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Stem Cell Therapies

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1. Introduction

Both development and adult homeostatic maintenance of different tissues and organs are dependent on the activity of a specific cell type named stem cell (SC). In recent years, SCs has been tested for clinical therapies against complex diseases. For many of these diseases, SC treatments offer a glimmer of hope. Fortunately, in some other instances, these therapies are an interesting reality.

In this chapter, we aim to introduce the readers to the nature and diversity of SC. We will describe their origin and location in the human body and the main SC therapies used in clinical practice. Finally, we will propose a standard goal of current applications to convince readers about future avenues in these treatments.

2. Stem cells

2.1 Concept and types

A stem cell is an undifferentiated cell that is able to proliferate, giving rise to various types of differentiating cell lineages. Through unequal divisions, SCs may predominantly give rise to two different cells: one SC and one progenitor cell which continues cell division to finally initiate cell differentiation. These are the two inherent features of the lineages of these cells, self-renewal and differentiation. Depending on their location and un-differentiation state, these cells are able to generate/restitute specific tissues, organs or complete embryos (Figure 1). This capacity is named potency. A stem cell shows a high potency when many different cell lines can be obtained in vitro or in vivo. SCs show reduced potency when very few cell lines can be obtained.

According to this potency concept, SC can be classified into the following groups:

Totipotent stem cells: Cells able to differentiate into any embryonic or extra-embryonic cell line. Blastomeres from zygote to morula are totipotent cells.

Pluripotent stem cells: Cells able to differentiate into any cell line derived from any of the three embryonic sheets. Cells of the Inner Cell Mass, the embryoblast, and cells of the germinal ridges are pluripotent SCs.

Multipotent stem cells: Cells able to differentiate into any cell type derived from only one embryonic sheet. Ectoderm, endoderm or mesoderm stem cells are multipotent.

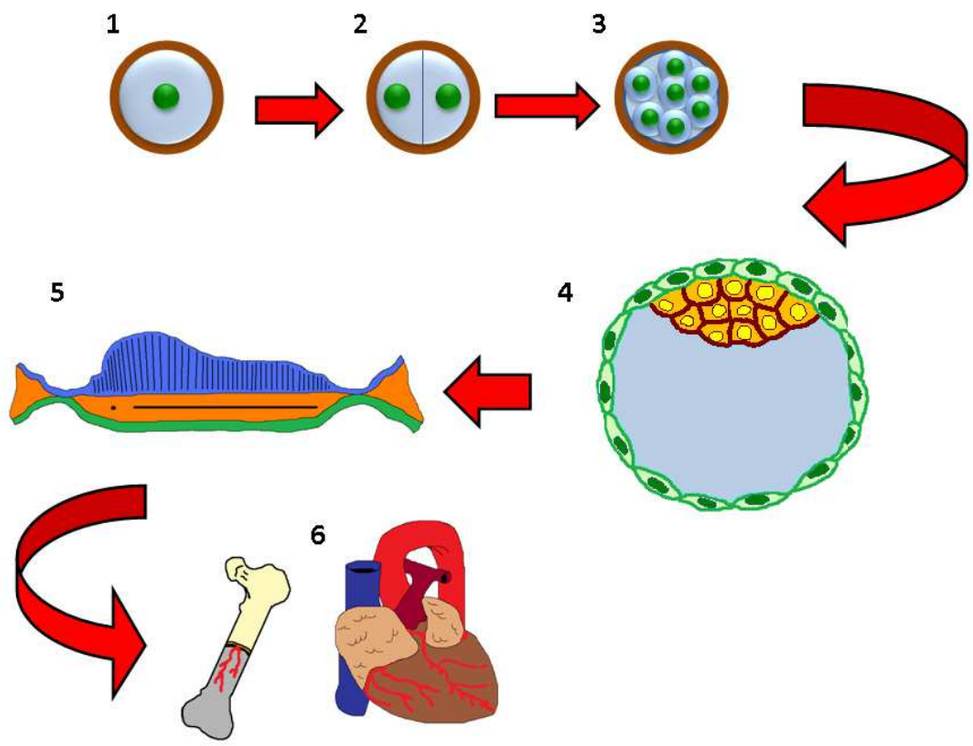

Fig. 1. **Cell potency varies during human embryogenesis and organogenesis**.
The zygote (1) and blastomeres at two cell stage (2) and morula (3) are totipotent cells. Cells in the inner mass of the blastocyst (4) are pluripotent. Trilaminar disc (5) cells are multipotent. Stem cells at heart or large bones (6) are either multipotent or unipotent. Green and yellow cells in the blastocyst respectively are trophoblast and inner cell mass.

Under this classification, the embryo appears as a very important source of many different types of stem cells. Cells of the Inner Cell Mass are named **Embryonic stem cells** (**ESCs,** Thomson et al., 1998) and show a high potency. Besides, **Adult (or Somatic) Stem Cells (ASCs)** are resident cells in adult tissues all along the complete life span. These cells show a smaller potency than ESCs but lesser risks and bioethical problems (see below) during their clinical use.

ASCs are responsible of adult tissue **homeostasis**. This homeostasis is established as a balance between cell death and cell proliferation. A clear example can be found in the skin. Superficial cell layers in the epidermis are continuously dying and being lost. In order to

maintain epidermis stability, basal cell layer continuously proliferate. This proliferation is carried out by a population of epidermal stem cells.

