSCs to repair meniscus injuries in terms of avoiding the shortcomings of cell-based methods. Stimuli like high pressure or rather tensile stresses should be administered in able to preserve the meniscus healthy and strong (M. Chen et al., 2018). Despite encouraging outcomes in animal models, MSCs' ability to create enduring tissues such as the meniscus has yet to be proved in people.

In athletics, tendon injuries are most prevalent. The JNK/SMAD1-peroxisome proliferator-activated receptor signaling pathway enhances BMSC myogenic differentiation by BMP-14 (Wang et al., 2018). Studies on tendogenic differentiation of MSCs have been focused on mechanical stimuli as well as factors or even scaffolds which encourage MSC differentiation into

tendon lineages. Yet, since MSCs were exposed to uniaxial cyclic straining, they did not cure tendon damage, but just slowed the progression of the damage (Güleçyüz et al., 2018).

4.5 Reconstruction of the Central Nervous System

Damage to the mature central nervous system (CNS) is irreversible and has no cure. Hence the main focus of MSC-based CNS regeneration treatment is on CNS dysfunction or damage induced by significant laceration and prolonged ischemia. To this day, the most thoroughly investigated cell sources for CNS repair are BMSCs and ADSCs, with both exhibiting similar neuronal differentiation capacity (K. S. Bae et al., 2011). ADSCs might just be a superior cell source than BMSCs because of the simplicity of harvest and quantity of these cells, which have been demonstrated to reduce the development of scar tissue around spinal cord injury (SCI) and associated lesions. According to studies, ADSCs procured from neural stem cells, lowers nervous system inflammation which accelerate the recovery of function (Jahanbazi Jahan-Abad et al., 2018). Dental pulp stem cells (DPSC) along with UCB MSCs develop into neuron cells *in vitro* and can both enhance differentiation into axonal and neurons regeneration.

MSC-based treatment has even been tested in animal models with ischemic brain damage/SCI and traumatic brain injuries. In 1568, an analysis of SCI rats found that MSC therapy greatly enhanced locomotor recovery (Oliveri et al., 2014). Patients with spinal cord injuries and/or traumatic brain injuries who received MSC-based treatment have been shown to be efficacious. When treated with hypoxia in rats, MSCs migrate to the peri-cerebral damage zone producing growth factors spectrum. These promote neurogenesis and neurological repair. Non-expanded MSCs are utilized to treat MS and Amyotrophic Lateral Sclerosis (ALS) while expanded MSCs are used to treat stroke and Parkinson's disease. More and more clinical investigations are looking into the immunomodulatory and neuroprotective effects of MSCs. Also, more research is

being done on local injections of either non-expanded or expanded autologous MSCs, with positive results.

4.6 Reconstruction of the Cornea

In addition to being transparent yet devoid of blood vessels, the cornea also serves as the eye's primary line of protection against infection and mechanical damage responsible for the refractive power of the eye. As cornea's fragile and vulnerable to direct contact with the outside world, it is prone to damage from a wide range of medical conditions, including Stevens-Johnson syndrome, aniridia, and chemical, mechanical, and thermal insult. Regeneration of human corneal epithelial cells is possible using limbal epithelial stem cells (LESCs) found in the cornea's periphery. Injury to the entire corneal layer can cause scarring and opacification, as well as reduced vision and even blindness if the entire corneal layer is affected (Murphy et al., 2013). As donor tissue is scarce and immunological rejection occurs after surgery, keratoplasty is the procedure of choice for corneal repair at the moment.

Upon corneal epithelial stem cell transplantation into limbal allografts, some people with severe ocular surface abnormalities were however possible to see (Tsubota et al., 1999). Cell immunotherapy for corneal illnesses using limbal epithelial stem cells (LESCs) grown on an amniotic membrane effectively reduced irritation also rebuilt the injured ocular surface, according to clinical and pre-clinical research. After a thriving transplant, it has been frequently used in clinical practice, despite a lack of improvement in visual recovery MSCs used in the restoration of cornea included UCB type MSCs, ADSCs, and BMSCs. Although autologous MSCs' impact of immunosuppressant character in pre-sensitized rat corneal transplant models

have been widely publicized, there are no comparability studies looking at the influence of various MSC types on the reconstruction of cornea.

For corneal repair, methods are mainly based on the anti-inflammatory effects of MSCs, although their metamorphose efficiency and purity need to be enhanced. MSC transplantation into a corneal surface in a rat model which is damaged, repaired it, although the pharmacologic advantage of MSCs may not have been metamorphose of epithelia, and more a reduction in inflammation and angiogenesis following transplantation" (Ma et al., 2006). Using amniotic fluid-derived MSCs creates an anti-fibrotic as well as anti-inflammatory environment in the body.

