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Collage of Biotechnology

Transplantation of rat bone marrow derived mesenchymal stem cells into severed induced burns to accelerate the healing process

A graduation project

Submitted to council of the department of Molecular and Medical Biotechnology / collage of Biotechnology / Al-Nahrain University as partial fulfillment of the requirements for the Degree of B.Sc in Molecular and Medical Biotechnology

Submitted by

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1442

Supervisor Certification

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Date: / / 2021

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Date: 9/06/2021

Committee Certification

We, the Examining Committee, certify that we read this Graduation project **"Transplantation of rat bone marrow derived mesenchymal stem cells into severed induced burns to accelerate the healing process"** and have examined the student **"Alyaa Hussain Tarad"** in its contents and that, in our opinion; it is accepted for the Degree of B.SC in Biotechnology.

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Date:

Signature:
Name:
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Dedications

The study locomotive has gone through many obstacles, yet I tried to surmount them steadily, with the grace of Allah, in order to reach this place.

I would love to dedicate this research to everyone who;

The evening spirit, light of moon, life bloom, earth paradise and my happiness, my soul "mother".

All the kindness of life in his looks, every laughter from him blossomed in my heart “father”

To my "brothers", which they are security that is not tainted by fear, support that does not tilt or vibrate, and love that does not disappoint.

My "little princess", she is a piece of me, without it everything loses its color.

Thanks to those who leave us happy things that make us smile when life seems bleak.

I’m proud that Dr. Zahara was my supervisor at one days in this graduate research, where she was and still a cloud raining with constant giving.

Alyaa

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We also extend our sincere thanks to the Biotechnology Research Center for their kind treatment and the persons who are working in the center for all the information and facilities which they are provided to me for the success of our research topic.

How did I get so lucky to have a friend like you. Thank you.

To my bestfriend whom I consider my sister, that my mother did not give birth to

Alyaa

Summary

Stem cells are typically found in multicellular living beings and have the one of a kind individual with respect to self-regeneration. They go through mitotic cell division and can distinguish one-self into various cell types., stem cells can take shape cells of every one of the three germ layers like; mesoderm, endoderm, and the ectoderm. Stem cells must have two primary attributes; stem cells must have the efficiency of unrestrained self-regeneration to reproduction offspring carefully equivalent to the beginning cell. Wound burns are known as; by how profound they are and how enormous zone they cover it. An enormous burn injury is probably going to incorporate burned region of various profundities. Burns destroy the skin's defensive barrier, which means microbes (bacteria) and other external invaders can penetrate it easy. Infections cannot only hold in the harmed territory, yet in additionally other organs like the lungs (pneumonia) and bloodstream (sepsis), whereas they are possibly deadly in some case. So in this research, we discuss the possibility of treating burn wounds through mesenchymal Stem cells which are taken from the bone marrow in the thigh of rat. Stem cells were used to speed up the process of treating burns. A rat was used as a model for the experiment. Researchers basically worked with two sorts of Stem cells from animals and humans, Embryonic stem cells, and Non-embryonic "somatic" or "adult" stem cells. Mesenchymal stem cells are an illustration of tissue or 'adult' stem cells. They are 'multipotent', which means they can deliver more than one kind of specialized cell of the body, however not all various types of mesenchymal stem cells can make a various sorts of cells belonging to our skeletal tissues, like cartilage, bone and fat. Scientists are examining how mesenchymal stem cells may be used to treat bone and cartilage sicknesses. Mesenchymal stem cells were at first described as adherent cells with a fibroblast-like appearance that can differentiate into osteocytes, chondrocytes, adipocytes, tenocytes and myocytes. There has been much progress in understanding Mesenchymal stem cells over the years, and there is a strong foundation for

future scientific research and clinical applications, but also some important questions remain to be answered. For instance, they can distinguish − or specialize − into cartilage cells (chondrocytes), bone cells (osteoblasts) and fat cells (adipocytes). Skin is the soft outer tissue which covers vertebrates. It protects our internal organs from the environment using a multi-layered system of cushioning and a cellular barrier. The skin is comprised of three main layers The epidermis, the dermis and The hypodermis. A burn is an injury to the skin or other organic tissue primarily caused by heat or due to radiation, radioactivity, electricity, friction or contact with chemicals. Burn depth is generally categorized as first, second, or third degree. The treatment of burns depends on the depth, area, and location of the burn, Gunshot injuries, explosions, chemical exposures, nuclear heat, and any other agent of warfare can cause severe damage to the skin. Depending on the type and extent of an injury if these injuries occur only in three layers of the skin the body regenerates itself through h the process of wound healing, a dynamic process that involves SCs, progenitor s, parenchymal cells, extra cellular matrix, blood cells and soluble mediators in three phases: inflammation, proliferation and remodeling this is happened in first and second degree of burns, third degree burns are considered full thickness and are the most difficult to treat. These require the regeneration of the entire skin structure. Apart from structural regeneration, restoring the function of the original skin is critical. Multiple preclinical models have demonstrated the efficacy of mesenchymal stem cells based treatments for promoting the repair and regeneration of thermal burns and radiation exposure. These studies have reported more expedient wound closure, decreased incidence of infection, increased vasculogenesis, increased elasticity and reduced scar formation.

List of Contents

List of Table

List of Figures

List of Scheme

List of Abbreviation

Abbreviation	Meaning
SCs	Stem cells
MSCs	Mesenchymal stem cells
ESCs	embryonic stem cells
US FDA	Food and drug administration
iPS cells	induced pluripotent stem cells
SSG	split skin graft
AFIRM	US armed forces institute of regenerative medicine
NIH	National institutes of health
USAISR	US army institute of surgical research
ESS	Engineered skin substitute
DMEM	Dulbecco`s Minimum Essential Media
PBS	Phosphate buffer saline
DW	Deionized water
FBS	Fetal bovine serum
BM-MSCs	Bone marrow mesenchymal stem cells

Chapter One Introduction and Literature Review

1.1. Introduction

Stem cells (SCs) are typically found in multicellular living beings and have the one of a kind individual with respect to self-regeneration. They go through mitotic cell division and can distinguish one-self into various cell types. During early stage up growth (i.e. embryonic development), stem cells can take shape cells of every one of the three germ layers like; mesoderm, endoderm, and the ectoderm (Donnelly *etal.*, 2018; Hall, 2018).

SCs must have two primary attributes; SCs must have the efficiency of unrestrained self-regeneration to reproduction offspring carefully equivalent to the beginning cell. This characteristic is likewise for malignant growth cells (cancer cells) which split in an uncontrolled way though undifferentiated, whereas in SCs division is very highlyregulation (Teng *et al.*, 2018; Capp, 2019). However, it is imperative to take note of the extra prerequisite for SCs; they must be having ability to give rise of a specific cell kind which turns out to be important for the healthy animal (Lee *et al.*, 2020).

SCs play a major role in advanced fields of regenerative medicine and other research areas. They are involved in the regeneration of damaged tissue or cells, due to their self-renewal characteristics (Reddy *et al.*, 2018). Tissue or cells can be damaged through a variety of diseases, including hematologic and nonhematologic malignancies. In regard to this, SC transplantation is a cellular therapeutic approach to restore those impaired cells, tissue, or organs. SCs have a therapeutic potential in the application of SC transplantation (Samadi *et al.*, 2020).

Also, in sickle cell anemia; is an inherited blood disorder caused by a genetic defect that alters the structure of hemoglobin, the oxygen-carrying protein found in red blood cells. The defective hemoglobin (also called hemoglobin S) causes red blood cells to become stiff, sticky, and

sickle-shaped (Azar and Wong, 2017). These deformed cells can block blood flow, causing symptoms ranging from severe pain to organ damage and stroke. SC transplant, or replacing the stem (precursor) cells that give rise to red blood cells, is the only treatment currently available to cure the disorder. Stem cells can be obtained from a donor's bone marrow (the site within the bones where SCs are produced), peripheral blood (blood in the veins), or umbilical cord (the cord that transports oxygen and nutrients from a mother to her baby) at the time of birth (Fitzhugh and Walters, 2017).

In addition, Mesenchymal stem cells (MSCs) are now known to display not only SC multipotency, but also robust antiinflammatory and regenerative properties. After widespread in-vitro and in-vivo preclinical testing, autologous and allogeneic MSCs have been applied in a range of immune mediated conditions, including graft versus host disease, Crohn's disease, multiple sclerosis, refractory systemic lupus erythematosus and systemic sclerosis (Riordan *et al.*, 2019).

Current data suggests that MSCs may not only replace diseased tissues, but also exert several trophic, regenerative and antiinflammatory effects. While the clinical outcome in case reports and phase I-II trials seems occasionally striking, these limited results point to the need to perform controlled multicenter trials. Future advances from stem cell science can be expected to pinpoint significant MSC subpopulations and/or stem cell markers for improved regenerative or immunoregulatory properties (Redondo-Castro *et al.*, 2017).

Wound burns are known as; by how profound they are and how enormous zone they cover it. An enormous burn injury is probably going to incorporate burned region of various profundities (Johnson, 2018; Abdel-Sayed *et al.*, 2019).

Deep burns remedy all the more gradually, are more hard to treat when it's deeper, also they are more inclined to complication like infections and scarring. Very deep burns are the most perilous of all and may require removal (cutoff, in severe case) (Brownson and Gibran, 2018). Infection is likewise a significant concern. Burns destroy the skin's defensive barrier, which means microbes (bacteria) and other external invaders can penetrate it easy (Yan *et al.*, 2020).

Burns in additionally debilitate the immune system, so the body is less ready to ward off the dangers (Jafaryparvar *et al.*, 2018). Infections cannot only hold in the harmed territory, yet in additionally other organs like the lungs (pneumonia) and bloodstream (sepsis), whereas they are possibly deadly in some case (Shao *et al.*, 2020).

So in this research, we discuss the possibility of treating burn wounds through mesenchymal SCs which are taken from the bone marrow in the thigh of rat.

1.2. Aim of study

The ability to use bone marrow (MSCs) to treat some establish burns in rat model.

1.3. Stem Cells

1.3.1. Timeline of SCs

In 1956, First successfully bone marrow implant between a donor and recipient is performed by Dr. E Thomas in New York. The patient, who has leukemia, is given radiotherapy and afterward treated with solid bone marrow from an identical twin.

In 1960, Researchers find out bone marrow contains at least two kinds of SCs – blood or haematopoietic SCs that structure every one of the kinds of platelets in the body and stromal SCs that structure bone, ligament, fat, and connective tissue. Additionally, first examination report to demonstrate that the cerebrum may generate new nerve cells is distributed, yet not generally acknowledged.

In 1968, First bone marrow transplant for non-malignancy treatment. Dr. Robert Good uses a bone marrow transplant to treat an eight-year-old kid with SCID. The donor is a HLA-matched sister. Also, Robert Edwards and his student, Barry Bavister, became the first to fertilize a human egg in the test tube. This is the start of in vitro fertilization (IVF) technologies.

In 1973, First bone marrow transplant unrelated patients. A five-year-old patient in New York with SCID is treated with multiple infusions of bone marrow from a giver in Denmark.

In 1978, The first IVF baby is born in England and, Blood stem cells are discovered in human umbilical cord blood.

In 1981, Mouse embryonic stem cells are derived for the first time from the inner cell mass of a mouse blastocyst and grown in vitro.

In the period 1984 – 1998, Pluripotent stem cells are isolated. When exposed to retinoic acid, these cells differentiate into neuron-like cells and other cell type.

In 1990, Bone marrow donor programme initiated. Dr. Thomas receives the Nobel Prize in Physiology or Medicine for his pioneering work

on bone marrow transplants.

In 1995, Scientists at the University of Wisconsin derive the first embryonic stem cells from non-human primates.

In 1998, Scientists at the University of Wisconsin, led by James Thompson, isolate and grow the first stem cells from human embryos. The embryos used in these studies were created by IVF.

In 1999, Researchers discover that stem cells can be made to differentiate into different cell types.

In 2001, President George W Bush permits federal funding of ESC research, but only on the 64 existing stem cell lines.

In 2004, Researchers in South Korea claim to be the first to clone a human embryo and then harvest the SCs for research. The research is later found to have been fabricated. California becomes the first state in the USA to provide its own fund for embryonic stem cell research.

In 2005, George W Bush’s restrictions on ESC research are loosened.

In 2009, President Barack Obama reverses George W Bush's executive order and issues a replacement order removing barriers for research.

In 2010, The United States Food and Drug Administration (US FDA) gives approval to test human ESC treatments for degenerative eye disease.

In 2013, The world's first SCs burger, grown from cow muscle cells, is cooked and eaten. The 142g patty took 3 months to create.

In 2014, US and Japanese researchers discover that "any cell can be potentially rewound to a pre-embryonic state" using a short, simple technique.

In 2015, The cost of a lab-grown burger drops from USD$325,000 per patty to a more reasonable USD$11.36. In addition, Japanese scientists grow and transplant a functioning kidney into a living organism.

In 2016, Lab-grown mouse eggs result in 11 apparently healthy live

births. The research began with more than 300 embryos.

1.3.2.Embryonic SCs and Non-embryonic "somatic" or "adult" SCs

Until newly, researchers basically worked with two sorts of SCs from animals and humans (Saini *et al.*, 2020);

Scientists find approaches to get embryonic SCs (ESCs) from early mouse embryos almost 40 years ago. SCs are the cellular putty from which all tissues of the body are made Since the time human ESCs were first mature in the lab, researchers have vision for utilizing them to fix harmed tissue or make new organs, yet such medical uses have additionally pulled in discussion. Recently, the capability of SCs to revolt in medicine got an enormous boost with information on a super flexible sort of SCs from adult mouse cells utilizing an astoundingly straightforward strategy. This course of events shows you the SCs improvement approaches (Mishra, 2018; Poulos, 2018).

Adult SCs, are undifferentiated cells found living within specific differentiated tissues in our bodies that can renew themselves or generate new cells that can replenish dead or damaged tissue. You may also see the term “somatic stem cell” used to refer to adult stem cells. The term “somatic” refers to non-reproductive cells in the body (eggs or sperm). Somatic SCs are typically scarce in native tissues which have rendered them difficult to study and extract for research purposes (Mobley, 2019).

Resident in most tissues of the human body, discrete populations of Adult SCs generate cells to replace those that are lost through normal repair, disease, or injury. Adult SCs are found throughout one’s lifetime in tissues such as the umbilical cord, placenta, bone marrow, muscle, brain, fat tissue, skin, gut, etc. The first Adult SCs were extracted and used for blood production in 1948. This procedure was expanded in 1968 when the first adult bone marrow cells were used in clinical therapies for

blood disease (Alhattab *et al.*, 2019).

Studies proving the specificity of developing Adult SCs are controversial; some showing that Adult SCs can only generate the cell types of their resident tissue whereas others have shown that Adult SCs may be able to generate other tissue types than those they reside in. More studies are necessary to confirm the dispute (Leberfinger *et al.*, 2017).

