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## Stem Cell Enriched Fat Transfer

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### 1. Introduction

Recently, medical techniques have been proposed for tissue regeneration using autologous adult fat stem cells obtained with liposuction to restore the regular volumes of adipose tissue in the body and especially in the face.

The regeneration of the face adipose tissue follows the outline of a technique already in use: the lipofilling. This process is based on fat cells obtained with low-pressure suction and further centrifugation to separate fat cells from the stroma-vascular connective tissue.. The aim is to infiltrate only intact cells, able to survive in their new home [1].

For the technique of regeneration we basically do not use only normal fat cells but mainly **fat stem cells**. These are sown in small amounts to stimulate the formation of new adipose tissue (**liposowing**) [2]

The fat is rich of stem cells [3]. An average rate of fat stem cells in adipose tissue is one of every 50 normal fat cells (compared to bone marrow that contains 1 for 10000).

Today there is a huge discussion about the use of stem cells present in adipose tissue. [4] The large numbers of stem cells in adipose tissue means that clinically relevant stem cell numbers could be extracted from the tissue, potentially eliminating the need for in vitro expansion. To utilize these characteristics of adipose tissue fully, Cytori Therapeutics Inc. has developed a closed system called Celution to isolate and concentrate stem cells and regenerative cells automatically from adipose tissue. [5]

J. Victor Garcia and Maurizio Ceccarelli have developed a simple technique to enrich stem cells in the area of collection for Liposowing. This technique has been presented for the first time at BioBridge Event of 2008 in Geneva Palais des Nations and you can find references about it on [www.ijcs.org](http://www.ijcs.org) and on [www.aephymed.org](http://www.aephymed.org). [49] [2]

The explanation of why fat is so rich in adult stem cells, can be sought in the biological function of this tissue: the energy storage. The adipocyte is able to significantly increase its volume to collect energy in the triglycerides form. [6] But when its volume is very high (higher than 170% of normal volume) the adipocyte stimulates the formation of new adipose tissue by activating the differentiation of stem cells in the stroma-vascular connective tissue. [7] The liposintetic stimulus leads to adipocyte hypertrophy that, at a certain volume, stimulates perivascular stem cell propagation and differentiation. The stimulus for preadipocytes mitosis and differentiation, follows mainly the increase of the insulin rate for the receptor down regulation and the liberation IGF-1 in the hypertrophic adipocyte. [8]

The insulin receptor down regulation (internalization of the insulin receptor for excess of adipocyte volume) creates insulin resistance. [9](Kim E). The insulin concentration increasing leads to stem cells proliferation with the new pre-adipocytes formation. [10]. The volume increase of adipocytes activates, also, paracrine secretion of IGF-1 and stimulates the preadipocytes formation. [11]

The liposowing differs therefore from lipofilling mainly because we implant fresh fat stem cells to prevent stem cell eventual differentiation and transformation when cultured.

Based on the foregoing, the Liposowing uses the following protocol:

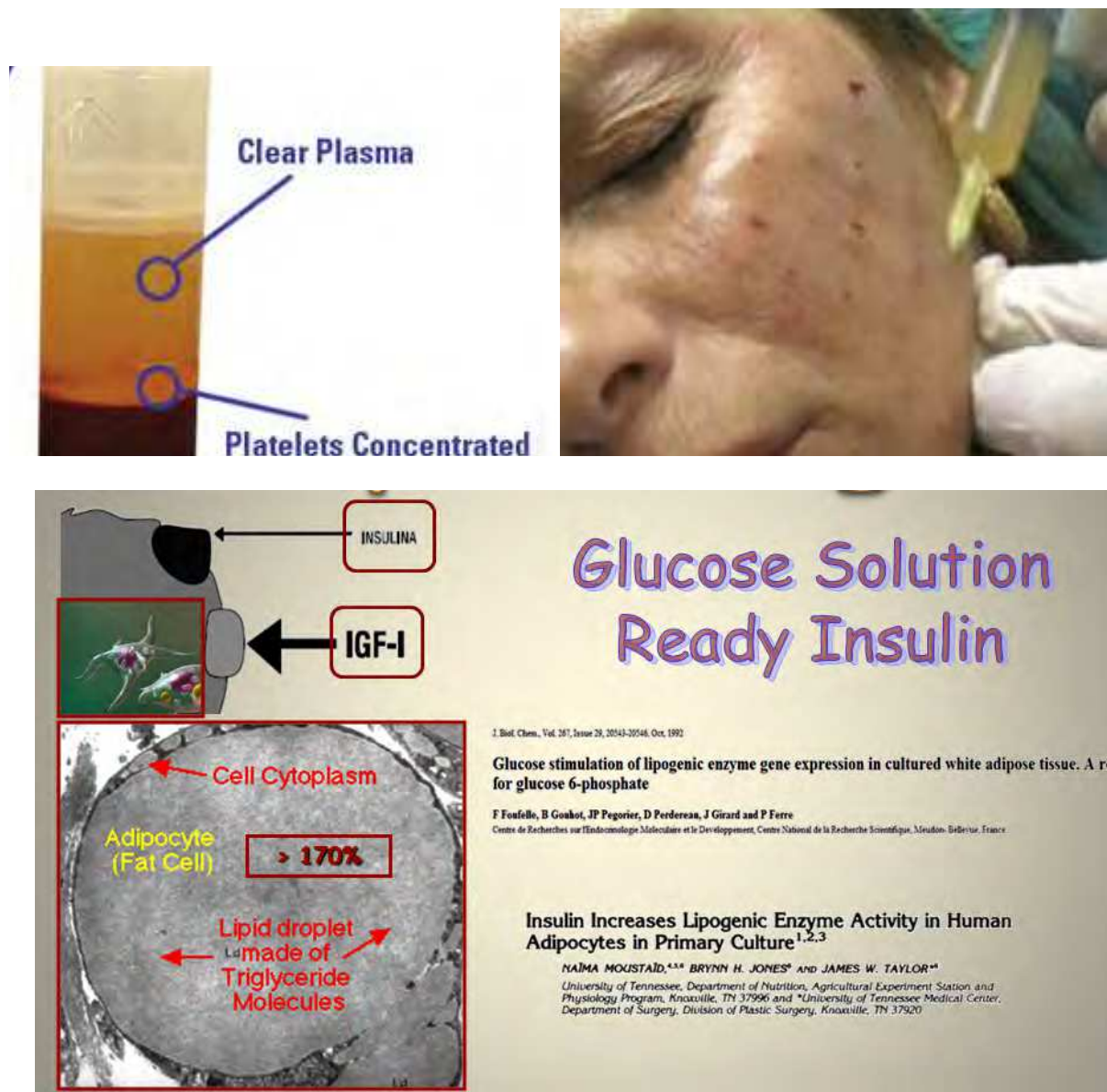
1. Stimulation of the fat donation area in the body with a ready insulin and glucose solution to increase the adipocyte volume.
2. Insulin, the main lipogenetic hormone, stimulates the function of lipoproteinlipase allowing the fatty acids uptake from the circulating lipoprotein and the glucose entry in the adipocyte. Glucose is the precursor of glycerol phosphate. [12] The latter binds fatty acids to form triglycerides. [13] We use an amount corresponding to 1 IU of insulin per kilo of fat to stimulate. [14]
3. 100 units of insulin are diluted in 250 cc of saline solution or glucose 5%. The resultant solution contains insulin 0.4 U.I. per milliliter.
4. We use one milliliter of this solution diluted in 200 ml of 5% glucose to inject it and stimulate a body area containing about 400 cubic centimeters fat. Usually, we use the abdominal area ease to handle.
5. We inject 0.5 milliliters of the prepared solution per one square centimeter in an area of stimulation.
6. 4 hours after the infiltration we do an infiltration with local anesthetic (1% lidocaine and adrenaline) to the same area.
7. When bleaching of the tissue is visible (bleaching is a sign of vasoconstriction and anesthesia), we aspirate fat from the area with 14 G needle which allows to collect rich stromal fraction of stem cells. The collection of stromal-vascular fraction is important because it is rich on stem cells.
8. To transfer and replant the fat rich on stem cells we use a small cannula with a diameter of 2.1 mm. The fat cells are inserted into the face fatty tissue in small amounts (rice grain technic - Fischer). We can use an automatic gun to maintain constant low volumes.

The liposowing, using amplified fat rich on stem cells, allow regeneration of the adipose tissue. Moreover, the presence of CD31 and CD34 positive cells could also induce a regeneration of skin tissues. [15]

## 2. Methods

The first clinical work and basic research of skin bio-stimulation with PDGF, made by Prof. J. Victor Garcia and Dr. Antonio González-Nicolás gives us important histological information. [16]

- After 7 days of biostimulation with PDGF we have a maximum of angiogenesis. [16] The improvement of vascularization allows for better engraftment of fat cells that we will insert. To increase the concentration of adipose stem cells we must induce the proliferation of these in the donor area. Insulin and IGF-1 have this feature and act when the adipocyte volume exceeds 170%.



To stimulate the increase in adipocyte volume that stimulates the new formation of triglycerides we infiltrate the donor area with Ready Insulin and Glucose Solution.

We perform an initial dilution using 100 IU of Insulin Ready in 250 cc of glucose solution 5% to get a concentration of 0.4 IU of insulin per milliliter.

Considering that the normal amount of insulin used for stimulation is 1 IU per kg, if we want to stimulate an drawing area of 20 cm for 20 cm corresponding to a total area of 400 square centimeters, we use 0.4 IU i.e. 1 ml of solution that we have prepared.