In order to maintain tissue homeostasis, ASC show specific cell cycle properties. These cells are in a quiescent state. Under a wound stimulus, they proliferate and give rise to differentiated cell lineages. Cell proliferation and differentiation are compensated in such a way that new differentiated cells are proportionate to the original induction by wound signals. Following a tissue injury and under new inducing signals released from the wound, ASC leave the quiescent state, proliferate and differentiate to replace damaged or dead cells. This natural process can restore absent or wounded tissues in few days.

Modern Cell Biology technologies have also been able to induce potency in adult differentiated cells. By specific in vitro culturing conditions, differentiated cells can dedifferentiate and proliferate to initiate several newly-differentiating cell lines. These cells are named **induced pluripotent stem cells** (**iPS**, Takahashi et al., 2007). All above-mentioned stem cells can be used in a potential cell therapy.

2.2 Locations and functions

Since the earliest stages of embryogenesis, stem cells can be inferred to occur. Blastomeres in all pre-morula stages are able to generate a complete organism when isolated from the rest. Thus, these blastomeres are the source of totipotent embryonic stem cells. A well-known example of this is both groups of blastomeres isolated to form two monozygotic twins during early development. Both resulting individuals are genetically identical and generate independent chorion, placenta and amniotic cavities. Thus, these monozygotic twins are also diamniotic, dichorionic and diplacental.

Just before implantation, blastocysts form pluripotent stem cells in the Inner Cell mass or embryoblast. At this stage, a first cell commitment can be observed during human embryogenesis, the embryoblast is able to generate a complete organism but not the extraembryonic tissues. During embryoblast formation, the isolation of two inner cell masses also generates two monozygotic and diamniotic twins, but they are monochorionic and monoplacental. These unique structures are generated from a different embryonic tissue named trophoblast.

Following this stage, the inner cell mass further specifies and moves their cells to form the embryonic sheets in two consecutive structures, the bilaminar and the trilaminar discs (see Figure 1). These three embryonic sheets, the ectoderm, the endoderm and the mesoderm, are formed by multipotent stem cells, committed to generate specific cell fates. Stem cells from any of these sheets are unable to differentiate into the typical fates of cells from other sheets (Thomson et al., 1998).

The ectoderm forms the nervous system and the integument. In the adult, a group of ectoderm-derived stem cells can be found in the basal layer of the epidermis, the epidermal stem cells, and in sub-ventricular positions within the central nervous system, the neural stem cells. The former regulate a continuous epidermal renewal, whereas the latter maintain the cellular structure of the nervous tissue against a classical paradigm of nervous proliferative quiescence. Also associated to ectoderm, a derived embryonic source of stem

cells is found in the neural crest. During neural crest fusion, a group of ectodermal cells trans-differentiate into migratory derived cells and generate different cell types all along the body. These cell fates are skin melanocytes, facial bones and sensory neurons among others. This large migratory pathway can provide ectoderm-derived stem cells in peculiar locations such as carotid body, from which stem cells can be obtained to use them in therapies for Parkinson's disease.

On the other hand, the endoderm gives rise to the respiratory and digestive systems. Endoderm-derived stem cells are multipotent and can be found in mucosae basal layer in both systems. These cells show a high cell proliferation rate and are continuously in risk to be affected by carcinogenic reagents. These reasons may have led these systems to show the highest neoplasia frequency of all human organs. In the human liver, another endoderm-derived stem cell type can be found, the oval cells. These cells have been recently shown to be involved in hepatic-regeneration. A number of cell types can be differentiated from these oval cells, such as the hepatocytes or biliary duct cells.

Finally, mesoderm is involved in the formation of many different apparatus or systems: the skeletal apparatus (bones, cartilage, tendons), the vascular system (blood, blood vessels and heart), excretory (nephric systems and kidney) and reproductive (gonads) systems and the whole connective tissue in the organism. Thus, many mesoderm-derived stem cells are also located in many locations in the body to form all these tissues. From a clinical point of view, optimal stem cells are those forming the blood, the haematopietic stem cells in the bone marrow, and mesenchymal stem cells which differentiate into muscle (myoblast), bone (osteoblast), cartilage (chondroblast), fat tissue (adipoblast) or fibroblast progenitor cells. These cell types can be found in any mesoderm-derived tissue. A special type of mesenchymal stem cells is umbilical cord stem cells that can be differentiated into blood or mesenchymal cells. These stem cells from umbilical cord are a handy and bloodless source of highly proliferative stem cells useful for clinical purposes.

2.2.1 Stem cell genetic program

The knowledge of a genetic program for stem cells was initiated by two German embryologists, Theodor Boveri and Hans Driesch. In the late XIXs, both scientists respectively showed that the program was both located inside the nucleus and dependent on cell-to-cell interactions. Hans Driesch also proposed that the early embryo is a harmonic system in which all, or many, cells show the same potency which is dependent on cell-to-cell interactions. Modern Molecular and Developmental Biology are providing important information on the nuclear location of the genetic program and the cell-to-cell interaction-dependence of animal embryogenesis.

Any animal or human cell is also under the control of a genetic program. This genetic program is regulated by a hierarchy of transcription factors that either enhance or silence the activity of a single enzyme named the RNA polymerase II (Figure 2). This enzyme generates a heterogeneous RNA using as a substrate a DNA template in the open reading frame of the gene. This heterogeneous RNA is processed by RNA splicing and editing to generate the messenger RNA. In the ribosome, this messenger RNA is translated into protein. Protein synthesis requires a complete set of other specific proteins and RNAs to either form the ribosome or regulate its activity.

