UNIVERSITY OF PARDUBICE

FACULTY OF CHEMICAL TECHNOLOGY

BACHELOR THESIS

2021 Natasha Brito

UNIVERZITA PARDUBICE

FAKULTA CHEMICKO-TECHNOLOGICKÁ

BAKALÁŘSKÁ PRÁCE

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University of Pardubice
Faculty of Chemical Technology

Bone marrow mesenchymal stem cells

Bachelor thesis

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Univerzita Pardubice
Fakulta chemicko-technologická
Akademický rok: 2020/2021

ZADÁNÍ BAKALÁŘSKÉ PRÁCE
(projektu, uměleckého díla, uměleckého výkonu)

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Osobní číslo:	**C20371**
Studijní program:	**B3912 Speciální chemicko-biologické obory**
Studijní obor:	**Zdravotní laborant**
Téma práce:	**Mezenchymální buňky kostní dřeně**
Zadávající katedra:	**Katedra biologických a biochemických věd**

Zásady pro vypracování

1) Prepare a review focused on mesenchymal cells in bone marrow. In the Introduction part, describe an overview of cells occurring in bone marrow with noting their function. Then, focus on the description of mesenchymal cells, e.g. their development, function in processes occurring in the bone marrow, role in hematopoiesis, etc. The aim of the bachelor thesis ought to be also recognized in the use of the mesenchymal cells in *in vitro* testing.

2) Use medical and scientific databases for preparation of the bachelor thesis, e.g. *ScienceDirect, HighWire, NCBI Pubmed*, etc.

Rozsah pracovní zprávy: **25 s.**
Rozsah grafických prací: **dle potřeby**
Forma zpracování bakalářské práce: **tištěná**

Seznam doporučené literatury:

Vedoucí bakalářské práce: **doc. RNDr. Tomáš Roušar, Ph.D.**
Katedra biologických a biochemických věd

Datum zadání bakalářské práce: **18. prosince 2020**
Termín odevzdání bakalářské práce: **2. července 2021**

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V Pardubicích dne 26. února 2021

DECLARATION

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ACKNOWLEDGMENT

I wish to express my deepest gratitude to my supervisor, doc. RNDr. Tomáš Roušar, Ph.D. who gave me the opportunity to work on such a fascinating topic, for his persistence, patience, motivation, and scientific knowledge not only through the course of my bachelor thesis but also through my academic years.

To my family, for their unconditional love, never-ending support, and understanding. Words will never be enough to express my eternal gratitude for everything my family has done and keep doing for me.

ANNOTATION

This thesis aims to establish a literature review based on mesenchymal stem cells within the bone marrow. Bone marrow mesenchymal stem cells have the ability to self-renew, extensive differentiation potential, and also present a high secretory potential, making them an exceptional tool in the field of regenerative medicine and cell therapy. This theoretical work deals with the known information about mesenchymal stem cells, such as their trilineage differentiation, role in hematopoiesis, and their potential use in *in vitro* culture.

KEYWORDS

Bone marrow, stem cells, mesenchymal stem cells, hematopoietic stem cells, hematopoiesis, cytokines, *in vitro* culture.

ANOTACE

Cílem této práce bylo vypracovat rešerši o mezenchymálních kmenových buňkách v kostní dřeni. Mezenchymální kmenové buňky kostní dřeně mají schopnost samoobnovy, mají rozsáhlý diferenciační potenciál a také vysoký sekreční potenciál, což z nich činí výjimečný nástroj v oblasti regenerativní medicíny a buněčné terapie. Tato teoretická práce se zabývá již známými informacemi o mezenchymálních kmenových buňkách, jako je jejich potenciál diferenciace na osteocyty, chondrocyty a adipocyty, role v hematopoéze a jejich potenciální využití v kultuře in vitro.

KLÍČOVÁ SLOVA

Kostní dřeně, kmenové buňky, mezenchymální kmenové buňky, hematopoetické kmenové buňky, hematopoéza, cytokiny, *in vitro* kultivace.

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ABBREVIATIONS

ALP	alcaline phosphatase
ASCs	adult stem cells
BAT	brown adipose tissue
β-CATENIN	protein that regulates and coordinates cell-cell adhesion and gene transcription.
bFGF	basic fibroblast growth factor
BM	bone marrow
BMMSCs	bone marrow mesenchymal stem cells
CBF-1α	core-binding factor subunit alpha-1
CCR	C-C motif chemokine receptor
CD	cluster of differentiation
C/EBP-α	c – enhancer-binding protein alpha
CFU-F	colony forming unit fibroblast
COL2-α1	collagen type 2
CXCR	C-X-C motif chemokine receptor
DCs	dendritic cells
DMEM	Dulbecco's modified eagle's medium
EGF	epidhermal growth factor
ESCs	embryonic stem cells
FBS	fetal bovine serum
FGF	fibroblast growth factor
FOXC2	forkhead box protein C2
GVHD	Graft-Versus-Host-Disease
HGF	hepatocyte growth factor
HLA-DR	human leukocyte antigen-antigen D related
HIF-1α	hypoxia-inducible factor 1-alpha
HOXA3	homeobox A3
IFN-γ	interferon-gamma
IGF-1	insulin-like growth factor-1
IL	interleukin
iPSCs	induced pluripotent stem cells
LIF	leukemia inhibitory factor

MCP-1	monocyte chemoattractant protein-1
MIP-1α	macrophage inflammatory protein-1 alpha
MITR	myocyte enhancer factor-2 interacting transcriptional repressor
MSCs	mesenchymal stem cells
NTSCs	nuclear transfer stem cells
NK	natural killer
Oct-4	octamer-4
PBS	phosphate-buffered saline
PDGF	platelet derived growth factor
PGE1-α	prostaglandin E1 – alpha
PGE2	prostaglandin E2
PGF2-α	prostaglandin F2 – alpha
PL	platelet lysates
PPARγ	peroxisome proliferator-activated receptor gamma
PRDM16	coding protein 16
RSCs	reprogrammed stem cells
SCF	stem cell factor
SDF-1	stromal cell-derived factor-1
SMAD3	mothers against decapentaplegic homolog 3
SOX9	SRY-box transcription factor 9
SSEA-4	stage-specific embryonic antigen-4
STRO-1	monoclonal antibody to identify mesenchymal stem cells
TF	transcription factor
TGF-β	transcription growth factor-beta
Th1	type 1 T-lymphocyte
Th17	type 17 T-lymphocyte
TNF-α	tumor necrosis factor-alpha
TWIST	embryonic transcriptor factor that plays a vital in the early stages of development
VCAM-1	vascular cell adhesion molecule-1
VEGF	vascular endothelial growth factor
VSELs	very small embryonic-like stem cells
WAT	white adipose tissue
YAP	yes-associated protein

1. INTRODUCTION

Bone marrow is soft and fatty tissue found within axial and long bones' central cavities. The bone marrow contains undifferentiated stem cells. Unlike other cells found within the human body, stem cells are unique, owning that, regardless of their source, they have two main properties: the potential to self-renew for an extended period and the capacity to differentiate. Their potential to self-renew ensures that sufficient stem cells are accessible for a lifetime's requirements. Stem cells found within the bone marrow can later differentiate and ultimately become hematopoietic or mesenchymal stem cells. Hematopoietic stem cells are directly involved in producing blood cells such as red blood cells that deliver oxygen throughout the body, white blood cells that fight pathogens in the blood, like bacteria, and platelets that help with blood clotting in case of injury. While mesenchymal stem cells are indirectly involved in hematopoiesis, they provide a microenvironment that influences hematopoietic stem cells' function and differentiation.

In the past few years, stem cell therapy is considered one of the most advanced and innovative treatments to approach several disorders such as musculoskeletal, cardiovascular, autoimmune diseases, and neurological diseases. Bone marrow mesenchymal stem cells are multipotent adult stem cells with exceptional application in regenerative medicine as they repair and generate new cells. They also play a critical role in supporting other cells' proliferation and differentiation. Mesenchymal stem cells secrete many factors that affect the migration, proliferation, differentiation, or even apoptosis of other surrounding cells. Furthermore, these cells possess unique immune modulation properties that affect the immune system's cells. Many studies have shown that some biological properties of these cells vary depending on their source, remarkably different levels of secreted cytokines and growth factors.

As well as mesenchymal stem cells positively affect the human body and regenerate damaged tissues within the organism, the organism also affects mesenchymal stem cells. Therefore, it is vital to consider the patient's medical condition before undergoing any mesenchymal stem cell therapy procedure. In this bachelor thesis, an overview focusing on the description of mesenchymal stem cells and their respective properties, development, and their crucial role in the processes that occur within the bone marrow would be highlighted. This thesis also focuses on mesenchymal stem cells' potential use in *in vitro* cultivation and their interaction with other cells, such as HSCs.

2. BONE MARROW STRUCTURE

In the human body, bone marrow is the primary site of effective hematopoiesis and is composed of bone, vascular structures, and hematopoietic tissue. There are two types of bone marrow tissue: red marrow and yellow marrow. From birth to adolescence, most of the bone marrow is red marrow, and with time the red marrow is systematically replaced by the yellow marrow (Travlos, 2006).

2.1 RED MARROW

The red marrow contains hematopoietic stem cells that can differentiate into myeloid or lymphoid stem cells (Fig.1).

Myeloid stem cells mature into red blood cells, platelets, or myeloblast cells which can later develop into granulocyte and monocyte white blood cells (El Barky et al., 2017).

Red blood cells, also referred to as erythrocytes, transport oxygen to the body cells and deliver carbon dioxide to the lungs. Platelets, also called thrombocytes, develop from megakaryocytes. They play an essential role in the blood clotting process and tissue healing. White blood cells, granulocytes mature from myeloblast cells and defend the organism against foreign invaders such as bacteria, viruses, and other pathogens. Myeloblast granulocytes include neutrophils, eosinophils, and basophils. On the other hand, monocytes are large white blood cells that migrate from blood to tissues and mature into macrophages and dendritic cells. Macrophages eliminate foreign substances, damaged cells, and cancer cells from the body by phagocytosis, while dendritic cells play an essential role in primary immune responses (Penka, 2001).

Lymphoid stem cells develop into lymphoblast cells, which generate other types of white blood cells referred to as lymphocytes. Lymphocytes differentiate into natural killer cells, B-lymphocytes, and T-lymphocytes (Morrison and Scadden, 2014).

