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UNIVERSITE MOHAMMED V DE RABAT
FACULTE DE MEDECINE ET DE
PHARMACIE
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Doctor of Pharmacy

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Dedication

This thesis is wholeheartedly dedicated to the many people who made it possible.

To my Mother

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A gentle and strong soul who taught me to believe in hard work, trust in Allah, look at the bright side and enjoy little things in life.

To my Father

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Head of Biotechnology Lab and Professor of Medical Biotechnology

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Professor Madam Naima EL HAFIDI

Professor of Pediatrics

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Professor Mister Jaouad HARTI

Professor of Therapeutic Chemistry

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List of Abbreviations

CAR	: Chimeric Antigen Receptor
COBRA-FISH	: Combined Binary Ratio labelling - Fluorescence in situ hybridization
CPDs	: cumulative population doublings
DMSO	: Dimethyl Sulfoxide
DNA	: Deoxyribonucleic Acid
ESCs	: Embryonic Stem Cells
FBS	: Fetal Bovine Serum
FDG	: Fluorodeoxyglucose
HLA	: Human Leukocyte Antigen
HSCs	: Hematopoietic Stem cells
iPSCs	: Induced Pluripotent Stem Cells
IVF	: In Vitro Fertilization
L-DOPA	: L-3, 4- dihydroxyphenylalanine
LIF	: Leukemia Inhibitory Factor
MAPCs	: Multipotent Adult Progenitor Cells
MIAMI	: Marrow Isolated Adult Multilineage Inducible Cells
MSCs	: Mesenchymal Stem Cells
NIH	: National Institute of Health
NK	: Natural killer
NOD	: Non Obese Diabetic
PBMCs	: Peripheral Blood Mononuclear Cells
PBS	: Phosphate Buffered Saline
PET	: Positron Emission Tomography

RNA : Ribonucleic Acid

SCID : Severe Combined Immunodeficiency

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Introduction

Cell culture is a very useful and versatile technique in the study of basic scientific research issues. Its origins date back to the early 20th century, when it has been introduced to study the growth and maturation of tissues, the role of genes in health and disease, virus biology and vaccine production, and the use of large scale hybrids of cell lines to develop biopharmaceuticals. The experimental uses of cultured cells are as various as the types of cells that may be grown in vitro. There is a rising interest in optimizing stem cells culture, not only because cell culture has become widely used in fundamental research to study stem cells biology, but also because of the potential therapeutic applications of cultivated stem cells. Stem cell culture parameters are crucial and require continual refinement as our understanding of stem cell biology advances and the latest technological developments.

Stem cells have a unique potential to self-renew and to differentiate into different specialized cell types in the organism. Recent advances in stem cells biology offered new hopes in treatment of disorders and diseases that can not be treated yet. Therefore, in recent years, stem cells therapy is becoming a very promising and advanced topic of scientific research.

There are various interesting types of stem cells with their own advantages and disadvantages. Embryonic stem cells have a great proliferation and differentiation potential. Despite their potential contribution to science, the use of embryonic stem cells in research raised sharp ethical, legal and religious concerns.

The use of stem cells in therapeutic applications requires defining optimal conditions for these cells amplification in vitro and in vivo, in order to ensure the absence of tumorgenic potential as well as their functional integration within the damaged tissue.

The objective of this work is to focus on the discovery of various stem cells and the potential therapies that are based on these cells as well as the perspectives, issues and limits of stem cells biology.

The first part of this work is an explicit presentation of cell culture.

The second part is a study of different stem cells.

The third part is an explanation of cell therapy and its therapeutic applications. In particular, the application of this therapy in dermatology, Parkinson’s disease, type I diabetes, heart failure and cancer.

The fourth and last part discusses the national and international legislation, ethical and religious issues concerning the use of stem cells.

First part : cell culture

I. The history of cell culture

Cell culture development has passed through three important periods.

In the early 1900s, the professor Ross Harisson developed the first method for culturing tissues outside of the body. A biologist, Harisson was able to grow frog neuroblasts on a culture dish and thus opened the door for current stem cell research.

Beginning in 1902, the biologist and surgeon Alexis Carrel attempted to develop the state of animal tissue cultures with experiments on a chick embryo tissue culture. He improved tissue harvesting techniques, developed aseptic principles, and studied nutritional requirements [1, 2].

In 1952, the biologist Aron Mosconaintroduced trypsinization of tissues for cell culture. Moscona developed a method for digesting a chick embryo tissue using trypsin in order to obtain dissociated cells that are capable of dividing in vitro [3].

II. Cell Culture

1. Definition, Concepts, and Characteristics of Cell Culture

Cell culture is the process through which cells are raised under particular conditions outside their natural environment. The term « in vitro » is used for tissue and cell cultures under artificial physiological conditions.

Cell culture provides a sufficient cell number to serve as an experimental material. The main challenge of cell culture is that it must be realized under sterile conditions because it is contamination-sensitive.

After cells have been isolated from living tissue, they must be installed in

an appropriate medium that supplies the essential nutrients and growth factors under controlled conditions (temperature, pressure, pH, humidity, etc). Cell isolation varies depending on cell type. That is to say, cells purified from bloodare isolated by ficoll density gradient centrifugation whereas cells obtained from solid tissues are isolated by enzymatic digestion using trypsin [4].

Cells can be grown either free floating in suspension or on a surface adherent or monolayer cultures.

Cellular proliferation happens in four phases in the cell culture. Firstly, the lag phase, in which no cell division is observed because cells adapt to the culture conditions. In the subsequent phase, the cells actively divide. At this phase, cell population is considered to be the most viable. Then comes the plateau or stationary phase in which the cell death rate is balanced with the cell division rate due to the cell population becoming confluent and nutrients depletion. Lastly, there is the decline phase in which the number of viable cells decrease and cell death increases due not only to nutrient reduction but also to the natural evolution of the cellular cycle [5].

In-vitro cells are characterized by two fundamental properties, their proliferative capacity and their differentiation function. These properties tend to vary regardless of the culture method used.

Usually, cells maintain their division potential which can be stimulated at the beginning of the culture by growth factors whereas cells in the culture often change or lose their differentiation function. This de-differentiation phenomenon can be morphologically visible. However, some cell types reveal their specific function only when grown slowly by gradually depleting nutrients in the culture medium or by using a cell division inhibiting substance [6].

Most isolated primary cells go through the process of senescence and can no longer divide. The life span of most cells is limited, but some cells have been transformed into immortal cells and acquired the ability to reproduce indefinitely [7].

2. Primary culture and establishing a cell line

There are different types of culture which can be divided into primary culture and established cell lines culture.

Primary culture is a cell culture method that is established when culture cells are directly derived from their tissue of origin. Primary culture starts with the removal of cells from pieces of tissue and then depriving cells via mechanic or enzymatic digestion techniques from this biopsy. Despite the fact that primary cells provide a heterogeneous population, have a low proliferation rate, and the difficulty of the techniques used, cells in primary cultures are significantly closest in form to cells present in normal tissue. Cells that are reproduced with primary culture have a limited life span and their replication stops when one nutrient of the culture medium is depleted. In this situation, cells of primary culture are transferred to a new culture medium in appropriate conditions and continue to reproduce. This cell culture is referred to as secondary culture. At a certain time, secondary culture cells lose the capacity to proliferate and survive due to the changes that happen physiologically and pathologically. This process is similar to cellular senescence that occurs in the organism.

The drawbacks of primary culture are that when cells are removed from the in vitro environment and transferred into the culture medium, they could lose their structural and functional properties. At this point, cells that have a different morphology at the tissue level can present similar morphologies in the culture

medium. Another important problem is the cells' limited lifespan and consequently, these cells must be replicated with serial passages and saved. Nonetheless, in some cases the number of cells of interest might be less than other cells and for this reason their reproduction could be pressed. In this situation, providing special mediums and growth factors might be required [5].

The second type of cell culture is the established or immortal cell lines culture. Most of these cells are obtained from tumors or transformed cells in vitro. Cell lines have the potential to divide indefinitely and to be grown for an extended period of time, retaining certain phenotypes and functions.

These cell lines have assumed an important role in studying the control of cell cycle and vaccine production. Cell lines have a number of advantages that make them useful in vitro. The obtained cells display a homogenous population, are easy to grow, and can be subcultured continually via an acceptable number of passes to produce a large cell number in a short time. This has made cell lines an attractive model for high-throughput drug screening. Nevertheless, the main drawback is that these cell lines are transformed which can alter their functional levels compared to those found in primary cells and some important functions are even missed entirely. Furthermore, cell lines cannot be used to evaluate genotypic and phenotypic differences in response to drugs in humans because they originate from a single ancestor cell [8].

Recently, cell lines have been evolved with the aim of retaining a normal phenotype combined with the capacity to grow the cell indefinitely in culture. This can be achieved by establishing the cell line from stem cells that can be induced to proliferate into a terminally differentiated cell type in culture. These lines are more difficult to handle in vitro [9].

For both scientific and economic reasons, cells might be needed again in separate studies, which reveal the need for freezing and storing cells (cryopreservation). Cells that provide a sufficient growth cycle can be frozen. Through this process cells are frozen in liquid nitrogen in the presence of cryoprotectant agents such as DMSO (dimethyl sulfoxide) or Glycerol. Thawing frozen cells is fast at 37 °C [5].

3. Applications of Cell Culture

Cell culture is the key technique for cellular and molecular biology. Cell culture is used as a model system to study cell functions and their normal physiology and biochemistry as well as the effects of drugs, cosmetics, chemicals, and toxic compounds on the cells and mutagenesis and carcinogenesis mechanisms.

Animal cell cultures are fundamental to the production of viral vaccines and important genetically engineered proteins (insulin, hormones…). These cell cultures are also used to insert new genetic material (DNA or RNA) into the cell, which can be used to study its effects on the cell. Furthermore, cell culture is utilized in drug screenings and development to study the cytotoxicity of new drugs.

Human stem cell cultures are used to increase the cell number and differentiate the cells into different types of somatic cells for transplantation which can be used to treat a variety of medical conditions [10, 11].

4. Cell Culture experiment

Experiment Design:

Cell culture samples can be obtained from different sources such as peripheral blood, adipose tissue, or the umbilical cord blood.

This experiment is about isolating cells and conducting a culture of human peripheral blood mononuclear cells (PBMCs) by density gradient centrifugation using ficollhistopaque.

Materials and Reagents:

All materials and reagents that come into contact with cells must be sterile.

- Freshlycollectedheparinizedblood.
- Phosphate Buffered Saline (PBS).
- Auto pipettes.
- Cell medium: 10% Fetal Bovine Serum (FBS), 1% L-Glutamine and 1% Antibiotic.
- Ficoll Histopaque.
- Trypanblue.
- Malassez counting chamber or automated cell counter.
- Culture flasks.

Equipment :

- Laminar air flow cabin.
- CO_2 incubator.

- Air conditioner, the lab temperature should be at 17 °C.

- Centrifuge machine with swing-out bucket rotors.

- Inverted microscope connected to a computer.

- Sterile centrifuge tube

Methodology:

The method for PBMC's isolation is based on density differences between PBMCs and different components of the blood. The most commonly used density gradient medium is Ficoll. This medium is less dense than granulocytes and erythrocytes, but denser than lymphocytes, monocytes, and platelets.

After receiving the heparinized blood sample, dilute the blood with the same volume of PBS. Prepare a sterile tube filled with an equal blood volume of ficoll, then hold this tube at a tilted angle and place the tip of the pipette against the tube wall and carefully layer the diluted blood over ficoll without intermingling the two phases. Centrifuge for 20 min at 400g without a brake. During this time, four layers are formed with each one containing different blood cell types. From bottom to top you find erythrocytes and granulocytes, followed by Ficoll, the layer containing PBMCs which appears white and cloudy, and the uppermost layer which is plasma. Collect the cloudy-looking material by pipetting and transfer PBMCs into a new tube and add PBS. Centrifuge for 10 min at 300g two times to wash off any remaining platelets. Remove the supernatant using the auto pipette and resuspend the pellet in the cell medium that was prepared beforehand. Take a drop of cell suspension using a micropipette and transfer it to paraffin paper, and then mix it with the trypan blue solution which isolates viable cells from dead cells. Take a drop from the

mix and put it in the malassez counting chamber to count the cells using the inverted microscope that is connected to a computer to facilitate cell counting. Finally, mark on the flask the date and number of cells, then culture the PBMCs in a flask of 25 cm ² and incubate the cells at 37 °C and 5% CO_2.

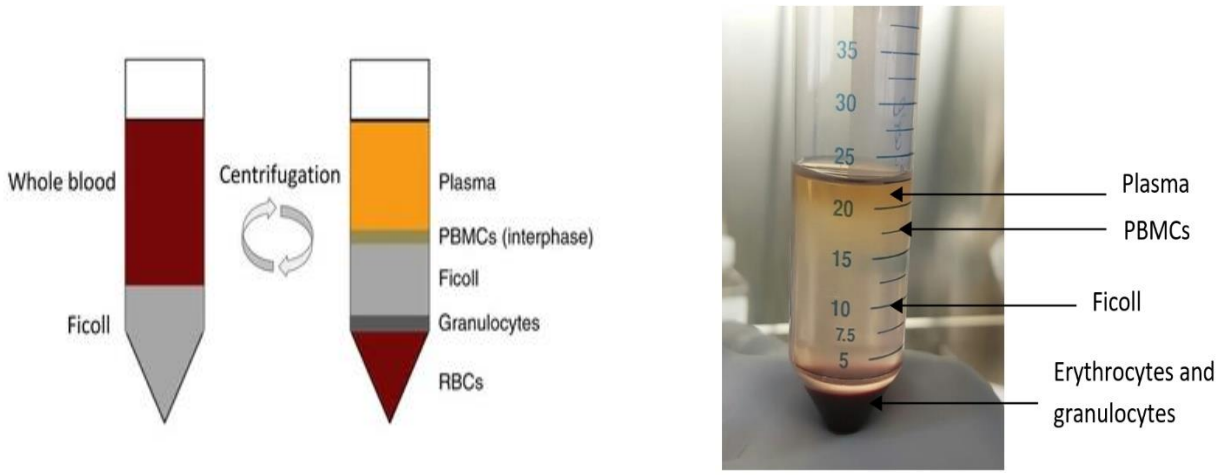

Figure 1: Isolating mononuclear cells from peripheral blood by density gradient centrifugation using ficoll [12].

Figure 2: The pellet obtained after double centrifugation with PBS to wash off any remaining platelets.

Figure 3: Malassez counting chamber to count the cells.

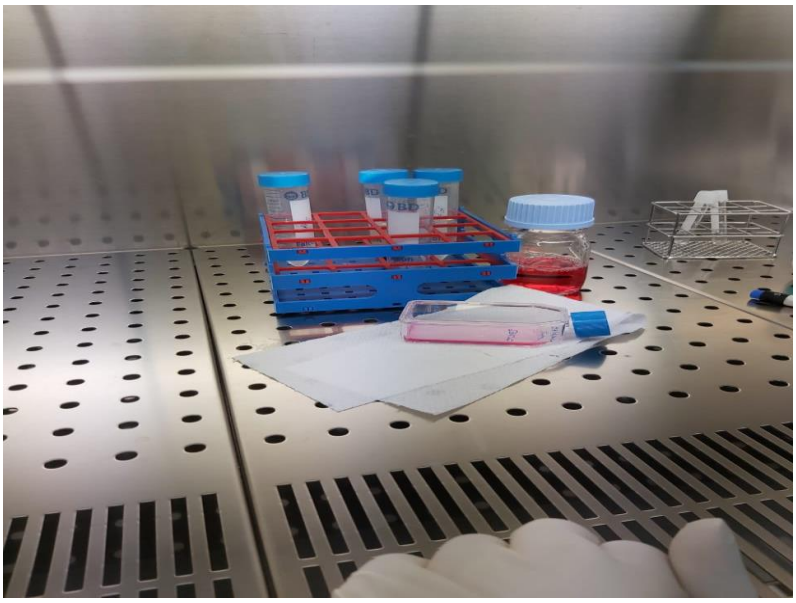

Figure 4: cultivation in a flask of 25 cm^2.

Results:

In the early stages of the culture, the mononuclear cells appear round and floating. After incubating the cells for 3 days, the culture medium color turns to purple which means that the culture medium is depleted. The viable cells appear adherent on a surface.

At this stage, the viable cells can be transferred into a new culture medium through a trypsinization process and pass from primary culture to secondary culture.

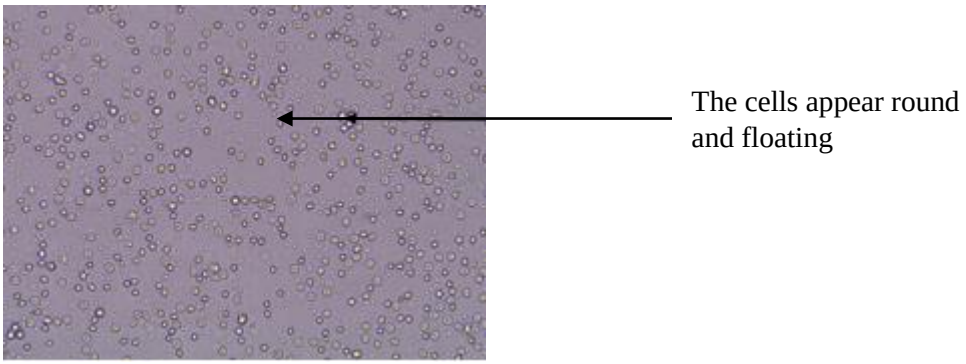

Figure5: Microscopic image of the early stages of the culture.