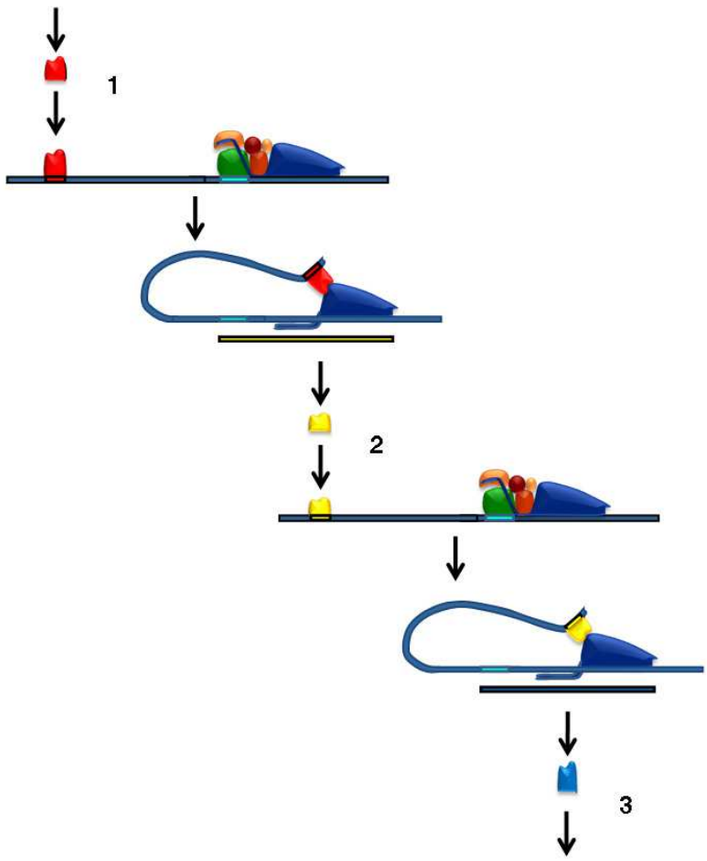

Fig. 2. **A gene transcriptional hierarchy controls cell specification**.
At each stage, blue proteins to the right are RNA polymerase II enzymes. Protein complex in various colors is the general transcription complex. At each stage, isolated color boxes to the left (1 to 3) are specific transcription factors. Longer blue narrow rectangles are DNA strands and shorter yellow and dark blue narrow rectangles are messenger RNAs codifying for transcription factors in the same color. Explanations can be found in the text.

The activity of the RNA polymerase II depends upon two different regulatory protein-complexes, the general and the specific transcription complexes. The latter complexes are formed by the transcription factors (Figure 2) and co-factors. These proteins are able to bind DNA and step-by-step activate new downstream genes. Some of these new genes may codify for other transcription factors that activate further new downstream genes. Some of these transcription factors which act during animal embryogenesis are codified by genes named *Hox, achaete, scute, engrailed, myoD*, *Dorsal*, *cubitus interruptus*, *apterous*, *Pannier* or *Iroquois*. These animal proteins are ordered in a transcriptional hierarchy, the so-called genetic program (Figure 2). This hierarchy is so complex that almost all embryonic cells can be supposed to be different at the transcription level (See Alberts et al., 2007). Finally, this genetic program also transcribes mRNAs codifying for other protein types. These new proteins may be structural or collaborate in the regulation of many cell properties, such as cell division, apoptosis or differentiation. This completes the genetic control of embryogenesis or tissue maintenance.

Two additional features can be observed in animal development, cell-to-cell interaction dependence and *pleiotropism*. In animals, transcription regulation is balanced among all cells. This balanced regulation is able to generate cell diversity, organ size or pattern formation during embryogenesis. By this process, the transcription regulation of a given cell depends upon the transcription in surrounding cells. In order to balance these transcription activities, a number of different signal transduction pathways have been found (see Figure 3). Wnt, Sonic hedgehog, FGFs and BMPs are ligands that activate some of these transduction pathways. This balanced transcription can be traced back to the genome from the mother to regulate follicle cells signaling. Moreover, these proteins can interact with many other proteins in other cells to regulate many cell processes. Thus, the same ligands may *pleiotropically* regulate cell differentiation in ectoderm, endoderm and mesoderm-derived stem cells. The embryonic-sheet specificity is provided by the genetic program of each cell.

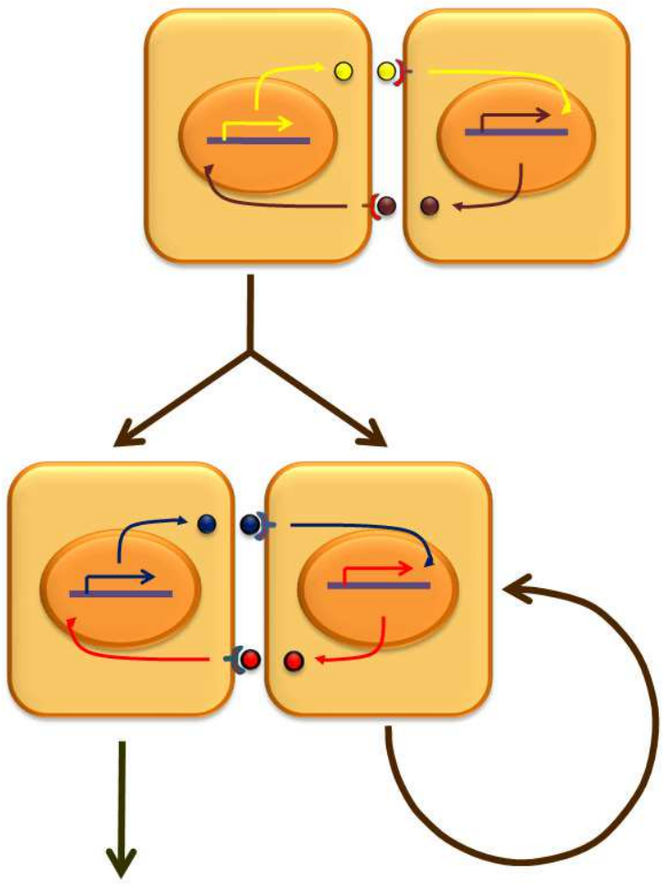

Fig. 3. **Cell-to-cell interactions regulate gene transcription within each human cell**. Signals can travel along varying distances. The upper left cell is a stem cell. The lower left and right cells respectively are a differentiating cell and a new stem cell. Details are described in text.