Types of Adult Stem Cells (Gurusamy *et al.*, 2018):

- Hematopoietic SCs (Blood Stem Cells).
- Mesenchymal Stem Cells.
- Neural Stem Cells.
- Epithelial Stem Cells.
- Skin Stem Cells.

1.4. Classification of SCs

1.4.1. Classification of stem cells on the basis of potency

SCs can be classified by the extent to which they can differentiate into different cell types (Barky *et al.*, 2017). As show in scheme (1-1).

* Totipotent;

The capacity to differentiate into all possible cell types. Such as, are the zygote formed at egg fertilization and the first few cells that outcome from the division of the zygote (Boiani *et al.*, 2019). Figure (1-1).

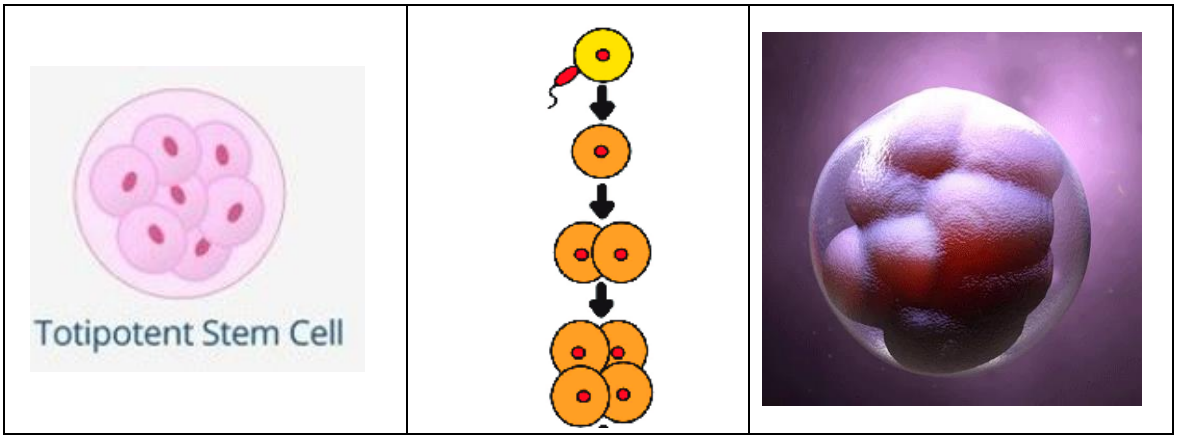


Figure (1-1) Totipotent SCs

* Pluripotent;

The capacity to differentiate into nearly all cell types. Examples include ESCs and cells that are derived from the mesoderm, endoderm, and ectoderm germ layers that are formed in the early stage of ESC differentiation (Baillie-Benson *et al.*, 2020). Figure (1-2).

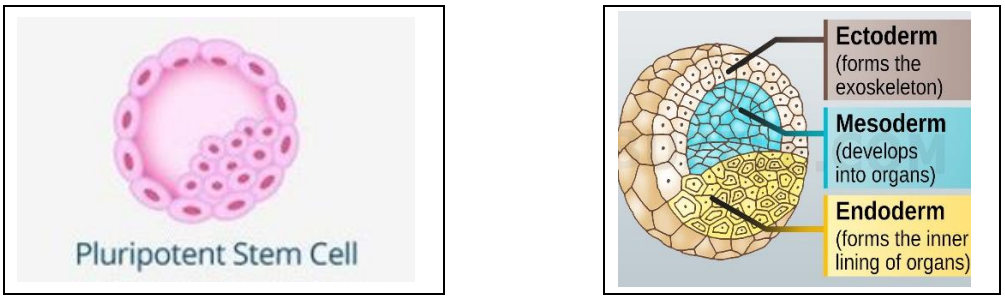


Figure (1-2) Pluripotent SCs

Germ layer, any of three primary cell layers, formed in the earliest stages of embryonic development, consisting of the endoderm (inner layer), the ectoderm (outer layer), and the mesoderm (middle layer) (Technau, 2020). The germ layers form during the process of gastrulation, when the hollow ball of cells that constitutes the blastula begins to differentiate into more-specialized cells that become layered across the developing embryo (Randolph *et al.*, 2018). The germ layers represent some of the first lineage-specific (multipotent) SCs (e.g., cells destined to contribute to specific types of tissue, such as muscle or blood) in embryonic development figure (1-3). Hence, each germ layer eventually gives rise to certain tissue types in the body (Saba and Balwan, 2021).

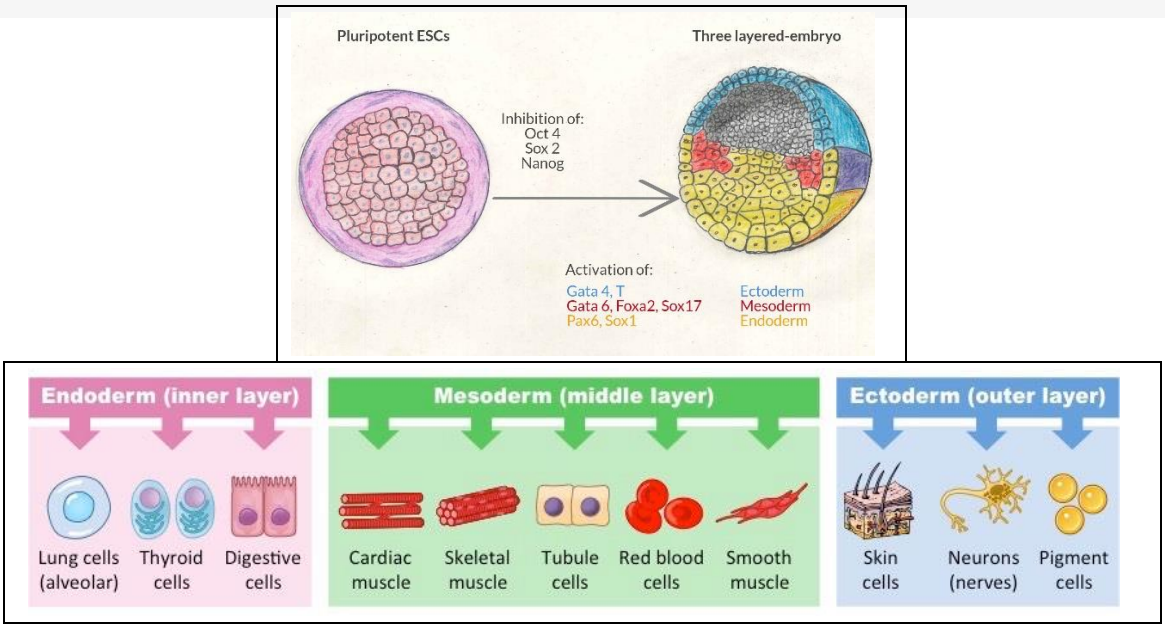


Figure (1-3) Pluripotent SCs differentiate to the three germ

Because the germ layers can differentiate into a vast variety of organs and tissues, they are of particular interest to the study of human development and to SC research (Corsini *et al.*, 2018). A pluripotent SC is one that can become any of the three germ layers. The multipotent SCs that then constitute the germ layers give rise to specific tissue lineages (e.g., a specific dermal layer or even one lineage within a dermal layer) (Ferretti and Hadjantonakis, 2019). The study of SCs and cell differentiation has enabled scientists to reliably produce specific types of cells from human ESCs as well as from induced pluripotent SCs (genetically reprogrammed adult cells), which has furthered knowledge of embryonic development and facilitated the development of novel cell-based therapies (Coll *et al.*, 2018).

* Multipotent;

The ability to differentiate into a closely related family of cells. Examples include hematopoietic (adult) SCs that can become red and white blood cells or platelets (Grinenko *et al.*, 2018). Figure (1-4).

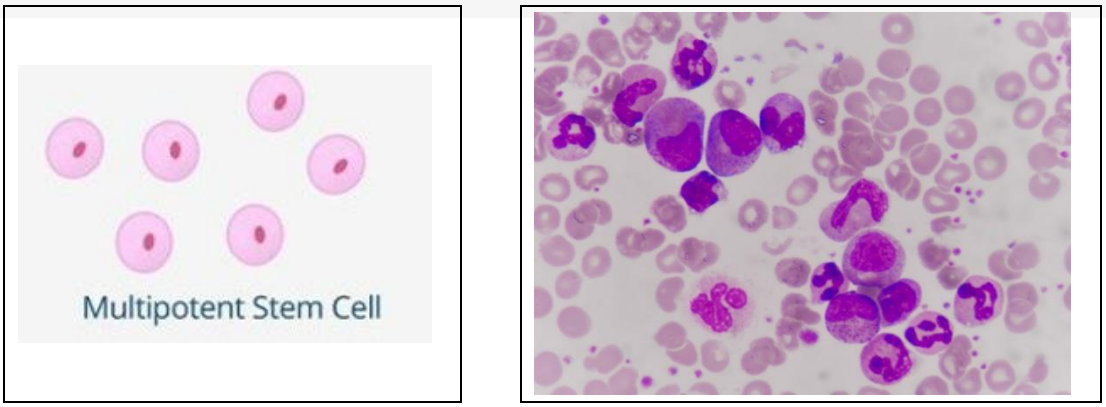


Figure (1-4) Multipotent SCs

* Oligopotent;

The ability to differentiate into a few cells. Examples include (adult) lymphoid or myeloid SCs (Gandhi *et al.*, 2018). Figure (1-5).

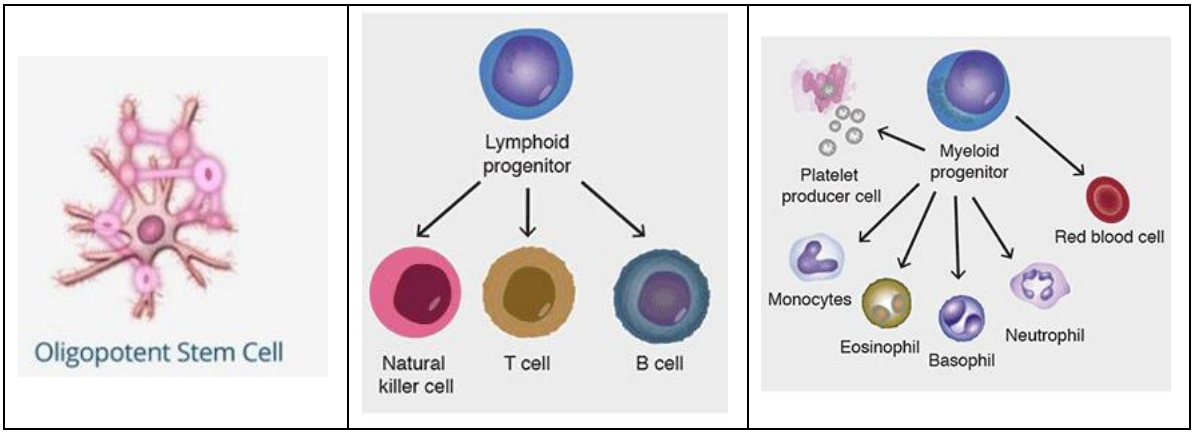


Figure (1-5) Oligopotent SCs

* Unipotent;

The ability to only produce cells of their own type, but have the property of self-renewal required to be labeled a stem cell. Examples include (adult) muscle SCs (Clevers and Watt, 2018). Figure (1-6).

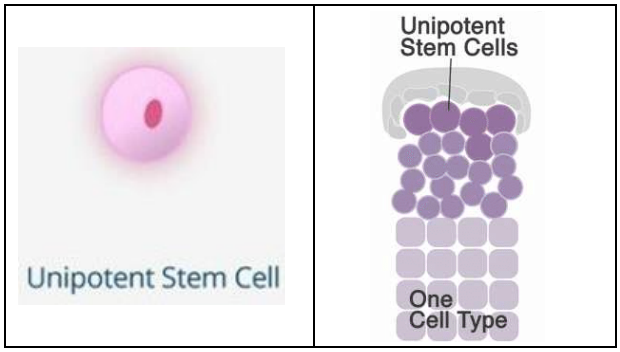


Figure (1-6) Unipotent SCs

1.4.2. Classification of stem cells on the basis of their sources

The easiest way to categorize stem cells is by dividing them into two types: Early or embryonic and mature or adult (Gandhi *et al.*, 2018). Early SCs, often called ESCs, are found in the inner cell mass of a blastocyst after approximately five days of development (Hassani *et al.*, 2019). Mature SCs are found in specific mature body tissues as well as the umbilical cord and placenta after birth (Thomi *et al.*, 2019). As show in scheme (1-1).

* ESCs

ESCs are self-replicating pluripotent cells that are potentially immortal. They are derived from embryos at a developmental stage before the time of implantation would normally occur in the uterus (Yang *et al.*, 2019). The embryos from which human ESCs are derived are typically four or five days old and are a hollow microscopic ball of cells called the blastocyst (Jagiri *et al.*, 2019). Figure (1-7).

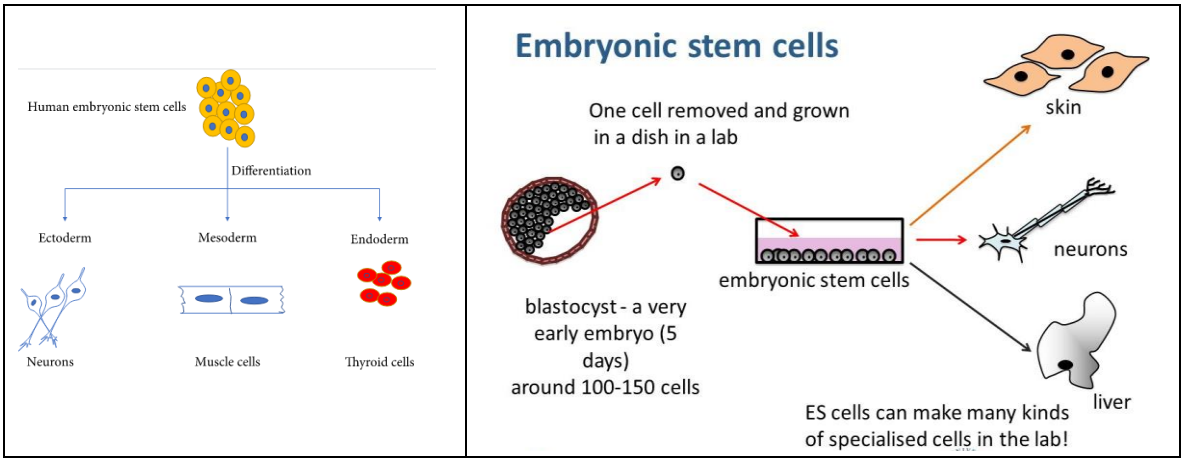


Figure (1-7) Embryonic Stem Cells

* Adult SCs

Adult SCs are undifferentiated totipotent or multipotent cells, found throughout the body after embryonic development, that multiply by cell division to replenish dying cells and regenerate damaged tissues. The primary roles of adult SCs in a living organism are to maintain and repair the tissue in which they are found. Unlike ESCs, which are defined by their

origin (the inner cell mass of the blastocyst), the origin of adult SCs in some mature tissues is still under investigation (Clevers and Watt, 2018). Figure (1-8).