Natural killer cells contain enzymes that cause apoptosis in infected and diseased cells. B-lymphocytes recognize molecular signals from pathogens and generate antibodies against specific antigens. They are one of the significant components of humoral immunity. T-lymphocytes help identify and destroy damaged, cancerous, and infected cells. They activate other immune cells such as B-lymphocytes and produce cytokines, and they also play an essential role in the adaptive immune response (Penka, 2001).

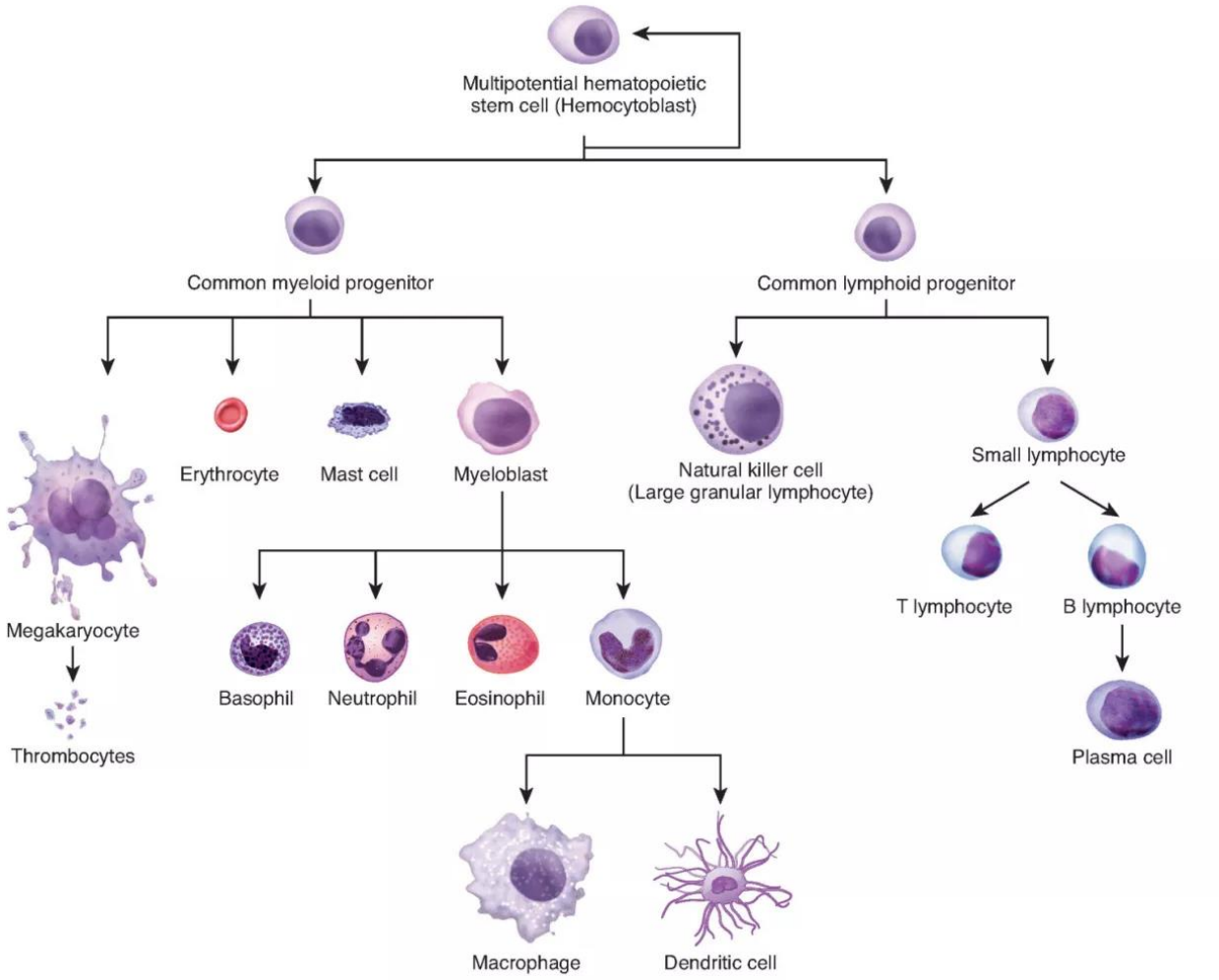

Figure 1:: Red marrow cells (Bailey, 2020): *Differentiation of hematopoietic stem cells into myeloid and lymphoid stem cells.*

2.2 YELLOW MARROW

Yellow marrow consists primarily of fat cells and is non-vascular. The yellow marrow is composed of mesenchymal stem cells that generate cartilage, fat, and bone (Fig.2). It plays an essential role in hematopoiesis: in the case of low blood supply, yellow marrow can be converted into red marrow to generate more blood cells (Horowitz et al., 2017). Mesenchymal stem cells or mesenchymal stromal cells can differentiate into several specialized cell types of skeletal tissues, such as bone cells- osteocytes, cartilage cells- chondrocytes, and fat cells- adipocytes (Sousa B. R. et al., 2014).

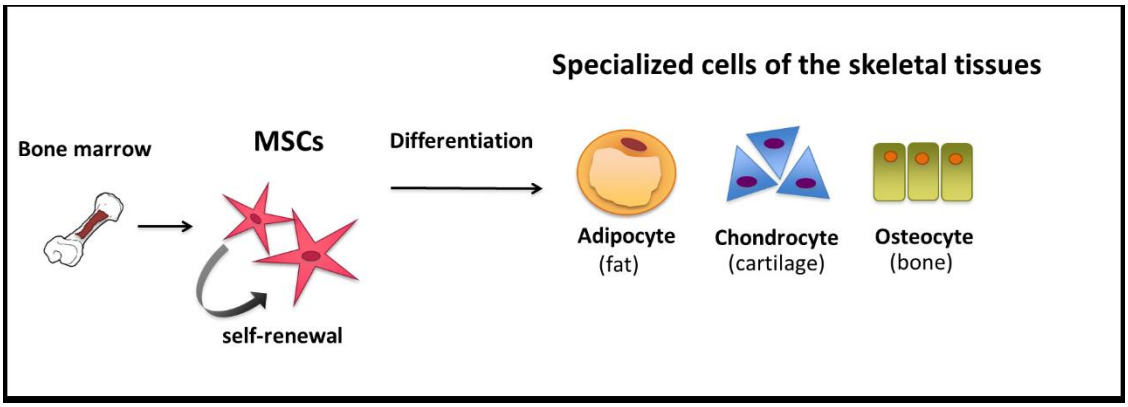

Figure 2: Yellow marrow cells (EuroStemCell, 2016): *Mesenchymal stem cells differentiation into skeletal tissues' specialized cells.*

3. STEM CELLS

3.1 HISTORY

Throughout history, many significant events have driven progress in stem cell research. For over more than half a century ago, in 1961, stem cells were discovered by biophysicist James Till and cellular biologist Ernest McCulloch at the University of Toronto in Canada (Till and McCulloh, 1961). Till and McCulloh found out that stem cells acquired from mouse bone marrow cells could differentiate into a diversity of cell types and were therefore called pluripotent stem cells.

According to Liu. G. et al. (2020), decades later, in 1996, Keith Campbell, Ian Wilmut, and colleagues from the Roslin Institute in Scotland were able to demonstrate the validity of the somatic cell nuclear transfer based on their research and experiment about the cloning of Dolly, the sheep.

By 1998, in the United States of America, biologist James Thomson was a pioneer who isolated the first human embryonic stem cells (James A. Thomson et al., 1998).

Years later, in 2006, researchers made another advance by identifying conditions that would allow specialized adult somatic cells to be genetically reprogrammed into assuming a stem cell-like state, with only four essential transcription factors. This new type of stem cell is now known as induced pluripotent stem cells (Takahashi K. et al., 2007).

In 2012, Doctor Shinya Yamanaka, alongside his colleague and biologist John Gurdon, were awarded the Nobel Prize for Physiology or Medicine after their studies proved that mature cells could be reprogrammed to become pluripotent. Researchers have detected inborn adult stem cells inward many organs (Sousa B. R. et al., 2014).

Presently, five primary stem cells exist based on stem cell research's systematic review. Such categories are embryonic stem cells, very small embryonic-like stem cells, nuclear transfer stem cells, reprogrammed stem cells, and adult stem cells (Liu G. et al., 2020). Even though nuclear transfer stem cells have been used to create an entire organism (Liu Z. et al., 2018), embryonic stem cells, induced pluripotent stem cells, and adult stem cells have only been used to generate tissues and organs (Liu G. et al., 2020).

Stem cell research has prospered into a thrilling and promising field in the last decade. Stem cells, specially ESCs, and MSCs have shown excellent application promise in significant areas such as regenerative medicine and disease therapeutics.

3.2 BIOLOGICAL BACKGROUND OF STEM CELLS

Stem cells are undifferentiated cells capable of self-replicating indefinitely and differentiate into mature cells with specialized functions (Kim, 2004). They can be classified as embryonic, fetal, or adult based on their origin. Embryonic SCs are found in the early stages of embryo development (Martin G.R., 1981), while fetal SCs are primitive cell types of the fetus that can give rise to various organs in the human body (Bond A.M., 2015). Adult SCs occur in different tissues, such as bone marrow and the brain. They retain the ability to self-replicate and differentiate into cell types from the origin's tissue throughout the organism's life (Liu G. et al., 2020). However, the use of embryonic SC involves ethical problems, and its carcinogenic potential is a serious risk factor for clinical applications. Several SC populations in adult tissues could work around this problem. Besides, several studies have shown that adult SCs have remarkable plasticity and can generate other cell types in addition to those of the tissues in which they originated, so they are called multipotent (Krause et al., 2001).

When it comes to cell therapies, "ideal" cells must be easily accessible, immunologically inert, capable of expanding rapidly in culture, surviving for long periods, and capable of integrating with host tissue. Among the most promising candidates are the bone marrow mesenchymal stem cells, which can be easily obtained through bone marrow aspiration (Daley G.Q., 2015).