These studies in animal species provide a good tool to now find human genes candidate to be regulating any cell process. By sequence similarity between any animal and human gene,

an orthologous human gene can be found. When compared with the animal gene, this human gene may also show similar functions in the human organism.

When studying human stem cells, all these regulatory features can also be found. Notch or Wnt signaling pathways have been shown to control the cell-to-cell-interaction-dependent decision between cell proliferation and cell differentiation.In humans, additional functions have been shown for these signaling pathways. The same can be found when other signaling pathways are studied. Transcription factors acting in stem cells are Bmi1, Oct4, SOX2, Klf-4, cMyc, FoxD3, or EGR1. An interesting effort might completely relate SC gene transcription to embryonic genetic program.

In summary, the differentiation of any stem cell type depends on a hierarchy of transcription, traced back to maternal information. This hierarchical regulation also influences, in a balanced way, the differentiation of other surrounding cell types. These neighboring influences, such as the so-called stem cell niche, are under profound analysis.

2.2.2 Stem cell niches

Stem cells require a very specific chemical and physical environment for a correct function. This environment is provided by **the Niche, a group of cells providing signals to stem cells for a balanced activity within the tissue** (see Becerra et al., 2011).

SC niche is defined as the microenvironment formed by SC, non-SC niche resident cells and specific signaling and ECM molecules surrounding them. The niche controls SC divisions. This SC proliferation can be regulated to fulfill the homeostatic needs (Becerra et al., 2011).

Although the information on niche structure is still scarce, a recent increase in scientific publication on this topic is being issued. Drosophila testis or germarium, hair follicle, intestine crypts, bone marrow and brain sub-ventricular zone are niches under intense study (Fig. 4).

Thus, the molecular/cellular paradigm is providing a new solution to one of the oldest unsolved questions in current developmental biology. Potency is now being understood under the concepts of genetic program and cell-to-cell interactions, and this may also help clinical practices.

3. Cell therapies

3.1 Introduction and concept

An ancestral question, traced back much before ancient Greece, is to understand why human body does not regenerate. A Greek example is Prometheus legend. Prometheus was immortal but stole fire from Zeus and was condemned to be devoured by an eagle. The eagle ate his liver every day, but Prometheus continuously regenerated his organ back and back again. The ancient observation of living beings led to the idea of animal regeneration. Indeed, sea urchin, triton or fishes are able to regenerate their limbs to restitute previous amputations. This probably inspired the legend.

The discovery and study of human stem cells brought about new hopes of recovering absent or injured organs. This was the birth of Cell therapy. **Cell therapy is the treatment of**

human diseases by application of cells. Although this discipline is at its beginnings, many current therapies aim to use stem cells to treat an important variety of human diseases.

