

# Isolation of Stem Cells from Human Adipose Tissue: Technique, Problems, and Pearls

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## 2.1 Introduction

*If you are looking for volume, take the leaves. If you are looking for leaves, flowers and fruits, take the stem and the branches. With Stem Cells it is the same.*

Plastic surgery is essentially the search for form. And form means volume (or its absence). When the volume is insufficient, it is necessary to look for alternatives to increase it, either by the inclusion of a material in the deficient area, or by the transposition of tissue from donor sites of the body; when the volume is excessive, the removal of a part of it is a viable option. The use of such techniques may either last or be abandoned depending on the tolerance of an inclusion by the human body and/or the cost–benefit relation that the surgical methods may offer.

Such mentality is currently applied in plastic surgery, as in many other fields of Medicine: removal of excessive tissue and replacement of absent tissue. Restoration is a rare option. Some time ago, scientists began to seek the real possibility of regenerating the human body in its deficient sites, based on a concept in which the cure is contained in the body itself. The essence of such treatments would fall within the simplicity of putting back what is missing, using the patient's own body as the source.

Certainly, the idea of partially or integrally restoring a missing organ dates back to ancient times. After all, it was observed that, after a gecko had lost its tail to escape a predator, leaving the still-shaking lost organ behind, the lizard would surprisingly grow back a new tail in a few days after the episode, being identical in functionality, texture, color, and vascular and nerve supply, bearing

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self-limited growth and without mutations. Why would it not be the same with humans? Millennia have passed, and the search for a magical treatment that would permit the restoration of tissues, organs, and their functions is still continuing.

The miraculous expression that seems to encompass this almost-divine gift is *stem cell*. Ultimately, what are living creatures, if not the perfect engineering originated from the union of only two cells? From a tiny code resulting from the mixture of two sources of DNA (ovum + spermatozoon = zygote), there will be a unique individual, bearing its own characteristics, and with a body full of rich details, in the end complete.

## 2.2 Basic Concepts of Stem Cells

This chapter is not intended to be a thorough source of learning about stem cells, but indeed a guide for the plastic surgeon to build a notion of this new edge of human knowledge, thus instigating the curiosity of those who identify with scientific research, and also offering the more pragmatic ones a panorama that permits them to deal with these new challenges that have entered plastic surgery, therefore enriching it and consolidating it as a state-of-the-art medical specialty.

The ability of two gametes to originate the whole universe of more than 200 different types of tissues present in the human body gives wings to wondering how far the power of human intervention in Nature may get, as long as man is capable of coordinating the pathways to be followed by those cells, which carry the potential to develop everything within a species, hence justifying their name – *totipotent cells*. And they will maintain this faculty during their first divisions. The early blastocyst, when still made up of eight cells, is the stage that allows a relatively easy harvest of embryonic cells, since they still have loose bonds between them, being almost free before compaction occurs. Those cells that are in the inner cell mass (around 4 or 5 days after fertilization), *if harvested and cultivated in vitro*, will originate the *totipotent embryonic stem cells*. The external layer of the blastocyst (trophoblast or trophoctoderm), in its turn, will originate the placenta. With the progression of mitoses, cells continue differentiating and, between the fifth and eighth weeks, there will be *embryonic germinal cells*, or *pluripotent embryonic stem cells*. This coincides with the end of the embryonic stage; from that

phase onward, the concept is considered to be a *fetus* (ninth week until birth).

*Totipotent stem cells*, as mentioned above, originate all fetal tissues (placental cells and body cells); *pluripotent stem cells* may originate *all body tissues*: ectoderm (neurons, skin), mesoderm (muscles, cartilage, bones, blood, fat), and endoderm (endocrine glands). Stem cells will keep undergoing mitoses and will become more and more specific, therefore losing their ability to differentiate.

*Adult stem cells* can be defined as those that are extracted after the embryonic stage. Those can be divided into *mesenchymal stem cells* (MSCs) (which will originate osteoblasts, chondroblasts, myoblasts, adipocytes), *hematopoietic stem cells* (HSCs) (which will originate blood cells), and *tissue-specific stem cells* (also known as *precursor cells* or *unipotent cells*, they will originate specific tissues, from which they can be extracted). *Adult stem cells* can be called *somatic stem cells* as well; besides the aforementioned unipotent cells, some may be *oligopotent* or *multipotent*, depending on how many types of cells may be originated. Such cells might be present in *all human tissues*.

Stem cells show the following features:

- They are undifferentiated (i.e., not specialized).
- They may multiply for long periods of time without differentiating, which can lead to a great population of similar cells.
- They are able to perform *asymmetric divisions*, which means that a part of their descendant cells will be identical to them; the other part will carry the characteristics of specialized cells.