BMMSCs are multipotent cells capable of differentiating mainly in mesodermal origin cells, such as cells in the bone, cartilage, fat, and muscle tissues (Kolios and Moodley, 2013). Nevertheless, mesenchymal stem cells have great plasticity and differentiation capacity. Several authors have reported that these cells can differentiate into central and peripheral nervous tissue cells if provided adequate stimuli and culture conditions (Bond et al., 2015; Kolios and Moodley, 2013; Liu G. et al., 2020).

Also, BMMSCs are particularly attractive for future cell therapies due to several other factors, such as they do not trigger ethical problems to be manipulated and maintained in the laboratory, do not require immunosuppression, can be efficiently expanded, constitute an abundant and accessible reserve for clinical use and high tolerance to genetic manipulation.

3.2.1 Stem cells classification based on their potential to differentiate

Nowadays, all stem cells can be classified based on their potential to differentiate into five major classes: totipotent, pluripotent, multipotent, oligopotent, and unipotent (Fig.3).

Totipotent stem cells have the maximum potential to give rise to a new organism. A totipotent stem cell can divide and differentiate into all possible cell types within the organism and generate fertile offspring. Thus, a fertilized oocyte and the first few divisions' cells are totipotent stem cells as they differentiate into embryonic and extraembryonic tissues, thereby forming the embryo and the placenta (Kolios and Moodley, 2013).

Pluripotent stem cells can proficiently self-renew and differentiate into embryonic stem cells and cells derived from the three germ layers – ectoderm, endoderm, and mesoderm, which differentiate to form all tissues and organs within the organism (El Barky et al., 2017). Embryonic stem cells were first derived from the inner cell mass of mammalian blastocysts, but in 2006, Takahashi and Yamanaka generated pluripotent cells by genetically reprogramming adult somatic cells. These cells are known as induced pluripotent stem cells and share the same characteristics as ESCs.

Multipotent stem cells can differentiate into multiple specialized cell types present in a specific tissue or organ. Mesenchymal stem cells are an excellent example of multipotent cells, as they can self-renew and undergo differentiation into osteocytes, myocytes, adipocytes, and chondrocytes (Koolios and Moodley, 2013). All these cell types are diverse in their characteristics. Thus, MSCs are considered multipotent cells.

Oligopotent stem cells are similar to multipotent stem cells, but they are more restricted when it comes to their capacity to differentiate. These cells can only differentiate into closely related cell types. Examples include hematopoietic stem cells that can differentiate into myeloid and lymphoid cells (Kalra and Tomar, 2014).

Unipotent stem cells are the least potent and the most limited cell type. These cells can only differentiate into cells of their kind but still have self-renewal property required to be considered a stem cell (El Barky et al., 2017). An example of this stem cell type is muscle stem cells, myocytes.

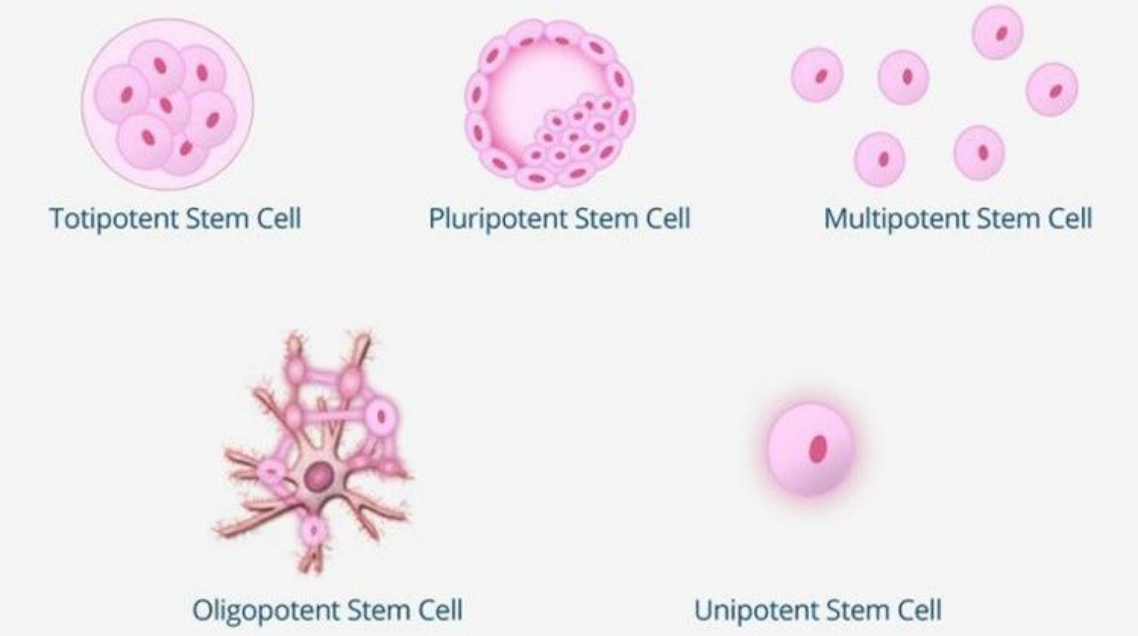

Figure 3: Stem cells (Hildreth, 2021): *Stem cells classification based on their potential to differentiate.*

3.2.2 Stem cells classification based on their origin

Stem cells are also classified based on their origin or source. According to their origin, we can divide them into two broad types, adult and embryonic stem cells.

Embryonic stem cells are self-renewing pluripotent cells found in blastocysts' inner cell mass approximately five days after fertilization (Khan et al., 2018). ESCs have remarkable plasticity. Therefore, they can generate different types of cells. This property of ESCs is due to the fact that the blastocyst can generate all the organs within the organism (Fig.4). After fertilization, the zygote divides and differentiates until it produces an adult organism consisting of more than two hundred cell types, thus giving rise to any tissue within the human body (Kalra and Tomar, 2014, Koolios and Moodley, 2013).

Adult stem cells are multipotent cells found throughout the organism after embryonic development, mainly in the bone marrow, adipose tissue, and umbilical cord blood. Their vital role in any living organism is to maintain, repair, and regenerate tissue. The adult stem cells primarily used in cell therapy clinics are hematopoietic stem cells and mesenchymal stem cells. Hematopoietic stem cells can originate all types of cells found in the blood-red blood cells, white blood cells, and platelets- and have been used in bone marrow transplantation for more than fifty years. MSCs, on the other hand, are considered multipotential since they can differentiate into different types of tissues with a mesodermal origin (Fig.5), such as fibroblasts,

osteocytes, chondrocytes, and adipocytes, but also into the muscle or neural cells (El Barky et al., 2017).

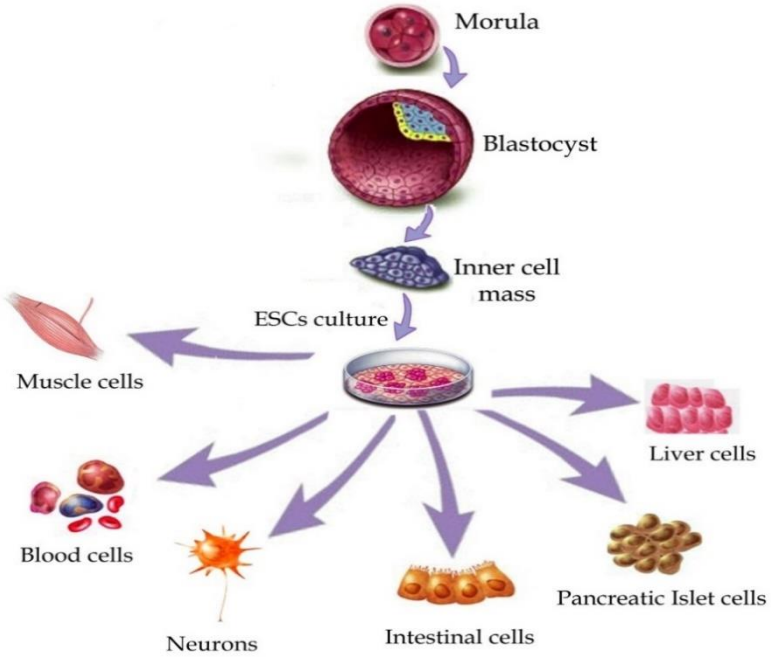

Figure 4: ESCs differentiation (Meregalli et al., 2011): *Embryonic stem cells can potentially differentiate into different types of cells.*

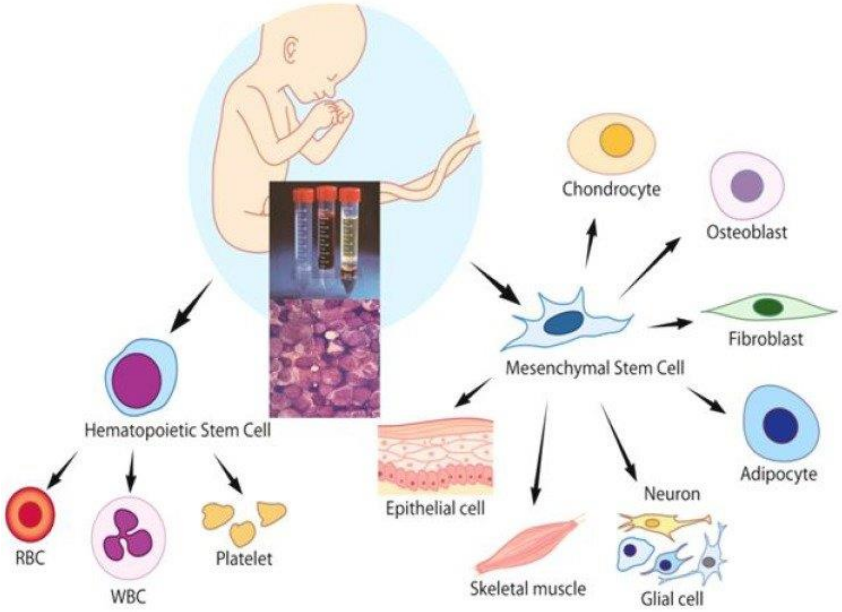

Figure 5: HSCs and MSCs differentiation (Lee, 2014): *Adult stem cells derived from umbilical cord blood can differentiate into multiple cell types.*

4. MESENCHYMAL STEM CELLS

MSCs were first described by Friedenstein and colleagues in the 1970s when they proved that the BM consists of two types of stem cells: hematopoietic cells, responsible for producing and replacing all types of blood cells, and stromal cells or mesenchymal stem cells, a self-renewing multipotent cell population progenitor of several cell lines. Friedenstein et al. (1970) described MSCs as a set of adherent, nonphagocytic, and fibroblastic cells capable of *in vitro* proliferation into bone, cartilage, and adipose tissue (Fig.2). Initially, they were called Colony Forming Unit- Fibroblasts (CFU-F) based on their efficiency to form colonies, strain generation, and differentiation potential.