4.7 Rebuilding of the Tracheal Tract

Tracheal stenosis and other significant disorders necessitate tracheotomy plus repair. There are already clinical trials using transplants, homografts, and prosthesis through an initiative to help with trachea healing. A number of studies on the effectiveness of MSC-based tissue regeneration for tracheal restoration have been conducted (den Hondt & Vranckx, 2017).

Preclinical investigations on animals have shown that tracheal healing based on MSC is fairly secure. It appears that the tracheal engineering potential of MSC seeding on decellularized trachea scaffolds has been established in rats, whereas acellular tracheal matrix seeded with BMSCs displayed minimal side effects and controlled release. Four rabbits were able to successfully repair partial tracheal lesions utilizing bioprinted artificial scaffold in 3D covered with fibrin seeded MSCs (Chang et al., 2014). Tracheal restoration using an acellular amniotic membrane (Jorge et al., 2018) was found to improve neovascularization, epithelialization, and regeneration of the cartilage and lower the likelihood of postsurgical problems, like as stenosis of

the trachea. Laser micropore technology has been employed to boost the porosity and mechanical strength of the scaffolds since the acellular cartilage matrix was discovered to be lacking in porosity. Artificial biomaterial scaffolds, such as electro spun constructions based on Col and core-shell nanofibrous structures, can be used to replace injured tracheas. Despite the presence of MSCs, scaffolds loaded with these cells do not induce cartilage production in vivo, just epithelium and angiogenesis (S. W. Bae et al., 2018).

Using MSCs for tracheal tissue engineering in clinical trials was backed by a tissue regeneration experiment in which tracheal restoration was performed. One of the earliest transplants of a trachea which was engineered with tissues was performed on a middle aged woman in 2008 who had advanced left main bronchus Malacia and received the transplant of an epithelial cell- and chondrocyte-rich trachea created from decellularized donor trachea (Macchiarini et al., 2008). However, the patient's lungs, ciliary, cough, and mucus function remained alright after five years of follow-up with no stem cell-related tumors or antibodies to the donor. The tissue-engineered tracheal prosthesis with BMSC performed well in a young child throughout a two-year follow-up period, proving that cell-seeded scaffolds are clinically safe and practicable (Elliott et al., 2012).

Studies hence unveiled that, to generate a functional tracheotomy based on differentiated MSCs and matrix components, stem cell biology, biomaterials, and tissue engineering approaches need to be combined.

4.8 Regeneration of the dermis

Due to the fact that the epidermis is the primary defense system against pathogens also bodily injury, skin anomalies may result from infectious agents and abrasion. Skin tissue availability limits autologous transplantation in cases of severe skin injury, making it difficult to provide

adequate treatment. Aside from that, allogeneic skin grafts almost often result in immune rejection or the transmission of infectious diseases (Murphy et al., 2013).

A multitude of components, comprising soluble mediators, the extracellular matrix (ECM), also circulating blood cells, should indeed make concerted efforts for a wound to heal correctly. A coupling of MSC-based treatment and artificial scaffolds is a possible approach for wound healing or full-thickness skin restoration (Figure 5) (Laverdet et al., 2014). Pressure ulcers were successfully treated using TGF-3, albumin-based scaffolds, and MSCs by Feldman et al. (Feldman & McCauley, 2018). Current investigations have demonstrated that MSC-based treatment can dramatically enhance wound closure via angiogenesis. Keratinocytes heal with MSC-secreted TSG-6 and VEGF. MSC-secreted VEGF prevents macrophages from combating infection. Angiotensin II promotes BMSC differentiation into keratinocytes by activating mitogen-activated protein kinase or Janus kinase signaling pathways (Jiang et al., 2019). Gene-modified MSCs, on the other hand, may be able to help regenerate the skin. MSCs in the cutaneous lesions display antimicrobial effect as employed in association with tactile stimulation such as laser treatment, ectodysplasin-modified MSCs, stromal cell-derived factor-1-transfected BMSCs, EGF-transfected MSCs and VEGF-modified human UCB type MSCs. Hybridized chitosan in 3D, poly(caprolactone) (PCL). Fibrin hydrogels, electro spun nanofibrous silk fibroin and ColCS, sodium carboxymethylcellulose and are by far the most intriguing scaffolds for MSC-based skin regeneration (Murphy et al., 2013).