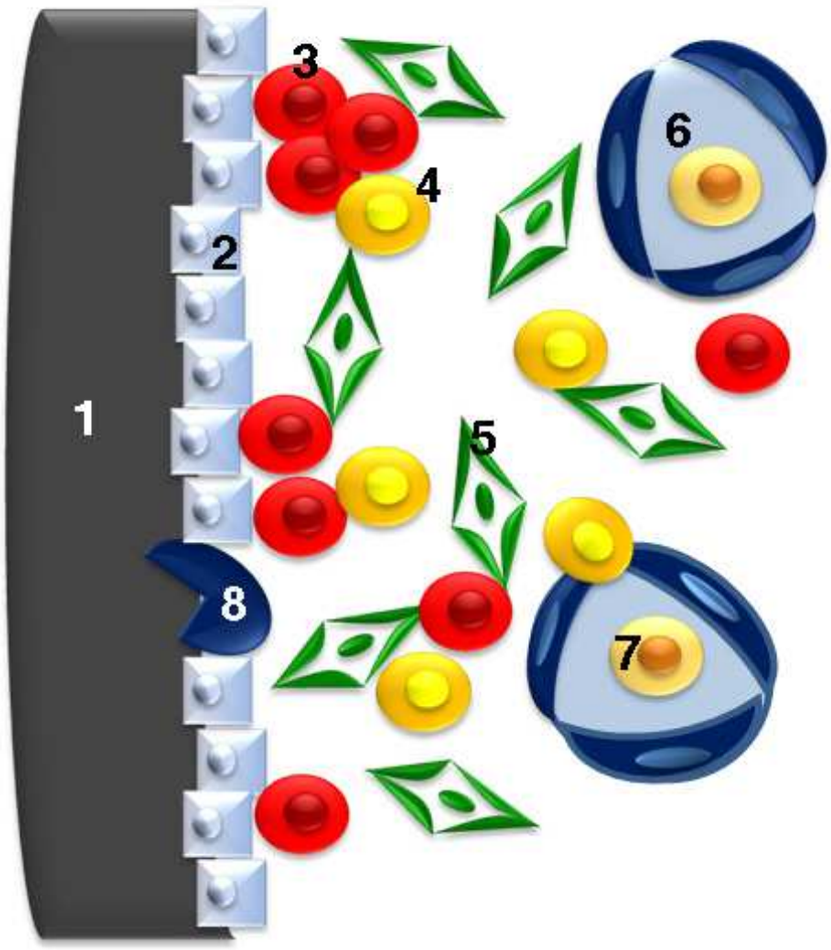

Fig. 4. **Simplified schematic representation of the niche of stem cells in the bone marrow**. MSC and HSC interact in the endosteal niche, around the bone, and the vascular niche, close to the blood vessels. A lot of factors have been identified related to the regulation of hematopoiesis and the fate of the MSC. (1) spicules of bone, (2) osteoblasts, (3) hematopoietic stem cells (HSC), (4)Hematopoietic progenitor cells, (5) mesenchymal stem cells (MSC), (6) fenestrated capillaries, (7) progenitors inside capillaries, and (8) is an osteoclast.

The above-mentioned cells, neural, respiratory or digestive mucosae stem cells, haematopoietic, epidermal, mesenchymal or umbilical cord stem cells are currently used to treat diseases. However, the methods for obtaining these cells and treatment effectiveness vary depending upon cell type or potency.

In 1998, embryonic stem cells were first discovered (Thomson et al., 1998). Inner cell mass cells from a blastocyst were cultured in vitro. An empirical device was used to succeed in

this task. This empirical device was being used since 1960s for in vitro culture of animal cells. Fibroblasts were killed by irradiation inducing extensive mutations in their nuclei DNA. A lawn of irradiated fibroblasts was deposited in a Petri dish, the feeder layer, in order to feed the non-irradiated embryonic cells. During culturing, these cells spontaneously dissociated from one another. These living cells were sub-cultured in another feeder layer to obtain embryonic stem cell colonies. These cells showed an important pluripotency when induced to differentiate in vitro under a variety of different stimuli and eventually produced teratomas when grafted into a mouse.

More modern techniques have been used to culture stem cells by in vitro amplification. Some of these cells can be obtained by egg enucleation and subsequent nuclear implantation from a differentiated somatic cell. If a pre-treatment of nutrient deprivation is carried out, nuclear re-programming may occur. This re-programming is able to de-differentiate the somatic cell nucleus and re-induce early embryogenesis in vitro. Under these conditions, a population of cultured cells from the patient can be obtained. Following these techniques, **Asexually Produced Totipotent (APT) Cells** were obtained. This technique shows a great advantage as these cells are not immunologically rejected when clinically used.

Two experimental trials are used to verify the quality of embryonic stem cells:

i. Teratome formation

If ESCs are experimentally implanted in animals, they generate tumors. These tumors may differentiate structures such as gastric glands, teeth or hairs. The nature of these structures suggests that these tumors may be originated from any embryonic sheet.

ii. Chimera induction

If embryonic stem cells are implanted into a genetically different inner cell mass, a chimeric organism is formed. Although classical terminology states that chimeric organism are mixture of different species,ESC-derived chimerism only implies genetic differences within a given species. In these mammalian organisms, the cells show two different genomes. This may be useful to trace the location of cells by simple histological observations, but it can also be used as a cellular treatment of diseases.

All these manipulations of human embryos or genes have precluded a direct application of these techniques due to bioethics reasons. Due to continuous argumentations in the society, an alternative to ESC use in cell therapies have arisen since the beginning. This technique use multipotent stem cells obtained from the adult, the above-mentioned ASC. Cell therapies using ASC has been widely expanded in clinical use around the world. However, although ASC are safe and very useful in many therapies, they show several practical problems that have to be considered.

A first problem is related to methods to obtain ASCs. Almost any stem cell type has to be obtained by a different experimental protocol. Thus, there are simple ways and very difficult protocols of obtaining stem cells. The easiest protocol is bone marrow aspiration to obtain mesenchymal and haematopoietic stem cells. This is clinically used in everyday treatment of leukemia or blood cell diseases. The most difficult protocol is to obtain neural stem cells from sub-ependymal layers. The clinical protocols using this latter technique are far away from being regularly used at hospitals due to its low benefit/risk level.

Beside stem cells, these techniques normally require further auxiliary material. Among them, bioactive molecules and artificial scaffold are currently necessary for growth and cell differentiation to obtain tissue integration. All these auxiliary techniques are included in the so-called Tissue Engineering.

3.2 Current applications

Many different cell therapy techniques are currently under legalization process to be applied to treat modern diseases. Some of these diseases affect bone, cartilage, central nervous and immune systems, skin, heart or blood. Bone Marrow therapies to treat blood or skeletal diseases are widely spread treatments used all around the world. However, other cell therapies are still under intense research. These stem cell techniques are treating self-immune or neurological diseases, such as Parkinson, ELA or medullar traumas.

The most important factor boosting improvement of these treatments is the close-knit collaboration between basic and applied research. From basic research, model systems must be proposed to evaluate the effectiveness of cell therapy. These model systems require the definition of precise model species, organ, tissues and/or cell type to verify responses to treatments. Experimental conditions, such as transgenic mice, implanted animals or in vitro culturing, may also be necessary for this model system definition. In some instances, some of these techniques may also be applied to hospitals, where material useful for the definite cell treatment is necessary. During this process, biological research grants are substituted with pre-clinical trials, always under medical rigorousness and refereeing. Since most applications require ex vivo manipulation of cells collected from the donor, the European Union has regulated its use as a drug.