Over the years, several researchers explored MSCs and their properties. Hence, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) needed to establish a consensual definition of MSCs. The ISCT proposed the minimal criteria set to define this population of cells, based on the adherence to plastic during their culture, expression of specific surface markers, and multipotent differentiation potential.

4.1 PROPERTIES OF MSCs

According to Brighton and Hunt (1991), a typical irregular star shape with multiple long and thin processes characterizes mesenchymal stem cells. Their nucleus is large, oval, and contains a prominent nucleolus. MSCs cytoplasm is pale, and it has few organelles such as Golgi apparatus, rough endoplasmatic reticulum, mitochondria, and ribosomes. A jelly-like matrix that contains a small amount of reticular or collagen fibers forms the intercellular spaces.

As stated by the ISCT, for a cell to be considered an MSC, it must meet three criteria: adhesion to plastics, expression of specific surface markers, and multipotent differentiation potential. Firstly, it is a must that the cell is plastic adherent to preserve the standard culture conditions during the use of tissue culture flasks (Dominici et al., 2006).

MSCs can be identified through a set of surface antigens that are not specific for this population but rather expressed by other types of cells (Paniushin et al., 2006). Given that the cell population of MSCs is heterogeneous (Lv et al., 2014), to guarantee that they are not confused with other cell types, Dominic and colleagues (2006) advocated using hematopoietic antigens since MSCs do not express such antigens. Furthermore, the surface phenotype as defined by the ISCT is negative for markers CD14 or CD11b, CD45, CD34, CD79 or CD19, and HLA-DR and positive for surface markers CD73, CD90, and CD105 (Kim et al., 2013).

Also, the cell must differentiate into osteocytes, chondrocytes, and adipocytes- trilineage mesenchymal differentiation under standard *in vitro* tissue culture differentiating conditions.

Plasticity is one of the unique and well-illustrated properties that characterize the mesenchymal stem cells of the bone marrow. It actively demonstrates their capacity to trilineage differentiate into several tissue types with a mesodermal origin such as osteocytes, chondrocytes, and adipocytes under standard *in vitro* culture conditions (Pountos, 2016). One way we can verify the trilineage differentiation is by using cytochemical methods. Toluidine blue stain, also referred to as tolonium chloride, can display osteoblast differentiation. In contrast, adipocyte differentiation is primarily identified by oil red-O stain, and chondroblasts differentiation can be evidenced with Alcian blue stain (Henry and Bordoni, 2021, Dominici et al., 2006). On account of MSCs' adherence to plastics, they are one of the most critical cells in regenerative medicine. These cells are easily accessible and expanded, suggesting that the use of MSCs may be a functional therapeutic approach for various disorders.

Table 1:Minimal criteria to identify MSCs (adapted from Dominici et al., 2006)

1. Adherence to plastic in standard *in vitro* conditions		
2. Phenotype (expression of specific surface markers)	**Positive**	**Negative**
	CD105	CD45
	CD73	CD34
	CD90	CD14 or CD11b
		CD79α OR CD19
		HLA-DR
3. *In vitro* trilineage differentiation: osteocytes, chondrocytes, and adipocytes		

4.2 SOURCES

Mesenchymal stem cells can be isolated from different tissues in the human body. The most popular sources are bone marrow, adipose tissue, placenta, and umbilical cord blood (Kim et al., 2013). The bone marrow is the standard gold source of MSCs even though MSCs represent about 0.001% to 0.01% of the total bone marrow. Thus, it is challenging to calculate the exact amount of MSCs isolated from the bone marrow aspirate (Fig. 6a) since the collection methods affect the harvest. Furthermore, the amount and quality of MSCs isolated from the bone marrow decline with advancing age (Romanov et al., 2005). Adipose tissue is one of the richest reservoirs of MSCs. Adipose-derived MSCs (Fig. 6b) are easily isolated from

abdominoplasties and lipoaspirations, which are less invasive methods richer in stem cells when compared to bone marrow (Hass et al., 2011).

Also, MSCs can be isolated from fetal tissues, particularly the placenta, umbilical cord tissue, jelly-like ligament (Warton's jelly), amniotic fluid, and umbilical cord blood. In the case of placental and amniotic fluid sampling, the use of MSCs is different because it contains a mixed population of endothelial stem cells and HSCs (Mushahary et al., 2018).

Other potential sources of adult mesenchymal stem cells are dental pulp, peripheral blood, and dermis. Also, MSCs have been identified in the lungs, liver, and spleen during fetal development.

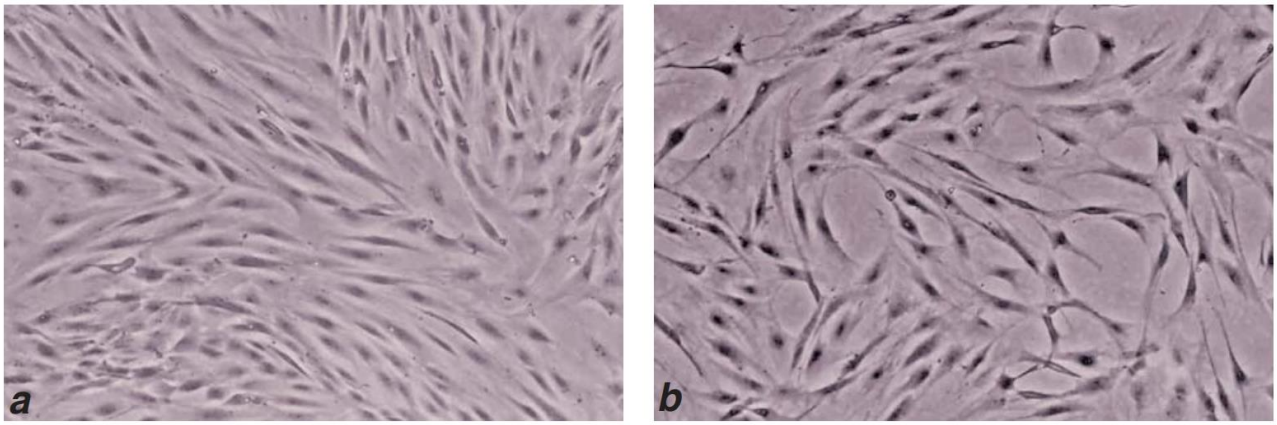

Figure 6: MSCs sources (Romanov et al., 2005): *a) bone marrow; b) adipose tissue lipoaspirate.*

4.3 TRILINEAGE DIFFERENTIATION POTENTIAL

In the light of MSCs being multipotent, they can give rise to a set of differentiated cells from the mesodermal germ layer, such as osteocytes, blastocytes, and adipocytes (Bruder et al., 1997; Johnstone et al., 1998; Sekiya et al., 2004). This trilineage differentiation constitutes the classical progeny of MSCs. Still, studies have shown that this cell population can also differentiate into several ectodermal and endodermal origin lineages under specific *in vitro* culture conditions (Ding et al., 2011). The differentiation process of MSCs is regulated by a series of genetic events, including transcription factors, hormones, cytokines, and growth factors.

In vitro studies involving the growth and differentiation of mesenchymal stem cells have been highlighted in this research field, thus contributing to the knowledge of mechanisms and factors related to the self-renewal and differentiation of these cells for their use in gene and cellular therapies.

4.3.1 Osteogenic differentiation

Osteogenesis or bone formation occurs during embryonic development after the proliferation of MSCs, leading to single colonies referred to as CFU. There are two crucial processes of bone transformation, and both require the modification of MSCs into the bone.

Most bones form per endochondral ossification, a process in which the hyaline cartilage is replaced with the bone (Fig. 7). MSCs first differentiate into osteochondroprogenitor cells that later evolve into chondrocytes, which are gradually replaced by osteoblasts (Ding et al., 2011). The endochondral process takes place in the physis, the epiphysis, and in the carpal and tarsal cuboidal bones.

In the course of endochondral ossification, MSCs proliferate and condensate into compact nodules, which later differentiate into cartilage cells, chondrocytes. The chondrocytes undergo rapid mitosis and produce a cartilage-specific extracellular matrix. Once the chondrocytes stop dividing, they mature, and their volume escalates drastically, giving rise to the hypertrophic chondrocytes, which signal the surrounding matrix to calcify. The calcified chondrocytes die by apoptosis, leaving spaces that will be occupied by osteoprogenitor cells that differentiate into osteoblasts. The osteoblasts remodel the bone matrix, and it becomes calcified on the surface of the degraded cartilage, and eventually, the bone replaces the cartilage (Hanna et al., 2018).

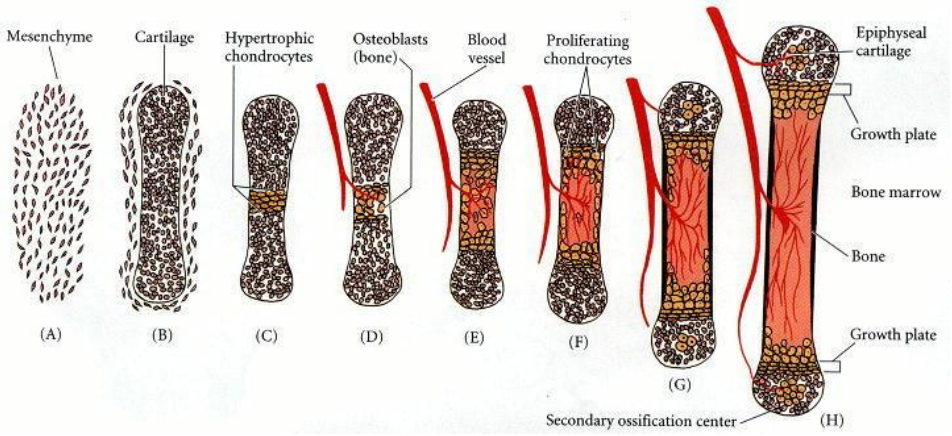

***Figure 7: Endochondral ossification (Gilbert, 2000)**: MSCs proliferate, giving rise to chondrocytes, which are eventually replaced by bone cells, osteocytes.*

Another ossification process is intramembranous ossification, in which MSCs directly convert into osteoblasts that sequently turn into osteocytes (Fig. 8). This process predominantly occurs in the skull's flat bones and the clavicles.