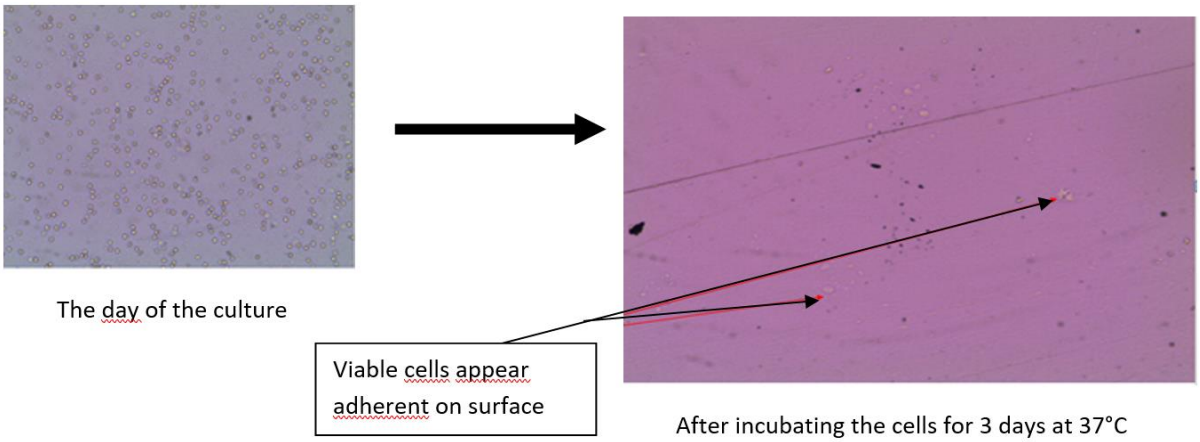

Figure 5: Microscopic observation

The system of cell culture is an important field of biotechnology. Currently, all kinds of cells can be isolated, reproduced, and manipulated in culture conditions. Furthermore, these cultured cells can be transplanted to treat some diseases. However, aseptic techniques are still key for cell culture, a simple contamination can easily ruin the study. Cell culture offers great potential for

innovative applications in cell therapy and regenerative medicine and for expanding our knowledge of human biology [5].

Second Part : Stem Cells

I. History of Stem Cells

Stem cells were first discovered as a consequence of the atomic bombs in World War II.

The atomic bombs dropped on Hiroshima and Nagasaki during the Second World War resulted in unusual destruction and loss of lives. The days to weeks after the bombing, victims began to die, not from burns or injuries, but from mysterious symptoms such as low white blood cell counts, hair loss, Anemia, in some cases, even destruction of the stomach and intestinal lining. This has caused scientists to look into the cause of death of these people, and they found out radiation to be the perpetrator [13].

This sinister report and the biological comparison of normal individuals with those affected by radiation has been able to further scientific work and lead to The concept of « Stem » cells, which are capable to divide when the rate of circulating cells decreases and gives daughter cell lines. By an intercellular message, each stem cell division helps to maintain the global capital of cells and stem cells, The concept of cell regeneration capacity of the skin and the liver in particular, and The concept of transferring bone marrow stem cells by transfusion [14].

Radiation damages cells, which causes cell death and interferes with the cell's ability to divide to give rise to new cells. As a result, body parts like blood and hair cannot be regenerated. Therefore, early radiation experiments lead to the discovery of stem cells, in particular hematopoietic stem cells [15].

The discovery of stem cells is attributed to Drs. Ernest McCulloch and James Till, who have published a number of publications describing the regenerative and multipotent characteristics of hematopoietic stem cells. They were able to demonstrate that a sole stem cell generates all three types of mature blood cells: white blood cells, red cells and platelets [16].

Despite their potential contribution to science, the use of embryonic stem cells in research is a debated topic. Some scientists declare the benefits of pluripotent cells for research. Embryonic stem cells have the potential to treat genetic disorders, neurodegenerative diseases, injuries, and even generate new organs for those requiring a transplant. However, the ethical issues of deriving embryonic stem cells from human embryos have hampered progress [16].

II. Human biological development

Embryology is the science that studies embryonic development from an egg cell to an adult human being.

The time required for the complete development of a fetus in the womb is called gestation. It can be divided into different periods of gestation. The first two weeks of pre-natal development are referred to as the preembryonic stage. A growing human being is referred to as an embryo during weeks 3–8 and a fetus from the ninth week of pregnancy until birth. The preembryonic and embryonic periods of development are both characterized by cell division, migration, and differentiation. At the end of the embryonic stage, all organ systems are structured in a rudimentary form, despite the fact that the organs themselves are either non-functional or only semi-functional [17].

1. Fertilization

Fertilization is a process in which the male-haploid gamete (sperm) and the female-haploid gamete (oocyte or egg), fuse together to generate a diploid zygote. It usually happens in the uterine tube. The function of Fertilization is to enables genes to be transferred from parents to offspring and the process of development to initiate [18].

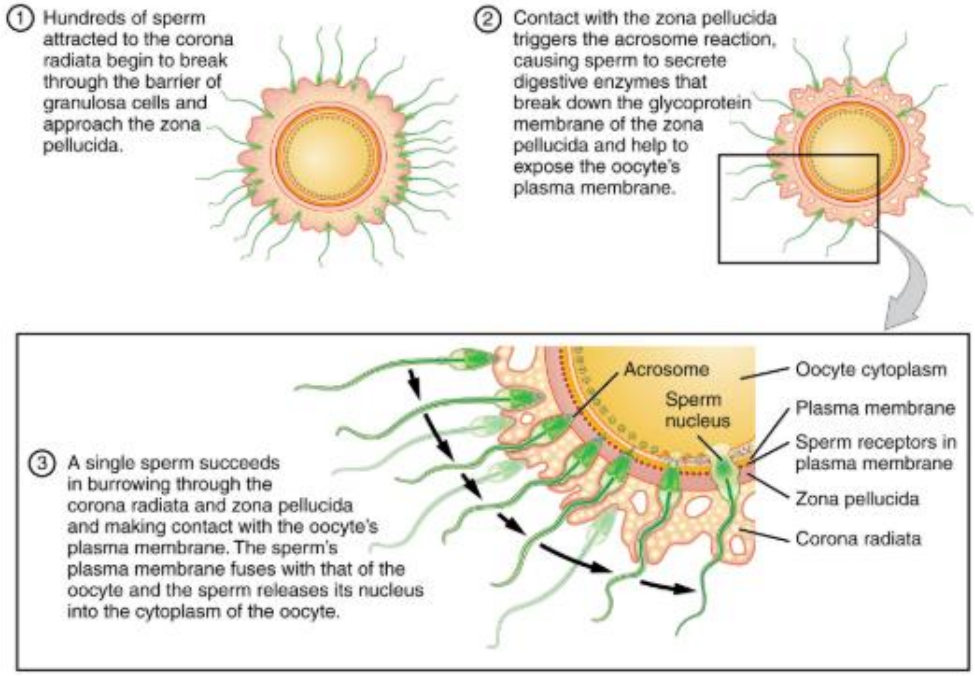

Figure 6: The process of fertilization [17].

2. Segmentation

After fertilization, the zygote continues to be propelled towards the uterus. As it travels to the uterus, the zygote passes through five or six mitotic cell divisions. Despite the fact that each cleavage generates additional cells, the overall size of the zygote stays the same. Each daughter cell generated is called a blastomer.

Mitotic divisions continue for about 3 days, a 16-cell conceptus reached the uterus. The cells that were loosely grouped together are now compacted and look more like a solid mass. This structure is called morula.

Once in the uterus, the morula continues to divide for four to five days, forming a ball of about 100 cells. The ball of now tightly bound cells begins to secrete fluid and organize themselves around a fluid filled cavity, called blastocoel. At this stage of development, the conceptus is referred to as a blastocyst. Within this structure, a group of cells is formed into an inner cell mass, which is destined to become the embryo. The cells that constitute the outer shell are called trophoblasts. These cells will develop into the chorionic sac and the fetal part of the placenta.

The inner mass of embryonic cells is totipotent in this stage, which means that each cell has the capacity to differentiate into any cell type in the organism. Totipotency lasts only a few days before the cells' fates are fixed as being the precursors to specific cell lineages [17].

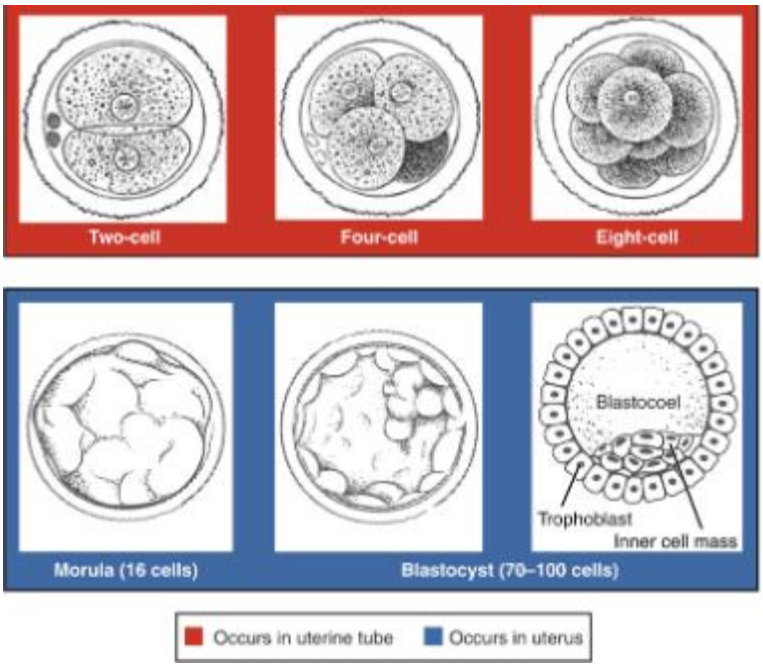

Figure 7: Pre-embryonic cleavages [17].

3. Gastrulation

The inner cell mass in the third week, the human embryo consists of two layers, epiblast and hypoblast. Gastrulation turns these two layers into three ones. An upper layer called ectoderm, a middle one called mesoderm, and a lower one referred to as endoderm [19].

Through the process of gastrulation, the cells go from totipotency to multipotency.

Each of these germ layers will differentiate into multiple human tissues in the embryo. The ectoderm develops into nervous system and brain, epidermis, sensory organs, hair, and nails. The mesoderm cells will become the muscles, bones, cardiovascular system, blood vessels, dermis, gonads, and the excretory systems. The endoderm proceeds to give rise to the inner lining of digestive tract, lining of lungs, and glands [17, 19].

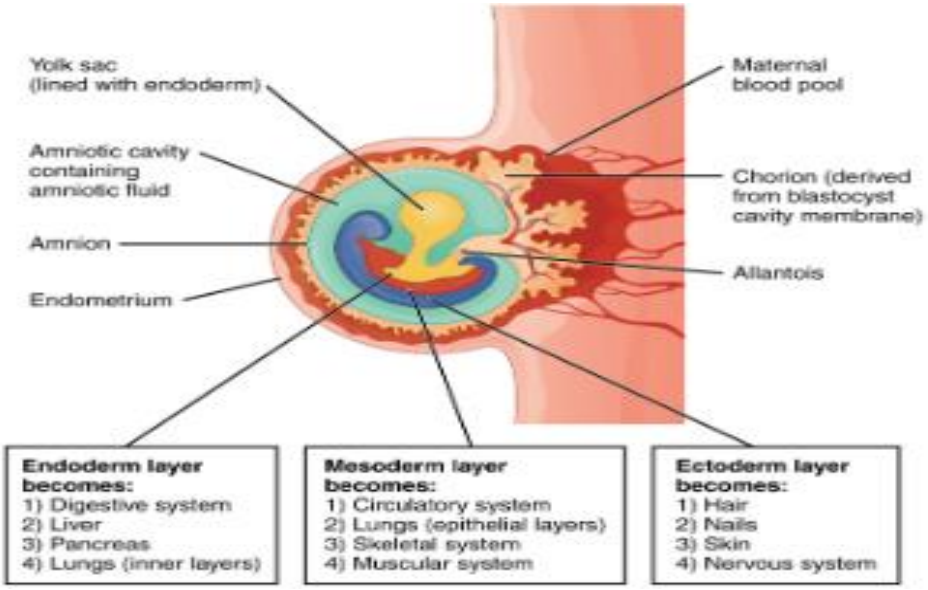

Figure 8: Germ layers fates in embryo [17].

4. Organogenesis

Organogenesis is the process of formation of the embryo's organs and tissues from the ectoderm, mesoderm, and endoderm. This occurs between the fourth and eighth week of gestation.

Through the process of neurulation, the neural tube is formed, which gives rise to the future brain and most of the length of the spinal cord. The ectoderm thickens which leads to the development of the neural plate- the precursor of the neural tube.

At this stage, most cells lose their ability to be pluripotent, and become unipotent. This means that the cells differentiate into a single cell type.

In this stage, the embryonic annexes are formed, which maintain the embryonic development and make the future placenta and the umbilical cord. These structures contain multipotent stem cells.

Within the eighth week, the organogenesis is mostly complete but the organs still need to develop to become functional. At this stage, the embryo is referred to as the fetus [17, 18].

III. Stem Cells

1. Definition of Cell

The cell is the fundamental structure of all living thing. It is the smallest element of life.

The cell is composed of cytoplasm, which is a jelly-like fluid consists of organelles such as mitochondria, ribosome…. That surrounds the nucleus. The nucleus contains the genetic material DNA and it is surrounded by a membrane named the nuclear envelope. The cytoplasm and the nucleus are enclosed in a membrane called the plasma membrane. It separates the cell from its environment and allows materials to enter and leave the cell.

The human body is composed of trillions of cells. The cells that divide to maintain the total number of cells and replace the ones who have died, are referred to as stem cells [20].

2. Stem Cells

Stem cells are defined by the ability to divide indefinitely in culture and the capacity to generate mature specialized cell types. Stem cells are characterized by two different properties, the potential to self-renew and to differentiate into a variety of specialized cell lineages. When stem cells divide, the daughter cells can either generate a differentiated specialized cell or self-renew to continue as an undifferentiated cell, a stem cell. This mode of cell division characteristic of stem cells is asymmetric and is a necessary physiological mechanism to maintain tissues and organs cells number in the body [21, 22].

Stem cells are classified as totipotent, pluripotent, multipotent, and unipotent:

- Totipotent stem cells have the ability to produce all cell types of the conceptus, including the fetus and placenta. These cells are able to form the whole organism. Totipotent cells exist in the organism during the first four days after fertilization [21, 23].
- Pluripotent stem cells have the capacity to form different cell types of all three germ layers (ectoderm, mesoderm, and endoderm) but not the entire organism. There are four categories of pluripotent stem cells which are embryonic stem cells, embryonic germ cells, embryonal carcinoma cells, and the multipotent adult progenitor cell [16, 21, 23].
- Multipotent stem cells have the potential to give rise to limited range of cells and tissues appropriate to their emplacement. For example, blood stem cells give rise to red blood cells, white blood cells and platelets, whereas skin stem cells produce several types of skin cells [21].
- Unipotent stem cells have the ability to renew itself but they can give rise to a single cell type [23].

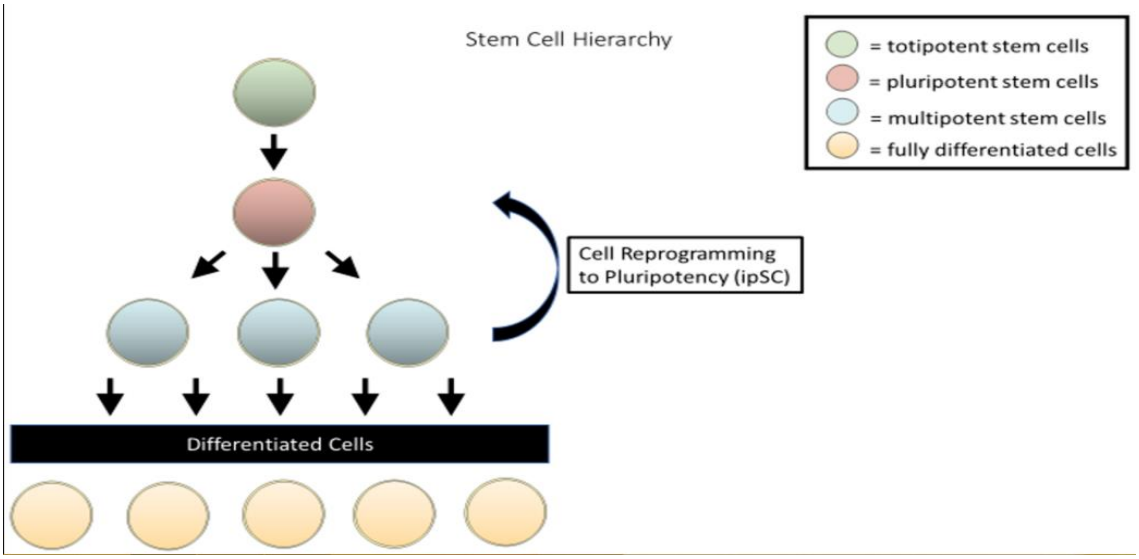

Figure 9: Different stages of stem cells differentiation [24].

3. Different types of human stem cells

3.1. Embryonic stem cells

Stevens is known as the innovator of embryonic stem cells research. Stem cell lines were formed from the teratocarcinomas called embryonic carcinoma cell lines and studied for their pluripotent characteristics [16].

Embryonic stem cells are pluripotent and are arised from the pre-implantation blastocyst that is composed of an outer layer of trophoblast cells and an inner cell mass. Embryonic stem cells are obtained from these cells at the fifth day of embryonic development. These cells can produce cells representative of all three embryonic germ layers (endoderm, mesoderm, and ectoderm) [25, 26].