Both autologous and allogeneic MSC injections help skin grow back and treat severe burns, perianal fistula, diabetic ulcers that don't heal, dystrophic epidermolysis bullosa, and radiation-induced skin lesions in people. Estrogen treatment significantly heals diabetic lesions by boosting MSC survival and proliferation and encouraging neovascularization. In 2009, Sheng et

al. (Sheng et al., 2009) reported using MSC implants to effectively regrow functioning sweat glands in patients presenting for the purpose of rebuilding appendages.

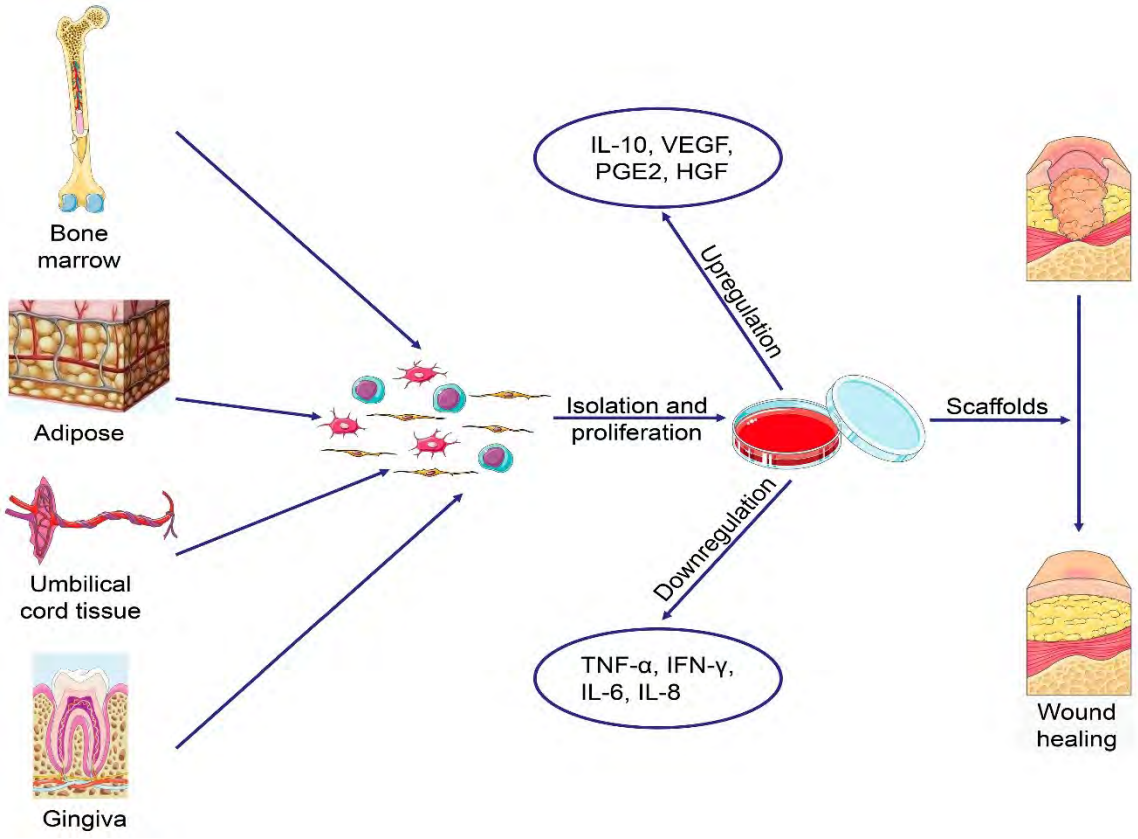

Figure 5: New varieties of mesenchymal stem cells are collected as well as defined, subsequently cultivated then sorted in a specialized environment to fix lesions. In attaining skin's elasticity, MSCs create a range of chemicals that control inflammation and stimulate angiogenesis (adapted from Laverdet et al., 2014).

4.9 Additional MSC-based therapeutics possibilities

Other disorders might benefit from the use of MSCs. One such possibility is the administration of MSCs systemically to repair kidney damage via paracrine and/or endocrine pathways. BMSCs can be injected into the lungs to treat lung illness (Dai et al., 2018), and another study found that

MSCs planted onto Polycaprolactone (PCL) or chitosan scaffolds can promote bladder regeneration. (Jiang et al., 2015). Bioprinted vascularized tissue including MSCs was thicker than that created by current bioprinting procedures, but it was only able to survive for a short length of time before needing replacement (Zhou et al., 2018). A diverse array of recent studies promote the use of MSCs for tissue regeneration (Murphy et al., 2013).

Chapter 5

Therapy with Mesenchymal Stem Cells: Benefits and Drawbacks

MSCs are superior than other cells since they create less immunological responses and less immune-suppressing debris. MSCs generate growth factors that help stimulate angiogenesis, decrease inflammation, and aid tissue regeneration (Wang et al., 2020).