Throughout intramembranous ossification, mesenchymal stem cells proliferate and

condensate into compact nodules. Some of these MSCs shapeshift and give rise to osteoblasts, bone precursor cells. Osteoblasts secrete a collagen-proteoglycan matrix that attaches to calcium salts leading to the osteoid's calcification. Once trapped in the calcified matrix, the osteoblasts differentiate into osteocytes, bone cells (Gilbert, 2000).

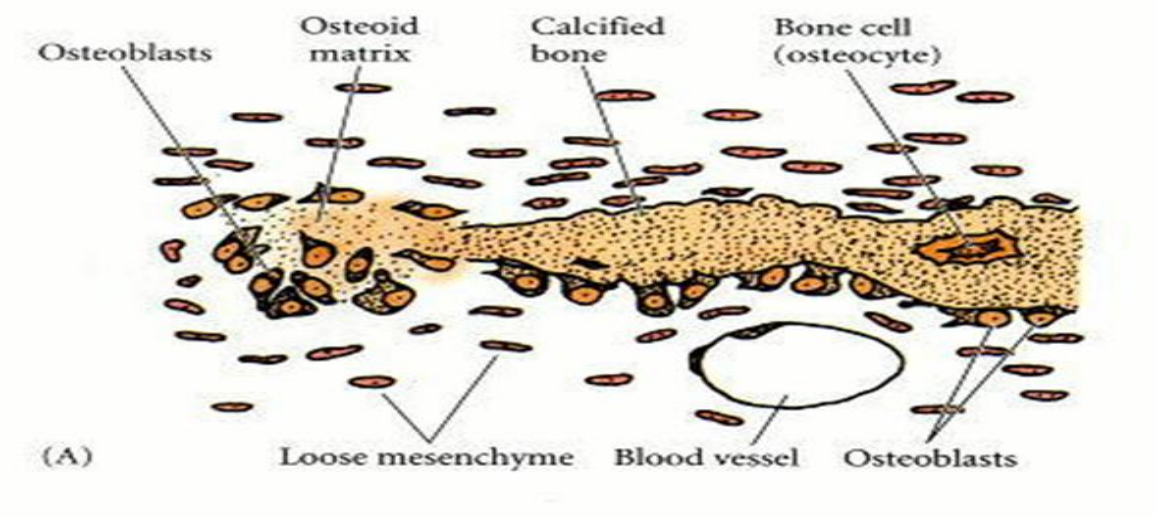

Figure 8: Intramembranous ossification (adapted from Gilbert, 2000): *MSCs proliferate, giving rise to osteoblasts that later differentiate into osteocytes.*

4.3.2 Chondrogenic differentiation

During the course of fetal development, the majority of the human skeleton is cartilaginous. Chondrogenesis, also referred to as chondrification, is an essential process for establishing the skeleton. The chondroblastic differentiation starts with the condensation of MSCs in which the chondroblast differentiate into chondrocytes, mature cartilage cells, giving rise to the extracellular matrix. As the matrix increases, the cells proliferate and undergo mitosis, referred to as interstitial growth. Once the interstitial expansion is completed, the daughter cells remain together in the lacuna forming the isogenous groups (Fig. 9) and gradually increase the cartilage's matrix (Kozhemyakina et al., 2015).

Mature chondrocytes have an oval to a round shape, contain many glycogen granules, and do not divide further. According to its composition, cartilage can be classified into hyaline, elastic, and fibrous. Most cartilage's surface is encircled by a layer of dense asymmetrical connective tissue referred to as perichondrium.

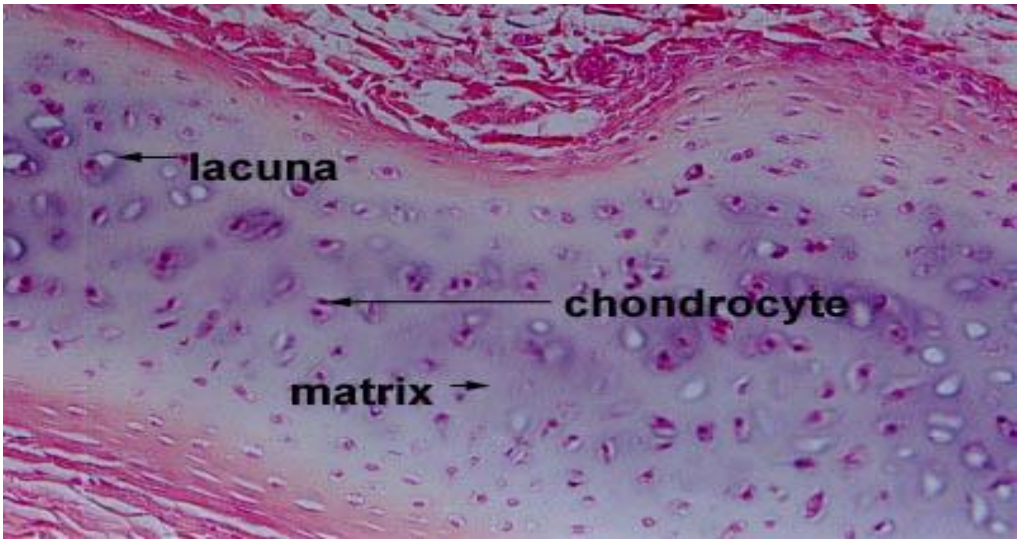

Figure 9: Histology of hyaline cartilage
(https://www.histology-world.com/factsheets/cartilage1.htm cit:23.03.2021)

4.3.3 Adipogenic differentiation

Adipogenic differentiation, also referred to as adipogenesis, is a process through which MSCs differentiate into adipocytes, fat cells. In adipogenic differentiation, MSCS gradually differentiate into pre-adipocytes, which are undifferentiated fibroblasts stimulated to give rise to mature adipocytes, leading to adipose or fatty tissue's origination (Berry et al., 2013). Adipocytes have a spherical shape with a diameter of up to 100 µm and are characterized by the existence of a single large lipid droplet (Fig.10), also known as adiposome -unilocular.

Fat cells consist of white adipose tissue as well as brown adipose tissue. WAT is typical of unilocular adipocytes and primarily found in adults, while BAT is multilocular because every single fat cell contains several adiposomes. The BAT is smaller than the WAT, and its color corresponds to the high content of mitochondria present in it (Horowitz et al., 2017). During the early stages of life, the bone marrow is primarily red, and as the adipocytes develop, the yellow or fatty marrow replaces the red marrow (Berry et al., 2013).

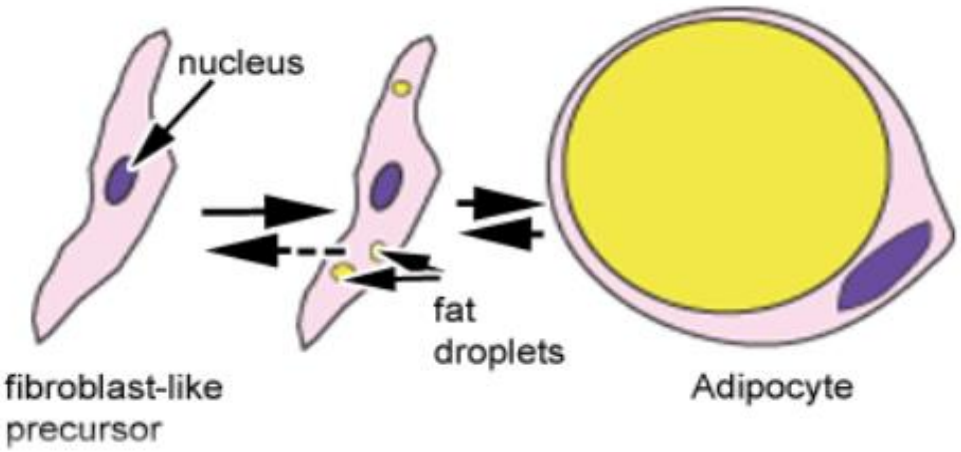

Figure 10: Adipogenic differentiation: *The lipid droplets come together to form one large droplet, and the nucleus is expelled to the outer edge.*
(https://www.histology.leeds.ac.uk/tissue_types/connective/connective_adipocyte.php cit: 24.03.2021)

5. MSCs *IN VITRO* REGULATION

MSCs in vitro proliferation and differentiation are regulated by a series of genetic events that include transcription factors, cytokines, growth factors, and even extracellular molecules. These cells can also influence other cells and tissues in their surroundings.

5.1 TRANSCRIPTION FACTORS

Bone marrow mesenchymal stem cells have the ability to differentiate into several functional types of cells, including osteoblasts, chondrocytes, and adipocytes. Such cells' maturation is determined by the expression of transcription factors found within the MSCs population. Transcription factors are proteins that regulate specific genes by binding to a particular DNA sequence. Their primary function is to up or downregulate gene expression and guarantee that these same genes are expressed in a suitable cell at the correct time and right amount.

The primary transcription factors that play crucial roles in the differentiation of MSCs into osteocytes are Runx2 and Osterix. *In vitro* studies based on mice lacking Runx2 have shown that the Runt-related transcription factor 2 is considered one of the most critical TF in the processes regarding bone formation, osteogenesis. During bone formation, the Runx2 transcription factor conducts MSCs differentiation into pre-osteoblasts while suppressing the adipogenic and chondrogenic differentiation. Besides, several *in vitro* studies have shown that Runx2 expresses osteoblastic markers, osteopontin, osteocalcin, ALP expression and increases mineralization, demonstrating that this specific transcription factor induces osteogenic differentiation in *in vitro* culture (Cook and Genever, 2013).