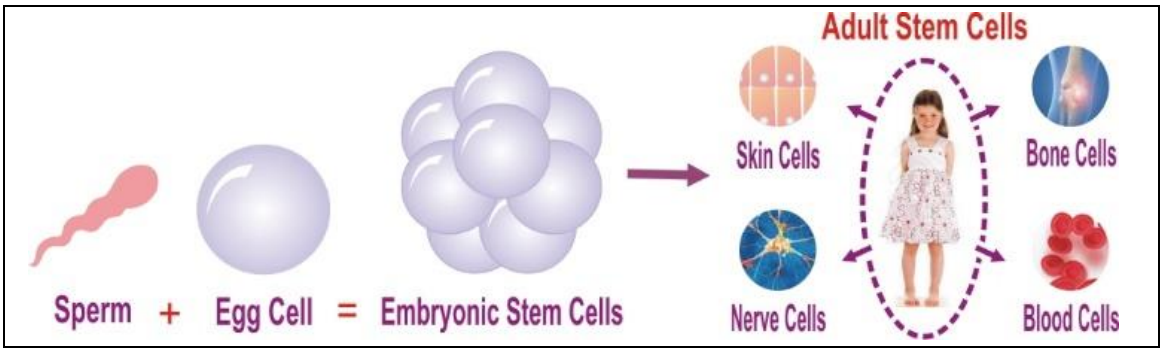


Figure (1-8) Adult Stem Cells

* Pluripotent SCs

Recently, a third type of SC, with properties similar to ESCs, has emerged. Scientists have engineered these induced pluripotent stem cells (iPS cells) by manipulating the expression of certain genes - 'reprogramming' somatic cells back to a pluripotent state (Lin *et al.*, 2017). Figure (1-9).

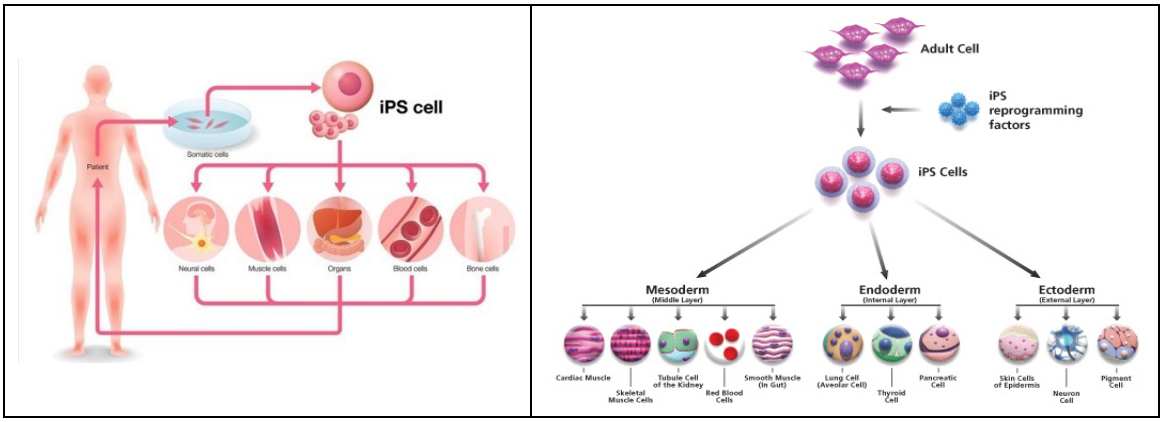
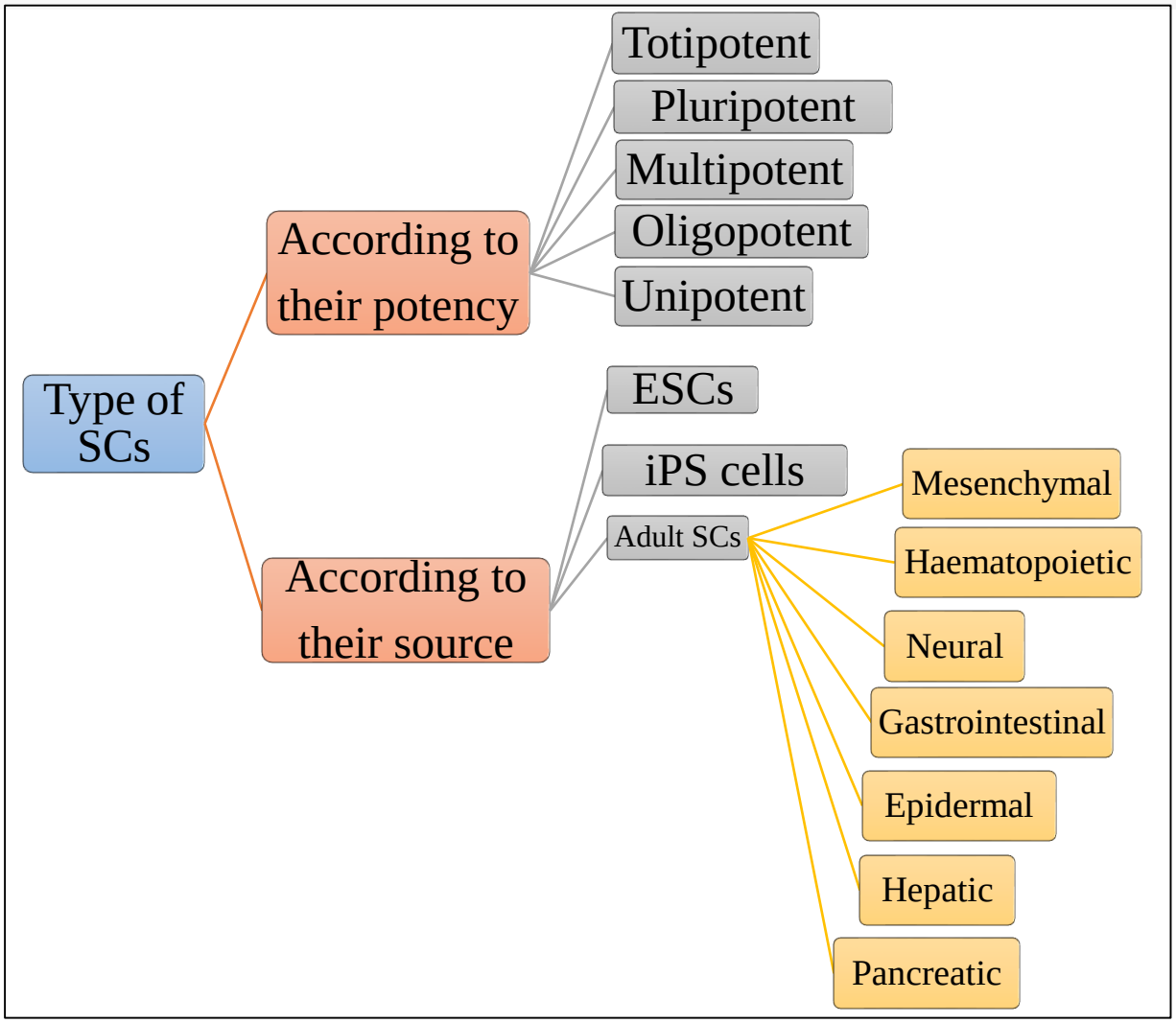


Figure (1-9) induced pluripotent stem cells (iPS cells)



Scheme (1-1) Classification of SCs

1.5. Mesenchymal stem cells (MSCs)

1.5.1. Introduction to MSCs

The terms MSCs have become the preferred acronym to describe a cell and a cell population of multipotential stem/progenitor cells commonly referred to as mesenchymal stem cells, multipotential stromal cells, mesenchymal stromal cells, and mesenchymal progenitor cells (Kozlowska *et al.*, 2019). The MSCs can differentiate to important lineages under defined conditions *in vitro* and in limited situations after implantation *in vivo* (Pavon *et al.*, 2018). MSCs were isolated and described about 30 years ago and now there are over 55,000 publications on MSCs readily available. Here, we have focused on human MSCs whenever possible (Hill *et al.*,

2019). The MSCs have broad anti-inflammatory and immune-modulatory properties (Wang, *et al.*, 2018).

At present, these provide the greatest focus of human MSCs in clinical testing; however, the properties of cultured MSCs *in vitro* suggest they can have broader applications. The medical utility of MSCs continues to be investigated in over 950 clinical trials (Simones *et al.*, 2018).

There has been much progress in understanding MSCs over the years, and there is a strong foundation for future scientific research and clinical applications, but also some important questions remain to be answered. Developing further methods to understand and unlock MSC potential through intracellular and intercellular signaling, biomedical engineering, delivery methods and patient selection should all provide substantial advancements in the coming years and greater clinical opportunities (Lee *et al.*, 2019).

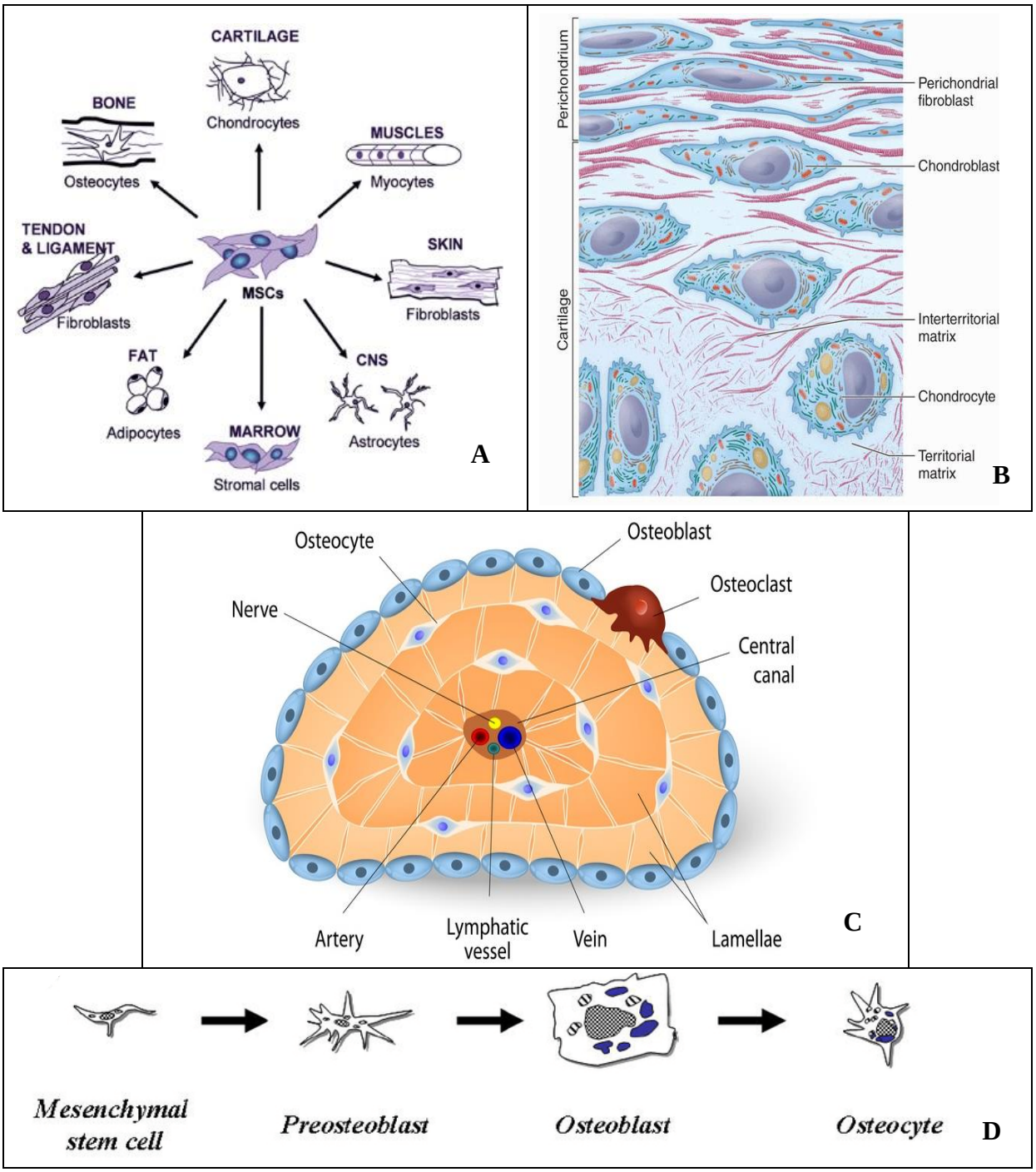
The expansive and growing field of MSC research is teaching us basic human cell biology as well as how to use this type of cell for cellular therapy in a variety of clinical settings, and while much promise is evident, careful new work is still needed (Saldanha-Araujo *et al.*, 2020).

MSCs can make a various sorts of cells belonging to our skeletal tissues, like cartilage, bone and fat. Scientists are examining how MSCs may be used to treat bone and cartilage sicknesses. Some MSC research is likewise investigating treatments for different illness (Shah *et al.*, 2018). MSCs were at first described as adherent cells with a fibroblast-like appearance that can differentiate into osteocytes, chondrocytes, adipocytes, tenocytes and myocytes (Andrzejewska *et al.*, 2019).

1.5.2. Definition of MSCs

MSCs are an illustration of tissue or 'adult' SCs. They are 'multipotent', which means they can deliver more than one kind of

specialized cell of the body, however not all various types (Hu *et al.*, 2017). MSCs make the different specialized cells found in the skeletal tissues. For instance, they can distinguish − or specialize − into cartilage cells (chondrocytes), bone cells (osteoblasts) and fat cells (adipocytes) figure (1-10). All these specific cells, each one has their own special shapes, structures and functions, and each belongs in a specific tissue (Szychlinska *et al.*, 2017).