MSC transplants, whether allogeneic (genetically distinct individuals of the same species undergoing stem cell transplantation) or xenogeneic (transplantation of stem cells between different species), have been shown to be safe and effective in restoring organ function without triggering severe immune rejection. Allogeneic and xenogeneic transplanting models showed excellent therapeutic benefits in 81% (13/16) of treated group in a large animal trial, which indicates tremendous promise of this therapy technique (Wang et al., 2020). Moreover, they can be easily grown *in vitro* and have the ability to differentiate into neo-angiogenic, inflammatory, and tumor-specific cells (Wang et al., 2020). MSCs are also free of teratoma danger or ethical concerns. As MSCs have all these characteristics, they are considered as an interesting prospect for regenerative medicine.

Regeneration medicine is a promising field, but there are numerous issues that need to be solved before this treatment approach can be used in clinical practice. Clinical investigations of MSC transplantation have demonstrated no apparent side effects during the previous decade. A number of preclinical research have shown that the long-term hazards of MSCs treatment cannot be determined by a short-term trial, even though the FDA has classified MSC transplantation safe. Mal-differentiation, immunosuppression, and cancer development are a few of the possible side effects (Aguilar et al., 2007).

The immunosuppressive features of MSC transplant, as well as the lack of immune-surveillance to infections, provide another risk. The powerful immunosuppressive qualities of MSCs, particularly allow for direct transplantation, are well-known. An investigation found that MSC transplanting may affect the host's ability to fight against infectious pathogens (Raposio et al., 2017). MSCs transplanted from healthy seropositive donors have a low chance of spreading herpesvirus. As a result, MSCs may get infected when they are infused with CMV or HSV-1. One other severe danger is the possibility for carcinogenic effects. MSCs become tumorigenic by direct transformation, chemotherapeutic drug metabolism, and inhibition of the antitumor immune response (Wang et al., 2020). Suppression of Wnt signaling in MSCs can lead to sarcoma development (Zhu et al., 2018).

Allogeneic host response to MSCs treatment, processes of direct cell homing and engraftment, and the response of MSCs to local differentiation signals must also be addressed, as well as many other factors related to the interaction between transplanted cell and host (Wang et al., 2020). .

Chapter 6

Conclusion and Future Prospects

Since mesenchymal stem cells (MSCs) were identified 50 years back, advancements in MSC-based tissue engineering owing to optimization of extraction, culture, and methods of differentiation have enabled MSCs to be utilized for disease therapy and tissue reconstruction. Earlier studies of regenerative medicine primarily focused on musculoskeletal tissues; however recent progress has expanded their applications into many other tissues, including the CNS, heart, liver, cornea and trachea. One of the critical factors affecting the outcome of MSC therapy centers around the induction factors that accelerates the repair process of MSCs on tissues. Additionally, scaffolds provide the environment for multiplication (proliferation) and differentiation of MSCs, resulting in production of mechanical stimulation to MSCs that can be further applied for the use of MSCs as regenerative medicine. In fact, scaffolds loaded with induction factors enhances the therapeutic effects of MSCs which warrants further study. Therefore, more research needs to be directed towards advanced materials as scaffolds and efficient induction factors that will lead to further applications of MSCs in regenerative medicine.

In spite of the advantages of MSCs, there are a number of challenges that needs to be overcome. Although the unique immunomodulatory properties of the MSCs are fundamental for performing their functions, the mechanism of MSC immune regulation needs to be investigated. Different culture conditions such as culture medium, supplements, cell seeding density and oxygen may affect cell proliferation and differential potential. As different laboratories and researchers have different methods of isolating and culturing MSC, although the primary medium is similar, there

is an imperative need to set up a standard protocol for the *in vitro* culture of MSCs. Moreover, cryopreserved MSCs have low viability that may negatively affect further applications of MSCs. Age of the donors is a significant factor that affects the proliferation and differential potential of MSCs with MSCs from young donors being more effective showing lesser damage and better proliferation.

Altogether, many factors influence the therapeutic potential of MSCs including induction factors, oxygen concentrations, donor age and mechanical stimuli. Therefore, it must be pointed out that optimizing the culture conditions along with choosing the appropriate scaffolds and induction factors is an effective way to improve the therapeutic potential of MSCs to be used as regenerative medicine. Development of new approaches to comprehend and unleash the potential of MSCs, as well as new delivery modalities and patient selection may lead to significant breakthroughs in the future years and more therapeutic opportunities. To sum up, despite the challenges associated with MSC based therapy, MSC-based tissue engineering represents a promising clinical strategy in the field of regenerative medicine.

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