An additional transcription factor that also plays an essential role in osteogenesis is Osterix. It belongs to the Sp1 family and contains three zinc fingers. According to Nakashima and colleagues (2002), Runx2 null mice do not express Osterix, indicating that Osterix downregulates Runx2. They also outlined that Osterix is a requisite for bone formation and the particular reason for this circumstance is that Osterix directs MSCs to osteoblasts. Another essential transcription factor that promotes the differentiation of MSCs into osteoblasts is β-catenin, which prevents MSCs from potentially differentiating into chondrocytes. CBF-α-1, referred to as core-binding factor subunit alpha-1, has a vital role in osteogenesis given that hypoxia boosts its expression in bone marrow mesenchymal stem cells leading to the differentiation of MSCs into osteocytes. Factors like TWIST and HIF-1α inhibit the differentiation of MSCs into bone cells, osteoblasts.

Furthermore, signaling pathways such as Wnt, BMP, and Notch control the expression of osteogenic transcription factors. For example, BMP-2 stimulates the expression of Runx2 and Osterix in pre-osteoblasts and osteoblasts. At the same time, Wnt signaling regulates the activity of β-catenin, while Notch signaling pathway actives CBF-α-1 in the nucleus, promoting bone formation (Almalki and Agrawal, 2016).

Throughout chondrogenic differentiation, Sox9 is the primary transcription factor considering that it plays an essential role in the condensation of mesenchymal stem cells and chondrogenic differentiation. Sox9 is an early regulator that extensively commands the expression of crucial marker genes such as collagen type 2, type 9, type 10, and aggrecan during chondrogenesis. In comparison, chondrogenic progenitors and all differentiated chondrocytes express Sox9, while hypertrophic chondrocytes only express collagen type 10. *In vitro* studies have shown that the absence of Sox9 prior to mesenchymal condensation can lead to undeveloped cartilage, while its lack afterward condensation interrupts chondrogenic differentiation. Besides, Sox9 is also known to have an inhibitor effect on Runx2 expression, thus, promoting chondrocyte differentiation and postponing hypertrophic chondrocyte differentiation (Nakashima et al., 2002).

Moreover, the triad of Sox genes constituted by Sox5, Sox6, and Sox9 also play a vital role in chondrogenic differentiation as they equally promote their condensation and differentiation (Cook and Genever, 2013). According to Akiyama and colleague's (2002) work, Sox5 and Sox6 are also expressed in all chondrogenic progenitors and differentiated chondrocytes, consequently activating chondrogenic markers such as COL2-α1, type 2 collagen. Sox9 does not depend on Sox5 and Sox6. Thus, the Sox9 levels remain identical in their absence.

Many other factors are known to have a restraining effect on bone marrow mesenchymal stem cells differentiating into chondrocytes, including Smad3, Hoxa3, and YAP. Also, chondrogenic differentiation can be controlled by different signaling factors like BMP, Wnt, FGF, and TGFβ. Throughout the years, several *in vitro* studies have demonstrated that peroxisome proliferator-activated receptor γ- PPARγ is the primary regulator of gene expressions in adipogenesis. PPARγ is considered a vital regulator in adipogenic differentiation due to its ability to interact with P2, an adipogenic gene.

PPARγ is known to have the capacity to increase gene expression during adipogenic differentiation, and its inhibition can cause the arrest of fat formation. This transcription factor consists of two isoforms, PPARγ1 and PPARγ2. Both play an essential role in the differentiation of MSCs into adipocytes, but PPARγ2 is considered to have a more substantial inducive effect

due to its restriction to adipose tissue (Almalki and Agrawal, 2016). Lately, some studies have reported that activated expression of PPARγ2 merged with C/EBP-α, C/EBP-β, or PRDM16 effectively stimulates adipogenic differentiation up to 90%. However, transcription factors such as TAZ and MITR have inhibitory effects when interacting with PPARγ2, decreasing its expression and suppressing the differentiation of MSCs into adipocytes (Nakashima et al., 2002; Cook and Genever, 2013).

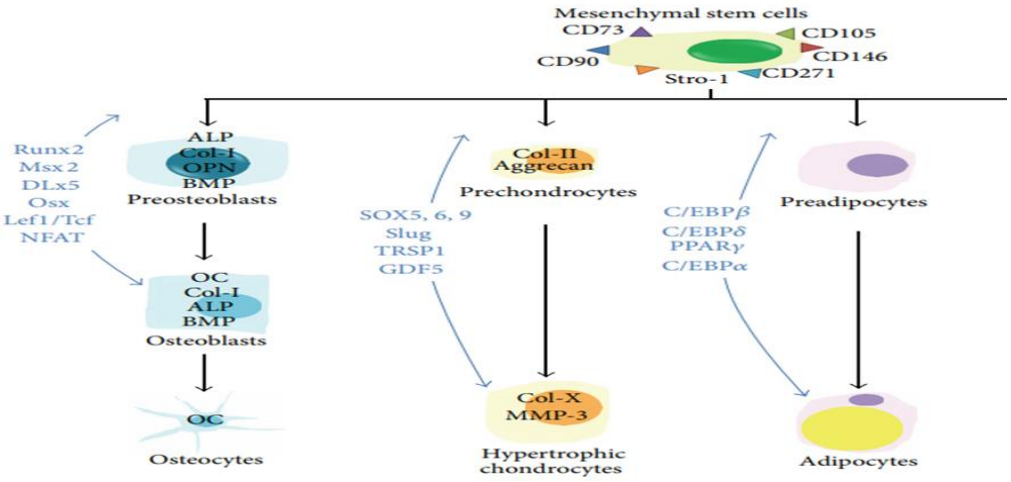

Figure 11: Trilineage differentiation of BMMSCs (adapted from Giuliani et al., 2013): *In vitro osteogenic, chondrogenic, and adipogenic differentiation regulated by specific transcription factors.*

5.2 CYTOKINES AND GROWTH FACTORS

Cytokines are small secreted proteins that act on specific cells through specific paracrine pathways, affecting cell interaction and communication. While growth factors, also referred to as a subset of cytokines, are signaling proteins capable of stimulating cellular proliferation and differentiation and tissue repair (Han et al., 2019).

One of the main reasons mesenchymal stem cells are considered one of the most effective treatment approaches in regenerative medicine is their capacity to repair tissue damages caused by diseases or injuries, secreting several soluble substances, which affect the microenvironment of surrounding tissues. These soluble substances known as cytokines and growth factors influence the proliferation and differentiation potential of BMMSCs. Cytokines and growth factors found in the bone marrow mesenchymal stem cells can impact biological

functions such as immunomodulatory, anti-apoptotic, and angiogenic properties and anti-inflammatory capacities (Hu and Li, 2018).

In 2008 Ren and colleagues demonstrated that the immunomodulatory property of MSCs is stimulated by an inflammatory cytokine (IFN-γ) when combined with TNF-α and IL-6. Preconditioning MSCs with IFN-γ induces the synthesis of indoleamine- 2,3-dioxygenase, which consequently inhibits the activation of natural killer cells (Ren et al., 2008). MSCS also secrete prostaglandins such as PGE1-α, PGE2, and PGF2-α. The prostaglandins play a vital role in the inflammatory process, as they mediate vasodilation, and they may also have an immunostimulatory effect on facilitating Th1 differentiation and proliferation of Th17 lymphocytes (Meirelles Lda et al., 2009).

MSCs are known for their migration effect on injured sites. Another essential property present in MSCs is a homing property defined as 'the arrest of MSCs within the vasculature of a tissue followed by transmigration across the endothelium' (Ley et al., 2007). Based on Honczarenko and colleagues' study in 2006, the homing property of MSCs results from the expression of three CC chemokine receptors- CCR1, CCR7, CCR9, and three CX chemokine receptors- CXCR4, CXCR5, and CXCR6. These chemokine receptors participate in the homeostatic leukocyte trafficking and cell compartmentalization within the BM and other lymphoid organs (Honczarenko et al., 2006). According to Honczarenko and colleagues, the set of chemokine receptors potentially stimulate the migration and in vitro chemotaxis of BMMSCs. In vitro culture of bone marrow mesenchymal stem cells has proved that these cells are a reservoir of chemokines. However, long-term cultivation significantly reduces the expression of chemokine receptors, and MSCs lose their ability to respond to such chemokines. Furthermore, the loss of chemokine receptor expression leads to a reduced regulation of other surface receptors related to MSCs (Rombouts and Ploemacher, 2003). Thus, long-term cultures of BMMSCs are associated with slow cell growth and an increase in spontaneous apoptosis. Different studies have shown that *in vitro* MSCs migration is also regulated by SDF-1 and its receptor CXCR4 and HGF (Rombouts and Ploemacher, 2003; Honczarenko et al., 2006; Ley et al., 2007).

As mentioned at the beginning of this topic, BMMSCs also have other essential features that constitute the anti-apoptotic, anti-fibrosis, and angiogenic properties. Mesenchymal stem cells produce cytokines and growth factors such as vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), macrophage inflammatory protein-1α (MIP-1α), and monocyte chemoattractant protein-1 (MCP-1), which influence their angiogenesis, migration, and even their apoptosis. MSCs can avoid apoptosis through the secretion of VEGF, IGF-1, and

HGF, reducing cell death. VEGF and HGF are also considered angiogenetic factors as they promote the proliferation and differentiation of endothelial cells (Maacha et al., 2020). Furthermore, MIP-1α and MCP-1 promote the migration of MSCs, while VEGF has the opposite effect. It inhibits the migration of such cells. For the anti-fibrosis effect, MSCs produce adrenomedullin, which inhibits the proliferation of fibroblasts and decreases the production of collagen and HGF. Flt3-L is a crucial growth factor for immature myeloid stem cells Flt3-L, it maintains the ability to proliferate and self-renew of HSCs while spreading hematopoiesis (Meirelles Lda et al., 2009). As mentioned previously, BMMSCs secrete several cytokines, chemokines, and growth factors that influence other cells' biological functions in their surroundings. The most relevant factors are present in table 2.

Table 2:Cytokines and growth factors secreted by MSCs (adapted from Meirelles Lda et al., 2009)

Cytokines and growth factors	*Angiogenetic effect*	*Anti-apoptotic effect*	*Anti-fibrosis effect*	*Immunomodulatory property*	*Chemoattractant effect*	*Hematopoietic supportive effect*
bFGF	x	x	x			
HGF		x	x	x		
IGF-1		x				
IL-6	x					x
LIF				x		x
PGE1α;PGE2; PGF2-α				x		
VEGF	x	x				
SCF						x
SDF-1						x
TGF-β		x		x		
CCR1;CCR7;CCR9					x	
CXCR4;CXCR5; CXCR6					x	

As stated in chapter 4.1, MSCs can be identified by expressing specific surface markers. However, based on the source of MSCs, they can also express other surface markers from the ones mentioned previously. Several studies (Gang et al., 2007; Greco et al., 2007; Jung et al., 2011) have shown that BMMSCs express a particular surface marker profile, comprising the expression of STRO-1, CD29, CD73, CD90, CD105, CD146, CD44, CD106 (VCAM-1), CD166, Sca-1, Octamer-4 (Oct-4), and stage-specific embryonic antigen-4 (SSEA-4).