Like every structure in living creatures, stem cells also undergo an aging process (*senescence*). After a certain number of mitoses, stem cells may simply halt the process of cell division or, as they multiply, they lose a small part of the distal portion of the DNA (*telomere*). When the DNA reaches a size too small to allow for the continuation of mitoses, the multiplication of cells is stopped. Another point to be considered in cell transplantation is *apoptosis* (programmed cell death): will an adult stem cell, once transplanted to another organism, follow its original path, or will it be able to live longer in its new host? This question emphasizes the following issue: for example, if tissues obtained from an adult with a hypothetical lifespan of thirty more years are transplanted to an 8-year-old child, will this define an “expiration date” of the transplant, therefore limiting the life expectancy of the host to 38 years?

**Table 2.1** Stem-cell yield according to donor sources

Source	Umbilical cord	Bone marrow	Liposuctioned fat	Sliced fat
Yield	200–20,000 CFU/mL	100–1,000 UFC/mL	3,600–10,700 UFC/mL	28,000 UFC/g

### 2.3 Sources of Stem Cells

*Embryonic stem cells* are usually obtained from the eight-cell blastula stage of mammal embryos. Such cells may have their original nucleus replaced by that of an adult cell, thus originating the process called *cloning*. This biomolecular engineering maneuver will create a new embryo, carrying the characteristics of the adult whose nucleus was transplanted.

Due to ethical, religious, and legal controversies, we will not present a deeper analysis of the methods used to obtain human embryonic stem cells. Another negative aspect regarding that type of stem cell is that there are studies on murines indicating that these cells display a strong tendency of developing tumors (*teratomas*) after a few divisions, owing to their ability of generating multiple tissues. The current lack of capacity to control their growth would be a predisposing factor for these conditions.

In adult humans, *adult stem cells* can offer a well-defined, relatively abundant extraction when harvested from bone marrow, peripheral blood, or fat (the three most frequent sources).

*Bone marrow* has been considered the usual source of *ASTs* (adult stem cells), although their harvest is a painful procedure, with significant morbidity and low yield [5]; it offers, according to several authors, *from 100 to 1,000 colony-forming units (CFUs)* per milliliter of extracted material. It has its greatest use in hematologic diseases, with well-established results in hematopoietic replacement. The basis for the treatment consists in storing bone marrow cells (either from the patient themselves or from a compatible donor), after selection and exclusion of tumor cells, sterilizing the cells and the sick bone marrow of the patient, and then reinfusing the hematopoietic stem cells (*HSCs*), which will adequately repopulate the bone marrow, without originating teratomas.

In order to harvest stem cells from peripheral blood, it is necessary to administrate stimulants (*hematopoietic growth factors*), with the purpose of releasing stem cells into the peripheral blood, which is easily collected. After collection, the blood is filtered to concentrate the greatest number possible of *HSCs* to be

reinfused. There are controversies regarding the best treatment option between direct harvest of bone marrow stem cells or their obtention from peripheral blood. Diseases in more advanced stages seem to better respond to peripheral blood stem cell transplant.

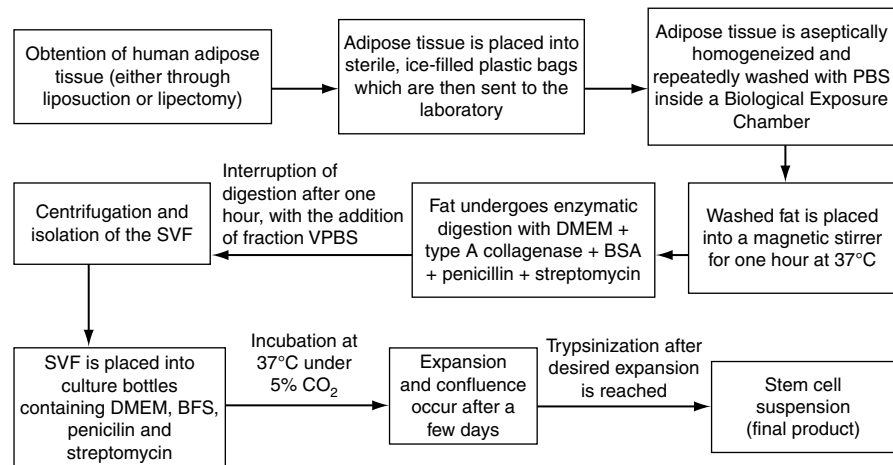
*Umbilical cord* is a source of stem cells that has been used for more than 2 decades after the first successful transplant in France. It has been known that both placental blood contained in the cord and Wharton’s jelly (*HUCM – human umbilical cord matrix*) are stem cell rich [5]. The storage of umbilical cord blood in specific blood banks has become frequent worldwide. A range of 10,000 samples can be enough to serve an entire population.