In 1998 Dr. James Thomson harvested embryonic stem cells and tested the accumulated knowledge of cell culture conditions for stem cells on human fertilized eggs. The eggs were donated by couples who were undergoing treatment for infertility [16, 27].

Embryonic stem cells can be obtained from spare embryos that have been fertilized in vitro and not held for a parental project, or by a technique called therapeutic cloning, through this process a cell from the patient is fused with an enucleated donor oocyte applying the nuclear transfer technique. These cells are isolated from the resulting clone, and then differentiated in vitro into different type of cells and tissues for transplantation to help patients with disorders such as Parkinson's disease, diabetes and cancer [23, 28].

Embryonic stem cells are defined by the possession of two key properties: embryonic stem cells can be grown in vitro and expanded in number indefinitely in the primitive undifferentiated state characteristic of the embryonic cells from which these cells are obtained, and throughout long periods of cultivation in vitro these cells retain a key property of those cells – pluripotency [27].

Furthermore, embryonic stem cells have the ability to self-renew and the capacity to preserve and expand the cells in culture for an extended time while maintaining their normal karyotype [29].

One major problem concerning the use of embryonic stem cells as a model system is their genetic and karyotypic integrity during long term culture and differentiation. Cytogenetic analysis of different human embryonic stem cell lines cultivated in different laboratories has shown that the euploid karyotype remains substantially stable, although recurrent gains of chromosome arms 12p and 17q or trisomy of chromosomes 12 and 17 have been described in some

subclones. Chromosomal abnormalities happen very frequently during embryonic stem cells derivation and culture [26].

The use of embryonic stem cells has major disadvantages. First, the use of human embryonic stem cells requires lifelong use of immunosuppressant drugs to prevent tissues rejection. Second, using embryonic stem cells can generate tumors from rapid growth when injected into adult patients. Finally, scientific progress is restricted by ethical issues about deriving human ES cells from aborted fetuses or from human embryos [30, 31].

An important stem cell discovery that may alter the ethical debate is induced pluripotent stem cells (iPSCs). This recent discovery has opened up new avenues of research, such as the induction of different somatic cell types into stem cells, comparisons of iPSCs to ESCs, and the potential of iPSCs for treating diseases [16].

3.2. Induced pluripotent stem cells

iPSCs are somatic cells that were reprogrammed to express specific genes, thereby causing them to transform from a differentiated state to a pluripotent state. The discovery was the result of many research groups working to determine what gave cell “stemness.” Shinya Yamanaka found out four genes that, when expressed together, could turn fibroblasts into iPSCs [16].

In 2006 Takahashi and Yamanaka launched the concept of induced pluripotent stem cells by producing stem cells that had having properties related to embryonic stem cells. iPSCs were generated by using a combination of four reprogramming factors, including Oct4 (Octamer binding transcription factor-4), Sox2 (Sex determining region Y) box 2, Klf4 (Kruppel Like Factor-4), and c-

Myc. These cells were demonstrated that they have the ability to self-renew and to differentiate like embryonic stem cells, and thus, could be used as an alternative for human embryonic stem cells in various research and clinics [32].

The production of iPSCs from somatic cells requires the incorporation of reprogramming factors into somatic cells. This can be realized by two types of methods— Integrative Viral Vector Systems and Non-Integrative Systems. Integrating systems are those in which the viral vector gets integrated into the host cell genome. The use of lentivirus and retrovirus comes under this category. Non-Integrating systems are those in which no integration in host cell genome happens. Various approaches such as viral vectors (Sendai virus and Adeno virus), Plasmid DNA, and the use of RNA and proteins come under this category.

iPSCs have been used since their discovery in many research and clinical studies including disease modeling, regenerative medicine, and drug discovery and or drug cytotoxicity studies. For disease modeling, somatic cells possessing the characteristics of patient's diseased cells are used for the production of iPSCs and are further used for studying the diseases. Similarly, iPSCs are widely used for the regeneration of tissue-specific cells for the transplantation to patients suffering from different injuries or degenerative diseases. Studies such as drug discovery which implies small molecules screening, toxicity testing for assessment of safety, have successfully utilizediPSCs based technique [32].

Induced pluripotent stem cells afford various advantages:

- ✓ **The ethical concerns are eliminated through the use of iPSCs**; these cells can be obtained from an adult patient instead of the destruction of the embryos resulting from the use of embryonic stem cells in research.
- ✓ **Reduced chances of immunorejection**; iPSCs are produced from the somatic cells of patient's own body and therefore the risk of immunorejection of these autologous cells is eliminated.
- ✓ **Throughput screening to predict toxicity and therapeutic responses of new drugs**
- ✓ **Reduce the global cost and risk of clinical trials;** The cost of clinical trials could be lowered by using iPSCs to provide details of the drug'stoxicity through different cytotoxicity assays, and could reduce the cost related to providing animal models which will ultimately lower the total costs of the clinical trials.
- ✓ **Gene targeting and correction technologies;**somatic cells reprogramming from any genetic background to iPSCs has enabled the generation of cell lines that possess diseasecausing mutations. The ability of modifying the specific sites in a genome to alter the genes of interest becomes very important here.

There are some drawbacks associated with iPSCs as well, the generation of iPSCs uses retroviral or lentiviral systems, so the concern is if the viral systems get incorporated with the host genome. The genetic material inserted by retroviral vectors could randomly integrate into the genome of the host which can lead to genetic aberration and teratoma formation. It has also been reported

that the altered expressions of the basal reprogramming factors can cause diseases. The overexpression of Oct4 can cause epithelial cell dysplasia. Furthermore, it has been reported that the aberrant expression of Sox2 cause mucinous colon carcinoma. Klf4 has a role in the development of breast tumors. C-Myc plays an important role in the formation of approximately 70% of human cancers. For this reason, retroviral insertion of c-Myc is also advised to be avoided for clinical applications [32].

3.3. Fetal stem cells

In 1998, John Gearhart and his team were the first who have been able to isolate and culture fetal stem cells which are known as primordial germ cells. These stem cells are the precursors of gametes, like eggs and sperms, and were isolated from fetal tissues such as the placenta, amniotic fluid, and umbilical cord blood, of 5-9 week old fetuses obtained by therapeutic abortion. The isolation of embryonic germ cells from fetal stem cells has two major problems. First, these cells are derived only from 8-9 week fetuses. Second, embryonic germ cells have limited proliferation ability [33, 34].

The concept of fetal stem cells is not new. Fetal stem cells have been used in clinics over the past years. Fetal neural tissue has been widely utilized therapeutically in Parkinson's disease. Fetal stem cells have many characteristics which make them better than adult cells for use in regenerative medicine applications, such as greater plasticity in differentiation capacity, rapid growth in culture, and increased survival at low oxygen tension. These cells have also the ability to make high levels of angiogenic and trophic factors, which enhance growth in vivo and facilitating the surrounding host tissues regeneration. Also the use of stem cells derived from umbilical cord doesn't make any ethical problems [34, 35].

The only problem associated with these stem cells is that the umbilical cord blood contains very few stem cells. The reason it is important to study these cells and their needs in order to improve the yield and the supply of those cells for therapeutic purposes [36].

3.4. Adult stem cells

Adult stem cells also called somatic stem cells are undifferentiated cells that take place in a differentiated tissue or organ. Adult stem cells are able to renew itself and can differentiate to yield all the specialized cell types of the tissue in which they reside. These stem cells exist in different tissues and organs such as the brain, liver, bone marrow, spinal cord, skin, epithelial lining of the digestive tract, skeletal muscle and heart. These cells are gathered in a microenvironment called niche. Somatic stem cells are multipotent or unipotent but they could exceed the limits and give rise to cell types of a different tissue, this phenomenon is referred to as plasticity [21, 33, 37].

Adult stem cells play an important role by replenishing dead or damaged cells to maintain the tissue or the organ. Some experiments in mice have shown that neural stem cells could differentiate into heart, blood, skeletal muscle, lung, and skin after transplantation. This phenomenon is called cell plasticity [33].

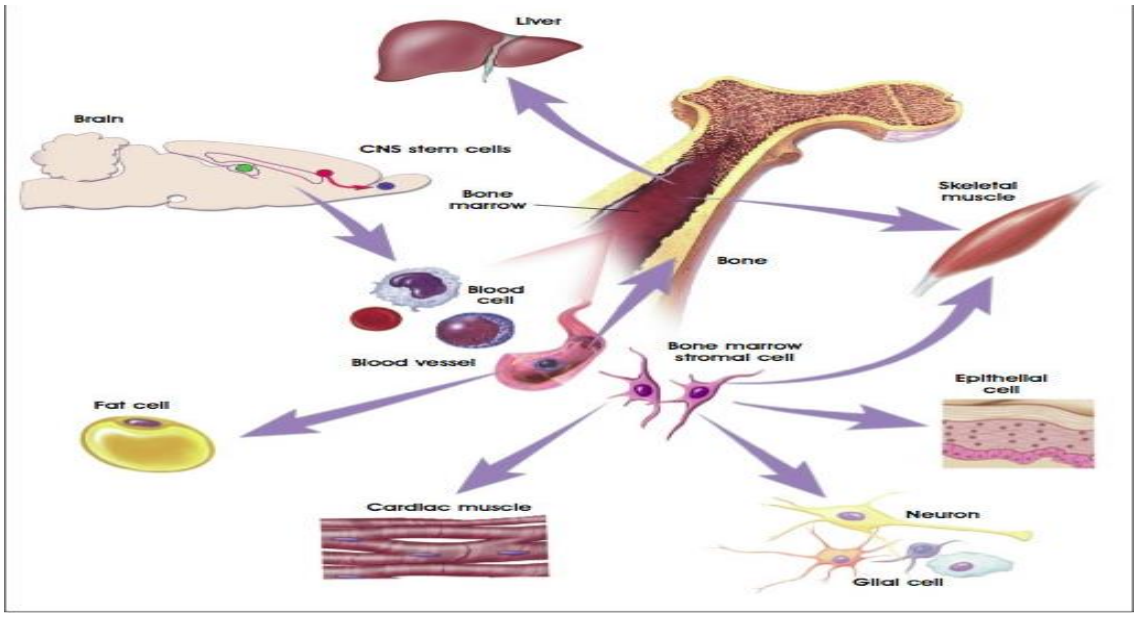

Figure 10: Adult stem cells plasticity [38].

There are three plasticity mechanisms of adult stem cells: transdifferentiation, dedifferentiation, and cell fusion.

The transdifferentiation phenomenon is defined by the conversion of a differentiated cell type into another cell type [39].

Through the process of dedifferentiation, a differentiated cell transforms into a differentiated cell of another cell type in condition that it dedifferentiate into progenitor [40].

The cell fusion mechanism is often observed in the organism: for example, myoblasts fuse to form multinucleated myofibers. When a differentiated cell fuse with an undifferentiated cell, this last one is as if reprogrammed by the differentiated cell.

Therefore, there could be confusion between the suspected plasticity phenomenon and the observed cell fusion phenomenon. In fact, transplanted stem cells showed a high fusion frequency with parenchyma cells acquiring the phenotype of these cells, and giving the illusion of plasticity or a transdifferentiation. As an example, replenishing a sick mouse's liver by hematopoietic stem cells obtained from a normal mouse. Nevertheless, researchers estimate that the frequency of cell fusion phenomena is very low, at least in normal tissues and does not seem able to explain all published results [41].

Adult stem cells reside in a cell microenvironment referred to as niche which controls stem cell self-renewal and differentiation. These niches are consisted not only of stem cells but also of other adjacent differentiated cell types which generate a rich environment of extracellular matrix and other factors that allow maintaining the undifferentiated state of stem cells.

There is considerable change in niche design, in the world of stem cell niches. Most of stem cells reside within complex niches, but some stem cells are not surrounded by this cell environment. Stem cells of adult muscle called satellite cells are quiescent and attached to the basal lamina that enclose each muscle fiber bundle, and only reactivate to proliferate and fuse into differentiated myotubes when damaged fibers need repairing.

The niche has an anti-tumor role, by controlling stem cells self-renewal and division, in normal conditions. However, mutations in stem cells which make these cells insensitive to the niche control or a change in the signals emitted by the niche could contribute to a cancer development [42].

Scientific researchers are making significant efforts to explore the niche, which offers an opportunity to affect regenerative medicine and anticancer treatments.

Nowadays, it appears that most if not all adult organs contain stem cells.

The bone marrow incorporates different populations of adult stem cells.

❖ Hematopoietic stem cells (HSCs)

HSCs were the first stem cells to be characterized, isolated and used clinically. These stem cells are capable to make all the types of blood cells, and are isolated from the bone marrow and umbilical cord blood. HSCs and many of the cytokines and growth factors are widely used clinically. For example, G-CSF helps mobilize HSCs from the bone marrow into the blood where they can be collected and used for the treatment of cancer patients [43].

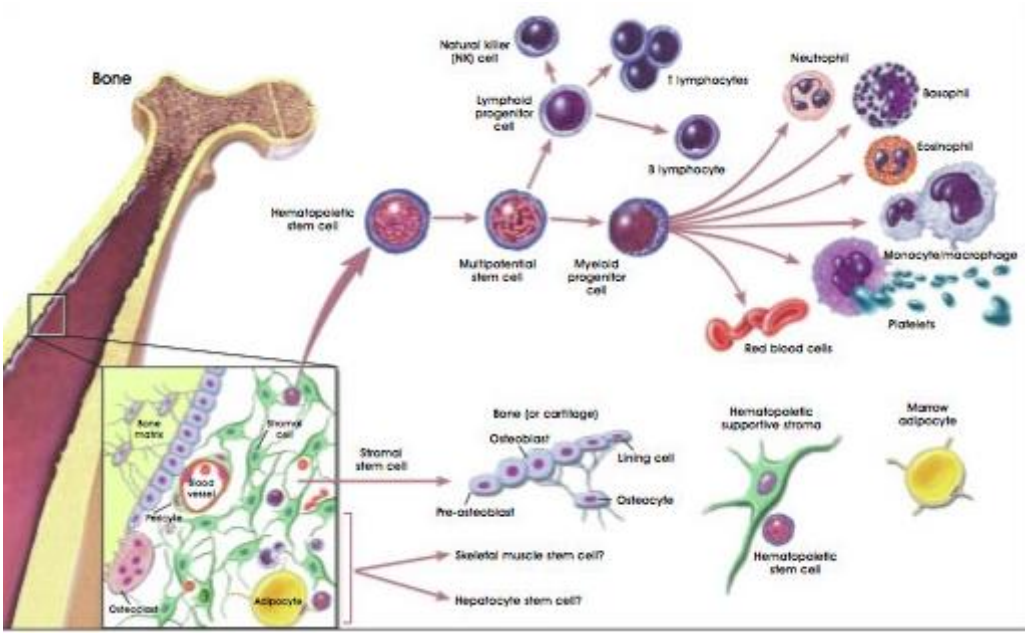

Figure 11: Hematopoietic stem cells differentiation [38].

❖ Mesenchymal stem cells (MSCs)

MSCs are of embryonic origin and exactly from a structure of the embryo called mesenchyme. MSCs are distributed in the connective tissues of the body and have been derived from bone marrow, adipose tissue, placenta, lung, blood, and the umbilical cord. These stem cells have the capacity to stimulate blood vessels establishment, produce cytokines and growth factors, restrict T-lymphocytes activation and modulate innate immune response, and differentiate into different cell types such as chondroblasts, osteoblasts, neuroectodermal cells, adipocytes, and hepatocytes [44].

❖ Endothelial progenitor cells

Endothelial progenitor cells have been found in bone marrow and blood. They have the ability to differentiate into blood vessels. Endothelial progenitor cells are useful due to their properties of neovascularization and restoring blood flow, in brain, retinal and heart damage [45].

Some reports claimed that there are rare cells within mesenchymal stem cells cultures which are pluripotent and can produce not only mesodermal but endodermal tissues. These cells are referred to as multipotent adult progenitor cells.

Multipotent adult progenitor cells (MAPCs) exists not only in bone marrow but in all organs. MAPCs are nonhematopoietic stem cells and have similar properties to those of embryonic stem cells. However, these cells seem not to give rise to tumor when injected into immunodeficient adult mice. MAPCs have the ability to grow extensively and to produce cells with phenotypic characteristics which distinguishes MAPC from mesenchymal stem cells, and

therefore suggests that MAPCs might be an ideal cell for in vivo therapies for damaged tissues and organs [46].

Recently another pluripotent cells population has been identified, from adult bone marrow referred to as marrow-isolated adult multilineage inducible cells (MIAMIs). MIAMI cells express the transcription factors oct-4 and rex-1 and telomerase. Also, they have the ability to differentiate into mesodermal, neural and pancreatic cells [47].

Adult stem cells offer different advantages:

- Adult stem cells are not rejected by the immune system as a consequence of performing an autologous transplant.
- Adult stem cells are chromosomally stable which does not cause a problem for therapeutic efficiency.
- Harvesting techniques and where most of adult stem cells are obtained have little effect on the individual, which causes less ethical problems.
- These stem cells are able to be reprogrammed and transformed into pluripotent stem cells.

Adult stem cells seem the best choice for cell therapy. However, the use of these stem cells is challenging, it is hard to isolate these cells because their numbers are small, their locations are hard to predict, they are difficult to culture in vitro and hard to maintain, and there is a low risk of cancer development after autologous transplant of stem cells [48].