In order to provide examples of this tortuous journey, we will discuss three examples. These three cell treatment examples use the same stem cell type, the mesoderm-derived mesenchymal stem cells (MSC). We will show that these three cases are elements in a single routing process that can be used as stepping stones to be followed when similar strategies are expected to be applied (Figure 5).

The first therapy is widely used around the world. Every hospital has enough experience and tradition in its application. Moreover, citizens in modern societies have a clear knowledge of its existence and utility. This technique is Haematopietic stem cells implantation, or the well-known bone marrow transplantation. The second therapy is also well implanted in hospitals but it is rarer than previous ones. Although it has roots in ancient Egypt, there is not such a consensual application as HSC implantation. This is the use of mesenchymal stem cells in treatments of bone diseases. The third case is at pre-clinical trials stage in some hospitals but it is widely unknown in clinical environments. This is the use of MSC to treat psoriasis or other angiogenic diseases. The three cases can be understood as a gradual process of accepting a unique clinical success. At this stage, the reconstruction of the original environment of stem cells in the body is reproduced and then these cells are able to restitute homeostasis in the organism.

3.2.1 Cell therapy and haematopoiesis

The tissue that generates blood cells is the Bone Marrow. In the bone marrow, millions of HSC and MSC can be found. If after an accident, an important quantity of blood is lost,

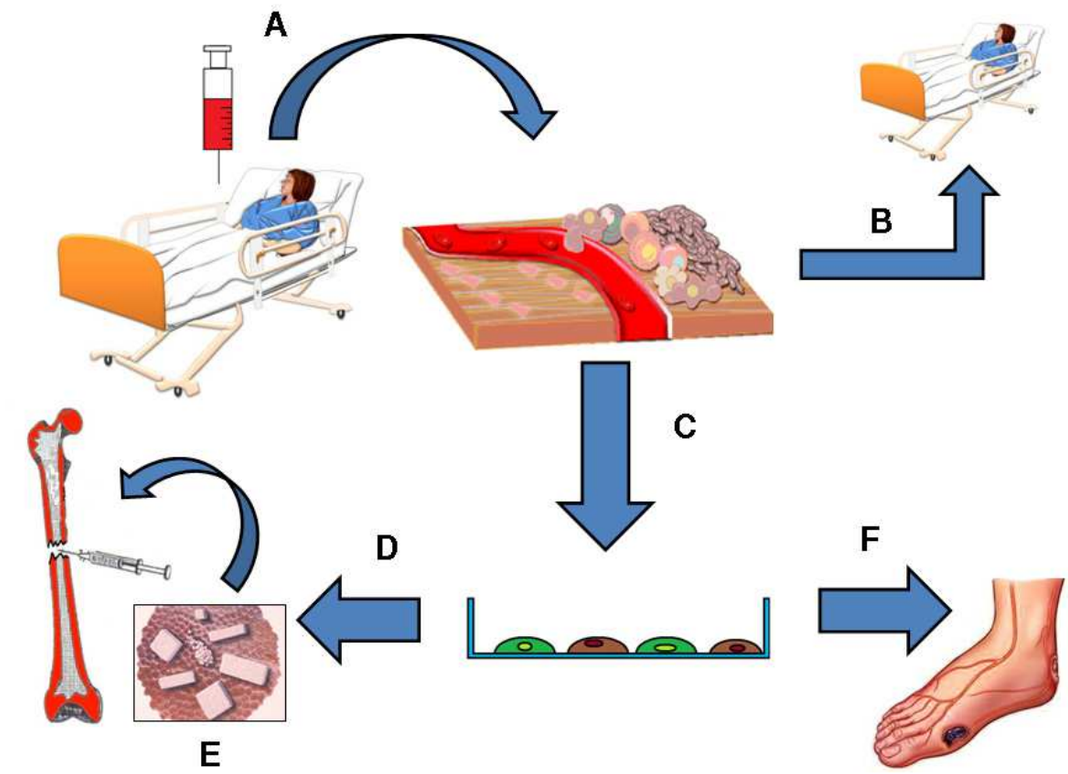

Fig. 5. **Bone marrow aspiration and liposuction provide mesenchymal stem cells**.
(A) bone marrow aspiration or liposuction, (B) direct grafting of mesenchymal stem cells (mononuclear cell fraction), (C) in vitro amplification of progenitor cells, (D) grafting of amplified stem cells, adsorbed on pieces of biomaterial (E) and (F) potential treatment of a diabetic foot.

signals are released at bone marrow to activate HSC. This activation induces cell proliferation and differentiation of the haematopoietic lineage to restore all blood cells. The restoring of blood cells is mandatory for reconstitution of blood in the injured patient.

Following experiments with irradiated dogs, bone marrow transplantation was shown to be useful to restitute blood homeostasis. The physiological knowledge of the process has directed a clear improvement of treatments against hematological diseases. This treatment obtains bone marrow from healthy donors and implants them in compatible patients. Even though blood transfusions are commonly used due to mobilization of marrow progenitors to peripheral blood, bone marrow sources are iliac crests, sternum, femur and humerus. Bone marrow is the residence of millions of HSCs able to restitute these cells.

Effectiveness of bone marrow transplantation can be easily studied in hematological diseases, such as congenital aplastic anemia, thalassemia, platelet defects or coagulopathy. In these instances, HSC at transplanted bone marrow restitute absent or defective blood cells in the patient (Figure 5). However, leukemia treatments provide additional information on this clinical strategy. This disease mostly affects white blood cells. In these diseases, white blood cells are present in the patient but they are in variable quantities suggesting loss of

regulation by uncontrolled proliferation. Bone marrow transplantation has also been important to treat leukemia. This cell therapy restores red blood cells and platelets previously reduced by chemical treatments. But this therapy also regulates abnormal HSC by substituting them with healthy HSC from donors. These healthy HSCs regulate patient haematopoiesis to homeostatic conditions.