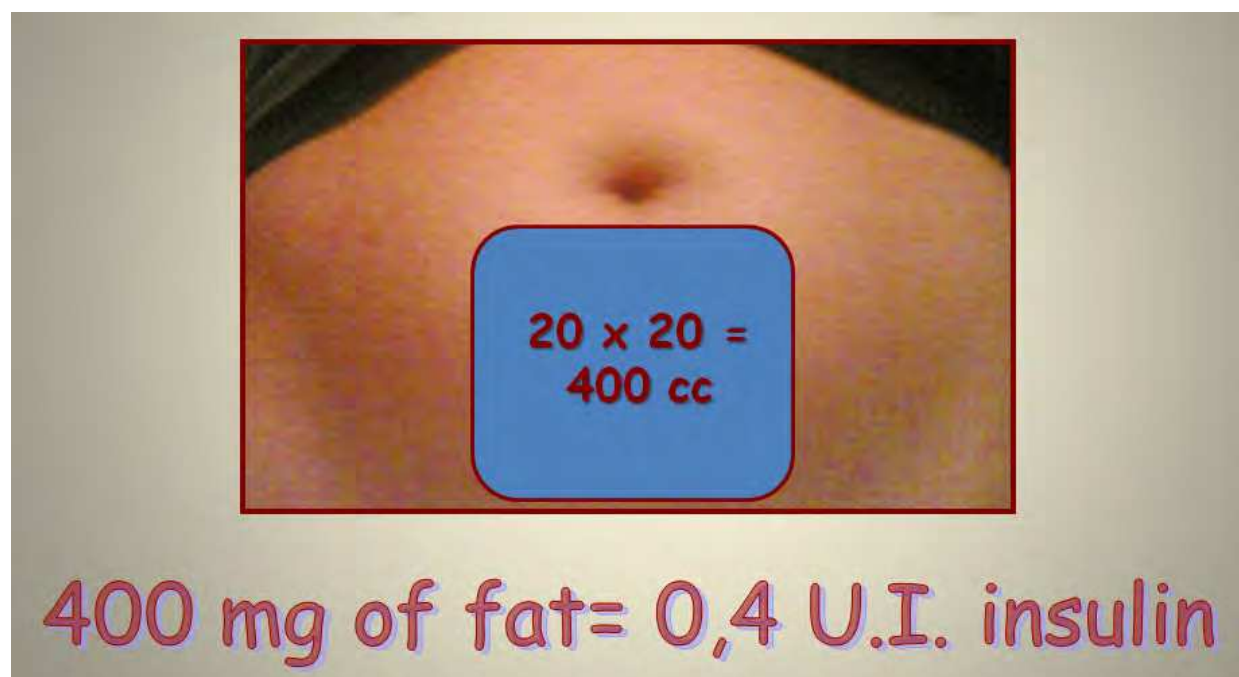
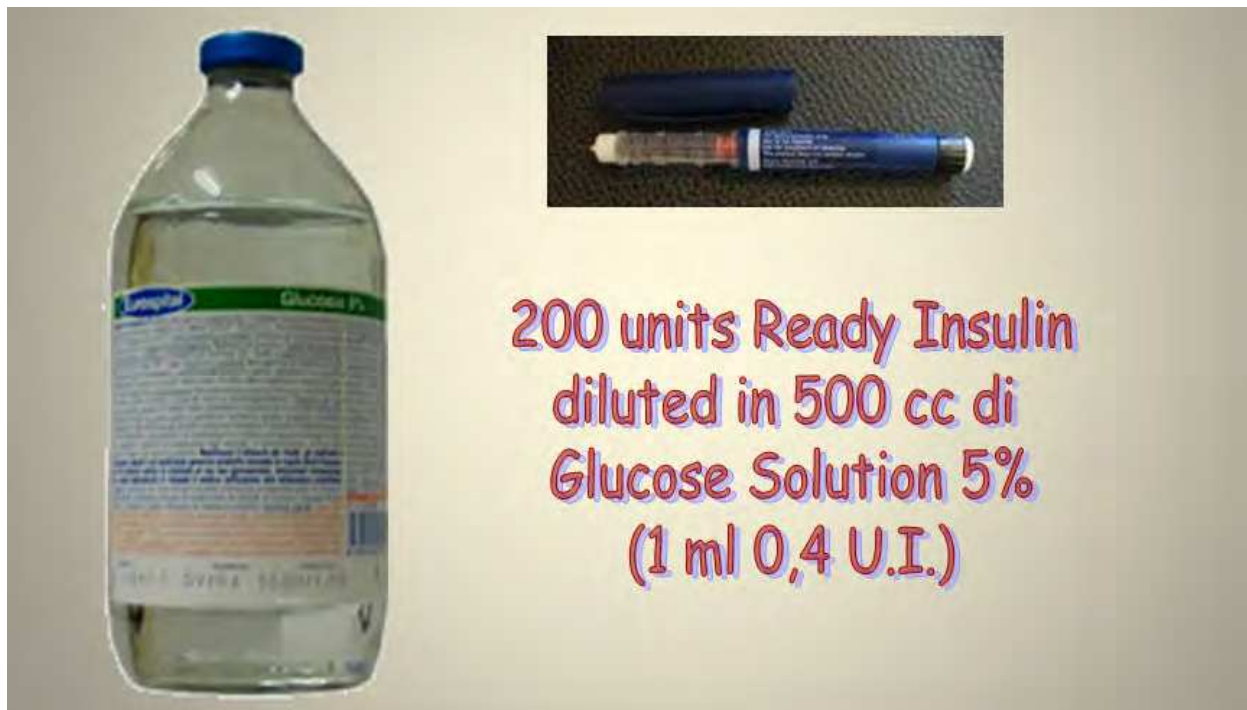
Solution for infiltration and stimulation of 400 cc fat:

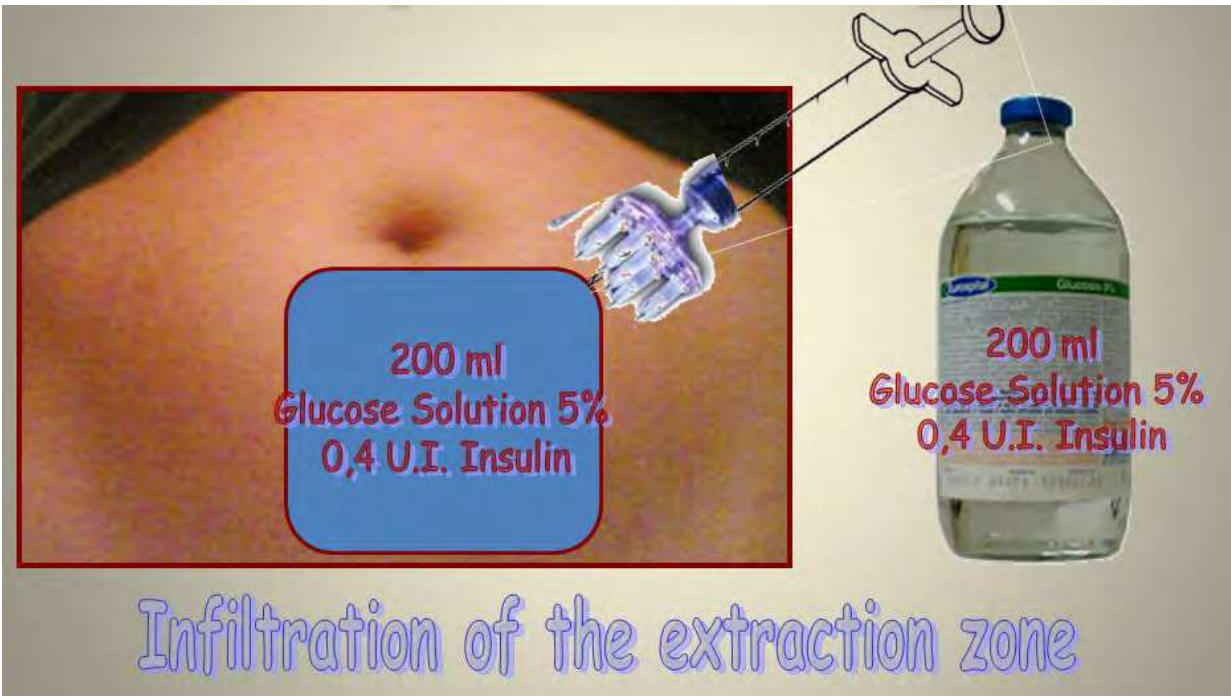
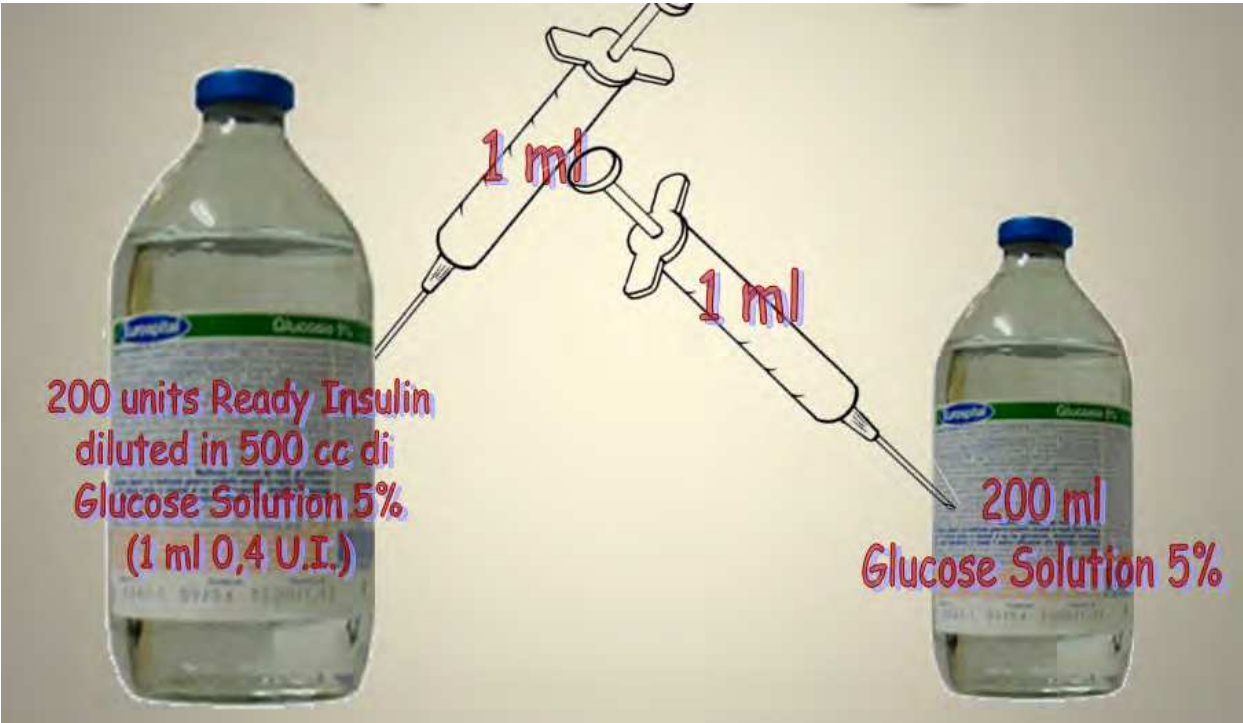
We prepare the solution to infiltrate in 400 cc of fat, like this:

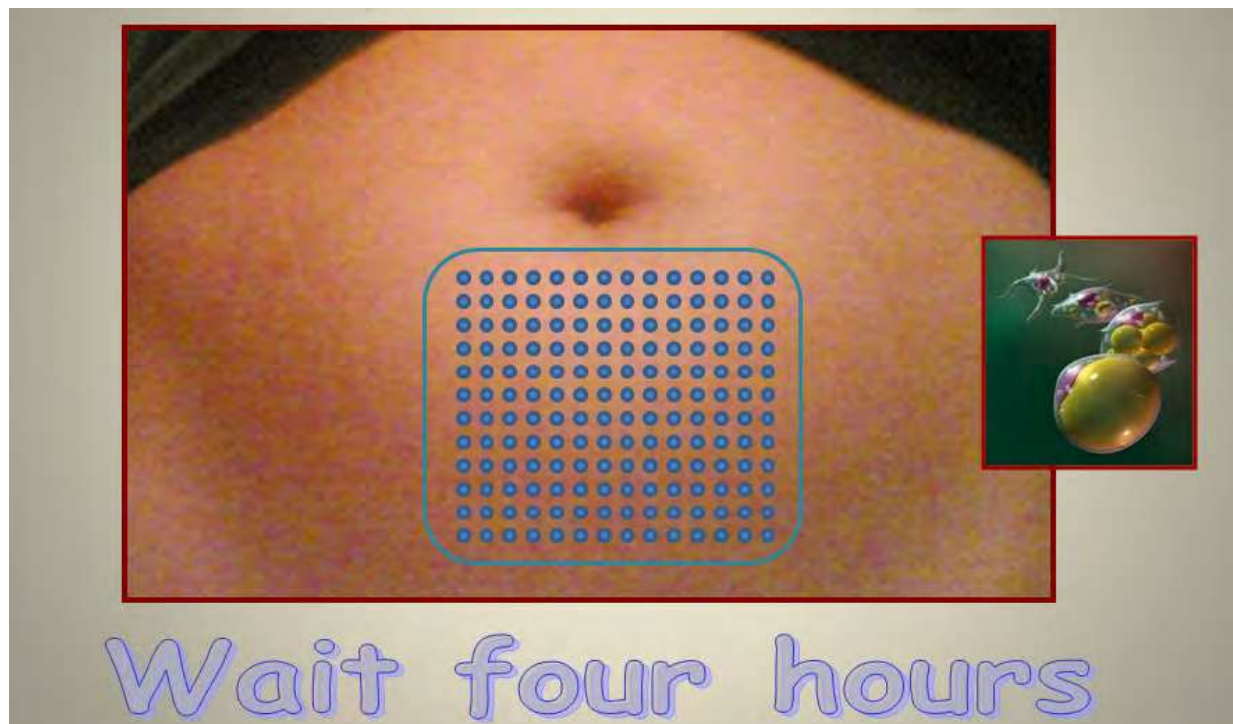
- We take 1 ml from the solution already prepared (containing 0.4 IU of insulin per milliliter),
- Than we dilute the same one milliliter with 0.4 IU insulin in 200 ml of glucose 5%.
- The new solution (200 ml sol. Glucose 5% + 0.4 IU of insulin) we use to infiltrate the donor area of 400 square centimeters. This is equivalent to a volume of 0.5 cc of the new solution per cubic centimeter of fat.