Throughout the years, researchers have debated on the dilemma of MSCs properties and their capacity to secrete cytokines, and different factors being dependent or not on their source. Many authors describe BM and AT as mesenchymal stem cell sources with very similar properties, including proliferation capacity, differentiation potential, and expression of surface

markers. However, these two sources of MSCs conflict in a few critical biological capabilities. Having that said, BMMSCs secrete higher levels of cytokines and growth factors such as VEGF, SDF-1, MCP-1, and TGF-β than ATMSCs. Another feature that differs these sources from each other is the fact that BMMSCs express SSEA-4 (Petrenko et al., 2020). Furthermore, the BMMSCs population ages faster under specific *in vitro* conditions in comparison to the ATMSCs population.

6. MSCs ROLE IN HEMATOPOIESIS

Hematopoiesis is the process by which HSCs differentiate into mature, functional blood cells (chapter 2.1). During embryonic development, hematopoiesis begins in the yolk sac, while in adults, this process takes place in the bone marrow. In case of any pathological condition, hematopoiesis may occur in the liver, spleen, and eventually even in the heart, brain, lung, or kidney (Charbord et al., 1996). The bone marrow also contains another type of stem cells, known as mesenchymal stem cells. Based on Dexter's (1982) *in vitro* studies, these cells are essential supporters of HSCs' long-term growth and hematopoiesis. MSCs support hematopoiesis by secreting different molecules that impact their biological functions, including migration, self-renewal potential, homing property, proliferation, and differentiation.

6.1 HEMATOPOIETIC NICHE IN THE BONE MARROW

Throughout the different stages of life, hematopoiesis demands a particular microenvironment known as the stem cell niche, which comprises soluble molecules and cell-cell interactions. The niche is mainly derived from progenitors of a mesenchymal origin, but it also includes endothelial cells and hematopoietic progenitors such as macrophages and megakaryocytes. The primary function of the niche is to preserve specific characteristics of hematopoiesis, such as HSCs survival, self-renewal potential, and differentiation (Wilson and Trumpp, 2006).

HSCs are found mostly alongside sinusoids within the bone marrow (Fig.12). MSCs support HSCs' long-term growth and hematopoiesis by regulating the immune system and producing cytokines and growth factors such as CXCL12, IL-6, LIF, osteopontin SCF, SDF-1, thrombopoietin, TGF-β, TNF-α, among many others, as shown in figure 12 (Meirelles Lda et al., 2009).

The BM niche is hypoxic, and this low oxygen tension (below 1%) in the niche and up to 6% in the sinusoidal cavity increases the proliferation potential. It also maintains the naive state and the plasticity of MSCs. In HSCs, the hypoxic environment, which takes place closer to the bone surface and distanced from the blood capillaries, is associated with a more inactive state (Morrison and Scadden, 2014). In the bone marrow, MSCs are an essential element of the hematopoietic niche. Together, MSCs and HSCs ensure hematopoietic and skeletal homeostasis

In the last few decades, knowing signaling pathways, which play a crucial role in the complex system of cell-cell communication and take part in the maintenance and regulation of HSPCs, is a fundamental step into finding new treatments for several diseases and the

development of HSPCs transplantation. Hematopoietic stem and progenitor cells give rise to all hematopoietic and immune cells, unceasingly. Due to their immunomodulatory potential, MSCs can affect the immunity system by secreting different factors or via cell-cell contact.

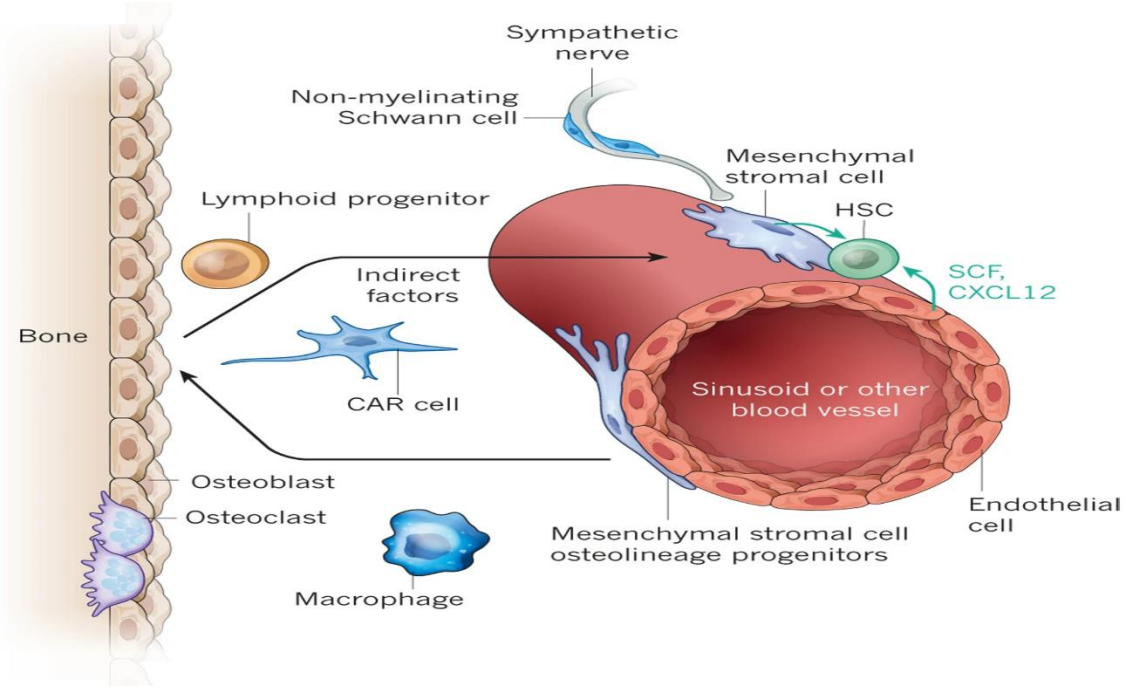

Figure 12: Bone marrow niche of MSCs and HSCs (Morrison and Scadden, 2014)

6.2 MSCs IMMUNOMODULATORY POTENTIAL ON HSCs

One of the most attractive features of MSCs that makes these cells exceptional in the regeneration field is their immunomodulatory potential. Through the secretion of a large scale of factors or direct cell-cell interaction, MSCs interact with several immune system cells.

Considered the most critical cytokine in co-culturing HSCs with MSCs, IL-6 induces the maturation of megakaryocytes, from which blood platelets are formed, increasing the proliferative capacity of HSCs by affecting IL-3, which stimulates their differentiation potential. Thrombopoietin also affects the proliferation and differentiation of HSCs, regulating the growth of hematopoietic progenitor cells through MPL receptors (Meirelles Lda et al., 2009).

Throughout the years, it was found that the MSCs' response to the same antigens, non-specific mitogens, CD3, and CD28 antibodies *in vitro* suppresses the proliferation and

activation of T-lymphocytes (Aggarwal and Pittenger, 2005). The inhibition of T- lymphocyte proliferation depends on the arrest of cell division mediated by MSCs in the G0-G1 phase of the cell cycle, which is related to the inhibition of the expression of cyclin D2. This phenomenon is known as condition arrest anergy, a condition in which T cells cannot react to a specific antigen. MSCs through Th1 cells temporarily reduce the production of interferon-gamma (Ren et al., 2008) and increase the expression of IL-4 by Th2 cells, leading to a change from the pro-inflammatory to the anti-inflammatory state. Hypoxic conditions also improve the inhibition of T cell proliferation, and hypoxia conditions occur prior to the inflammatory process (Meirelles Lda et al., 2009).

Several *in vitro* studies indicate that MSCs block the proliferation of B cells via division arrest anergy at G0-G1 phases. Besides, MSCs have been shown to have the ability to dimmish B cell differentiation, preventing them from becoming plasma cells and regulating the secretion of antibodies. MSCs also modulate the chemotactic properties of B cells by downregulating the expression of CXCR4, CDCR5, CCR7, as well as their chemotaxis response (Corcione et al., 2006; Honczarenko et al., 2006).

The immunomodulatory feature of MSCs also affects the dendritic cells. Mesenchymal stem cells block the *in vitro* differentiation of monocytes and $CD34^+$ into DCs, suppressing their ability to stimulate T cell proliferation. Furthermore, MSCs suppress the proliferation of dendritic cells by the inhibition of TNF-α secretion from myeloid dendritic cells. These mature dendritic cells upregulate the expression of IL-10, therefore generating Th2 and regulatory T cells. Moreover, factors like IL-6, MCSF, and cell-cell interaction may also regulate the effects of MSCs on dendritic cell functionality (Aggarwal and Pittenger, 2005).

Lastly, MSCs' immunomodulatory feature affect the natural killer cells as well. Studies have demonstrated that MSCs have the ability to restrain their maturation, cytotoxicity, and cytokine production when stimulated with IL-2 or IL-15. The inhibition of NK cell proliferation is associated with the production of the soluble factor TGF-β (Aggarwal and Pittenger, 2005). The bone marrow mesenchymal stem cells regulate blood production and immunity, as they can divert immune recognition and influence the different functions of the immune system.

7. MSCs *IN VITRO* CULTIVATION

MSCs are considered mesoderm-derived multipotent stem cells based on their ability to differentiate into osteoblasts, chondrocytes, and adipocytes under specific *in vitro* culture conditions. MSCs also present immunomodulatory properties, displaying the ability to escape immune responses through the secretion of cytokines. Such cellular features make these cells an excellent tool for the progress of regenerative medicine and cell therapy, as they repair and generate cells. In addition, the adherence to plastic and the presence or absence of specific surface markers also take part of the minimal criteria to identify MSCs under specific *in vitro* conditions (Dominici et al., 2006).