Millions of MSC, adipocytes or pericytes are still present in the irradiated host bone marrow. Some of these auxiliary cells may also help to generate a correct signaling ambient for the proliferation of cells to be up-regulated (Figure 4). Indeed, some of these cell types have been proposed to act as HSC Niche to provide a fine regulation of the restituting process. In the end, this fine regulation is crucial for homeostatic renewal and health restitution. This fine regulation of stem cells is the real standard goal of this technique. A tentative hypothesis would extend this standard goal to any other cell therapy method. Since this type of cell therapy does not perform ex vivo manipulation, they do not impose regulatory standards of drug quality.

3.2.2 Cell therapy and chondrogenesis/osteogenesis

In modern societies, a number of diseases have appeared associated to the new life style. The increase in life span brings about additional degenerative diseases, such as osteoporosis or arthritis, which affect bones and joints of an increasingly number of citizens. Moreover, traffic accidents or the massive play of sports also generates an important number of bone and joint diseases. These ostearticular patients are thus widely these ostearticular patients are widely distributed in the population and very different age groups can be affected by similar diseases. Although some evidence of limited digit regeneration has been reported in children, treatments for these diseases must be adapted to the special conditions of the patient.

Cell therapy of partial bone or cartilage-defects, requires the in situ administration of osteoblast and chondroblast progenitor cells. These cells can also be obtained from bone marrow or other origins, amplified and induced in vitro into a osteo- or chondrogenic lineage. Then, these cells can easily be applied to the injured tissue (Figure 5). But, when surgeons transplanted these cells, they did not obtained proper results. Scaffolds to support cells and appropriate signals were not present and the skeletal tissue was not completely restored. A classical clinical practice was to obtain bone powder or small bone fragments and apply them to the operation field. Following this technique, a very important increase in bone regeneration was obtained. Then, surgeons transplanted a mixture of this powder and osteoblast progenitor cells to significantly improve bone regeneration. Even in these treatments, small or even larger pieces of bones were also transplanted in the mixture to gradually obtain better results. At this stage, a clear conclusion can also be obtained. Stem cells cannot fulfill a correct treatment by themselves, and a potential stem cell niche, either natural or artificial, to provide a necessary cell induction, must be present.

Indeed, artificial biocompatible materials have also been extensively developed in this task. These biomaterials show superficial properties of special adherence to progenitor cells. Moreover, a number of different proteins, many in the TGF-beta super-family, can also be applied. These molecular treatments aim to reproduce the natural regulation of progenitor cells in the body and are combined with the cell therapy. This type of Tissue Engineering is one of the most promising techniques in this area. Pre-clinical and clinical trials are well

advanced and are showing significant results. As expected, these "artificial" treatments will be compared with "natural" ones after bone fragments transplantation, where cells, signals and extracellular matrix components are present. As the latter cannot always be applied due to the type of bone lesion, artificial scaffold treatments could be a candidate cell therapy in most hospitals in a short future.

In general, these therapies take advantage of easy methods to obtain MSC. Bone Marrow aspiration is a well-founded medical technique. This technique can be applied to the patient and an important number of MSC obtained in vitro. These cells and the appropriate natural or artificial scaffold can hopefully collaborate in the regeneration of osteocartilage injuries. Recent alternatives to bone marrow aspiration can be found in liposuction techniques. Vasculo-stromal fraction from fat tissue obtained by this technique can be use as an in vitro source of MSCs. Potency and quality of these cells are enough to obtain osteoblasts or chondroblasts under the appropriate stimuli. But liposuction is less invasive than bone marrow aspiration and can further help the cosmetic surgery of the patient. In any case, cell therapy for skeletal repair is one of the most promising applications of regenerative medicine.

3.2.3 Cell therapy and angiogenesis

Angiogenesis is the generation of new capillaries by a process of sprouting of pre-existing microvessels. In health, vessel proliferation is under stringent control and occurs only during embryonic development, endometrial regulation, reproductive cycle and wound repair. On the contrary, a persistent and deregulated angiogenesis is related to diseases such as proliferative retinopathies, psoriasis and rheumatoid arthritis, and seems to be essential for tumor growth and metastasis (Carmeliet, 2005). On the other hand, the concept of *therapeutic angiogenesis* has emerged as an approach to ischemic diseases in which stimulation of new vessels growth is intended to restore blood supply to ischemic tissue (Carmeliet, 2005; Tirziu and Simons, 2005).

Promising results in pre-clinical studies prompted the initiation of a number of clinical trials in patients with advanced coronary and peripheral arterial disease who had no other treatment option (Lachmann and Nikol, 2007; Tse et al., 2007). In spite of the recent advances in medical therapy, coronary artery disease remains the major cause of morbidity and mortality in the developing countries. In patients with severe coronary artery disease, persistent myocardial ischemia in hibernated myocardium can result in progressive loss of cardiomyocytes with development of heart failure. On the other hand, peripheral arterial disease is a common manifestation of systemic atherosclerosis that is associated with a significant limitation in limb function due to ischemia and high risk of cardiovascular mortality. The lower limb manifestations of peripheral arterial disease are classified into the categories of chronic stable claudication, critical leg ischemia, and acute limb ischemia. Lower limb ischemia is a major health problem since, in the absence of effective pharmacological, interventional or surgical treatment, amputation becomes the only solution to unbearable symptoms at the end-stage.