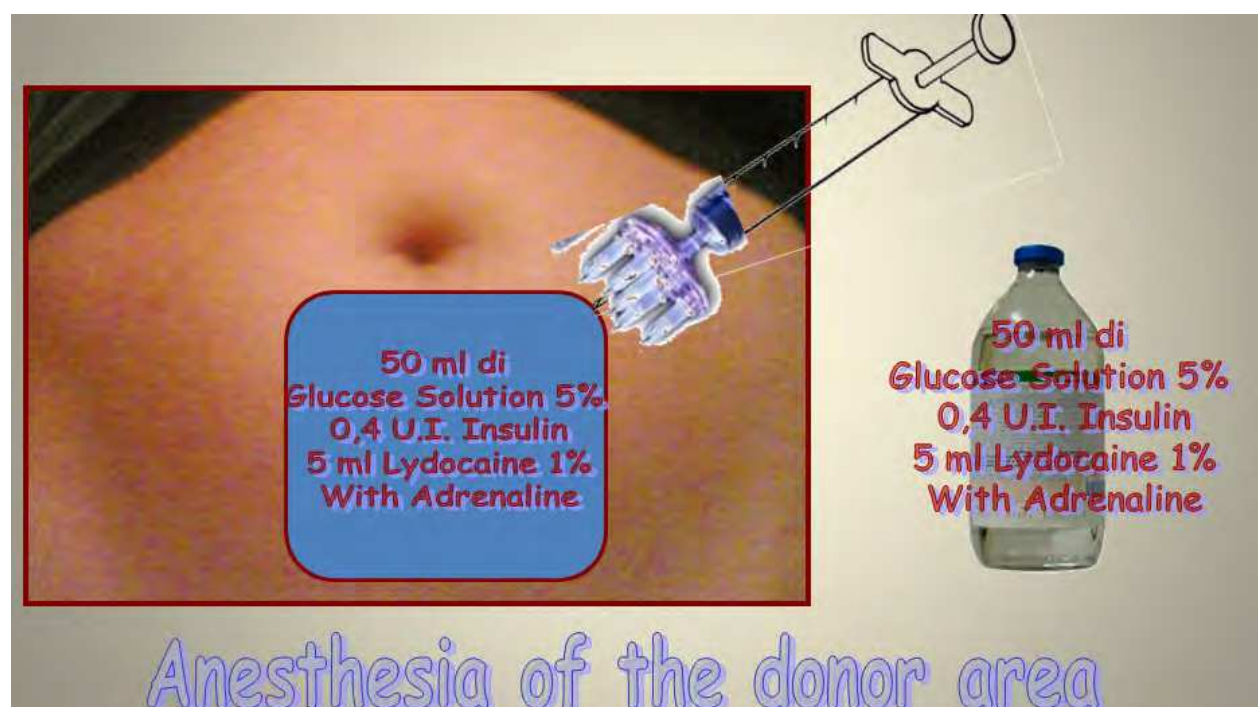
- We wait for four hours to allow the formation of new triglycerides intraadipocyte and the resulting proliferation of stem cells by insulin and IGF-1. [17]







- After 4 hours we perform a new infiltration with a solution of 200 ml of Sol 5% glucose with 0.4 IU insulin and adding 5 ml of 1% lidocaine with 1:50,000 Adrenalin.



- We wait for the sign of complete bleaching of the zone confirming the action of the anesthetic and vasoconstrictor.