7.1 ISOLATION OF MESENCHYMAL STEM CELLS

In the bone marrow, MSCs differ from other cells by their unique plasticity property. Most of the other cells present within the bone marrow are not plastic adherents, making it possible to remove them from the culture flasks by the washing process. Bone marrow is obtained by a procedure referred to as aspiration, in which a small amount of the bone marrow is removed, exceptionally from bones like the hip bone, breastbone, or thigh bone (Hoffmann et al., 2017).

According to Hoffmann and colleagues (2017), bone marrow can also be acquired by scraping from bones alongside anticoagulants such as heparin or acid citrate dextrose.

Lately, a diversity of methods for the following isolation of MSCs exist, such as low and high-density culture techniques, frequent medium changes, enzymatic digestion approach, and antibody cell sorting (Baghaei et al., 2017). During the isolation of these cells, MSCs must form compact fibroblastic colonies. Thus, these colonies are referred to as CFU-F.

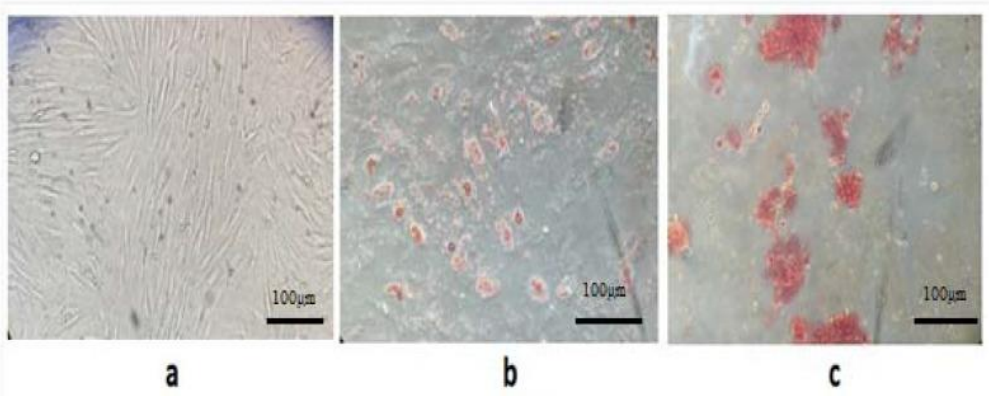

Figure 13: BMMCs under microscope (Baghaei et al., 2017): *a)CFU-C; b) adipogenic differentiation stained with oil red-O; c) osteogenic differentiation with Alizarin staining.*

7.2 CULTURE AND EXPANSION OF MSCs

Based on Hoffmann and colleagues comparative studies (2017), after bone marrow aspiration, the isolated MSCs can be cultured in different media and media additives, including Dulbecco's modified eagle's medium (DMEM) with high- 4.5 g/l and low glucose content- 1 g/l, Iscove's Medium, Ham's F12 or a mixture of DMEM and F12 (1:1). Osteogenesis and chondrogenesis are conducted in a medium with high glucose content, while the adipogenic induction medium of MSCs within the bone marrow contains low glucose content. MSCs also express antimicrobial activities for Gram-positive and Gram-negative bacteria. Thus, their culture should be avoided in the presence of antibiotics.

The most commonly used proteinaceous medium additive is a fetal bovine serum (FBS) due to its low cost. Innumerous studies use 10% FBS, but in some cases, the use of 20% FBS has been reported. Another source of proteinaceous medium additive is the preparations of human platelet lysates. PL preparation accumulates and releases growth factors like TGF-β, IGF-1, EGF, and PDGF, supporting the growth of MSCs (Schar et al., 2015).

In 2017, Baghaei and colleagues cultured bone marrow-derived mesenchymal stem cells on tissue-treated culture plates in DMEM medium supplemented with 10% FBS and penicillin, after the plates were incubated for 48h under specific conditions such as 37°C and 5% carbon dioxide. They used PBS to remove the non-adherent cells.

Cytochemical methods are generally used to verify if the isolated MCSs could differentiate, as mentioned in chapter 4.1. Alizarin staining confirms the formation of calcium oxalates on the differentiated MSCs, which indicates osteogenetic differentiation. Intracellular lipid droplet staining with oil red- O demonstrates the adipogenesis of BMMSCs, while Alcian blue stain is used to visualize the presence of chondrocytes.

7.3 FLOW CYTOMETRIC ANALYSIS

Flow cytometry is a laboratory technique used to identify and measure specific physical and chemical features of different cell populations. The flow cytometric analysis gives information based on physical features and markers found on the cell surface. These features are unique to each cell type (Bartůnková and Paulík, 2011).

According to the International Society for Cellular Therapy, one of the minimal criteria to identify MSCs is their positive or negative expression of specific cell markers (Dominici et al., 2006). Flow cytometry accesses the immune profile of MSCs, demonstrating that these cells

positively express surface markers such as CD73, CD90, and CD105 while being negative for CD34, CD45.

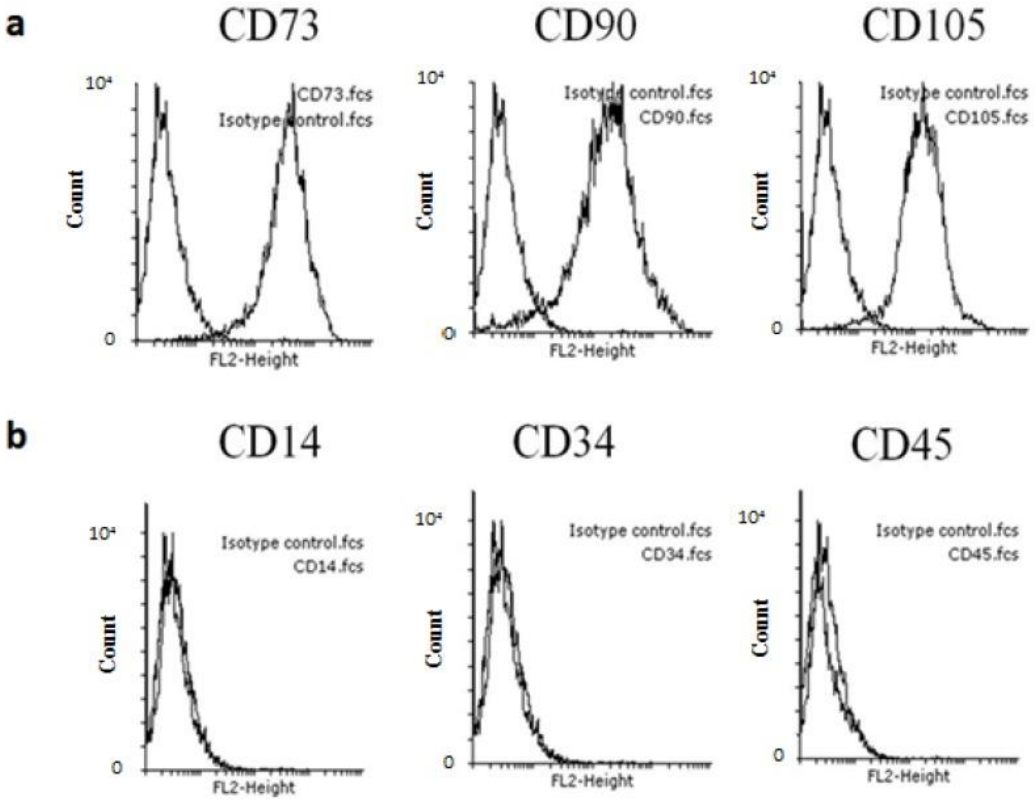

Figure 14: Flow cytometric analysis of BMMSCs (Baghaei et al.,2017): *a) positive markers; b) negative markers.*

As mentioned throughout this thesis, for the past decades MSCs have been one of the most subject matter, worldwide in numerous fields concerning medicine. MSCs' ability to interact with the immune system and effectively modulate the immune response constitutes one of the most propitious ways to treat the Graft-Versus-Host-Disease (GVHD). This disease occurs post allogeneic hematopoietic cell transplantation when the donor T cells attack the recipient cells, producing pro-inflammatory cytokines such as TNF-α and later leading to the effector T cells demolishing tissues found in the liver, lungs, and skin (Socié and Blazar, 2009).

Bone marrow mesenchymal stem cells can be very useful to control this disease, considering that they can suppress the production of pro-inflammatory cytokines, regulate the T cells response and also upregulate the production of IL-4 (Ren et al., 2008; Socié and Blazar, 2009). Not only can BMMSCs be used to treat the Graft-Versus-Host-Disease, but they are also

extremely beneficial to the engraftment of HSCs when co-transplanted with the same. The immune properties of MSCs have also been a successful tool for the treatment of many other diseases like multiple sclerosis, type 2 diabetes, and Crohn's disease (Lysak et al., 2015).

8. CONCLUSION

In this bachelor thesis, I deal with the known information about bone marrow mesenchymal stem cells. I mainly focused on their description, properties, development, role in hematopoiesis, as well as their potential use in *in vitro* culture. MSCs secrete a large spectrum of cytokines and growth factors, which affect the microenvironment of the surrounding cells. Co- cultivating HSCs with MSCs under specific *in vitro* condition affect the proliferation and differentiation potential of HSCs, thereby influencing their biological functions.

For the past decades, bone marrow mesenchymal stem cells are considered one of the most effective treatment approaches for several disorders such as musculoskeletal, cardiovascular, autoimmune diseases, and neurological diseases, making them one of the most innovative tools in the field of regenerative medicine. BMMSCs have the unique ability to repair, replace, or even generate new cells from different types of tissues within the human body.

Even though the bone marrow is the standard gold source of mesenchymal stem cells, MSCs found within the bone marrow only represent 0,001-0,01% of the entire BM. Thus, it is challenging to calculate the exact amount isolated from the bone marrow aspirate. Another disadvantage of these cells is that the amount and quality of MSCs isolated from the bone marrow decline with advancing age. Therefore, I would suggest considering adipose tissue-derived MSCs as a source. After all, they are easily isolated from less invasive methods.

Bone marrow mesenchymal stem cells' primary function is to support the proliferation and differentiation of their surrounding cells. Knowing how BMMSCs can affect or influence other cells is crucial for developing an extensive range of treatments to approach several diseases such as Graft-Versus-Host-Disease and Crohn's disease. In general, the use of MSCs in cell therapy is a promising approach to treating numerous diseases and significantly improving the quality of life.

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