A part of therapeutic angiogenesis approaches to treat coronary and peripheral artery diseases is based on cell therapy. Bone marrow consists of multiple cell populations, including endothelial progenitor cells, which have been shown to differentiate into

endothelial cells and release several angiogenic factors and thereby enhance neovascularisation in animal models of hind limb ischemia. The promising results from various preclinical studies provide the basis for clinical trials using bone marrow-derived cells or non-bone marrow cells, like cells from the peripheral blood or other tissues (Figure 5). However, the mechanisms by which these cells exert their positive effects are poorly understood (Lachmann and Nikol, 2007). Furthermore, although the initial pilot clinical trials showed potential clinical benefit of bone marrow derived cell therapy for therapeutic angiogenesis, the long-term safety, the optimal timing and treatment strategy remains unclear (Tse et al., 2007). This might explain why, up to the moment, some controversial results have been obtained. While a meta-analysis of randomized, controlled clinical trials of therapeutic angiogenesis published in 2009 concluded that patients with peripheral arterial disease -and in particular those with critical ischemia- improved their symptoms when treated with cell therapy with acceptable tolerability, a more recently published article concluded that stem cell or progenitor cell therapy did not reveal clinical benefit in patients with peripheral artery disease (De Haro et al., 2009; Nikol, 2011). Additional controlled clinical trials are in progress. When data from large randomized placebo-controlled trials will be available, it will be possible to evaluate properly the actual impact of this therapeutic approach.

4. Risks and bioethics problems

As any other medical treatment, cell therapy techniques may show intrinsic risks or lead to unexpected damages. First, to obtain adult MSC is bloody. Although it varies with the used technique, all methods show morbidity. Improvement can be found from Bone marrow aspiration to liposuction. The first technique is painful and shows potential surgical risks, whereas the second operation shows lesser morbidity although certain potential surgical complications can still arise. In any case, other alternatives show even worse side effects. Neural stem cells or liver oval cells can be obtained from an adult, but a complete neurosurgical or hepatic operation is necessary with many additional drawbacks.

Obtaining the ASCs is not the whole problem. Stem cell implantation is another risky part of the protocol. When manipulating biological material during the surgical operation, the infection risk is very high. In order to reduce this risk, the surgeon and auxiliary manipulators has to follow strict conditions and actuations. All these risk does not preclude the application of these techniques. However, **all implicated manipulators have to strictly follow many security protocols at each stage.** Good Manufactured Practices (GMP) must be observed at all times.

Moreover, postsurgical risks must also be considered. When heterologous grafts (donor and host are different persons) are carried out, immunological rejection is always important. Indeed, very severe diseases can appear such as Graft-against-Host disease or Systemic Inflammatory Response Syndrome which can drive to death to the patient. A continuous risk of cell treatments is to produce an uncontrolled growth in grafted cells to generate neoplasia. Statistically, the risk to suffer neoplasia is much lower when treated with ASC than using ESC. In ASC treatments, this probability is similar to that observed in untreated patients due to other carcinogenic factors. Recent findings indicate the MSCs produce recovery by trophic and immunomodulatory effects (Caplan, 2009). This has boosted numerous clinical trials that may hopefully lead to new healing therapies.

Another limiting question is posed by bioethics commissions. A big controversy arose with embryonic stem cells. To obtain these cells, embryos must be destroyed or their genetic material manipulated. This leads to moral problems which involves both the scientific community and the non-scientific society. Some countries approved these protocols whereas others did not. So, besides the moral questions, other economical-political reasons also influenced the natural development of these protocols. All this has led scientists and physicians to avoid the use of these cells in favor of ASCs.

5. Future perspectives

Besides all these improvements, Cell therapy has a long journey ahead. Among all obvious perspectives, to obtain Stem Cells through a lesser bloody operation, to find the appropriate stem cell source and to investigate the best signaling cocktail for any tissue differentiation, are clear options. In order to reach this, a continuous interaction between scientists and physicians must occur. If these therapies are approved for use in every hospital, GMP labs would be installed near the operating room. On the contrary, if marketing criteria are imposed, "therapeutic cells are drugs", the situation will be very different.

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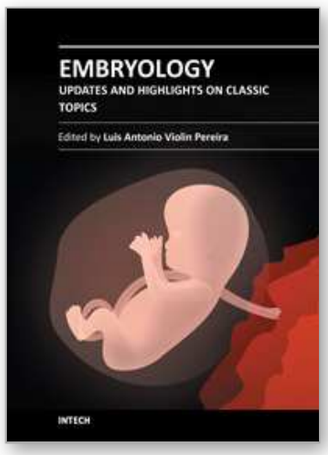

Embryology - Updates and Highlights on Classic Topics

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Embryology is a branch of science concerned with the morphological aspects of organismal development. The genomic and molecular revolution of the second half of the 20th century, together with the classic descriptive aspects of this science have allowed greater integration in our understanding of many developmental events. Through such integration, modern embryology seeks to provide practical knowledge that can be applied to assisted reproduction, stem cell therapy, birth defects, fetal surgery and other fields. This book focuses on human embryology and aims to provide an up-to-date source of information on a variety of selected topics. The book consists of nine chapters organized into three sections, namely: 1) gametes and infertility, 2) implantation, placentation and early development, and 3) perspectives in embryology. The contents of this book should be of interest to biology and medical students, clinical embryologists, laboratory researchers, obstetricians and urologists, developmental biologists, molecular geneticists and anyone who wishes to know more about recent advances in human development.

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