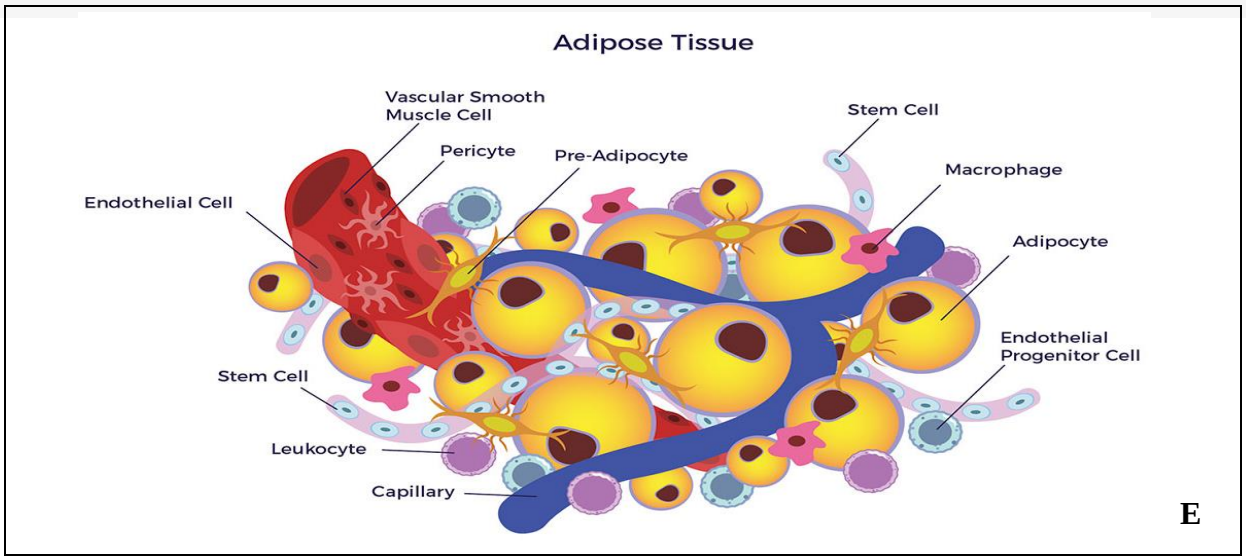


Figure (1-10) A) MSCs can distinguish − or specialize − B) cartilage cells (chondrocytes), C and D) bone cells (osteoblasts) and E) fat cells (adipocytes)

1.5.3. Source of MSCs

MSCs are not only found in bone marrow. MSCs have been isolated from multiple tissues such as skeletal muscle (Pantelic and Larkin, 2018), adipose tissue (Alonso-Goulart *et al.*, 2018), synovial membranes (Zhu, *et al.*, 2017), dental pulp (Kunimatsu *et al.*, 2018), periodontal ligaments (Zhang *et al.*, 2020), umbilical cord, amniotic fluid and placenta (Asgari *et al.*, 2017). figure (1-11).

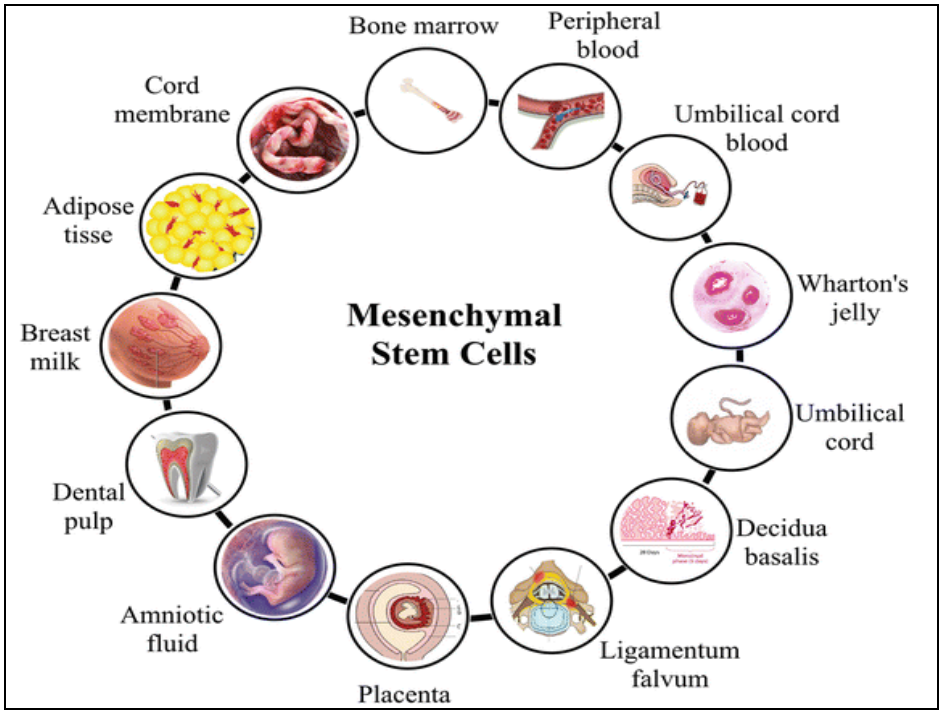


Figure (1-11) Source of MSCs

1.5.4. Properties of MSCs

MSCs have many properties, some of them are (Mushahary *et al.*, 2018; Wang *et al.*, 2018; Yang *et al.*, 2018; Lu Yang *et al.*, 2019)

1. Possess Immunomodulation and tissue repair - privileged phenotype (provide allogeneic transplantation without Immune ejection).
2. No serious side effects so far.
3. Having innate migration potential to the injury sites.
4. Having very great paracrine effects.
5. Many sources of isolation (i.e. bone marrow, placenta, adipose tissue). Possibility for autologous therapy.
6. Capability to be expanded efficiently and easily *In vitro*.
7. Immune - modulatory capacities and high immunosuppressive properties.
8. More resistant to oxidative insult (ROS).
9. No ethical or side effects problems.
10. Possess multi – lineage differentiation potential.
11. Self-proliferation and multipotent.
12. Chemotaxis and homing function.

1.6. Bone marrow

1.6.1. Introduction of bone marrow

Bone marrow have ability to produces about 200 billion new red blood cells every day, along with white blood cells and platelets. Also, bone marrow contains mesenchymal and hematopoietic stem cells (Wu *et al.*, 2018), figure (1-12)

Around 10,000 people in the US are diagnosed each year with diseases that require bone marrow transplants, and several diseases pose a threat to bone marrow and prevent bone marrow from turning stem cells into essential cells (Johnstone *et al.*, 2021).

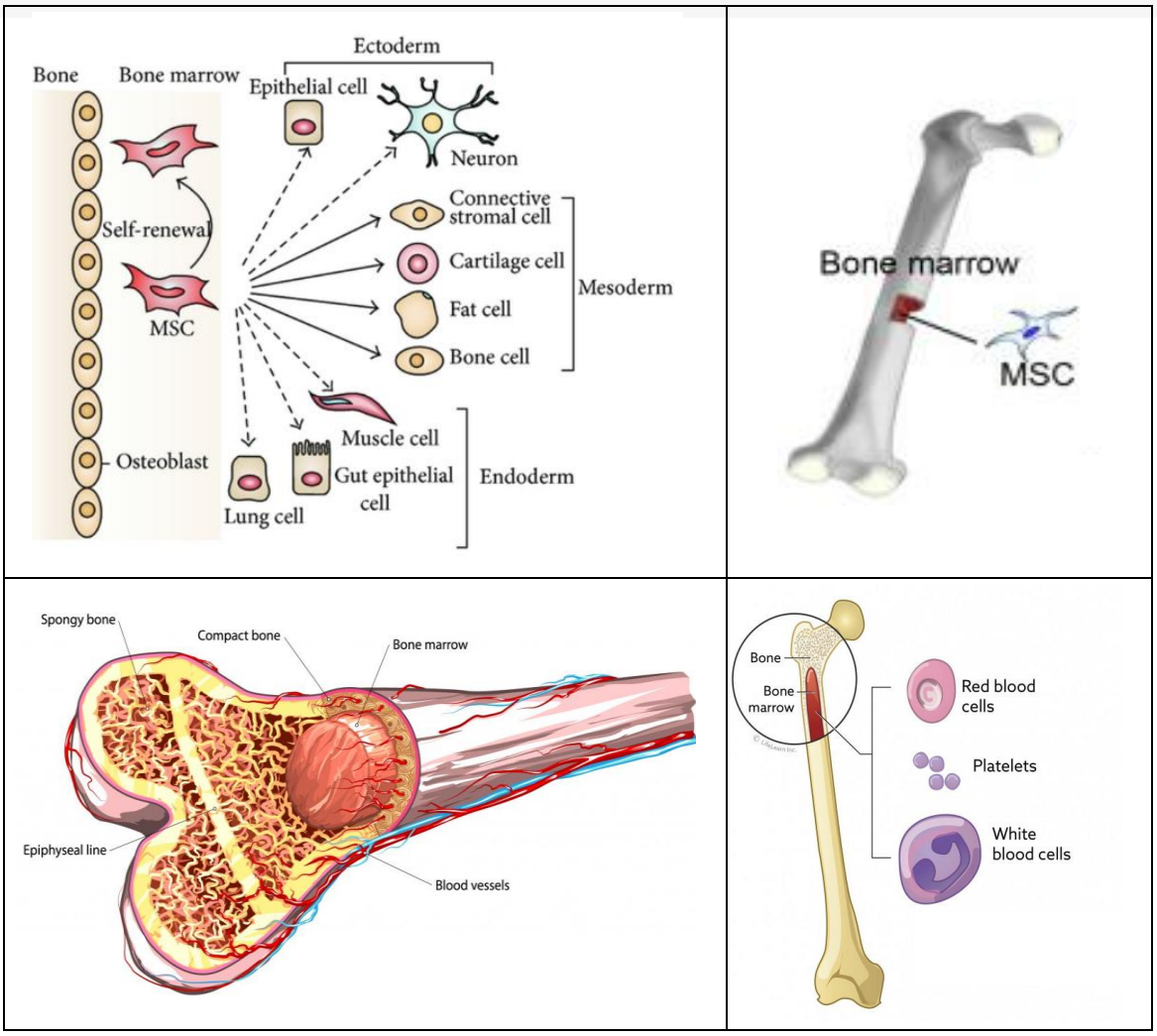


Figure (1-12) Bone marrow structure

1.6.2. Sources of stem cell transplant

Depending on the type of transplant that's being done, there are 3 possible sources of stem cells to use for transplants (Chander and Gangenahalli, 2020);

- Bone marrow (from you or someone else).
- The bloodstream (peripheral blood – from you or someone else).
- Umbilical cord blood from newborns.

In this research we talk about bone marrow. So, Bone marrow is the spongy liquid tissue in the center of some bones. It has a rich supply of stem cells, and its main job is to make blood cells that circulate in your body. The bones of the pelvis (hip) have the most marrow and contain

large numbers of stem cells. For this reason, cells from the pelvic bone are used most often for a bone marrow transplant. Enough marrow must be removed to collect a large number of healthy stem cells (Abdel Meguid *et al.*, 2018).

The bone marrow is harvested (removed) while the donor is under general anesthesia (drugs are used to put the patient into a deep sleep so they don't feel pain). A large needle is put through the skin on the lower back and into the back of the hip bone. The thick liquid marrow is pulled out through the needle. This is repeated until enough marrow has been taken out (Meyerson, *et al.*, 2019).

The harvested marrow is filtered, stored in a special solution in bags, and then frozen. When the marrow is to be used, it's thawed and then put into the patient's blood through a vein, just like a blood transfusion. The stem cells travel to the bone marrow, where they engraft or "take" and start to make blood cells. Signs of the new blood cells usually can be measured in the patient's blood tests in a few weeks (Selvi, 2017).

1.7. The skin

1.7.1. Introduction to the skin

The skin is one of the largest organs and constitutes 16% of the human body weight. It weighs around 5kgs and covers an area of about 1.8-2 m^2 (Al-Japairai *et al.*, 2020). The thickness of the skin differs all through the body. It relies upon how much use we make of that region (Turgul and Kale, 2017).

For instance, since we use our feet for moving, it is thickest on the soles of our feet. We use our hands for doing numerous everyday tasks like getting things and write, so it is likewise thick on our palms (Holowka *et al.*, 2019).

1.7.2. Definition of skin

Skin is the soft outer tissue which covers vertebrates. It protects our internal organs from the environment using a multi-layered system of cushioning and a cellular barrier (Rembiesa *et al.*, 2019).

1.7.3. Skin structure

The skin is comprised of three main layers, as show in figure (1-13) (Kim *et al.*, 2019):

* The epidermis: Its main job is to protect the body acting as a barrier and help control body temperature. It is made up of four types of cells: keratinocytes (which comprised the 90% of the epidermis), melanocytes, Langerhans cells and Merkel cells, provides a waterproof barrier and creates our skin tone, figure (1-14A).
* The dermis is much thicker than the epidermis. It contains hair roots, blood vessels, lymph vessels, glands, and nerve endings. Blood and lymph vessels in the dermis bring nutrients to the dermis and epidermis. Glands make fluids the body needs, and the connective tissue (which builds an extracellular matrix) holds all these structures in place and allows the skin to stretch, figure (1-14B).
* The hypodermis is the subcutaneous tissue, which means “below the skin”. It is mostly made of fat and connective tissue. It connects the skin to muscles and bones and it also saves body heat, stores energy, and absorbs shock to protect the body from injury, figure (1-14C).