4. Timeline of stem cell research

These are some important dates which mark advancements in human stem cell research:

- 1860 – 1920: The existence of stem cells is discovered from the analysis of embryo development and bone marrow microscopy [49].
- 1948 – 1958: Greater understanding is reached about stem cell mechanisms from sperm development and intestinal epithelium replacement [50].
- 1961: The first blood stem cells are discovered [51].
- 1964: Stem cells are detected in the embryonal carcinoma [52].
- 1968: The first bone marrow transplant is conducted [53].
- 1981: scientists begin obtaining and culturing mouse embryonic stem cells [54].
- 1992: The presence of nerve stem cells are recognized in the adult human brain [55].
- 1994: Scientists distinguish stem cells from the majority of other cancer cells. Doctors begin using corneal stem cells to treat patients with damaged cornea [56].
- 1994: Scientists begin the isolation and culture of inner cell mass cells from human blastocysts [57].
- 1995: Scientists begin the isolation of a primate embryonic stem cell's line. These embryonic stem cells are diploid, pluripotent and have a normal karyotype. These cells have the potential to develop into derivatives of all three embryonic germ layers. Primate embryonic stem cells resemble

human embryonal carcinoma cells which have led to the possibility to produce and maintain human embryonic stem cells in vitro [58].

- 1996: The beginning of mammalian cloning and the birth of the cloned sheep dolly [59].
- 1998 – 2000: The beginning of the production of human embryonic stem cells from the inner cell mass of a human blastocyst donated by a couple who had undergone IVF treatment. Embryonic stem cells proliferate in vitro over extended periods while maintaining a normal karyotype. These cells have the ability to developinto somatic cell lines derived from the three embryonic germ layers and form teratomas when injected into immunodeficient mouses [60].
- 2000: The first production of human embryonic stem cells and their differentiation into neurons [60].
- 2003: The first production of human embryonic stem cells from human exfoliated deciduous teeth [61].
- 2004: Human embryonic stem cells were obtained by transferring a woman’s somatic cell nucleus into her enucleated ovum [62].
- 2004: The first successful bone marrow transplant was carried out at CHU Ibn Rochd of Casablanca [63].
- 2006: Human embryonic stem cells are derived from human embryos considered as naturally dead [64].
- 2008: The first production of stem cell lines from nuclear transferred and parthenogenetically activated mouse oocytes for therapeutic cloning [65].

- 2010: Scientists reprogram adult cells directly into neurons, heart muscle cells, and blood cells [66].
- 2010: Scientists successfully isolate human pluripotent blood stem cells that have the ability to form all types of blood cells [54].

Currently, stem cell research focuses on the mechanisms of intercellular communication, cell differentiation, and specialization. Scientists are studying the conditions under which these stem cells are harvested, cultured, proliferated, and administered locally or systemically. This effort seeks to understand the regenerative potential of these cells which allow many therapeutic applications like tissues and organs regeneration.

Third part : CellTherapy

I. Definition and perspectives

Cell therapy can be defined as an approach which uses a biological product that has a therapeutic effect derived from human or viable animal cells. Cell therapy is a therapy in which viable human cells are injected in order to prevent and treat a disease. Cellular therapy is based on regenerating damaged tissues with new functioning cells [67].

Regenerative medicine is a field of medicine which deals with the process of replacing and engineering damaged tissues with cells or tissues obtained from stem cells or modified biological materials [68].

The cell types used in cell therapy are differentiated or mature cells obtained from an organ and precursor cells derived from stem cells. These cells have the best differentiation and proliferation capabilities, stem cells are the center of research and development of new treatments in cell therapy [67].

Many diseases cause cell destruction, and the only solution would be organs and tissues transplantation. This solution is challenging because of compatibility problems between donors and patients and because patient demand for donors outpaces availability. Cell therapy is an alternative to organs and tissues transplantation, which can not treat all pathologies relative to cells degeneration or cell destruction. Through this therapy, tissues could be produced from adult stem cells obtained from the patient (autologous cells), and thus the donors problem is solved and the risk of rejection is avoided.

The main issues encountered in cell therapy are to control the culture and to use adult stem cells, to induce their proper transformation; in terms of quality and quantity. Also, there is a need to develop an injection technique that will ensure the cells survival once implanted.

Cell therapy has already achieved many advances, and this therapy still offers therapeutic possibilities for many severe pathologies [69].

II. Stem cells in cell therapy

1. Principle and process of cell therapy

Cell therapy is based on cell's transplantation. The principle of this therapy involves collecting cells which are either directly reinjected into the patient or in most cases undergo some manipulations in the laboratory (sorting, amplification, selection or depletion of certain cell populations). Some cells transplants can be stored at an extremely low temperature in stem cells banks, then thawed when needed for therapies.

Cell therapy can be done in five steps.

Cell therapy starts by harvesting the cells. The cells can be taken from the same patient (autograft).

If needed, a purification step of the collected cells is realized in order to keep only one cell type. Then these cells are modified or treated according to the therapeutic objective aimed.

If the collected cells are from a patient whose disease is genetic. In this situation, the collected cells should be corrected by gene transfer.

The cultured cells must be amplified in order to proliferate and then reimplant them in the patient at the damaged tissue or cells.

The last step is the administration of the biological product into the patient and its follow-up [70].

2. Cell therapy laboratory

Cell therapy laboratory should include a production laboratory, a research and development laboratory, a cold room, a freezing room, and the bank where cells are stored in liquid nitrogen. Monitoring of environmental conditions (oxygen concentration, storage spaces temperature, levels of liquid nitrogen) is necessary to ensure that cell therapy processes run smoothly and safely.

Manipulation of cell for clinical use requires respect of good manufacturing practices to guarantee the safety of cell transplants, individuals and the environment, which implies the use of closed systems. The closed system is a process where the biological material is not exposed to the open environment, in order to reduce the risk of contamination.

Closed culture system is a system that employs:

- ✓ Bags for samples and for manipulation of different products.
- ✓ Culture bags made of a special plastic allowing gas exchange between cells and the incubator atmosphere.
- ✓ Sterile connection system, which connect the different bags by tubes that can be sterilely cut and reattached by welding at 320 °C.

The use of a closed culture system for production of therapeutic cell products batches has proved its efficiency concerning the risk of contamination [71].

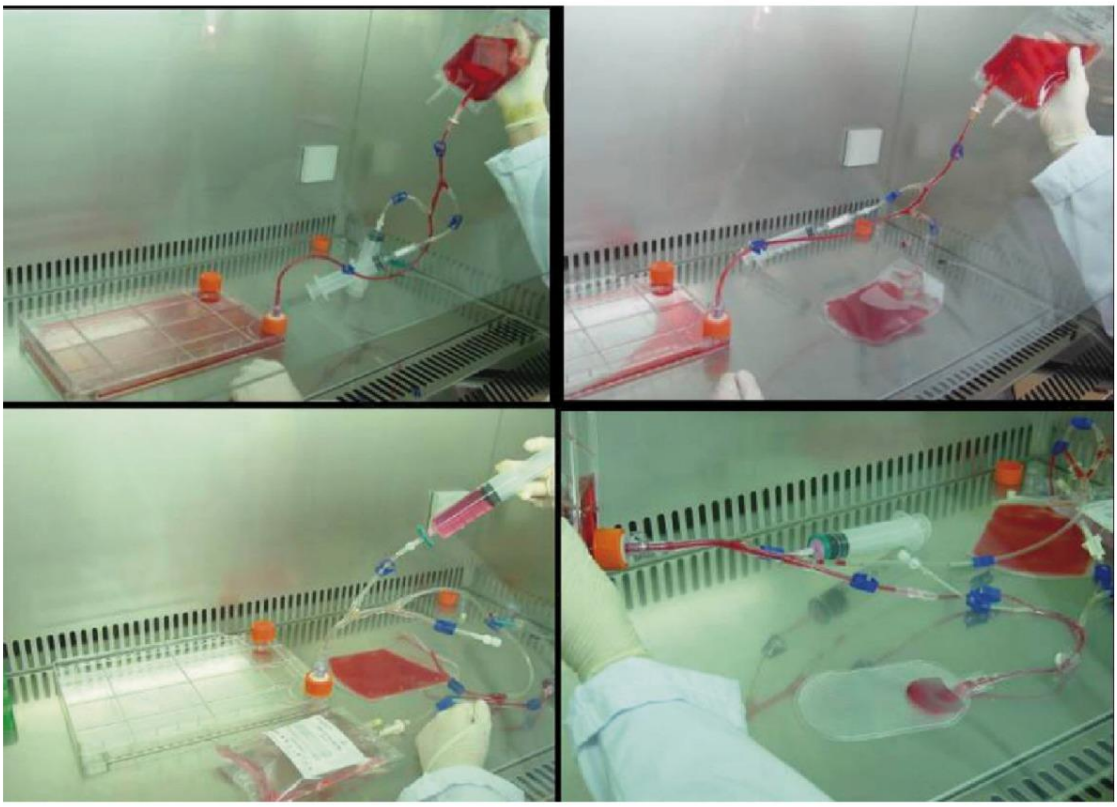

Figure 12: Example of manipulations in a closed system [71].

3. Sources of stem cells samples

3.1. Sources of embryonic stem cells

Embryonic stem cells are important for cell therapy as an unlimited cells source. Embryonic stem cells can be isolated by three different methods. These cells are isolated from aborted embryos or those lost in miscarriages, or by therapeutic cloning, or in vitro fertilization (IVF).

IVF, used for research purposes, is ethically inacceptable because this method ends up establishing embryos industrialization. However, the law of bioethics allows the use of supernumerary embryos systematically produced during IVF or abortion, and that are no longer the subject of a parental project and with the agreement of the parents. The point is not to create embryos but to use the cells of these spare embryos [72].

3.2. Sources of fetal stem cells

Fetal tissue contains a significant number of stem cells and progenitor cells which makes it useful for certain treatments.

Fetal stem cells are harvested after voluntary termination of pregnancy or after abortion at a later stage (5-9 weeks) than the blastocyst stage. The three most accurate sources of fetal stem cells are amniotic fluid, the placenta and umbilical cord blood. These sources are interesting in that their stem cells are collected in a less invasive way from the fetus [73].

3.3. Sources of adult stem cells

Adult organism always holds stem cells as mentioned above. These cells obtained from adult tissues do not generate any controversy from ethics committees. Research on other tissues that contains stem cells is still in progress [74].

3.4. Therapeutic cloning

Therapeutic cloning is the transfer of nucleus obtained from the patient somatic cell into an enucleated egg cell. Six days after in vitro nuclear transplantation the blastocyst is formed. At this stage, embryonic stem cells can be harvested and cultured.

Somatic cell nuclear transfer products are genetically and immunologically compatible with the nuclear donor which circumvents the problem of rejection. The harvest of stem cells from transnuclear unfertilized oocyte seems to generate fewer ethical problems than harvest of embryonic stem cellsfrom fertilized eggs derived from fertility treatment.

Therapeutic cloning, via the production ofan histologically compatible stem cells, offers considerable therapeutic potential and great promises for regenerative medicine [75].

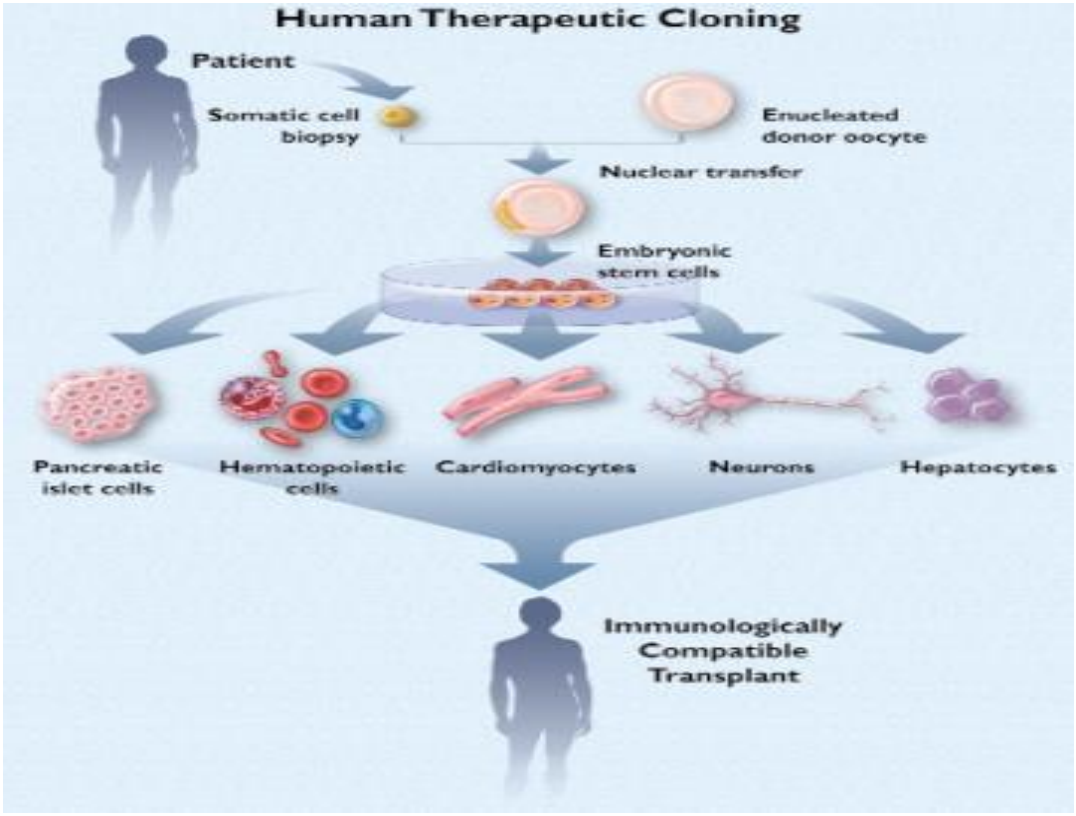

Figure 13: Therapeutic cloning [28].

4. Culture and amplification of stem cells

The establishment of stem cells population follows the rules of animal cell culture. Primary cultures are obtained either by cells migration from explant tissue or by tissue dissociation with proteolytic enzymes followed by culturing the cell suspension obtained. Some cells of the suspension adhere to the plastic of the culture bags, then proliferate under the effect of serum growth factors.

The resulting cell population will proliferate exponentially performing a limited number of cumulative population doublings (CPDs) of primary cells until reaching the crisis phase. This phase is characterized by a slow down (presenescence) and then a cessation of proliferation (senescence) of cell population. The maximum number of CPDs, or hayflick's limit, attained by cells in culture before reaching the crisis phase depends on the species, the tissue/organ and especially on the age of the donor.

In fact, the younger the donor, the more CPDs number of a certain cell type is high. For stem cells cultures, if the culture conditions required to maintain the stem cells population are met, then a high CPDs number could be recorded without major signs of senescence. If, on the other hand, the culture conditions are not optimal, passage after passage, the number of stem cells within the population decreases [76, 77].

4.1. Optimal conditions for culture

Optimal culture conditions have to be defined and standardized in order to maintain reproducible cultures in various laboratories. Furthermore, the cells have to maintain a stable karyotype and phenotype under these conditions, as well as the ability to differentiate.

❖ **Plating density**

Plating density is a critical parameter to ensure an optimal cell expansion as well as preserving the cells potential to differentiate. Prockop's team has shown that most stem cells develop at low seeding densities. Increasing the seeding density per unit area can decrease the cells expansion rate [78].

❖ **Passage numbers**

The passage number is a simple manner to reduce the samples volume collected from the donor. Each passage increase the culture surface area, the cell passage number is a technique based on cells dissociation by the action of a proteolytic enzyme (trypsin), then replating these cells on a new culture dish at lower density, the successive passages are enough to ensure stem cells purification because of the elimination of other initial adherent cells (macrophages, endothelial cells). This technique has its limits, the passages numbers affect the cells differentiation potential and can lead to cell senescence [79].

❖ **Culture medium and growth factors**

Stem cells end use is important to define the appropriate medium for the culture and growth factors for the desired application [78].

4.2. Stem cells' culture limits

▪ **Cell senescence**

Senescence is the sum up of the cell mistakes in biosynthesis mechanisms. Senescence is an irreversible phenomenon. The senescent cells phenotype was described as the nuclear DNA replication machinery that is unable to complete the neostrands synthesis at telomeric ends of chromosomes, cycle after cycle,

The telomeres at the ends of chromosomes shorten slightly. The shortening of these chromosomes is seen by the cell as a genotoxic stress.

Telomeres shortening and other DNA damages (point mutations, double-strand DNA breaks…) lead to the activation of p53 tumor suppressor protein (proto-anti-oncogenes family). The p53 protein will stop the cell cycle in the G1 phase to allow the DNA repair systems to correct the damage before the chromosomes replication. If the damage is severe, the long-term p53 protein overexpression will end the cell cycle definitively and leads to cell senescence or even cell apoptosis [80].

- **Cell transformation**

Cells proliferation contributes to the accumulation of DNA mutations which lead to the cells transformation. There are tests that allow to assess the degree of cell transformation. These tests should be applied on secondary cultures in which the presence of stem cells is assumed. The amplification of stem cells in vitro leads to their transformation and alters stem cells properties [81].

➢ Hayflick limit

The experiments should be done on culture before the crisis phase, for which the number of CPDs is evaluated according to the passages number. Therefore, a three phases curve can be developed, phase I named primary culture, phase II when cells proliferate exponentially (secondary culture). After doubling for a certain period of time the cells reach phase III called senescence, where cell division stops [82].

Hayflicklimit [83].