*Fat* obtained from humans is a rich source of *mesenchymal stem cells (MSCs)*, also known as *ADSCs – adipose-derived stem cells*, which, due to their *plasticity* (ability to generate several types of tissues), show an extremely promising future as the first option for obtaining *ASTs* [3]. The low-morbidity extraction (through liposuction) and the high yield (*5,000 CFUs per gram of extracted material*) make human fat a potential weapon for stem cell therapy and engineering [7, 14, 15]. The storage of *ADSC* in tissue banks will permit every person to have a technical supply of their own stem cells for future use (Table 2.1).

### 2.4 Stem Cell Culture

Stem cells have well-standardized in vitro culture methods [9]. The obtention of stem cells from fat will be described, because it is comprehensive and concerns plastic surgeons. *ASTs* exhibit the feature of adhering to glass or plastic. That allows one to confirm their successful culture only after that process occurs. Once human fat is obtained, either through liposuction or lipectomy, it is placed in sterile, ice-filled plastic bags and sent to the laboratory. The extracted tissue is aseptically homogenized and then taken to a Biological Exposure Chamber, where the tissue is repeatedly washed with PBS (Phosphate Buffered Saline) until all visible blood and excessive fluids are eliminated, in order to leave fat as clean as possible. The washed fat is

**Fig. 2.1** Diagrammatic representation of stem cell culture



**Table 2.2** ADSC yield according to donor sites at the moment of harvest, according to author's projection

Donor site	Submental	Arm	Pre-axillary	Gynecomastia	Abdomen	Flank	Riding breeches	Inner thigh	Knee	Sliced abdominal fat
Yield	3,600 UFC/mL	3,800 UFC/mL	7,100 UFC/mL	5,700 UFC/mL	5,500 UFC/mL	5,300 UFC/mL	5,300 UFC/mL	4,300 UFC/mL	7,100 UFC/mL	28,000 UFC/mL

placed in a magnetic stirrer for 1 h at 37°C. Afterwards, the fat undergoes enzymatic digestion, with the addition of a solution prepared with DMEM (Dulbecco's Modified Eagle Medium), type A collagenase, Fraction V BSA (Bovine Serum Albumin), penicillin, and streptomycin. After 1 h, the digestion is interrupted with the addition of BFS (Bovine Fetal Serum). The resultant material is centrifuged, and the floating fraction is discarded. The portion sitting at the bottom of the centrifuged tube (called *pellet*), which is the one containing the so-called stromal vascular fraction (SVF), is transferred to culture bottles filled with DMEM, BFS, penicillin, and streptomycin. The cells are taken to an incubator at 37°C, under 5% CO<sub>2</sub>, where they will expand. The liquid content of the bottles is changed daily, and the cells are washed with PBS, resulting in their adhesion to the plastic bottom of the bottles. After washing, the culture medium is replaced. Within a few days, cells will proliferate (*expansion*) and adhere to one another (*confluence*). At this point, stem cells will have become *undifferentiated mesenchymal cells* (UMCs). When cells reach the desired expansion, they are treated with trypsin-EDTA (*trypsinization*), in order to rupture the intercellular junctions. This procedure results in a *stem cell suspension*, for clinical use or culture transfer (Fig. 2.1).

Based on studies conducted at the Hospital Santa Catarina and at the Laboratory of Medical Investigation of the Discipline of Human Structural Topography of the Department of Surgery at the University of São Paulo Medical School, Stocchero projected a Stem Cell Growth Curve based on fat obtained through liposuction of different topographic areas [10], coming to the conclusion that, on the day of collection, there are an average of  $5.3 \times 10^3$  UMCs per gram of extracted material, and that such UMCs multiply at an average rate of 18.5% per day. In another study conducted at the Division of Clinical and Toxicologic Analyses at the University of São Paulo School of Pharmaceutical Sciences [1] was concluded that fat donor sites located in the human torso could offer a 23.6% higher yield when compared to limb donor sites (Table 2.2).

## 2.5 Animal Models

Most laboratory studies are carried out using several murine species, owing to their easy obtention, confinement, and follow up. Examples well-known to the greater public have involved successful experiments on cattle and sheep. An excellent animal model of easy care was described as a source of ADSCs at the Second

Laboratory of Medical Investigation of the Department of Surgery at the University of São Paulo Medical School – the New Zealand white rabbit [12].