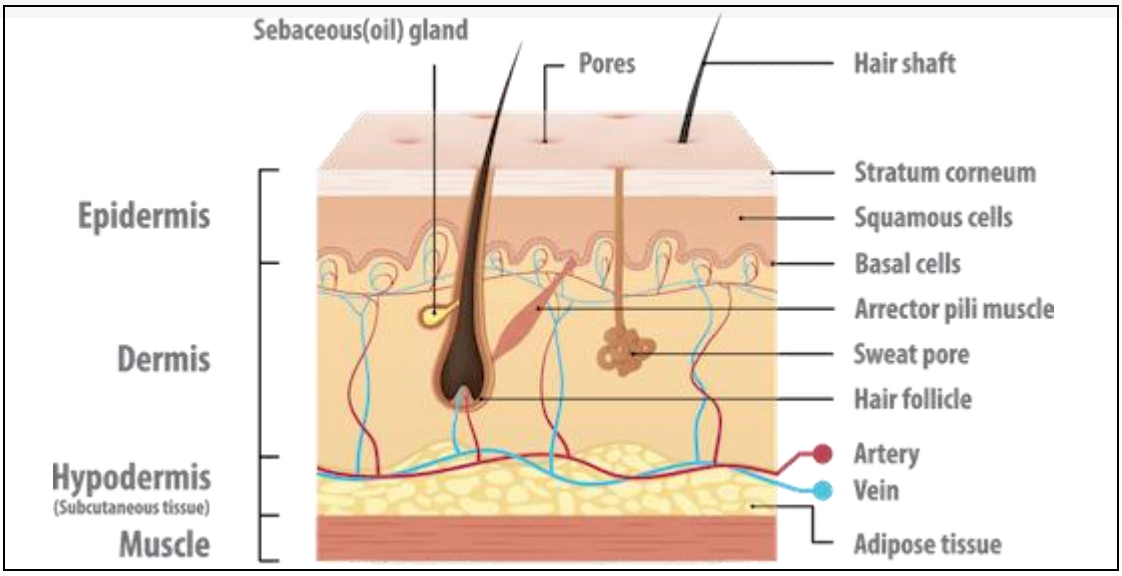


Figure (1-13) Skin structure

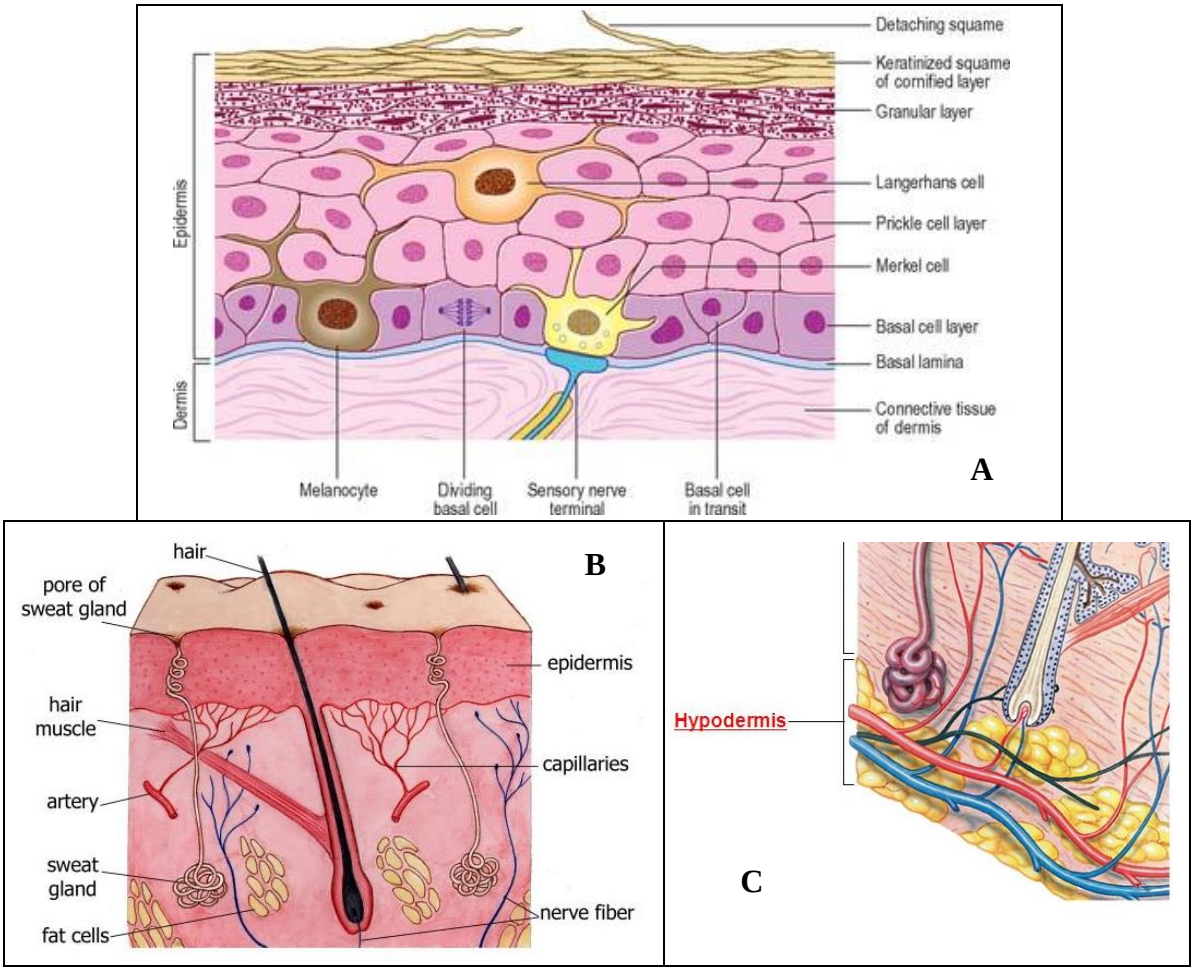


Figure (1-14) The skin is comprised of three main layers;
A) Epidermis, B) Dermis, C) Hypodermis

1.7.4. Characteristic of skin

The skin has many characteristic (Fernandes and Barreto Junior, 2017; Harding *et al.*, 2018; Malcov-Brog *et al.*, 2018).

* Thermoregulation

The skin helps us to maintain our body temperature. When we are hot, there is vasodilation (widening of blood vessels) at the skin surface. This cools us down by allowing more heat to escape. When we are cold, there is constriction (narrowing of blood vessels). This allows less heat to escape, helping conserve heat.

* Metabolism

When we are hot or exercising, sweat glands in our skin excrete water salts and proteins. Once on the surface of the skin, sweat evaporates into the air. This cools the skin and helps us control our body temperature.

* Sensation

There are many nerve endings and receptors that sense changes in the skin. This allows us to feel everyday objects, feel pain, determine hot from cold and also sense pressure.

* Protection

As the skin covers our whole body and is a continuous layer, it acts as a barrier and protects the body from injury and infection. It also shields against the sun's light and radiation and prevents us from drying up.

* Synthesis of vitamin D_3

When exposed to the sun's rays, the skin produces vit. D_3. This is essential for building strong, well-shaped bones.

1.8. The burn

1.8.1. Introduction of burn

Burn injury of the skin is describing by the damage to skin tissue from hot (scald, flash, fire, contact), electrical, chemical, sunlight, or

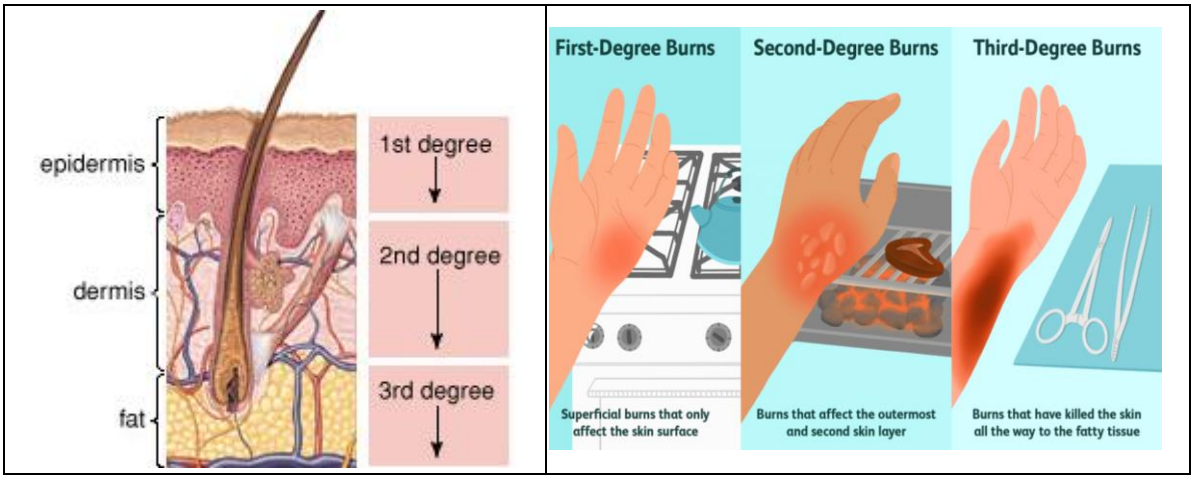
different sources (Ye and De, 2017). Burns frame one of the most possibly well-known reasons for morbidity and mortality around the world. They can bring about critical distortion, physical impairment, work loss, psychological issues, and extensive economic load (Girard *et al.*, 2017).

1.8.2. Definition of burn

A burn is an injury to the skin or other organic tissue primarily caused by heat or due to radiation, radioactivity, electricity, friction or contact with chemicals. Skin injuries due to ultraviolet radiation, radioactivity, electricity or chemicals, as well as respiratory damage resulting from smoke inhalation, are also considered to be burns (Lal and Sahu, 2017).

1.8.3. Degree of burn

Burn depth is generally categorized as first, second, or third degree. The treatment of burns depends on the depth, area, and location of the burn, as well as additional factors, such as material that may be burned onto or into the skin (de Barros *et al.*, 2017; James and Jowza, 2017) figure (1-15).



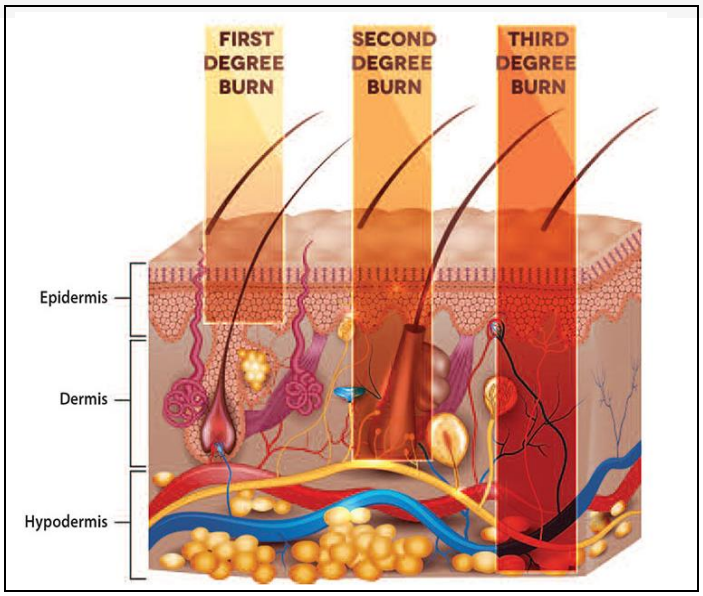


Figure (1-15) Explain the degree of burn

- First-Degree burns:

Are superficial and only involve the outer layer of skin and mild compared to other burns, figure (1-16). Signs to look for in determining a first-degree burn include;

- ❖ Red and tender skin.
- ❖ Similar to a sunburn.
- ❖ Heals over three to six days.

- Second-Degree burn:

Referred to as a "partial-thickness" burn, a second-degree burn is similar to a first-degree burn in that it also affects the outer layer of your skin. The increased severity of a second-degree burn impacts the inner layer of the skin and causes damage to that deeper layer, figure (1-16). Signs to look for from second degree burns include:

- ❖ Skin will be red and blistered.
- ❖ Severe pain is present due to damaged nerve endings.
- ❖ Healing time can range anywhere from seven to 28 days.

Recovery from a second-degree burn can be impacted by several factors:

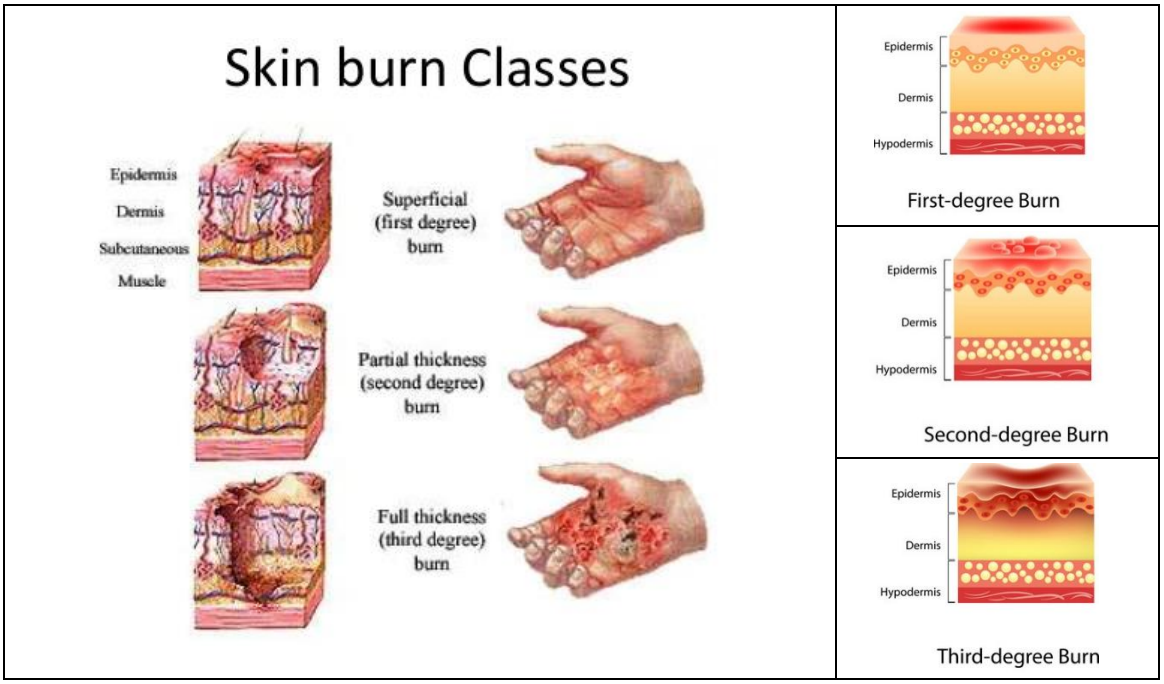
- Age. Medical History.
- Nutritional Status.
- Location of the burn.
- Size and depth of the burn.
- Complications, such as infection.

Third-Degree burn:

Referred to as a "full thickness" burn and destroys the outer and inner layer of the skin. Damage is extensive and can lead to scarring at the site of the burn, figure (1-16). Signs to look for include:

- Limited or no pain at the site of the burn due to damaged nerves.
- A whitish or charred appearance that has a tough, leathery feeling.
- The outer edges of a third-degree burn are often second-degree burns.

Healing usually requires skin grafts and you should seek immediate professional burn treatment in the event of a severe burn. Infection is highly likely with a third-degree burn if you are not careful and treatment should not be delayed.



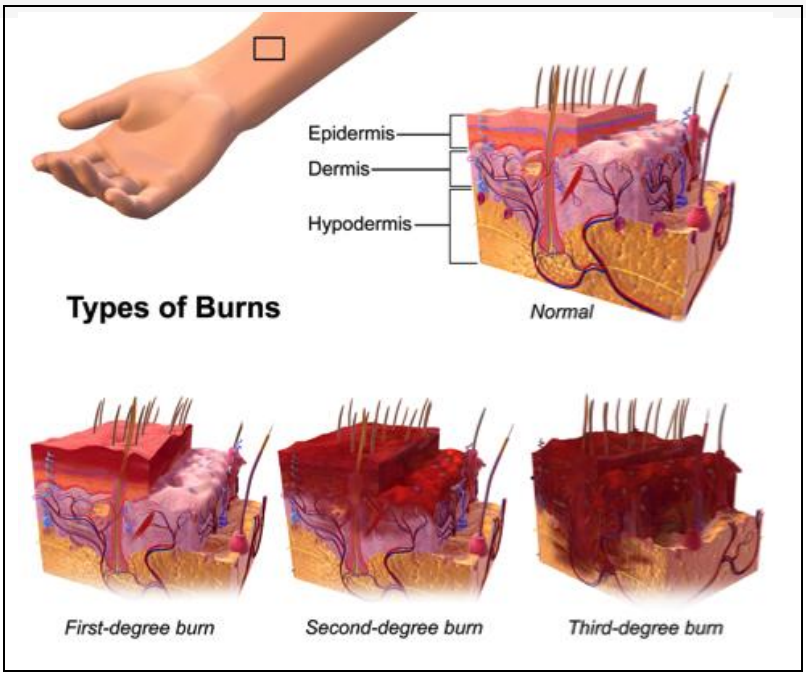


Figure (1-16) Degree of burn damage in comparing to normal skin tissue

1.8.4. Treatment of burn

In general, treatment options range from simply applying a cold pack to emergency treatment to skin grafts. Although first-degree burns are not as serious as higher-degree burns, but they can hurt quite a bit and can leave a scar if not properly treated (Csenkey *et al.*, 2019).