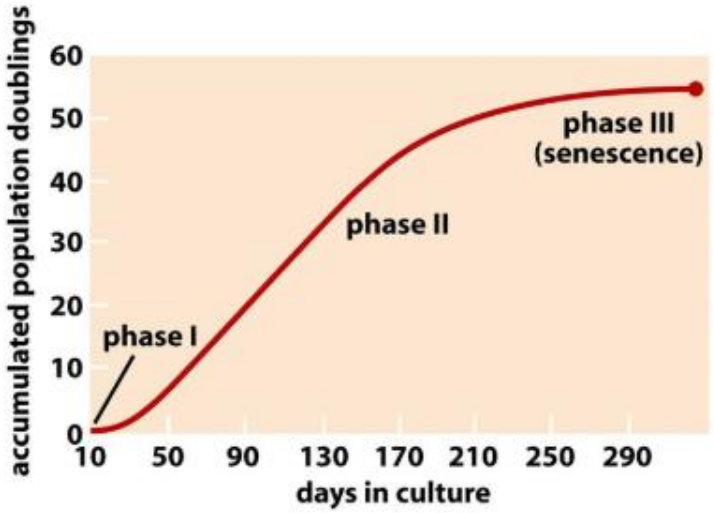

Figure 14: Hayflick limit [83].

➢ pH 6 beta-galactosidase activity

Senescent cell cultures can be identified by using beta-galactosidase activity at pH 6 as a cell marker. This enzymatic activity is identified by a classic method using cytochemical staining with X-Gal. The higher the number of cells displaying this activity, the more senescent the culture will be [84].

➢ Sensitivity to cell cycle regulators

Growth factors independence*;* normal cultured cells proliferate in response to growth factors in the serum until these cells reach confluence. These growth factors, via the generated signal by receptors tyrosine kinase activity, allow the cells to proliferate through G1 phase of cell cycle. Experimentally, there is a test in which the serum in the culture medium is either reduced or removed. If the cells continue to proliferate under these conditions, then the existence of oncogenic mutations through gain of function mutations is assumed within cell population [85].

Loss of contact inhibition; contact inhibition is a process through which cell growth is arrested when cells come in contact with each other. Normal cells stop dividing when these cells form a monolayer in a culture dish. This inhibition involves intracellular signal cascades starting from these proteins (cadherin and selectin) and ending with proteins repressing cell proliferation. If the cells continue to reproduce after confluency (which is characterized by formation of a multilayered cell foci), the presence of mutation such as loss of anti-oncogenic function mutations can be assumed [86].

- Karyotype

Stem cells secondary culture has to be normal, therefore cells must a normal euploid karyotype with 2n chromosomes. The most relevant test is the realization of karyotype by COBRA-FISH (Combined Binary Ratio FISH) technique, which is a multicolor fluorescence in situ hybridization (FISH) that enables to identify all human chromosome arms based on colour, and as a result facilitating cytogenetic analysis. This technique enables to identify small-scale anomalies [87].

- In vivo tumorigenesis

Tumorigenicity tests enable us to verify the capacity of a cell population transplanted into an animal model (irradiated NOD/SCID mice or RAG mice) to form a tumor at the site of transplantation by proliferation or by metastasis at a distant site [88].

4.3. Culturing adult stem cells

Adult stem cells proliferate rapidly in culture while preserving their properties. This is the case of neuronal, epidermal and mesenchymal stem cells. Certain stem cells do not have this capability, either because these cells lose their properties while dividing (hematopoietic stem cells), or because these stem cells proliferate slowly in vitro (muscle stem cells).

However, it is challenging to produce a proper culture medium to the proliferation of these cells, as well as storing them, but research is still in progress to solve all these problems [89].

4.4. Culturing embryonic stem cells

First case: producing human embryonic stem cells cultures.

Stem cells are obtained from the inner cell mass of a human blastocyst, culture conditions should be appropriate for:

i. *The undifferentiated state of embryonic stem cells*: LIF (leukemia inhibitory factor) is used to maintain these stem cells in an undifferentiated state. LIF is capable of suppressing stem cells differentiation. These cells culture is realized on a fibroblasts monolayer whose mitosis is suppressed, which enables to plate these cells on a culture dish and to maintain them in an undifferentiated state.

ii. *Embryonic stem cells proliferation*, by fulfilling all these cells nutritional requirements.

Second case: producing differentiated cells from embryonic stem cells.

Embryonic stem cells differentiation can be directed through specific

modification of culture conditions. Various selective growth conditions can be used to direct ESC differentiation towards a desired cell type [90, 91].

5. Stem cells banking

Cell banks are a storage centers for cell lines and embryonic, fetal and adult stem cells samples. A large number of cells will be required for future clinical applications and for that the establishment of stem cells banks will be needed. Cell banking involves the preservation of a cells stock in the state that these cells were originally obtained. These banks must guarantee the availability, quality and safety of the stocked cell products, especially when the stored cells are for clinical use. Cell banks enable to overcome the immunological barrier for human embryonic stem cell lines, covering a wide spectrum of possible human leukocyte antigen (HLA) combinations of genotypes [92].

These banks may one day be able to replace organ banks and solve the problem of organ shortage for transplantation. The first cell bank has opened in the United Kingdom in May 2004.

5.1. Bank of embryonic stem cells

Embryonic stem cell bank is a mainstay of stem cell research. It provides essential resources to sustain research advances in this field that promises large-scale cell and tissue therapy. It is important to produce good quality and well characterized embryonic stem cell lines that will be available for both clinical and research purposes. Furthermore, the cell lines that exist are exchanged free of charge between laboratories in the framework of research programs under strict legal controls.

The first source of human embryonic stem cells could be obtained from IVF clinics that freeze additional embryos after fertility treatmentand implantation. Despite the fact that the exact number of these spare embryos is unknown, more than 60,000 couples go through IVF treatment each year in the United States, and more than 400,000 embryos are frozen for possible future use [93].

5.2. Bank of fetal stem cells

Fetal stem cells can be isolated from different tissues and fluids including umbilical cord blood, amniotic fluid, and placenta. Fetal stem cells are a potential source of autologous and allogeneic cells for various therapeutic applications in cell therapy and regenerative medicine.

Cord blood contains hematopoietic stem cells and mesenchymal stem cells, that are similar to cells found in bone marrow and which is why these cells are used to repair certain degenerative diseases of bone marrow. Cord blood is collected from the umbilical cord and placenta after the cordis cut from the newborn.

There are approximately 134 private cord blood stem cells banks around the world with at least 240,000 units. These private banks in Europe are strictly forbidden in France, Italy and Spain. However, in these countries cord blood collection is allowed. The blood is then sent abroad, where the isolated stem cells are stored. Public cord blood stem cells banks stock free donated cord blood. There are about 54 public banks around the world with 230,000 units [94, 95].

5.3. Bank of adult stem cells

Adult stem cells have a great therapeutic potential inhuman care, however there are many challenges concerning the quality and safety of clinical applications and a significant of these challenges are related to the processing and banking of these stem cells. It is known that there is a competition between bone marrow and umbilical cord blood banks [96].

Currently, it is possible to produce embryonic and adult stem cells for clinical use under appropriate conditions for safety. Now, the challenge is to conduct clinical trials which will enable the validation of indications.

III. Applications of cell therapy

Cell therapy has demonstrated success in various clinical applications. However, the complex structure and function of cells, tissues and organs limit the perspectives [97].

Tableau 1 : The main applications of cell therapy [97].

Stem cellstransformedinto	Applications
Specialized nerve cells (neurons, glial cells…)	Parkinson disease, Alzheimer disease and other neurodegenerative diseases, spinal cord injury, multiple sclerosis ...
Cardiomyocytes (cardiac muscle cells)	Myocardial infarction (heart attack), heart failure, cardiomyoplasty in heart defects.
Insulin-producing cells (islets of langerhans)	Diabetes
Chondrocytes (cartilage cells)	Arthritis, osteoarthritis
Blood cells	Cancer, immunodeficiencies, leukemia, genetic blood disorders.
Hepatocytes (livercells)	Acute or chronic hepatitis, cirrhosis, liver cancer.
Skin cells	Burns, woundhealing
Bone cells	Bone loss (tumors, metastases), bone fractures, osteoporosis.
Retinalcells	Age-related macular degeneration, hereditary blindness.
Skeletal muscle cells	Muscular dystrophy, amyotrophies, muscle wasting from various causes ...

1. Cell therapy on the skin

The skin ensures a number of important functions for the organism survival; it provides a protective barrier against external aggressions. The skin is constantly engaged in various processes such as temperature regulation and water balance, signal perception, production and activation of hormones, neuropeptides and cytokines.

The skin is composed of three main layers; epidermis, dermis and hypodermis. The outer superficial layer is the epidermis which is thin and stratified and the major cell components of this layer are keratinocytes. Keratinocytes ensure keratinization due to their ability to differentiate starting in the basal layer. As keratinocytes differentiate and migrate towards skin surface, they become anucleated and form a clustered keratin, then they flatten and die.

The layer beneath the epidermis is the dermis which forms a thick layer composed mainly of the connective tissue and the extracellular matrix. Last but not least is the hypodermis or the subcutaneous layer which is formed by connective tissue and adipocytes. The hypodermis protects the internal organs and tissues from trauma and cold, provides energy and takes part in the synthesis of hormones.

Severe burns are treated by autologous skin grafting which is based on removing skin from a place and transplanting it into another place on the same individual. However, this esthetic technique is challenging in the treatment of skin injuries with limited donor sites for skin graft harvesting. To overcome the autograft shortage, cell therapy has become an interesting solution for extensive skin injuries through autologous epidermal sheets grafting or skin equivalents prepared in vitro.

Cells are the principal part of tissue-engineered skin used to treat skin injuries. They include stem cells and somatic cells and that can be divided into two main groups: autologous and allogeneic.

Keratinocytes stem cells and dermal fibroblasts are common cells used in products for burn injuries healing. Keratinocytes are the main cell component of the epidermis and are the cause of its stratified structure and form many tight intercellular junctions. Fibroblasts are the major cell type in the dermis and produce extracellular matrix components and secrete various growth factors, matrix metalloproteinases and cytokines, which ensure the formation of extracellular matrix and the proliferation and differentiation of keratinocytes. In 1980s, scientists developed techniques to proliferate human keratinocytes in vitro with fibroblasts feeder cells to form epidermal sheets that can be transplanted into individuals suffering from extreme burn injuries. Since then, cultured human keratinocytes and epithelial autograft sheets became the most used cell products around the world. Cultured epithelial autografts can be used as a sheet or in suspension.

Epidermal stem cells are of particular interest for the regeneration of skin tissue because they have a high rate of reproduction and easy access, and retain their potency and differentiation potential for long periods of time. Non-cultured autologous epidermal cells for treating wounds are a quick procedure that was reported. It consists of extracting epidermal cells from a small skin sample via enzymatic digestion, making a basal cell-enriched suspension and distributing the cells over the granulated wound area to ease permanet wound closure or to correct the loss of pigmentation.

Mesenchymal stem cells (MSCs) and induced pluripotent stem cells (iPSCs) have proved to have great potentials in healing both acute and chronic wounds and repairing the skin. MSCs including bone marrow – mesenchymal stem cells and adipose derived stem cells have a great differentiation potential and a certain plasticity degree and could give rise to mesodermal, ectodermal and endodermal cell lines. These cells can migrate to the damaged tissues, differentiate and regulate the regeneration of the tissue throught the production of cytokines, growth factors and chemokines. However, in tissue regeneration the greatest interest belongs to iPSCs; using somatic cell reprogramming enables to develop patient-specific cells with an adapted phenotype and apply them in clinics.

Epidermal cell suspensions and cultured keratinocytes promote wound healing and epithelialization, but have shown limitations in the treatment of deep burn wounds which lack dermal foundation.Artificial dermal regeneration models are used for dermal regeneration in the treatment of deep burns. These templates are porous bio-scaffolding structures formed by biodegradable polymers including proteoglycans and proteins.They offer structural support and a niche matrix for cell attachment, migration and reproduction.The scaffold guided co-culture of various skin cells synergistically improve the production of cytokines and bio-factors that promote wound healing [98, 99, 100].

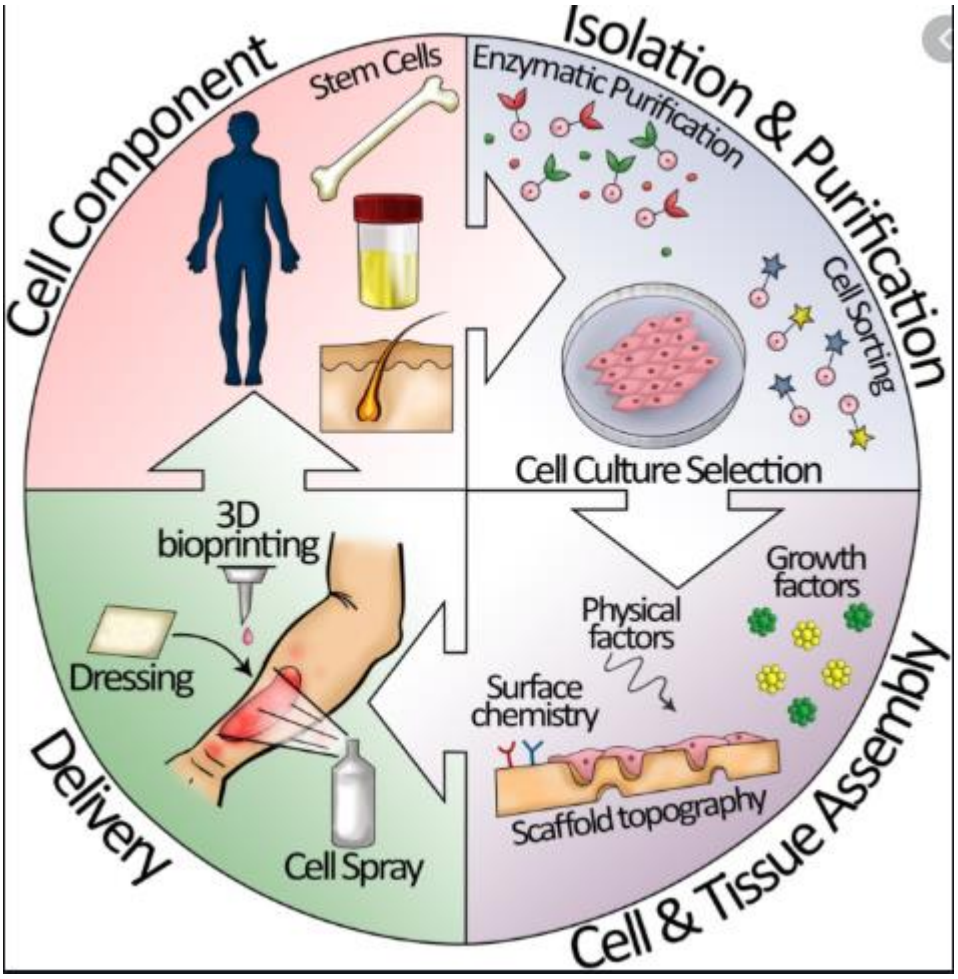

Figure 15: Autologous stem cell-based therapy on burn injuries [101].

2. Cell therapy and Parkinson's disease

Parkinson's disease is a common neurodegenerative disorder that affects one in 100 individuals over age 60. The disease is characterized by a motor syndrome which consists of bradykinesia, muscle rigidity and tremor. The disease is caused by a loss of midbrain dopaminergic neurons which results in a reduced amount of dopamine produced, which functions as a messenger between the brain parts that manage voluntary and involuntary movements. As a result, in the absence of this vital connection, the brain begins to lose the ability to control movements. It is believed that environmental factors, as well as genetic factors, could be responsible for nerve cells loss. Pharmacological treatments aiming to reduce Parkinson's disease motor symptoms involve the use orally of L-DOPA (L-3, 4-dihydroxyphenylalanine) and dopamine receptor agonists, and deep brain stimulation in globus pallidus and subthalamic nucleus by surgically implanted electrodes. These treatments have shown to be effective to some extent, but they could result in adverse effects, like L-DOPA-induced dyskinesias, and they do not prevent disease progression [102].

In the 1980s and 1990s, series of studies in patients suffering from Parkinson's disease with human fetal ventral mesencephalic tissue intrastriatal grafts have demonstrated the principle that cell therapy can be effective in patients with Parkinson's disease, and that dead dopamine neurons can be substituted with new neurons by transplantation. The grafts could provide striatum reinnervation and symptomatic relief.The most successful surgical cases have been able to withdraw from L-DOPA treatment. However, the further advancement of this approach was hampered by the occurrence of adverse effects, known as graft-induced dyskinesias, in a subset of patients. Due to

shortcomings with tissue availability and grafts standardization, it is doubtful whether transplantation of human fetal ventral mesencephalic tissue will become a mainstream therapy for Parkinson’s disease. In this respect, cell replacement therapy using stem cells represents a promising alternative therapy [103].

Embryonic stem cells are extremely proliferative and remain pluripotent after long periods of in vitro expansion.Since they can produce any cell type in the organism, including dopaminergic neurons, their potential to be used in a clinical setting appears to be great. Dopaminergic neurons derived from rodent and human embryonic stem cells have been shown to be able to survive transplantation into the striatum of Parkinson’s disease rats and to induce some degree of functional recovery. However, studies have demonstrated that the survival of dopaminergic neurons derived from embryonic stem cells post-transplantation is relatively low. The use of dopaminergic neurons derived from embryonic stem cells for transplantation in patients with Parkinson's disease poses a major problem ; the risk of side effects such as tumour formation, which has been reported in rats. Prolonged differentiation or Cell sorting and therefore depletion of undifferentiated cell pools in vitro prior to transplantation would potentially lower the risk of tumour formation.