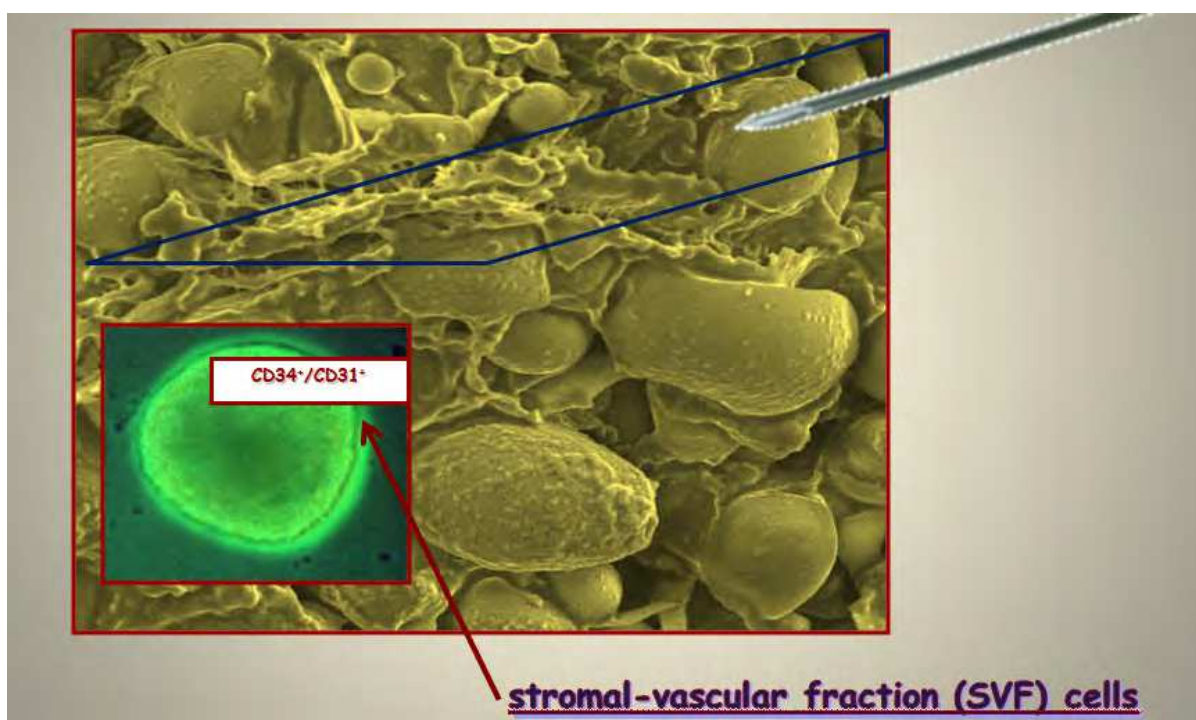


- We perform the aspiration of fat stem cells using 14 G needle mounted on a 5 ml syringe.





This is to collect the stromal-vascular fraction, where stem cells are located, [18] and to avoid injury to these very sensitive cells. The needle picks up real small fraction-vascular stromal cores. Stem cells (CD34 +/CD31-) differ into adipoblast (CD34+/CD31+) [19] that are implanted more easily, and with their propagation give rise to the formation of a new fat. [20] [21]



- The fat obtained with stem cells is retained in the syringe with glucose and insulin solution during the preparation of the recipient area (cleaning, disinfect and anesthesia).
- Lastly, the syringes are emptied of the solution and the fat with stem cells is injected with a cannula of 2.1 mm into the donor area. In areas where fat tissue is hypotrophic (Bichat fat pad area, nasolabial fold), the fat transplant is injected in small amounts (rice grain technique - Fischer).

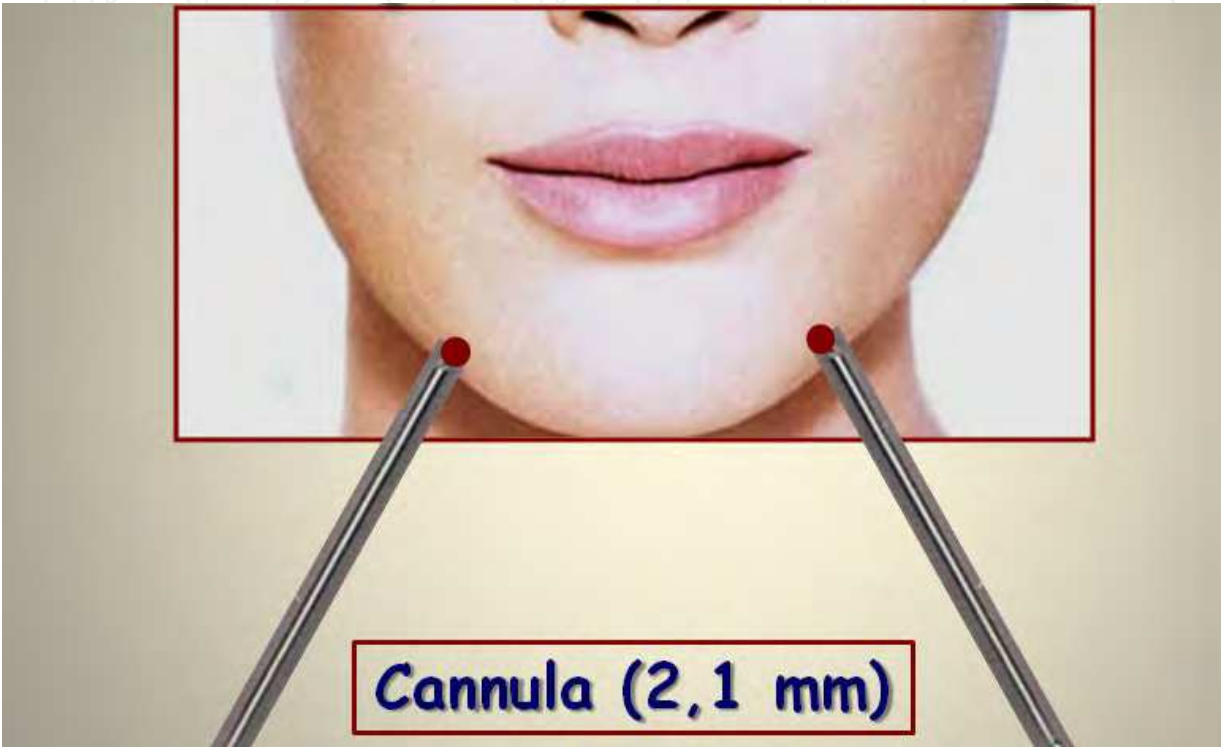
### 3. Discussion on safety in use of adult fat stem cells in regenerative medicine