In first degree, it sample to treated it by 2 step; Cool Burn "Use compresses if running water isn't available until the pain subsides" and Protect Burn "Cover with sterile, non-adhesive bandage or clean cloth".

In second degree, use cream or herbal medicine to decrease the harmful.

In third degree, required immediately medicine surgical.

1.8.5. MSCs used for treated the burn

Gunshot injuries, explosions, chemical exposures, nuclear heat, and any other agent of warfare can cause severe damage to the skin. Depending on the type and extent of an injury, the body regenerates itself through the process of wound healing, a dynamic process that involves SCs, progenitor

cells, parenchymal cells, extra cellular matrix, blood cells and soluble mediators in three phases: inflammation, proliferation and remodeling (Ude *et al.*, 2018).

The above processes can occur as long as any of the three layers of skin remain. Third degree burns are considered full thickness and are the most difficult to treat. These require the regeneration of the entire skin structure. Apart from structural regeneration, restoring the function of the original skin is critical. Improved understanding of wound healing mechanisms has aided emerging tissue engineering technologies in the generation of functional skin. The current method of split skin graft (SSG), which costs over $50,000 to cover burn areas on up to 40% of an average man, suffers from several limitations, such as donor site morbidity, extended healing times and significant expense. Efforts to overcome these limitations have led scientists to explore techniques for engineering skin substitutes (Ude *et al.*, 2018).

Multiple preclinical models have demonstrated the efficacy of MSC-based treatments for promoting the repair and regeneration of thermal burns and radiation exposure. These studies have reported more expedient wound closure, decreased incidence of infection, increased vasculogenesis, increased elasticity and reduced scar formation (Frueh *et al.*, 2018).

Clinical evidence that MSCs play a natural role in human skin regeneration has been documented. In one study, the number of MSCs circulating in the peripheral blood of thermal-burn patients was quantified and compared to the number of circulating MSCs in the blood of healthy volunteers (Golchin *et al.*, 2019). It was found that the percentage of circulating MSCs in burn patients was greater than 20% compared to healthy individuals, and the degree of increase correlated with the size and severity of the burn (Churchman *et al.*, 2020).

In 2008, the US Armed Forces Institute of Regenerative Medicine (AFIRM) began to harness stem cell technology to reconstruct new body parts, including skin. Over 2250 million dollars in public and private funds were allotted for the project's first 5 years, with the National Institutes of Health (NIH) teaming up with three other public universities for a progressive developmental project. The results were encouraging (Sui *et al.*, 2018).

The US Army Institute of Surgical Research (USAISR) has also developed a bio-engineered skin substitute for the treatment of burn patients with severe, life-threatening wounds. This treatment, named Engineered Skin Substitute (ESS), uses tissues made from autologous collagen-producing cells to replace the two top layers of skin (Boyce *et al.*, 2017). ESS was developed in conjunction with the California-based biotechnology firm Amarantus BioScience and Rutgers University. The use of the patient's own cells avoids the need for foreign substitutes, which lowers the chances of infection, avoids the use of immunosuppressants and reduces the number of surgeries required. USAISR has also investigated other treatments for severe burns, including the ReCell Spray device for skin, developed by British Avita Medicals (Girard *et al.*, 2017; Mahmood *et al.*, 2019).

This technology distributes autologous healthy cells, proliferating and suspended in a physiological solution, onto wounds after the removal of dead cells. Surgeons at USAISR have also explored the science of stratigraphy, involving the layering of skin cells developed from a patient's stem cells to grow new tissues (Tarassoli *et al.*, 2018).

Chapter Two

Materials and Methods

2.1. Materials

2.1.1. Apparatus and equipment

Table (2-1): The main Apparatus and equipment were used in this study;

Apparatus	Company and origin
Autoclave	K&K – Korea
Digital camera	Memmert – Germany
Electric oven	Binder – Germany
Incubator	Binder – Germany
Inverted microscope	Olympus – Japan
pH – meter	Fisher – USA
Disposable insulin syringes (1ml)	Hunan – China
Disposable syringes (5ml)	Hunan – China
Disposable petri dishes	Sterilin LTD – England
Forceps	Hide – Germany
Eppendorf tubes	Eppendorf – Germany
Micropipettes and tips	Island – Germany
Millipore filter, size 0.20µm	Asahi – Japan
Plastic tissue culture flask ($25cm^2$)	Jiangsu – China

2.1.2. Chemicals and biological materials:

Table (2-2): The main chemicals and biological materials were used in this study;

Apparatus	Company and origin
Absolute alcohol	Hiclean – Iraq
Antibiotics (Ampicillin and streptomycin) vials	Abbott – USA
DMEM media	US-Biological – USA
Fetal Bovine Serum	Cellgro – USA
Dulbecco`s Minimum Essential Media medium	BI – USA

2.1.3. Laboratory animals

Eight of four to eight-weeks rat male (albino male) obtained from the animal house of Biotechnology Research Center (Al-Nahrain University), Baghdad, Iraq were used in this study. An average weight of rat was (220 g) were used and maintained in a plastic cages under controlled conditions of temperature (23 C°), water and food were given.

2.2. Methods

2.2.1. Preparation of solution for culture media

2.2.1.1. Antibiotics solutions:

Streptomycin: 1 g of streptomycin was dissolved in 5m1 triple distill water, and 0.5m1 of it was added to 1 litter of culture media.

- Ampicillin: 1000000 IU of ampicillin was dissolved in 5ml triple distill water, and 1ml of it was added to 1 litter of culture media.
- Amphotericin B (Freshney, 2000): it was a ready-made solution.

2.2.1.2. Phosphate Buffer saline (PBS) (pH 7.2):

This solution was prepared by dissolving the 10.8 g of PBS powder in one liter of triple deionized water (triple DW). The solution was filtered through a Nalgene filter 0.20 pm, and then stored at 4°C, prior to use PBS was warmed to 37C°

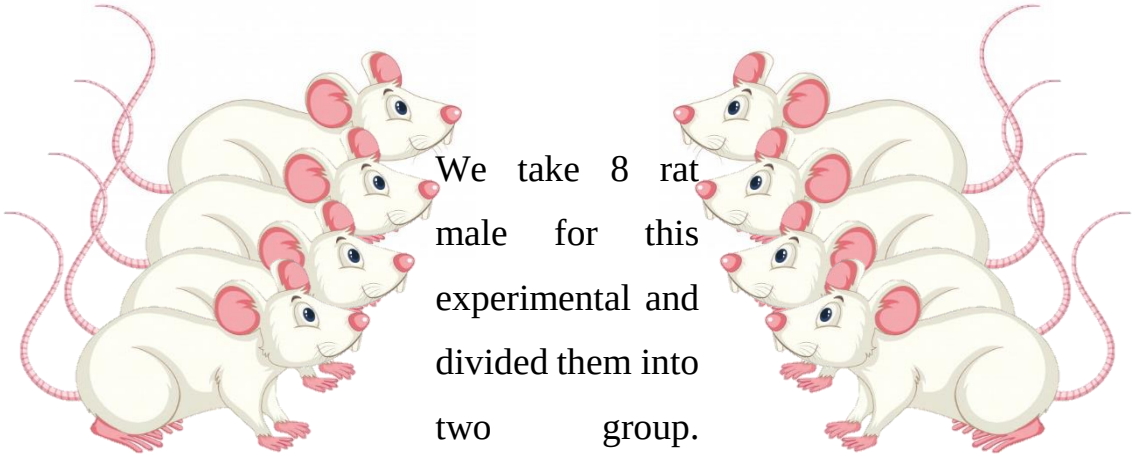
2.2.1.3. Fetal Bovine serum (FBS):

Frozen Fetal calf serum was first warmed at 45 C° in a water bath, and used for tissue culture media (Freshney, 2000).

2.2.2. Preparation of DMAM media

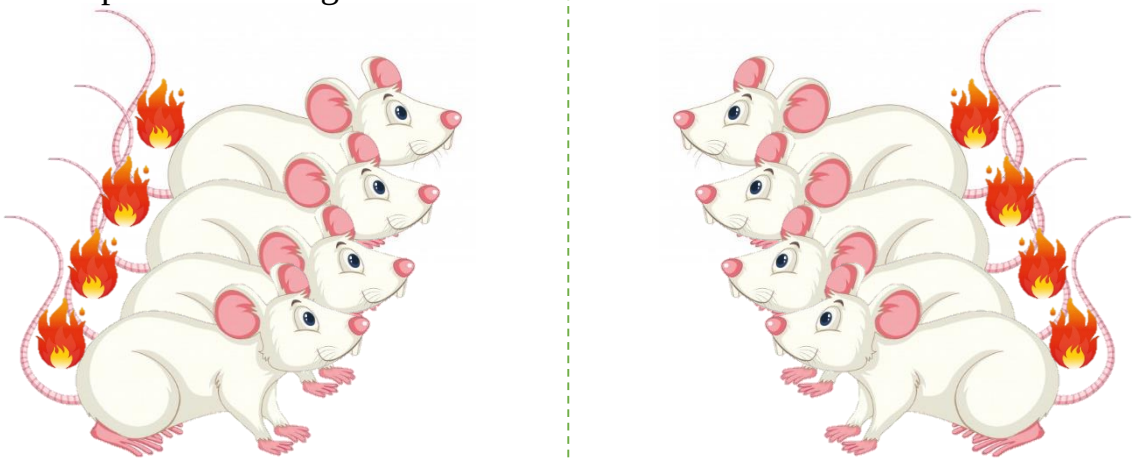
DMEM culture media was prepared mix 20ml of DMEM with 2ml FBS and add 0.1ml Antibiotics solutions.

2.2.3. Experimental design



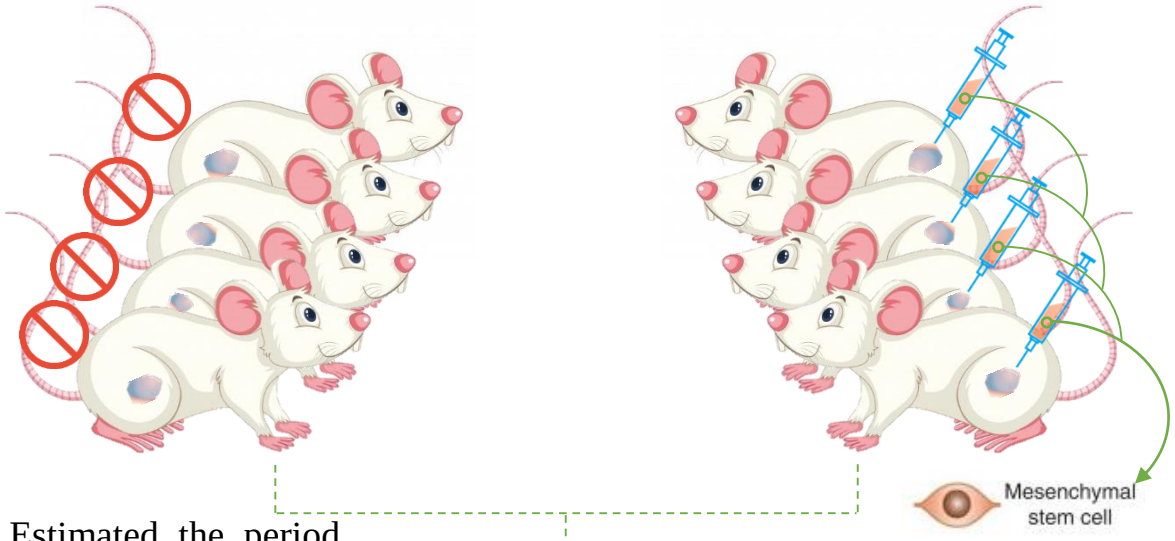
We take 8 rat male for this experimental and divided them into two group.

And then make both of them burn at the pried of healing surface (2nd degree) and estimated



which group of rats consider as control negative (without

Other group of rats treated with MSCs.



Estimated the period to full healing.

Scheme (2-1) Experimental design of the project

2.3. Induction of burns in Rat

The process most commonly used in small rodents to harvest bone marrow requires the excision of the femurs and tibias, resulting in the death of the animal. There are several methods to harvest bone marrow from rats without killing the animal, but these tend to allow for the withdrawal of only very small amounts of bone marrow - suitable mainly for microscopic examination, but not for culture. The authors describe a simple and minimally invasive technique for harvesting bone marrow from rats; the procedure involves widely available equipment, does not require femur excision, and allows the user to harvest quantities of bone marrow suitable for culture.

First, we shave the hair in the thigh of rat. Then we take a piece of metallic iron, heat it on the heater and put it on the area for about 15 seconds. Then we left the rat without any treatment about 4 days, and then we started the treatment.