Another source of stem cells is reprogrammed adult fibroblasts called iPSCs, then differentiated to dopaminergic neurons. The iPSC technology has opened up the possibility of generating an infinite source of Patient-specific dopaminergic neurons, which could theoretically be used for autologous transplantation. The dopaminergic neurons were first developed from mouse iPSCs, grafted into the striatum of a rat Parkinson’s disease model and showed to improve functional deficits. Lately, dopamiergic neurons have also been

generated from iPSCs derived from fibroblasts of adult human and patients with Parkinson's disease. These neurons have survived transplantation into the striatum of rodents with Parkinson's disease and have shown some degree of functional recovery. However, the risk of tumor formation as with embryonic stem cells must be minimized before dopaminergic neurons derived from iPSCs can be considered as a transplantation option in a Parkinson’s disease clinical setting. In addition, it is doubtful whether dopaminergic neurons that are delivered by autologous transplantation in Parkinson’s disease would be more sensitive to the pathology of the disease because genetic mutations may also be present in fibroblast derived cells.

Fetal brain neural stem cells derived dopaminergic neurons have a reduced risk of tumor formation and immune rejection than embryonic stem cells. Studies demonstrated that undifferentiated neural stem cells implanted into Parkinson’s disease primates were able to survive, migrate and have a functional impact. A limited number of neural stem cells progeny differentiated into dopamine phenotypes.

Mesenchymal stem cells and stromal cells derived from bone marrow present a potential cell sources for Parkinson’s disease transplantation. Studies have reported that undifferentiated murine mesenchymal stem cells are capable to differentiate into tyrosine hydroxylase positive neurons and enhance motor symptoms in mice. It has also been reported that cells with dopaminergic properties can be generated from rat and human mesenchymal stem cells, and that transplantation of these cells resulted in improvement of motor fonctions in an animal model of Parkinson’s disease. Recently, a clinical trial in patients with advanced Parkinson’s disease using unilateral transplantation of autologous

mesenchymal stem cells derived from bone marrow into the sublateral ventricular site showed modest clinical improvement without side effects like tumor formation [104, 105].

If these therapeutic strategies for cell replacement and regeneration prove to be successful in restoring normal nervous system functions, they could also be applied to stroke, spinal cord injury, cancer and other degenerative diseases.

3. Cell therapy for heart failure

Heart failure, also called congestive heart failure, occurs when the heart does not pump blood sufficiently to preserve blood to meet the organism's needs.Heart failure is becoming a major public health problem and, with increasing longevity, will become the leading cause of morbidity and mortality in the 21st century. In many cases, heart failure occurs as a result of a heart attack (myocardial infarction). Anoxia, followed by the development of major inflammatory sites, leads to the death or destruction of the adult cardiomyocytes responsible for the myocardial contractility. Heart failure can also occur in the absence of an acute ischemic event, some forms are of genetic origin (monogenic), mutations alter the function of proteins involved in the structure of the contractile apparatus or cell (myosin-binding protein C, myosin chains, dystrophin, sarcoglycan…).

Heart transplantation remains the most effective treatment for the most advanced forms of heart failure, but this approach is challenging with the limited number of grafts available, and many complications, particularly immunological as well as the clinical status of the patient. Alternative approaches developed, most of them tend to modify the shape of the dilated ventricle.Ventricular assist devices have improved durability and have enabled prolonged support for

patients waiting for heart transplant. In this respect, the concept of cell therapy has been developed over the last ten years by a large number of research teams involving clinicians and biologists [106].

The goals of cell therapy are numerous and not exclusive. The transplantation of cells should result in the formation of a tissue with greater functional capacity than that of the injured site. The transplanted cells must be less immunogenic; the autologous transplant option would be preferred. The first clinical trials were reported in the early 1990s; the approaches are multidisciplinary, involving cell biology, cardiology, surgery, immunology, imaging, physiology, molecular biology, histology [106, 107].

Regardless of the cell type examined, certain questions need to be resolved concerning the fundamental, technical and clinical aspects of the approach.

- ***Selection of animal models***

Both small and large appropriate animal models need to be developed. Ischemia could be induced by coronary ligation (permanent or transient), embolization or cryogenic. There are also animal models with heart failure of genetic origin (Syrian hamster developing sarcoglycanopathy, Golden Retriever dog model of Duchenne muscular dystrophy, transgenic mice). Cardipathies can alsobeinduced by injection of anthracyclines [106].

- ***Monitoring the fate of transplanted cells***

The selection of appropriate methodologies and markers to trace the fate of the implanted cells is crucial. Classical histology staining techniques provide morphological information. The detection of antigen-specific antibodies is used when the transplanted cells are from a different origin than the recipient heart

tissue. Metabolic labelling (tritiated thymidine, BrDU [5-bromodeoxy-uridine]), but these markers can be diluted during cell division. In addition, cells derived from transgenic animals expressing a particular transgene (e.g. beta-galactosidase) under the control of a promoter activated during cardiac differentiation could be used [106].

- ***Assessment of cardiacfunction***

This should be done before and after transplantation and compared with control animals. Several methodologies have been developed for small and big animal models, allowing the quantification of multiple parameters ex vivo (The Langendorff heart or isolated perfused heart) and in vivo (echocardiography, Doppler, sonomicrometry, tomoscintigraphy, nuclear magnetic resonance, PET-Scan [positron emission tomography]...) [106].

- ***Administration Protocols***

In most cases, especially in small animals, cell administration is achieved by the trans-epicardial route. Other catheter injection protocols are under validation [106].

There are different types of stem cells that could be used to enhance problems resulting from cardiac ischemia.

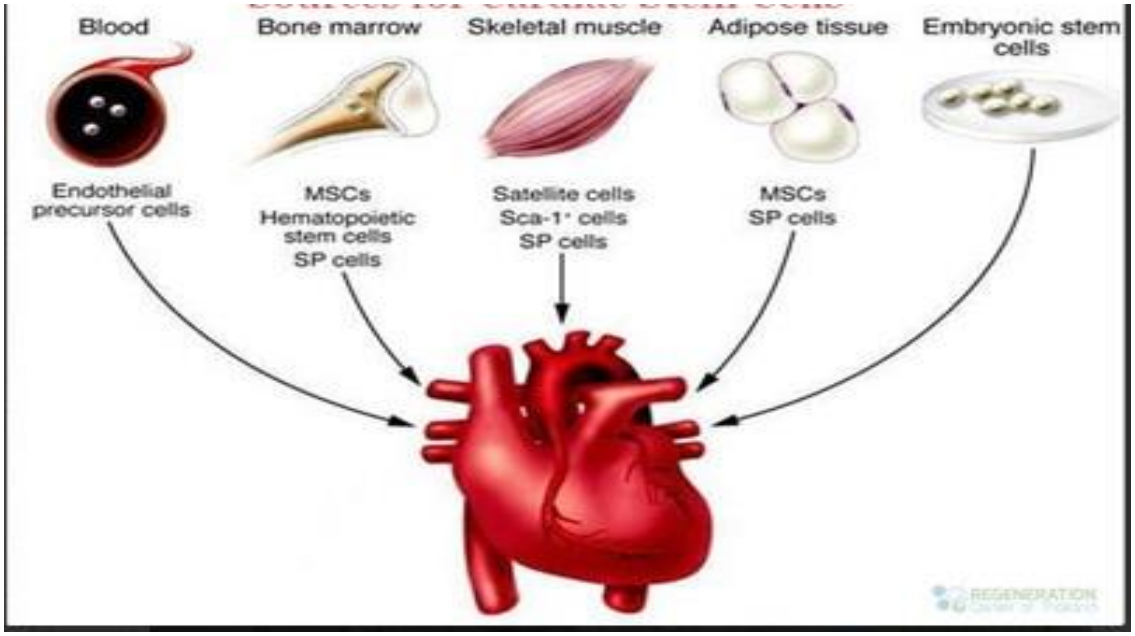

Figure 16: Sources for cardiac stem cells [108].

- ✓ ***Embryonic stem cells***

Embryonic stem cells have been considered a possible source of heart cells for a long time ago. In vitro, these stem cells are the source of many cell and tissue types, and special conditions have been developed for the preparation of cardiomyocytes. The resulting cells present similar phenotypic characteristics to those of adult cardiomyocytes, however an arrhythmogenic risk has been reported. The first in vivo studies of embryonic stem cell transplantation showed a development of teratomas, the transplantation of embryonic stem cells induced in vitro towards differentiation into cardiomyocytes and rigorously selected could make it possible to overcome this obstacle. The question of the expected immunological reactions due to the allogenicity of these cells is discussed, as they are not known to be very immunogenic.Research for such biotherapeutic

developments is achieved by using human embryonic stem cells in countries where it is legally permitted [106].

- ✓ ***Fetal or neonatal cardiomyocytes***

Fetal or neonatal cells were used in the transfer experiments because adult cardiomyocytes do not proliferate in vitro. Fetal or neonatal cardiomyocyte implantation has been used by many groups. Transplanted cells are able to integrate within the recipient tissue and, depending on the case, to form communicating junctions with resident cells, when these are accessible, or to gather in isolated clusters within the fibrous tissue.These cells have the ability to contract and to generate action potentials. They improve the function of the damaged myocardium in animal models of myocardial ischemia or toxic cardiomyopathy.

However, the use of fetal cells in a clinical context poses problems in terms of immunology (allografts), in terms of quantity, since the mass of fetal tissue that would be used to repopulate an adult myocardium must be large, and finally in terms of ethics, which is a real limitation that encourages the search for alternative sources [106].

- ✓ ***Adult stem cells***

Adult stem cells are found in bone marrow, skeletal muscle, blood, adipose tissue, and human's heart, so harvesting these stem cells for clinical application could be more straightforward.

- *Hematopoietic stem cells*

Bone marrow consists of differentiated cells such as lymphocytes, adipocytes, monocytes, fibroblast, chondroblasts, osteoblasts and osteoclasts, as

well as a small but diverse group of undifferentiated stem cells. Hemangioblasts are responsible for angiogenic (CD34+, CD31+...) and hematopoietic (CD34+, CD133+, CD45+, c-Kit+...) cells, which are precursors of blood and immune system cells. Hematopoietic stem cells promote angiogenesis and cardiogenesis. The injection of these cells into an infarcted myocardium leads to the formation of endothelial and cardiac cells and a spectacular regeneration. However, the use of purified cell population obtained from transgenic animals expressing marker genes under the control of a cardiac promoter demonstrated that these cells were unable to deviate from their hematopoietic lineage after intramyocardial implantation.

The injection of certain bone marrow cell populations (CD34+, CD133+) led to functional improvement (increased perfusion, improved contractility), without any histological characterization. In some cases, the approach could be deleterious: for example, certain cell types could promote endothelialization of large vessels, and lead to restenosis. The intracoronary route of administration chosen in some protocols could lead to the occurrence of microembolism, which is responsible for new ischemic events. Not all injected cells have the same ability to differentiate into cardiomyocytes. The CD34+ cells that present the best cardiomyogenic and angiogenic potential are not very present compared to the other cell types injected.

Current assays intend to isolate cells which have the best transdifferentiation potential, and to find the least harmful route of administration [106].

- *Mesenchymal stem cells*

Depending on culture or environmental conditions, mesenchymal stem cells derived from bone marrow, differentiate into osteoblasts, chondroblasts, myoblasts, adipoblasts, fibroblasts. Several studies have suggested that mesenchymal cells could differentiate into cardiomyocytes, either in vitro in the presence of 5-azacytidine, or in vivo after implantation in an infarcted myocardium (although in a very limited proportion).

The stromal fraction of adipose tissue, in vitro gives rise to cells with an automatic contractile activity, expressing several genes specific to cadiomyocytes. Adipose tissue could provide an impotant source of cells that canbe used in an autologous manner.

- *Endothelial cells*

Rather than restoring contractile function directly, some groups have attempted to restore or increase local blood perfusion by inducing neoangiogenesis. Endothelial precursors, contained in bone marrow and blood, can be prepared and amplified in vitro. The injection of these cells into ischemic cardiac sites increased blood perfusion and thus the use of dormant cardiomyocytes located in proximity to this zone. Studies have suggested a differentiation of endothelial cells into cardiomyocytes, in vitro by contact with fetal cardiomyocytes, or in vivo by direct injection into cardiac muscle.

- *Smooth muscle cells*

Smooth muscle cells have been studied for their spontaneous contractile properties. Injected into animal models, these cells improve cardiac function. However, the use of these cells is challenging when it comes to the accessibility of biopsies needed to prepare the cells [106, 109].

- *Skeletal myoblasts*

The regenerative capacity of skeletal muscle is well established. Every mature skeletal muscle fiber holds a small number of undifferentiated and non-active satellite cells called myoblasts. Skeletal myoblasts stay in a quiescent state till the muscle fiber is injured. These cells reproduce rapidly and fuse with each other and with the damaged muscle cell, re-establishing continuity of muscle fiber.

Skeletal myoblasts have the ability to differentiate and proliferate into muscle fibers, as well as replace and regenerate dying or damaged skeletal muscle cells. These cells are widely available, able to withstand ischemia, and are relatively easy to harvest, which make them a potentially suitable transplantation cell candidate.

On the flip side, under normal conditions, skeletal muscle cells are not able to perform repetitive, high-frequency, and synchronous contraction which are the hallmark of heart muscle function. Therefore, the major clinical problem with skeletal myoblasts is that these cells remain electrically and mechanically disconnected from the host myocardium.

In the multicenter clinical trials, 97 individuals received injections of autologous native cultured myoblasts into the epicardial surface and around the infarction site at the time of coronary artery bypass grafting.After a 4-year monitoring period, skeletal myoblasts implantation into the scar tissue of myocardium was believed to have resulted in some beneficial effect as shown by increased fluorodeoxyglucose (FDG) on PET scan, by an increase in tissue viability area by MRI (Magnetic Resonance Imaging), or by improving left ventricular ejection fraction on echocardiogram. However, the development of ventricular arrhythmias has raised a significant safety concern.

These studies demonstrated that cell cardiomyoplasty with skeletal myoblasts is moderately effective in preventing degradation of ventricular geometry and cardiac function in ischemic cardiomyopthy clinical trials. This therapy has shown limited clinical potential and is restricted by the risk of arrhythmias.

Cardiovascular disease and heart failure requires dedicated and inspired innovative physicians to develop new evidence-based knowledge to enhance short and long term clinical results and reduce the relentless march from heart failure to death [109].

4. Cell therapy for type I diabetes

Type I diabetes, also known as juvenile diabetes or insulin-dependent diabetes, this disease usually appears in children or adolescents. It is characterized by polyuria and polydipsia.

Type I diabetes is an autoimmune disease that results in destruction of insulin-producing β cells of islets of langerhans in the pancreas. Therefore the pancreas produces little or no insulin which is a hormone that enables glucose to enter cells in order to produce energy and therefore lowers the amount of glucose in bloodstream. Type I diabetes treatment involves insulin injections which provide patients with their daily insulin requirements. However, in some diabetic patients, insulin injections do not effectively control blood sugar levels, resulting in hyperglycemic or hypoglycemic episodes and related complications [110].

Alternatively, pancreatic islets transplantation appears to be more attractive. The success of Edmonton protocol for islets of langerhans transplantation and the development of non-invasive imaging of islet transplantation by using positron emission tomography have raised a new interest in insulin-producing cells transplantation. Nevertheless, the availability of donor islet tissue is limited and will only be able to treat a small portion of insulin-dependent diabetes patients.

A lot of research is being conducted in order to find a less restrictive treatment. Stem cell therapy offers a promising vision to treat type I diabetes [111].

The use of stem cells as a potential source of insulin-producing β cells solves the shortage problem of pancreas donors in clinical islets transplantation.

❖ ***Embryonic stem cells***

Embryonic stem cells have a significant therapeutic potential to produce a large number of cells with insulin-expressing phenotype for β cells replacement therapy. Kroon and al. Demonstrated that human embryonic stem cells derived pancreatic endoderm developed into a mature functional β cells in vivo and preserved against induced hyperglycemia by streptozotocin a few months after implantation. However, the use of insulin-producing cells derived from human embryonic stem cells in clinical trials is limited because of many ethical and scientific considerations. Recent studies have proved the possibility of identifying endodermal cells derived from embryonic stem cells by using cell surface markers. Kelly and al. Have used anti-CD142 antibodies to enrich the fraction of pancreatic endodermal cells, which have differentiated into all pancreatic lineages, with the functional insulin-producing cells included. Similarly, a four step, 14 day differentiation protocol has been developed for in vitro differentiation of commercially available human embryonic stem cells into a highly enriched Pdx1 population of pancreatic progenitor cell. Transplantation into diabetic mice led to a functional restoration and maturation of normoglycemia. However, the need to ensure the complete suppression of undifferentiated cells still remains, as does the uncertainty that the differentiated cells can revert to pluripotent state. Technologies like cell encapsulation are in progress which can theoretically allow transplanted encapsulated insulin-producing cells to function and survive without the need for immunosuppressive therapy [112].