## 2.6 Pitfalls

*Stem cells do not carry a GPS navigation device!*

Effective use of ASCs in clinical practice still faces some obstacles that have been traversed quite rapidly. One of the issues to be solved regards the *homing* (the *target*) of treatments. That means the ability to conduct ASCs to their adequate destination. Even though such cells seem to display a certain tropism toward their expected sites of action, it is fundamental that this is an absolutely precise process. It seems perfectly acceptable that HSCs seek the bone marrow as their main target due to their own nature. But when we want MSCs to differentiate into a more specific type of tissue (e.g., cardiomyoblasts), it is essential that the site of interest be reached.

Nowadays, among the greatest challenges, there is the search for an adequate *scaffold* to create three-dimensional structures that can contain stem cells in adequate size and shape, allow angiogenesis and innervation and, above all, be transplantable from the laboratory to humans.

Difficulties increase when the subject in question is the need to replace an organ (which is three-dimensional) starting from two or three imbricate germ layers. And in case of a limb that must be replaced (a situation that is currently not possible to reproduce in an animal model), the solution for this issue possibly relies on the implantation of a germinal bud in the remaining stump to grow back the missing part.

## 2.7 Pearls

*Stem cells suffer from personality disorders! They do not know exactly who they are or what they will be!*

Cell differentiation will occur by means of adequate cell differentiation inductors (*triggers*), which are well-known biomolecules capable of determining what tissues MSCs will originate. By linking the development of MSCs to growth factors, one would only have to wait for the desired amount of cells and then halt their proliferation. This would be valid for any tissue-

deficient site. In case of organs constituted by few differentiated tissues (e.g., breast), it will be possible to place an MSC *carrier* [4] in the desired place and, with the use of a trigger plus the interaction between neighboring cells, there may be an induction to cell differentiation, through microRNA (*μRNA*), which is a messenger displayed by the surrounding tissue cells. Therefore, plasticity depends on these factors. It is necessary to reach the target. And one can only be sure that the relocated stem cells have indeed differentiated into the desired cells if one uses *cell markers*, which will replicate along with the already transdifferentiated descendent cells [2, 6].

There are situations when stem cells *fuse* with the cells of the target tissue, without the occurrence of true differentiation. Although intended results may apparently be achieved in this manner, it was not owing to stem cell differentiation, but to their fusion to other types of cells. The stem cell-drenched carrier may be the best solution, since it will permit the development of stem cells directed in the site of implantation, using both vascularization and innervation available at the receptor site itself.

## 2.8 Conclusion

Throughout this chapter, whose intention is no more than that of giving plastic surgeons an idea about the names, practices, and tactics involved in this field of medicine which is still not a part of the daily work of most who practice plastic surgery, we cited some expectations for the future use of these little wonders that are stem cells, in their multiple intriguing features.

It is important to emphasize that the adipocyte in itself will not originate anything. When we discuss fat, we assume that there is a stroma supporting such tissue. MSCs are indeed located within blood vessels and the pericytes surrounding them, where stem cells are responsible for the turnover of fat cells, whose replacement is currently known to take from 2 to 10 years, depending on the body region where they are located. Stem cells work to promote angiogenesis when necessary (e.g., when fat mass increases), to restore the vascular endothelium, etc.

The fact that such stroma is the great source of MSCs can be confirmed by observing that sliced fat tissues obtained through lipectomy show a much higher stem cell yield when compared to liposuctioned fat samples, owing to the increased amount of supporting stroma present in the former.



Extreme care must be taken when referring to the use of stem cells in a treatment. What has been used in wound in healing and breast reconstruction is the *stromal vascular fraction*, which contains stem cells, sometimes enriched with the addition of a cell concentrate that can be obtained even inside the operating room, although it is not a pure stem cell composition [8, 11, 13].

Based on what is currently known about MSCs, it is possible to imagine that, within a relatively short period of time, there will be uses for stem cells such as

- Excellent-quality skin, cultivated from the patient's own cells, to cover burnt areas, or to replace unaesthetic scars and sequelae of tumor resection.
- "Custom-built" replacement bones and cartilage for accident victims and patients who have undergone large resections or suffered from degenerative diseases, or production of new limbs, in case of agenesis.
- Nerve repair after trauma or tumor excision.
- Insertion of collagen in resorption areas of the face, leading to facial rejuvenation through the laxity of folds and rhytids.
- Replacement of allogeneic prostheses using the patient's own tissues. Surely, it will be possible to induce growth in hypoplastic breasts, or to offer new ones to women who have lost them.

Although this seems to be a divine power, man's dedication to Science is making it closer to all of us. From small cells we will get great results (Y.-G. Illouz, 2003, personal communication).

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