2.4. Transplantation of isolated BM-Derived MSCs – into the burn site

We anesthetize each rat with an intraperitoneal injection of 60 mg/kg ketamine with 6 mg/kg xylazine, and then place the animal in dorsal recumbency. We then intubate the rat and ventilate it throughout the procedure with 100% oxygen. We shave the anterior face of the thigh and disinfect the area with ethanol. The investigator holds the rat's leg firmly in place with his or her left hand throughout the procedure. The investigator then uses the needle to pierce perpendicularly through the thigh skin and musculature on the anterior face, above the knee joint (avoiding the opening of the joint; figure (2-1)) until it reaches the solid surface of the femur. With the needle perpendicular to the bone, the investigator makes a hole in the femur at the junction between epiphysis and diaphysis by a firm, rotating, and grinding movement. Next, the investigator moves the needle so that it is

almost parallel to the bone and inserts the needle into the diaphysis channel. After attaching a syringe to the needle figure (2-1), the investigator advances

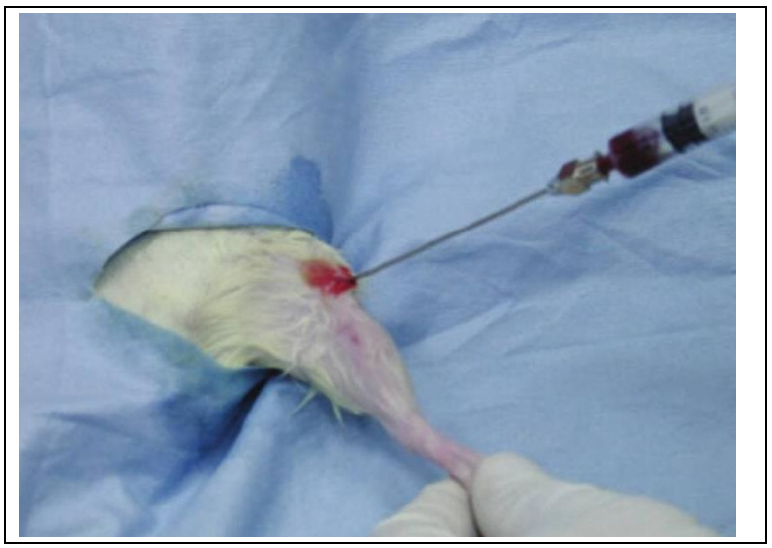


Figure (2-1) The needle pierces the anterior face of the thigh above the knee joint and is advanced into femur

it gradually up its cylinder (until the proximal epiphysis) by pushing and rotating the needle as it moves up, periodically aspirating the marrow. To prevent the coagulation of the diaphysis content one preloads the syringe with 0.1 ml heparin (5,000 μg/ml). One repeats the diaphysis aspiration two or three times, and the final volume of aspirate is ~0.6–1 ml, depending on the size of the animal. The investigator then cleans and disinfects the wound, and processes the bone marrow accordingly. One day after surgery the rat is able to use the leg normally. Alternatively, one can section the anterior thigh through the muscle down to the femur and pierce the bone with the needle under direct observation, but that is a more invasive procedure that requires suturing of the musculature and skin afterward. To confirm the presence of the needle inside the femur's diaphysis, we made radiographic images of the leg, figure (2-2) at 52 kV/32 mA on a Medicor SR-2 machine (Medicor Imaging, Charlotte, NC). We plated the bone marrow cells for culture in DMEM with 10% fetal calf serum to obtain an MSC-like cell population.

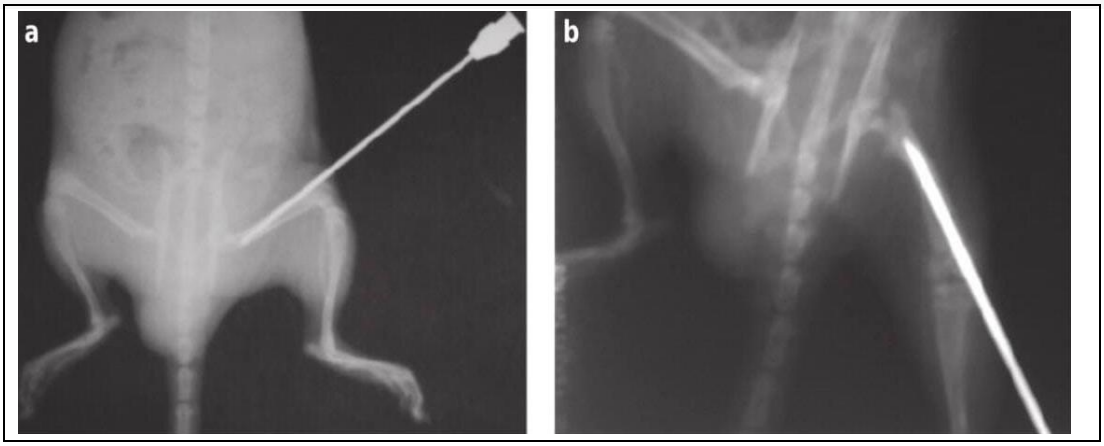


Figure (2-2) Radiographic image showing the needle inside the femu`s diaphysis while the animal is in a supine position (a) and while the leg is extended (b)

Chapter Three Results and Discussion

3. Result

3.1 Isolation and Culturing of rat bone marrow derived MSCs:

After 7-8 days of passage 0, number of adherent cells increased significantly and a colony formation unit of long spindle cells which were uniformly distributed was observed. After 7-9 days, adherent cells increased in number and gradually the morphology changed to polygon and spindle-shape. After 12-15 days of passage 0 (P0), a colony formation unit of uniformly distributed long spindle cells were observed (Fig. 3-2), this result agree with study by Friedenstein *et al*. (1976) have first demonstrated that fibroblast-like cells could be isolated from bone marrow due to their inherent adherence to plastic in culture. They described these cells as multipotential stromal precursor cells, which were spindle shaped and clonogenic in culture conditions, defining them as colony-forming unit fibroblasts (CFU-F).

The best candidate cells for this purpose are MSCs, because they are multi-potent and have a high proliferative capacity. Because there is no definitive marker to identify MSCs, the gold standard procedure to prove their stem cell identify is their adherence on cell culture plates after isolation, their expression of specific marker, and their differentiation potential to osteoblasts, adipocytes and chondrocytes *in vitro* (Wang *et al*., 2017).

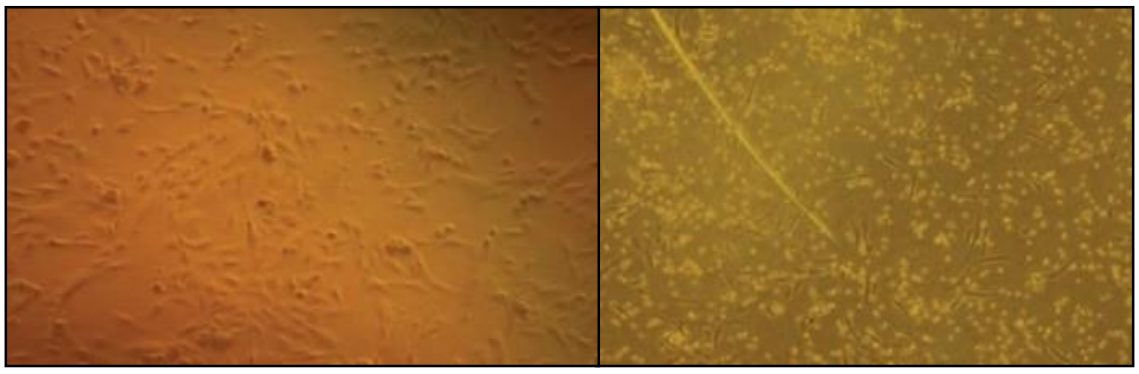


Figure (3-1) MSCs isolated from Rat bone marrow culturing on a flacon containing DMEM 10% FBS viewed by inverted microscope (100X)

3.2 Induction of severe burns and transplantation of rat BM-MSCs:

Severe burns were successfully induced using the modified method (fig 3-1), At 2021/01/21, in this day we were made burn in the surface of each rat leg and keep them few days until pus appear, and in 2021/01/24, the pus appear and then we start injection of MSCs treatment, and keep for few days under watching. Figure (3-2).

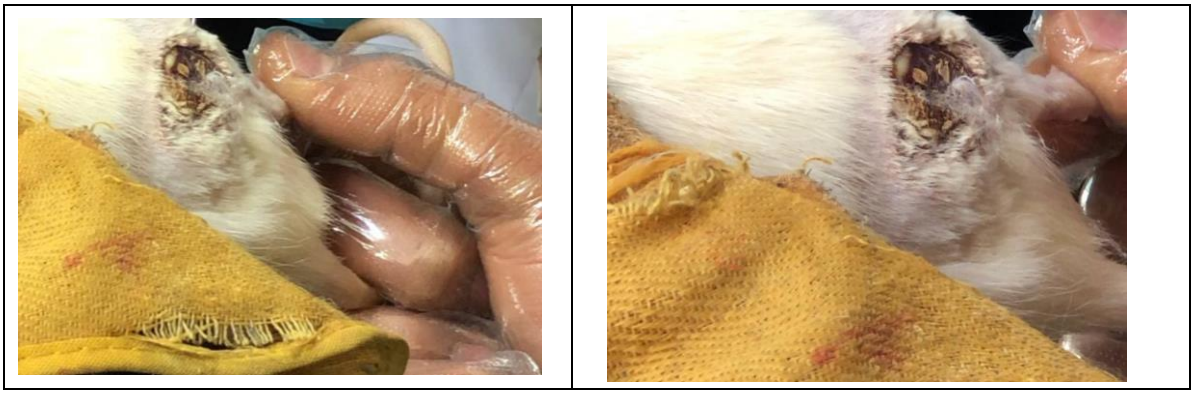


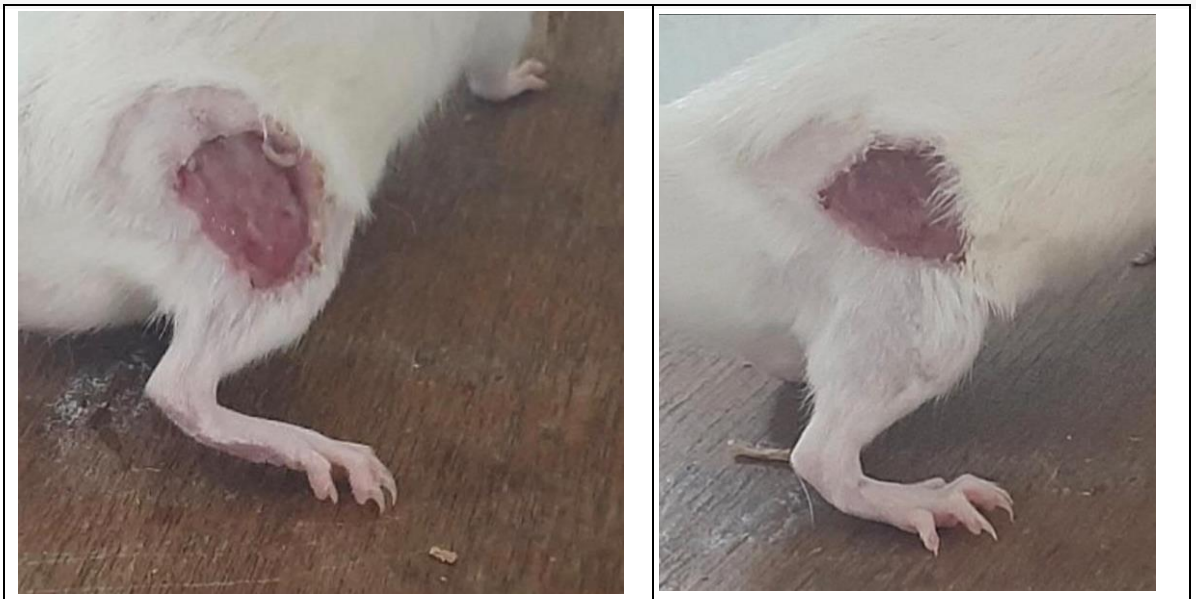
Figure (3-2) Pre-injection preparations

1) At 2021/02/01, after one week of lookout, we saw improved in the group of rat which treated with MSC which pus less. In contrast, the control negative had no improved. Figure (3-3).

A B

Figure (3-3) One week ago; A) Control negative (without treatment) B) Treated with MSCs

2) At 2021/02/04, after two weeks of treatment, we lookout the pus around the burn's area in the group MSCs were almost complete disappearance. In contrast, group of control negative (without treatment) had pus. Figure (3-4).



A B

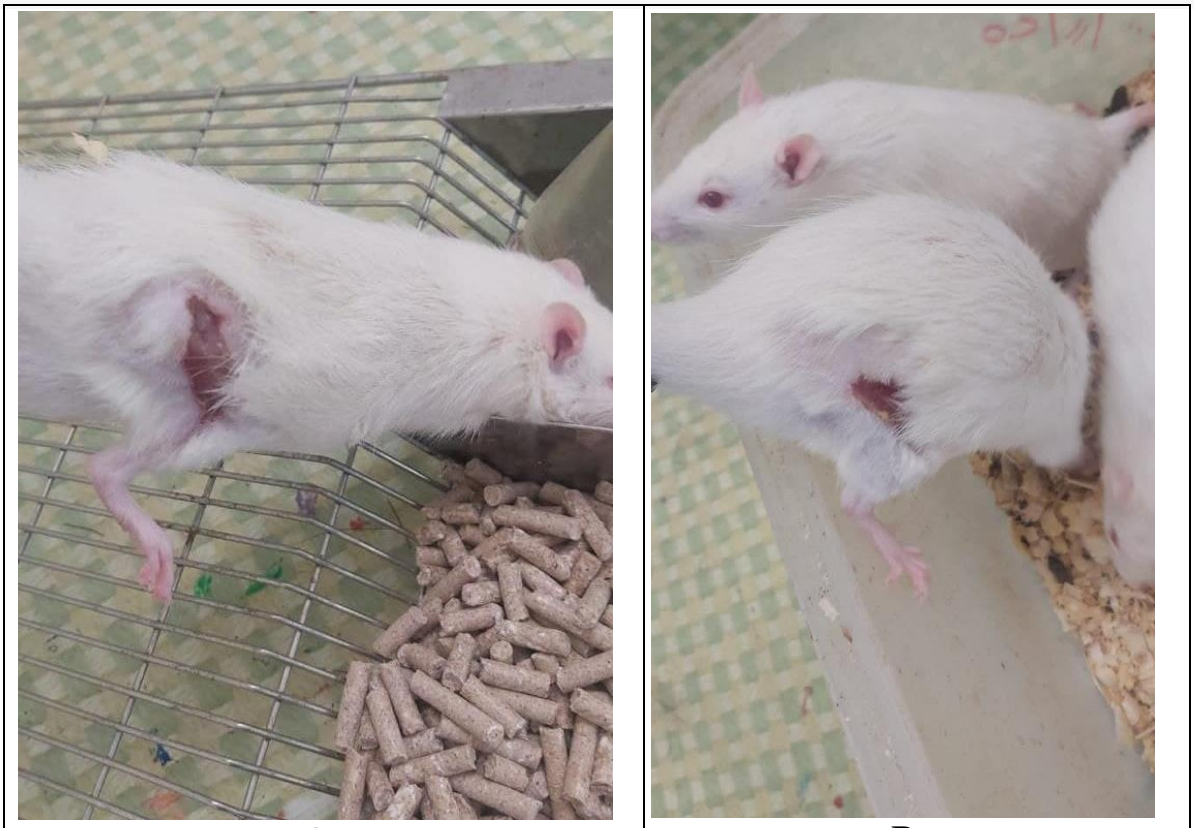
Figure (3-4) Two weeks ago; A) Control negative (without treatment) B) Treated with MSCs

3) At 2021/02/08, after half month of lookout, there was improved in the treatment in the rat which inject MSCs where hair began to grow again, but in control negative the hair wasn't grow, and pus almost disappearance. Figure (3-5).