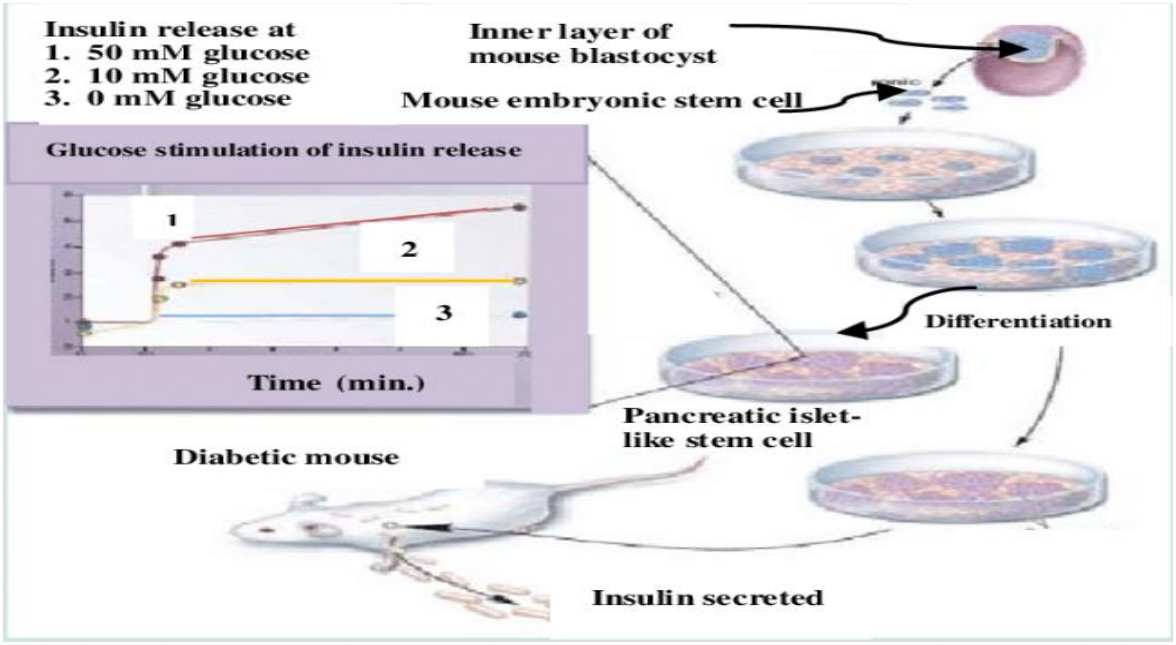

Figure 17: Production of insulin-secreting pancreatic-like cellsfrom mouse embryonic stem cells [113].

❖ *Induced pluripotent stem cells (iPSCs)*

iPSCs might as well be considered an appropriate source for the production of large numbers of β-cells from a nonembryonic autologous source, overcoming islet transplantation limitations. Reversal of hyperglycemia by using pancreatic β-like cells derived from induced pluripotent stem cells has been demonstrated in type 1 and 2 diabetes mouse models. iPSCs produced by reprogramming epithelial cells derived from pancreas of nonobese diabetic mouse have shown to give rise to insulin-producing cells that expressed different pancreatic β-cells markers and have normalized hyperglycemia following transplantation in diabetic mice. These cells have also been derived by reprogramming dermal fibroblasts from type I diabetes patients. However, iPSCs clinical usefulness is impeded by such factors like chromosomal

aberrations, incomplete differentiated cells maturation, and tumorigenic and or oncogenic potential. Different strategies are being trialed to circumvent viral vectors use. These involves the use of nonintegrating vector systems, like synthetic modified mRNA for Klf4, Oct4, c-Myc, and Sox2, nonintergratingepisomalcontrusts, transducible proteins, DNA minicircles, and small chemical agents in order to improve reprogramming and replace functionally some reprogramming factors. Nevertheless, regardless of the success of these strategies autologous induced pluripotent stem cells remain the subject to autoimmune response after transplantation. Recently, a study with a significant clinical utility has described has reported a new approach to induce iPSCs to produce transforming growth factor β and IL-10 secreting regulatory Tcells (Tregs) which are able to suppress host immune responses when transferred to mice [112].

❖ ***Adult stem cells***

Adult stem cells could have the capability to enhance and possibly even reverse type I diabetes effects. The possibility to generate β cells from isolated adult stem cells solve ethical problems related to the use of embryonic stem cells.

Recent study showed that mesenchymal stem cells are able to differentiate into insulin-producing cells in pancreatic islet in vitro and in vivo. After 38days of co-culture, these stem cells differentiated into insulin-producing cells, identified by secreted insulin and C-peptide staining.

Multipotent adult progenitor cells (MAPCs) are rare progenitor cells found in bone marrow that are able to differentiate into mesodermal, endodermal and ectodermal lineage cells both in vitro and in vivo. These cells present potential sources for islet cells, however the lack of reliable cell markers and testing system has made it difficult to characterize and validate such cells [112, 114].

Finally, cell therapy for type I diabetes represents a potential alternative to daily insulin injection, therefore improving the lifestyle conditions of diabetic patients.

5. Cell therapy and cancer

Cancer is the most common and dangerous disease, causing millions of deaths in the world. The treatment depends on cancer progression and type. The first option is surgery in order to remove directly solid tumors that are located in one area. Radiotherapy could kill tumors through damaging DNA of cancer cells. Chemotherapy helps to stop or slow down tumors growth by using very toxic drugs. Immunotherapy includes the use of monoclonal antibodies, adoptive cell transfer, and cancer vaccines. However, the major drawbacks of current therapies are inadequate and unspecific target to tumor sites besides for effector chimeric antigen receptor cells (CAR), which results in sub-optimal efficacy, resistance to therapy and later tumor recurrence. In the meantime, stem cell therapy has offered a great hope in the fight against cancer [115].

Human embryonic stem cells and iPSCs; both are a potential sources for the induction of T and NK effector cells and for production of anticancer vaccines.

Adult stem cells; can generate different specialized cell types of tissues and organs. This group includes mesenchymal stem cells, hematopoietic stem cells, and neural stem cells which are often used in cancer treatment.

Cancer stem cells; also called tumor cells immature progenitors or stem-like cells, are induced via epigenetic mutations in normal stem cells or progenitor cells. These cells are found in tumor tissues and play an important role in cancer growth and metastasis. Thereby, targeting cancer stem cells could offer a promise to treat different types of solid tumors [116].

Different strategies have been developed for treatment of cancer through stem cell therapy.

- ✓ ***Hematopoietic stem cells transplantation***

Hematopoietic stem cells transplantation has been mainly used as a standard process for treatment of leukemia, multiple myeloma, and lymphomas after a series of high dose chemotherapy or radiotherapy which results in damaging leukocytes and blood forming cells. This procedure is now widely studied in clinical trials combined with immunotherapy and chemotherapy, to treat other types of cancer, such as breast cancer, brain tumors, and neuroblastoma. Nevertheless, the development of graft versus host disease upon the use of allogeneic sources of hematopoietic stem cells stills a challenge [117].

- ✓ ***Mesenchymal stem cells transplantation***

The treatment of aggressive tumors involves high dose therapy which often results in damaging hematopoietic system and normal tissues. Studies have demonstrated that mesenchymal stem cells infusion helps to maintain hematopoietic stem cells undifferentiated state and proliferation, therefore

improving the treatment outcome. Furthermore, mesenchymal stem cells with immunomodulatory effects can reduce immune responses in patients with graft versus host disease. Mesenchymal stem cells have also been found to facilitate healing of damaged organs and can enable the body to tolerate high dose chemotherapy that is to enhance tumor-killing effects [118].

- ✓ ***Stem cell carriers***

Genetically modified stem cells; stem cells, including neural stem cells and mesenchymal stem cells, are transduced virally to increase the expression and secretion of prodrug converting enzymes or tumor toxic cytokines and chemokines. The first system is known under the name of « suicide gene therapy ». Enzymes released from stem cells can transform prodrugs into active molecules that are more toxic to cancer cells. For example, the infusion of cytosine deaminase expressing mesenchymal stem cells and neural stem cells converts 5-fluorocytosine into tumor toxic 5-fluorouracil. The expression of suicide gene and cytotoxic gene in stem cells have been proven to reduce tumor growth.

Nanoparticles carraying stem cells; nanoparticles have been used as carriers of anti-cancer medicines for a long time. However, their lack of targeting effect, rapid excretion from the organism, and uncontrollable absorption by normal cells are the major disadvantages. Interestingly, stem cells can be effective vehicles for transporting nanoparticles to tumor sites. In this approach, nanoparticles can be uplaoded into cells or conjugated to the cell surface.

Oncolytic viruses-carrying stem cells; oncolytic viruses could cause tumor cells lysis, and emit danger signals in order to stimulate immune system to kill tumor cells more effectively. However, naked oncolytic viruses could be easily identified by immune cells and eliminated rapidly from the organism. Stem cells offer potential carriers to protect and transport oncolytic viruses to tumor sites. However, stem cell carriers effectiveness depends on the relationship between tumor cells and vehicle cells as well as their therapeutic effects. For example, mesenchymal stem cells and neural stem cells have been found to promote viral replication, but a number of viruses can be released from neural stem cells [115].

Exosomes derived from stem cells as therapeutic carriers; exosomes were used to encapsulate different anti-cancer therapeutical materials, such as proteins, mi-RNA, and small drugs. Exosomes have many advantages over other synthetic nanoparticles, including unique stability, biocompatibility, high loading capacity, and better internalization into tumor cells. Furthermore, these natural carriers can easily functionalize with specific ligands and proteins on their surface to improve targeting effect to tumors microenvironment. Studies have demonstrated that stem cell derived exosomes are able to suppress tumor growth of myeloma, leukemia and pancreatic adenocarcinoma cell lines.

Viral transfection is an efficient and a common technique of modifying stem cells for gene delivery carriers. Nevertheless, this technique introduces the risk of a viral infection to patients [115].

- ✓ ***Immunotherapy***

Stem cells are a potential source for the production of immune cells. Chimeric antigen receptor T cells (CAR) and natural killer (NK) were

successfully utilized for anti-cancer immunotherapy. These clinical immune cells are often taken from the same patient, activated, genetically transduced with chimeric antigen receptor constructs, expanded, and finally reinfused into the patient. However, controlling the quality and quantity of those immune cells for immunotherapy is still challenging, especially in patients who have received heavy chemotherapy or who are older. Additionally, in vivo antitumor activity of these chimeric receptor immune cells is usually restricted because of their rapid differentiation to short lived effector cells. Human pluripotent stem cells, involving embryonic stem cells and iPSCs, can provide unlimited sources for the generation of chimeric antigen receptor.

It is interesting that the induction of chimeric antigen receptor on hematopoietic stem cells provides more benefits in cancer treatment. Implanted chimeric antigen receptor-hematopoietic stem cells would be grafted in bone marrow and produce different immune cells chimeric antigen receptor, such as NK cells, T cells, neutrophils, and monocyte. The combined effect of these immune cells could result in stronger immunity to kill cancer [119, 120].

- ✓ ***Stem cells based anticancer vaccines***

Cancer stem cells play an important role on formation and progression of tumors, treatments that target cancer stem cells would dramatically enhance therapeutic effectiveness in the battle against cancer. Among different approaches that target cancer stem cells, the production of anticancer vaccines offers a great promise due to their strong immunogenicity. Anticancer vaccines can be produced from oncofetal peptides or whole cells based on cancer stem cells/ embryonic stem cells/ iPSCs. The single use of vaccines based on oncofecal peptide can not offer significant immune responses toward tumors

because of tumor heterogeneity and fast escape mechanisms. To date, the isolation of a small population of cancer stem cells from tumors tissues is still challenging despite the fast progress in the identification of cancer stem cells, which hampers the use of these cells as a vaccine source.

In the meantime, vaccines developed by embryonic stem cells/iPSCs-derived various antigens could provide more applications for treating cancer. The possibility of teratomas formation and autoimmunity induction are the major concerns. Whereas, xenogeneic source, autologous iPSCs, and allogeneic embryonic stem cells based vaccines have been found to be more effective inpreventing tumor recurrence in mice, as demonstrated by increased immune cells proliferation and production of cytotoxic cytokine [115].

To conclude, in spite of the success in preclinical and clinical trials, the challenges of stem cell therapy need to be overcome. More experiments should be conducted to shed light on stem cells signaling on tumors growth and metastasis in specific conditions, therefore choosing an appropriate strategy to engineer stem cells [116].

Fourth Part: The Ethical, Legal, and Religious Aspects of Stem CellResearch

Stem cells offered great hope and optimism as an alternative to organ and tissuetransplants and the problem of graft rejection. However, alongside this hope, human stem cell research has also drawn up ethical, legal, and religious controversy.

I. The Ethical Context

In science, ethics aim to shed light on various possible dangers and to indicate the objectives and the progress that can be achieved through experiments. Ethics provide a statute of scientific limitations and prevent dangerous or reckless applications of science, but ethics must also support what is reasonable and necessary in the field. This is difficult to apply in reality since the problem is where to set these limitations.

There are five fundamental ethical principles:

- The principle of respect for human dignity
- The principle of individual autonomy, which requires informed consent, and respect for privacy and confidentiality of personal data.
- The principle of justice and beneficence more specifically, from the perspective of improving and protecting health.
- The principle of research freedom, which must be reconciled with the other fundamental principles.
- The principle of proportionality, especially the fact that the research methods are indispensable to the objectives pursued and that there are no other acceptable methods [121].

Therefore different ethical debates arise from different stages of stem cell harvesting. However, it is possible that these fundamental ethical principles be altered at certain stages of research more than others.

1. For the embryonic stage

Human embryonic stem cell research is a highly debated topic because harvesting these cells involves the destruction of embryos.

Two opposing ethical positions could be distinguished in human embryonic stem cell debate. First, those who are totally against research with stem cells derived from human embryos. According to their opinion, embryos have the same moral status as individuals and therefore have to be protected as individuals. They promote adult stem cell research and other research strategies as alternatives.

Second, there is the stance that young in-vitro embryos, irrespective of their origin, can be utilized for scientific research with the condition that embryos used in the experiments will not be implanted in the uterus after (unless the research has a therapeutic value for the embryo and the resulting person). The main requirements concerning human embryonic stem cellresearch are related to control and safety, proportionality, commercialization, and issues of informed consent. In Europe, the United Kingdom, Belgium, and Sweden have regulations that are in line with this point of view [122].

On the other hand, most countries adopt a middle stance. This intermediate position has two principal variants; one makes a distinction between the use of human embryonic stem cells and their derivation from embryo in vitro, and one between the use of spare in vitro fertilization embryos for research and the use

solely created embryos for research. Within these two points, there can be variations [123].

2. Fetal stem cells samples

Pluripotent stem cells may be obtained from fetal tissues after abortions. However, the use of fetal tissues is ethically controversial as it is related to abortion, a highly contested topic. Under federal regulations, research using fetal tissues is permitted on the condition that tissue donation for research is considered only after the decision to interrupt the pregnancy was made. This requirement reduces the chance that a woman's decision to terminate pregnancy may be affected by the perspective of contributing tissue to research [124].

3. Post-natal stage

Adult stem cells do not raise any major ethical or legal concerns and are widely used in scientific research and clinical care. This is particularly relevant in the context of autologous cell therapy. However, it is necessary to have a protocol that has the approval of the committee for the protection of each individual. When stem cells are harvested in allogeneic circumstances, the ethical issues raised are similar to those during the donation of human body parts. They essentially involve the free and informed consent ofthe donor as well as respect for their anonymity. From the perspective of the recipient, the first thing to consider is the issue of intervention and the safe harvesting of organs. It can be seen as a right of the patient [121].

4. Therapeutic cloning

Therapeutic cloning implies primarily the creation of embryos out of any parental project, which is against fundamental ethical principles. Some people

who oppose this technique believe that the creation of embryos in order to use them in research and to destroy them in the process violates respect for nascent human life. Even people who support obtaining stem cell lines from frozen embryos, which could otherwise be disposed of, sometimes object to the creation of embryos in order to be used for research. However, some people argue that pluripotent entities produced by somatic cell nuclear transfer are ethically and biologically distinct from embryos [124].

Cloning can raise major ethical concerns if it falls in the drift of reproductive cloning. Another issue concerning cloning is the possibility of commercializing oocytes. In fact, since the cloning technique requires oocytes, there is a risk of falling into oocyte trafficking, through the payment and or exploitation of female donors. This risk exists particularly for women in developing countries [125].

II. The Legal Context

1. Legal situation in Morocco

The only known point of concern regarding stem cells is related to the use of umbilical cord blood which implies a necessary revision to the organ donation law.

The law n° 16-98 related to organ donation does not permit altruistic donation, even if free. Nevertheless, collecting organs from a brain-dead individual is permitted for a future organ bank. It remains that the collection of umbilical cord blood, which is considered to be “surgical waste” and that it is ex-vivo manipulation and non-related administration, is simply forbidden even if the therapeutic need is well justified (especially in siblings with families with a history of hematological malignancies) [126].

2. Legal situation in European countries

Among the European Union, the convention on human rights and biomedicine was signed in 1997 in Oviedo, Spain. Article 18 of this convention states that it is up to each country to decide whether or not to authorize embryonic stem cell research. Each country is required to respect two conditions: “To ensure appropriate protection of the embryo,” which means to adopt a legislation that sets up the conditions and limits of embryos research and “To forbid the creation of human embryos for research purposes.”

Each country has adopted different regulating legislation:

- Ireland is the only member of the European Union whose constitution affirmed the right to life of the unborn fetus and that this right is equivalent to that of the mother.
- In certain member states there is no legislation on embryonic stem cell research. This is true of the Netherlands and of Belgium, where embryonic stem cell research is nevertheless performed. However, in Portugal, in the absence of legislation, no embryonic stem cell research appears to be carried out. This also appears to be the case in Italy despite the fact that artificial reproductive methods are widely practiced.
- In countries where embryonic stem cell research is regulated by legislation, which either forbids all kinds of embryonic stem cell research (i.e. Germany, Austria), or permits this research under certain conditions (i.e. Spain, Finland, Sweden, and the United Kingdom). In France, for example, embryonic stem cell research remains forbidden, however the law allows embryo study without prejudicing the integrity of embryos as well as pre-implantation diagnosis.

- In other countries, the law allows the possibility of creating embryos by nuclear transfer in order to be used in stem cell research, as is the case in Belgium and the United Kingdom. In the United Kingdom, legislation authorized the creation of embryos for research purposes, but limited their use to infertility treatment, contraception, or the prevention of genetic diseases [127].

3. Legal situation in United States

The situation in the United States differs from that of Europe. A fundamental difference is a clear distinction between the public and the private sectors.

Since 1995 the United States congress has adopted a provision each year in the appropriation Bill to forbid public funding for embryonic stem cell research. Therefore, the national institutes of health (NIH) cannot perform embryo research, which, in the absence of legislation, persists, unregulated in the private sector.

In 1998, new discoveries concerning stem cell cultures led to the reopening of the debate. The national bioethics advisory committee (NBAC) published a report in September 1999; hearings were held in 1999 and 2000 before the relevant committees of the United States Congress and lastly the Clinton Administration. This presidential administration suggested that, under certain conditions, the funding of research to obtain and study human embryonic stem cells could be authorized. The NIH released new guidelines in August 2000 according to which, human embryonic stem cell research could be publicly funded if two conditions are met. Firstly, the cells have to be harvested from frozen spare embryos from fertility clinics that are already destined to be

disposed. Secondly, federal funding cannot be used to destroy embryos to derive the cells; privately funded researchers will be required to pass them on to federally supported scientists [127].

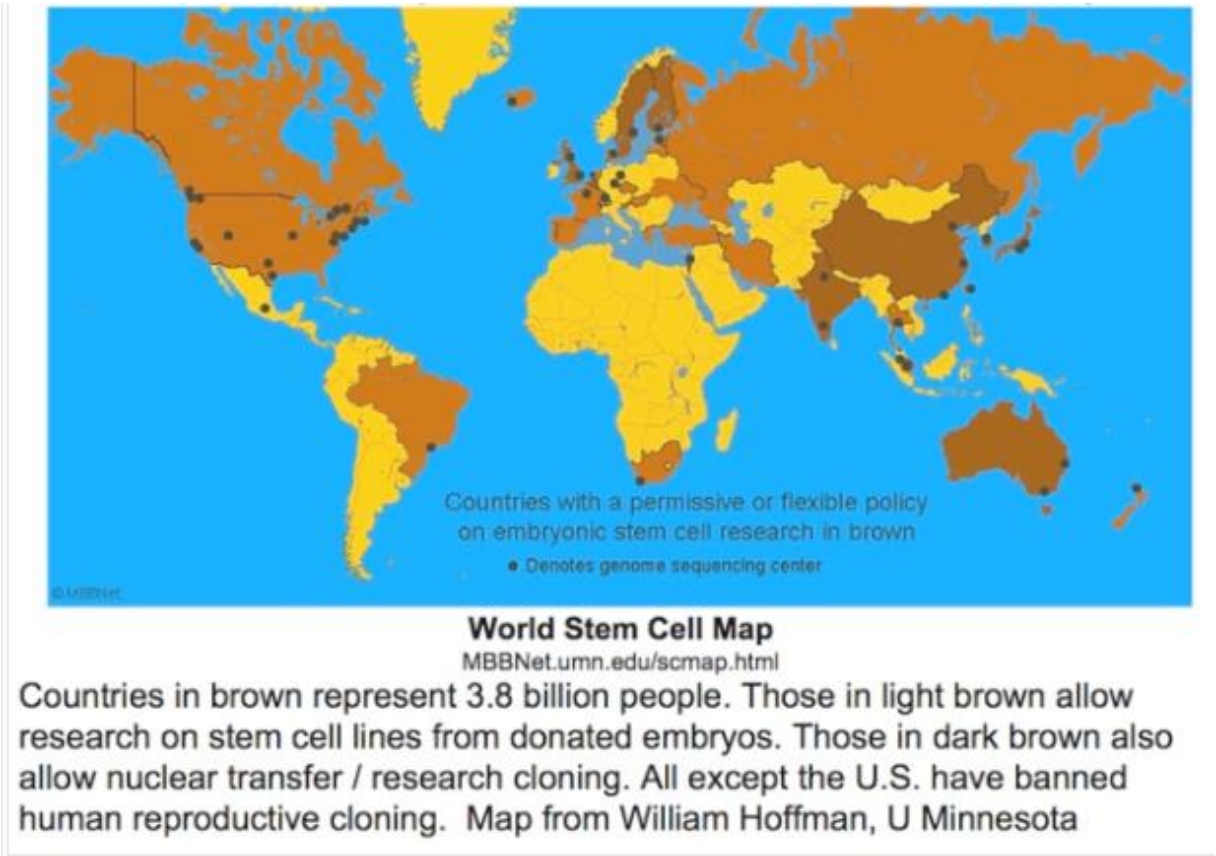

Figure 18: Human embryonic stem cells research policy [128].

III. The Religious Context

The perspectives of the three Abrahamic religions - Islam, Judaism, and Christianity - will be discussed in relation to stem cell research.

1. Islam

Islam has always encouraged scientific research, especially research directed towards developing cures for human diseases.

That being said, the sanctity of human life takes precedence in the approach to the embryo. This concept is based on the principle that life is a gift from God and that we are responsible for preserving it and not harming it.

Muslim scholars have introduced texts that supplement the Quran and the Hadith (according to the word of the Prophet Mohammed), in order to avoid any set guidelines and to allow the believer to adopt an evolutionary position in the face of new issues related to biotechnology. However, Muslims have to face these dilemmas and form their own decisions. Therefore, Muslims' attitude towards the topic remains delicate regarding bioethical issues.

Muslim scholars forbid harvesting stem cells from human embryos directly from the womb, since this leads to the embryo's death. However, they accept when stem cells are derived from spontaneous abortions or induced ones to save the mother's life that the embryo cannot be saved. Nonetheless, for spare embryos resulting from in vitro fertilization and generally from medically assisted procreation, the scholars' position differs. Some believe that these embryos should not be touched and should be left to their fate. While others think that if Islam allows the mother "trunk," in extreme need, to lose the child "branch," it would be better to sacrifice "branches," here spare embryos, to

allow "trunks" (thousands of patients) to heal. Furthermore, Muslim scholars prohibit the use of embryonic stem cells derived from embryos created by therapeutic cloning because it is a deviation of the egg from its natural route [129].

2. Judaism

In Judaism, the human being is considered in its unity, in other words, the body and the spirit form an inseparable unit. Respect of life is therefore sacred and absolute. Human life has an infinite value because it is a gift from God and man is made in the image of God. However, the Talmud – the central religious text - determines that the status of human being does not exist during fertilization. It is acquired only when the genetic materials are implanted in the womb which occurs until the fortieth day of gestation. Therefore, the fetus cannot be considered a human being in its own right. An embryo outside the womb has no legal status.

Consequently, this religion does not systematically prohibit the use of therapeutic cloning as long as it is beneficial and is considered under strict control. Embryos conserved in vitro without the prospect of implantation could be donated and used for research for therapeutic purposes. Such use would be in accordance with the primary obligation in Judaism, that is, to save a life [127].

3. Christianity

The strongest opposition to the use of embryos for research and therapeutic purposes arises in the Christian tradition, specifically within Catholicism. According to the bible, life is a sacred and an inviolable gift from God. Every Pope has consistently affirmed respect for nascent life from conception. Since

human being (in the process of becoming and not completed) exists upon fertilization, the embryo is considered a whole individual who has the right to life.

Therefore, the Catholic Church places a preponderant value on the sanctity of mankind in its unity of body and spirit. Thereby, every embryo must have the opportunity to develop and reach full term. This implies that egg cell fertilization in vitro must be strictly controlled and that the use of spare embryos for therapeutic purposes is not permitted. The embryo would then be treated as a pure research material [130, 131].

After discussing the ethical, legal, and religious aspects of stem cell research, it is difficult to know to what extent human embryonic stem cell research should be regulated. In fact, there are a number of arguments both for and against the use of embryos, especially for therapeutic purposes.

Conclusion

After decades of experiments, stem cell therapy has become a great game changer for medicine. Each experiment, stem cells capabilities are growing, even though there are still several obstacles to overcome. However, the significant impact of stem cells in transplantology and regenerative medicine is immense.

The translation of stem cells and regenerative medicine to clinical use raises sharp ethical, policy and religious issues. However, understanding these issues would help to place emerging scientific advances into proper context and to assure the appropriate clinical translation for promising therapeutics.

Currently, non treatable neurodegenerative diseases have the chance to become treatable through stem cell therapy. The induced pluripotency allows the utilization of the patient's own cells. Tissue banks are getting more and more popular as they collect cells which are the source of regenerative medicine in the fight against present and future disorders and diseases. Stem cell therapy and its regenerative advantages enable the prolongation of human life than at any other time in history.

Summaries

Abstract:

Title: Stem cells and cell therapy

Author: Drissi Jihane

Rapporteur: Pr. Ibrahimi Azzedine

Key Words: Cell culture, Stem cells, Cell therapy.

Stem cells are defined by two major characteristics, the ability to self-renew continuously and the capacity to differentiate into specialized cell types. These stem cells are classified into pluripotent, multipotent, totipotent, and unipotent which can either be of embryonic, fetal or adult origin.Stem cells offer great potential for tissue regeneration and repair.

Clinical applications using cultured stem cells have occurred for over 30 years and can help scientists and patients identify and expedite promising pathways for future therapies. There are three types of stem cells that are commonly developed in culture. Embryonic stem cells, adult stem cells and induced pluripotent stem cells.

Recently, stem cell therapy is becoming a very promising and advanced topic of scientific research. Cell therapy is based on cell's transplantation. The principle of this therapy involves collecting cells which are either directly reinjected into the patient or in most cases undergo some manipulations in the laboratory (sorting, amplification, selection or depletion of certain cell populations).

Stem cell therapy offered great hopes in treatment of disorders and diseases that can not be treated yet. However, stem cell therapy has to overcome many challenges to be accepted worldwide. Based on the studies conducted in this field, stem cells have found numerous applications in the treatment of diseases, such as skin injuries, heart failure, type I diabetes, Parkinson's disease, and cancer. A large variety of possibilities has made this cutting-edge therapy a major shift in modern medicine, offering hope for untreatable diseases.

Résumé

Titre: Les cellules souches et la thérapie cellulaire

Auteur: Drissi Jihane

Rapporteur: Pr. Ibrahimi Azzedine

Mots clés: Culture cellulaire, Cellules souches, Thérapie cellulaire.

Les cellules souches sont définies par deux caractéristiques majeures, la capacité à s'auto-renouveler continuellement et la capacité à se différencier en cellules spécialisées. Ces cellules souches sont classées en cellules pluripotentes, multipotentes, totipotentes et unipotentes, qui peuvent être d'origine embryonnaire, fœtale ou adulte. Les cellules souches offrent un grand potentiel de régénération et de réparation des tissus.

Les applications cliniques utilisant des cellules souches mises en culture existent depuis plus de 30 ans et peuvent aider les scientifiques et les patients à identifier et à accélérer des voies prometteuses pour de futures thérapies. Il existe trois types de cellules souches qui sont couramment cultivées. Les cellules souches embryonnaires, les cellules souches adultes et les cellules souches pluripotentes induites.

Récemment, la thérapie cellulaire est devenue un sujet de recherche scientifique extrêmement prometteur et avancé. La thérapie cellulaire est basée sur la transplantation de cellules souches. Le principe de cette thérapie consiste à recueillir des cellules qui sont soit directement réinjectées dans le patient, soit, dans la plupart des cas, soumises à certaines manipulations en laboratoire (tri, amplification, sélection ou épuisement de certaines populations cellulaires).

La thérapie cellulaire offre un grand espoir dans le traitement des troubles et des maladies qui ne peuvent pas encore être traités. Cependant, la thérapie cellulaire doit relever de nombreux défis pour être acceptée dans le monde entier. En se basant sur les études effectuées dans ce domaine, les cellules souches ont trouvé de nombreuses applications dans le traitement de maladies telles que les blessures de la peau, l'insuffisance cardiaque, le diabète de type I, la maladie de Parkinson et le cancer. Un large éventail de possibilités a fait de cette thérapie de pointe un changement majeur dans la médecine moderne, offrant un espoir pour les maladies incurables.

الملخص

العنوان: الخلايا الجذعية والعلاج بالخلايا

المؤلف: الإدريسي جيهان

المشرف: أستاذ إبراهيمي عز الدين

الكلمات المفتاحية: زراعة الخلايا ، الخلايا الجذعية ، العلاج بالخلايا

يتم تعريف الخلايا الجذعية من خلال خاصيتين رئيسيتين ، القدرة على التجديد الذاتي باستمرار والقدرة على التمايز إلى أنواع خلايا متخصصة. يتم تصنيف هذه الخلايا الجذعية إلى متعددة القدرات، وكاملة القدرة ، وحيدة القدرة والتي يمكن أن تكون إما جنينية أو من أصل بالغ. توفر الخلايا الجذعية إمكانات كبيرة لتجديد الأنسجة وإصلاحها

توجد التطبيقات السريرية التي تستخدم الخلايا الجذعية المستنبتة لأكثر من 30 عامًا ويمكن أن تساعد العلماء والمرضى على تحديد المسارات الواعدة للعلاجات المستقبلية وتسريعها. هناك ثلاثة أنواع من الخلايا الجذعية التي يتم تطويرها بشكل شائع في زراعة الخلايا. الخلايا الجذعية الجنينية والخلايا الجذعية البالغة والخلايا الجذعية المستحثة متعددة القدرات

في الآونة الأخيرة ، أصبح العلاج بالخلايا الجذعية موضوعًا واعدًا ومتقدمًا للغاية للبحث العلمي. يعتمد العلاج بالخلايا على زرع الخلايا. يتضمن مبدأ هذا العلاج تجميع الخلايا التي يتم حقنها مباشرة للمريض أو تخضع في معظم الحالات لبعض التلاعب في المختبر (الفرز والتضخيم والاختيار أو استنفاد مجموعة خلايا معينة).

قدم العلاج بالخلايا الجذعية آمالًا كبيرة في علاج الاضطرابات والأمراض التي لا يمكن علاجها بعد. ومع ذلك ، يجب أن يتغلب العلاج بالخلايا الجذعية على العديد من التحديات ليتم قبولها في جميع أنحاء العالم. بناءً على الدراسات التي أجريت في هذا المجال ، وجدت الخلايا الجذعية تطبيقات عديدة في علاج الأمراض ، مثل إصابات الجلد ، وفشل القلب ، ومرض السكري من النوع الأول ، ومرض باركنسون ، والسرطان. مجموعة كبيرة من الاحتمالات جعلت هذا العلاج المتطور تحولًا كبيرًا في الطب الحديث ، مما أعطى الأمل للأمراض التي لا يمكن علاجها

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Serment de Galien

Je jure en présence des maîtres de cette faculté :

D'honorer ceux qui m'ont instruite dans les préceptes de mon art et de leur témoigner ma reconnaissance en restant fidèle à leur enseignement.

D'exercer ma profession avec conscience, dans l'intérêt de la santé publique, sans jamais oublier ma responsabilité et mes devoirs envers le malade et sa dignité humaine.

D'être fidèle dans l'exercice de la pharmacie à la législation en vigueur, aux règles de l'honneur, de la probité et du désintéressement.

De ne dévoiler à personne les secrets qui m'auraient été confiés ou dont j'aurais eu connaissance dans l'exercice de ma profession, de ne jamais consentir à utiliser mes connaissances et mon état pour corrompre les mœurs et favoriser les actes criminels.

Que les hommes m'accordent leur estime si je suis fidèle à mes promesses, que je sois méprisée de mes confrères si je manquais à mes engagements.

قسم الصيدلي

بسم الله الرحمان الرحيم

أقسم بالله العظيم

أن أراقب الله في مهنتي

أن أبجل أساتذتي الذين تعلمت على أيديهم مبادئ مهنتي وأعترف لهم بالجميل وأبقى دوما وفيا لتعاليمهم.

أن أزاول مهنتي بوازع من ضميري لما فيه صالح الصحة العمومية، وألا أقصر أبدا في مسؤوليتي وواجباتي تجاه المريض وكرامته الإنسانية.

أن ألتزم أثناء ممارستي للصيدلة بالقوانين المعمول بها وبأدب السلوك والشرف، وكذا بالاستقامة والترفع.

أن لا أفشي الأسرار التي قد تعهد إلى أو التي قد أطلع عليها أثناء القيام بمهامي، وأن لا أوافق على استعمال معلوماتي لإفساد الأخلاق أو تشجيع الأعمال الإجرامية.

لأحضى بتقدير الناس إن أنا تقيدت بعهودي، أو أحتقر من طرف زملائي إن أنا لم أفي بالتزاماتي.

والله على ما أقول شهيد.

المملكة المغربية
جامعة محمد الخامس بالرباط
كلية الطب والصيدلة
الرباط

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الخلايا الجذعية والعلاج بالخلايا

أطروحة

قدمت ونوقشت علانية يوم: / / 2020

من طرف:

السيدة الإدريسي جيهان
المزدادة في 19 شتنبر 1995 بسلا

لنيل شهادة

دكتور في الصيدلة

الكلمات الأساسية : زراعة الخلايا - الخلايا الجذعية - العلاج بالخلايا

أعضاء لجنة التحكيم :

السيدة نعيمة علامي أوهابي أستاذة في البيولوجيا الجزئية والكيمياء الحيوية	**رئيسة**
السيد إبراهيمي عز الدين أستاذ في التكنولوجيا الحيوية الطبية	**مشرف**
السيدة نعيمة الحافظي أستاذة في طب الأطفال	**عضو**
السيد جواد الحارثي أستاذ في الكمياء العلاجية	**عضو**