A B

Figure (3-5) Half month of treatment; A) Control negative (without treatment) B) Treated with MSCs

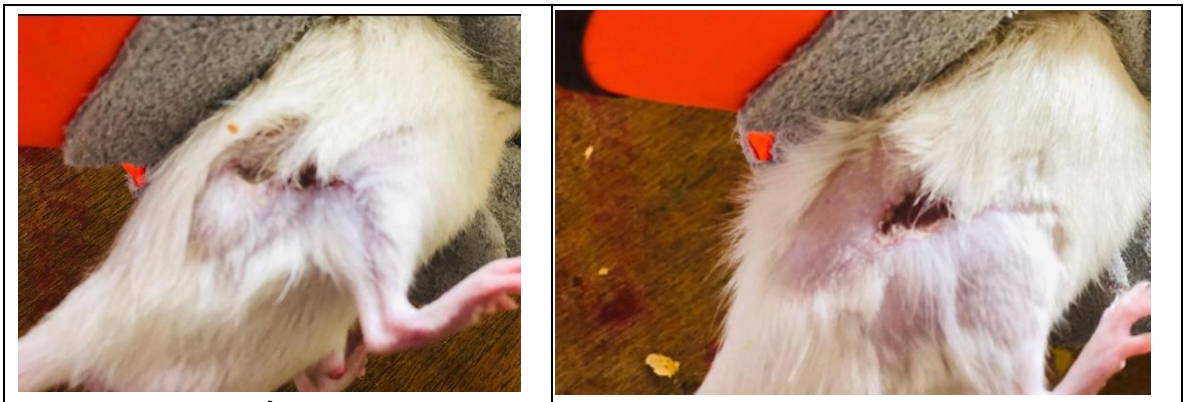
4) At 2021/02/11, we saw the hair had grown in control negative, but rat with MSCs make the burn's area small and grow the hair. Figure (3-6).



A B

Figure (3-6) After 18 days of treatment; A) Control negative (without treatment) B) Treated with MSCs

5) At 2021/02/15, After two thirds of the month had passed, we saw the rat with MSCs reached advanced stages in healing. In contrast the control negative at this period burn's area decrease in size. Figure (3-7).

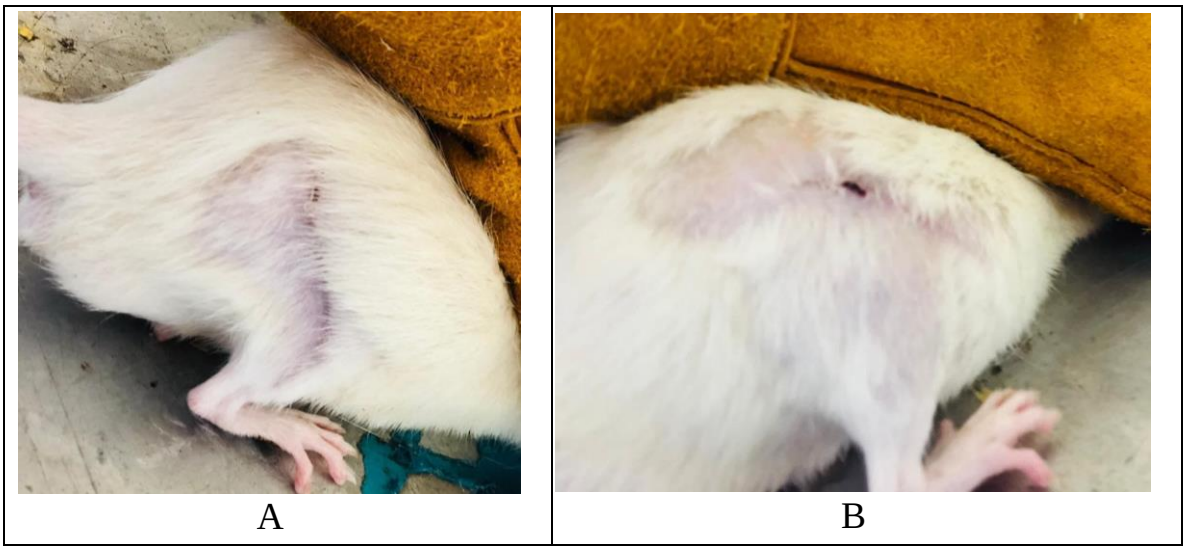


A B

Figure (3-7) Two thirds of the month; A) Control negative (without treatment) B) Treated with MSCs

6) At 2021/02/25, about after one month of the treatment, the rat with MSC make wound healing is almost complete, in contrast the control

negative need more time to complete healing. Figure (3-8).



A B

Figure (3-8) After one month of the treatment; A) Control negative (without treatment) B) Treated with MSCs

3.3. Discussion

Normal wound healing is a dynamic and complex process that involves a coordinated series of events, including bleeding and coagulation, acute inflammation, cell transmission, proliferation, differentiation, angiogenesis, epithelial remodeling, synthesis and remodeling of the extracellular matrix (Netam *et al.*, 2019). Many factors can impair wound healing including infection, tissue hypoxia, necrosis, secretions, and increased levels of inflammatory cytokines, which prolong one or more stages of inflammation, spread, or remodeling (Čoma *et al.*, 2020).

Functional characteristics of BM-MSCs that may be of benefit in wound healing include their ability to migrate to the site of injury or inflammation, participate in regeneration of damaged tissues, stimulate proliferation and differentiation of resident original cells, promote recovery of injured cells through growth factor secretion and matrix remodeling, and exercise Immunomodulatory and anti-inflammatory effects (Kucharzewski *et al.*, 2019).

In this study, implantation of BM-MSCs was shown to have a positive effect in promoting delayed wound healing. The main findings of this study are the following (Al-Shaibani, 2018; Ashraf *et al.*, 2019):

(1) Transplanted BM-MSCs either significantly migrated or found a habitat for wound tissue and contributed to tissue cells;

(2) Implantation of BM-MSCs promoted and remodeling epithelial remodeling.

(3) Transplanted BM-MSCs significantly enhanced angiogenesis and cell proliferation.

The wounds in our albino rat showed inflammatory properties, such as purulent fluid, redness, and swelling. Intraperitoneal injection of BM-MSCs can travel from muscles to injury sites in response to chemotropic signals to modulate inflammation and contribute to tissue remodeling. Several studies have found that grafted BM-MSCs can differentiate into keratinocytes, epithelial cells and endothelial cells of the skin (Braid *et al.*, 2018).

The primary method for wound healing in humans is granulation tissue formation and wound healing. Granulation tissue is essential for wound healing, as it forms on the surface of wounds to protect and nourish wounds, and it consists of fibroblasts, new blood capillaries and infiltrating inflammatory cells (Tottoli *et al.*, 2020).

This study was identical to each of the Lataillade in 2007 and follow him Bey in 2010, looked at burn wounds induced by radiation therapy. MSCs were shown to reduce inflammation and improve wound healing (Lataillade *et al.*, 2007; Bey *et al.*, 2010). Building upon the application of stem cells in irradiated human cutaneous burns, Zong et al, modulated SC application with human beta defensing, explained by (Hao *et al.*, 2009; Zong *et al.*, 2010). This was performed using adenovirus as vector.

These modified stem cells illustrated antibacterial properties advantageous in infected burn wound healing (Hao *et al.*, 2009; Zong *et al.*, 2010). The transplanted stem cell therapeutic group also demonstrated a faster rate to complete healing. This looked specifically at the microorganism pseudomonas aeruginosa, which is a commonly seen pathogen in burns patients (Hao *et al.*, 2009; Zong *et al.*, 2010).

Chapter Four Conclusions and Recommendations

4.1. Conclusions:

1- It is possible to isolate and culturing Rat bone marrow mesenchymal stem cells (BM-MSCS) using adherent property of MSCS.

2- Dulbecco`s Minimum Essential Media (DMEM) can be used successfully for isolation and maintenance of Rat BM-MSCS.

3- Bone marrow derived mesenchymal stem cells were successfully interfere with accelerating healing process of in vivo induced burns which maybe a hopeful process of using mesenchymal stem cells in severe burns patients to accelerate their healing process preventing them from exposure to infection.

4- BM-MSCS can be differentiated in to epithelial cells using to treated the wound.

4.2. Recommendations:

1- Investigation of the possible human mesenchymal stem differentiation into epithelial cells lineage which may open a new field in the clinical trials of using stem in damaged tissue treatments in Iraq.

2- Studying the possible role for the use of co-culture or conditioned media methodologies for tissue engineering applications.

3- Using other new cells source to study the healing capacity of stem cells like using the adipose tissue as a source of human mesenchymal stem cells.

4- Stem cell research offers fresh phenomenal for improving new treatments for debilitating diseases for which there are little or no medicament. Stem cells likewise present another approach to investigate major inquiries of science, for example, deciding the essential mechanisms of tissue development and specialization, which will be needed for the improvement of treatments.

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

وَمَن يَتَوَكَّلْ عَلَى ٱللَّهِ فَهُوَ حَسْبُهُۥٓ ۚ
إِنَّ ٱللَّهَ بَٰلِغُ أَمْرِهِۦ ۚ
قَدْ جَعَلَ ٱللَّهُ لِكُلِّ شَىْءٍ قَدْرًا

الطلاق الآيه ٣

الخلاصه

توجد الخلايا الجذعية عادة في كائنات حية متعددة الخلايا ولها نوع فريد من نوعه فيما يتعلق بالتجديد الذاتي. يمرون من خلال انقسام الخلايا الانقسامية ويمكنهم تمييز الذات الواحدة إلى أنواع مختلفة من الخلايا، يمكن للخلايا الجذعية أن تأخذ شكل خلايا من كل طبقة من الطبقات الجرثومية الثلاث مثل؛ الأديم المتوسط والأديم الباطن والأديم الظاهر.

يجب أن تحتوي الخلايا الجذعية على سمتين أساسيتين ؛ حيث يجب أن تتمتع الخلايا الجذعية بكفاءة غير مقيدة للتجديد الذاتي لتكاثر النسل الذي يكافئ بعناية الخلية الأولى. تعرف حروق الجروح: من خلال مدى عمقهم ومدى اتساع المنطقة التي يغطونها. من المحتمل أن تتضمن إصابة الحروق الهائلة منطقة محترقة بأعماق مختلفة. تدمر الحروق الحاجز الدفاعي للجلد، مما يعني أن الميكروبات (البكتيريا) والغزاة الخارجيين الآخرين يمكنهم اختراقه بسهولة. و لا يمكن للعدوى أن تصيب المنطقة المتضررة فقط ، ولكن أيضا في أعضاء أخرى مثل الرئتين (الالتهاب الرئوي) ومجرى الدم (تعفن الدم)، في حين أنها قد تكون مميتة في بعض الحالات. لذلك في هذا البحث ، نناقش إمكانية علاج جروح الحروق من خلال الخلايا الجذعية الوسيطة المأخوذة من نخاع العظم في فخذ الجرذ.

تم استخدام الخلايا الجذعية لتسريع عملية علاج الحروق. تم استخدام الفئران كنموذج للتجربة. عمل الباحثون بشكل أساسي مع نوعين من الخلايا الجذعية من الحيوانات والبشر، الخلايا الجذعية الجنينية، والخلايا الجذعية غير الجنينية "الجسدية" أو "البالغة".

الخلايا الجذعية الوسيطة هي توضيح للأنسجة أو الخلايا الجذعية "البالغة". إنها "متعددة القدرات"، مما يعني أنها يمكن أن توفر أكثر من نوع واحد من الخلايا المتخصصة في الجسم، ولكن لا يمكن لجميع الأنواع المختلفة من الخلايا الجذعية الوسيطة أن تصنع أنواعا مختلفة من الخلايا التي تنتمي إلى أنسجة الهيكل العظمي لدينا، مثل الغضاريف والعظام والدهون. يدرس العلماء كيف يمكن استخدام الخلايا الجذعية اللحمية لعلاج أمراض العظام والغضاريف.

تم وصف الخلايا الجذعية الوسيطة في البداية على أنها خلايا ملتصقة ذات مظهر شبيه بالأرومة الليفية يمكن أن تتمايز إلى خلايا عظمية وخلايا غضروفية وخلايا شحمية وخلايا الوتر وخلايا عضلية. كان هناك تقدم كبير في فهم الخلايا الجذعية الوسيطة على مر السنين، وهناك أساس قوي للبحث العلمي والتطبيقات السريرية المستقبلية، ولكن أيضا بعض الأسئلة المهمة لا تزال بحاجة إلى إجابة. على سبيل المثال، يمكنهم التمييز – أو التخصص – في الخلايا الغضروفية (الخلايا الغضروفية) ، والخلايا العظمية (بانيات العظم) والخلايا الدهنية (الخلايا الشحمية).

ويعد الجلد، النسيج الخارجي الرقيق الذي يغطي الفقاريات. إنه يحمي أعضائنا الداخلية من البيئة باستخدام نظام توسيد متعدد الطبقات وحاجز خلوي. يتكون الجلد من ثلاث طبقات رئيسية هي البشرة والأدمة واللحمة.

الحرق عبارة عن إصابة في الجلد أو الأنسجة العضوية الأخرى ناتجة أساسا عن الحرارة أو بسبب الإشعاع أو النشاط الإشعاعي أو الكهرباء أو الاحتكاك أو التلامس مع المواد الكيميائية. يصنف عمق الحرق عموما على أنه درجة أولى أو ثانية أو ثالثة. ويعتمد علاج الحروق على عمق، ومساحة، وموقع الحرق، وإصابات الطلقات النارية، والانفجارات، والتعرضات الكيميائية، والحرارة النووية، وأي عامل حرب آخر يمكن أن يتسبب في أضرار جسيمة للجلد. اعتمادا على نوع ومدى الإصابة إذا حدثت هذه الإصابات فقط في ثلاث طبقات من الجلد، يتجدد الجسم نفسه من خلال عملية التئام الجروح ، وهي عملية ديناميكية تتضمن الخلايا الجذعية، السلف، الخلايا المتنية، المصفوفة الخلوية الإضافية، الدم الخلايا والوسيطات القابلة للذوبان في ثلاث مراحل: التهاب وانتشار وإعادة تشكيل وهذا يحدث في الحروق من الدرجة الأولى والثانية، وتعتبر حروق الدرجة الثالثة سمكًا كاملاً وهي الأكثر صعوبة في العلاج. تتطلب هذه تجديد بنية الجلد بالكامل. بصرف النظر عن التجديد الهيكلي، فإن استعادة وظيفة الجلد الأصلي أمر بالغ الأهمية. أثبتت العديد من النماذج قبل السريرية فعالية العلاجات القائمة على الخلايا الجذعية الوسيطة لتعزيز إصلاح وتجديد الحروق الحرارية والتعرض للإشعاع. أفادت هذه الدراسات بإغلاق الجرح بشكل أكثر ملاءمة، وتقليل حدوث العدوى، وزيادة تكوين الأوعية الدموية، وزيادة المرونة وتقليل تكوين الندوب.

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كلية التقنيات الإحيائية

زراعه الخلايا الجذعية المنزشيمية المعزولة من نقي العظم للجرذ في الجروح الشديدة المستحثة لتسريع عملية الشفاء

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مُقدم من قبل

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