Today, more and more, we talk about stem cells and their possible use to regenerate tissues or organs. Even in physiological medicine, recently, treatments have been proposed based on cells defined as stem cell (**liposowing**).

In the light of any legislative issues, we should, make a clarification in this scientific field. Indeed, the generalization of the term stem cell can lead to include useful treatments and therapies, without biological damage, including treatments that must be rightly adjusted and maintained in appropriate environment.

Attitudes towards the use of stem cells for research or medical care vary from country to country. In Germany, for example, the extraction of stem cells from human embryos is illegal. In Britain is perfectly legal, but laws are strict. In many countries there is still no explicit laws designed to regulate research on human stem cells.

Since the use of embryos is a matter of great controversy in ethical terms, scientists around the world are looking for other sources of stem cells. The type of stem cells found in bone marrow of adults seems to be one possibility. Today, the discovery of the high numbers of adult stem cells present in adipose tissue resulted in vogue for conservation of this cell type. Moreover, scientists have begun to manipulate these adult mesenchymal stem cells so that instead of producing only one type of tissue, it became possible to give rise to cells of other tissues.





- After liposowing antibiotic cover is maintained for three days.





### 3.1 Stem cells

With the term *stem cells* are defined primitive cells and non-specialized capable subsequently to differentiate into many different cell types. A stem cell must have the ability to run an unlimited number of replication cycles, without differentiation.

Depending on the capabilities we can distinguish four types of stem cells:

- The **totipotent stem** cells capable to develop into a complete organism. [22]
- The **pluripotent stem** cells able to specialize in all types of cells that can be found in an individual. [23]
- The **multipotent stem** cells able to specialize only in certain cell types. [24]
- The **unipotent stem** cells that generate only one type of specialized cell. [25]

Stem cells are also classified according to the source of derivation, as embryonic, fetal, amniotic, and adult.

The **pluripotent stem cells** are induced, obtained in the laboratory to the regression of adult cells (already determined, for example, skin) in a state stem cell (pluripotent), using a pool of specific genes, placed via a viral vector. Therefore, in future these cells may be used to obtain adult stem cells already established, belonging to any tissue or organ.

The bulk of the regenerative work leading to the repair and/or to proliferation of tissues, is played by cells no-stem defined **progenitors** or **transit amplifying cells (TACs)**, [26] directly derived from stem cells, but partially differentiated with lack of ability to self-renewal [27] This replicative strategy, which limits the number of replication events that a stem cell can do, is based on the need to keep checking the number of stem cells and maintain the integrity of the genome of stem cells by reducing the risk of damage to DNA (i.e. mutations). [28] Mutations in stem cells are extremely harmful and dangerous, because are transmitted to all generations of daughter cells derived from stem cells. Unlike, a mutation in a TAC affects only a single generation of cells that after some time will be replaced, or may induce stem cells to develop into cancer, becoming a stem cell tumor, a type of cell that is probably responsible for the continuous supply of new cells that characterizes the development and especially the recurrence of cancer. [29]

### 3.2 The transit amplifying cells

Adult stem cells or transit amplifying cells are unspecialized cells that reproduce daily to provide certain specific cells, e.g. red blood cells are generated daily in the body from hematopoietic stem cells. [30] Until recently it was thought that each of these cells can produce only one type cell. [31] Today there is evidence that adult stem cells can become many different forms: it is known that stem cells in the stroma of the bone marrow can become liver cells, neural, muscle, kidney, and follicular. [32] Transformation of one type stem cell into another is called **transdifferentiation**. [33] Useful sources of adult stem cells are actually detectable in all organs of the body.

### 3.3 Cell differentiation

This is the process by which a less specialized cell becomes more specialized. Cell differentiation occurs during the development of a multicellular organism, but also common in adult stem cells during tissue repair and during normal cell turnover. [34] The differentiation changes dramatically the size of shape cell the membrane potential, activities and metabolic response to signals. [35]

The main types of molecular processes that control the cell differentiation, involve the cellular signals. Many of the signaling molecules used to transmit information from one cell

to another are called **growth factors**. Typically, a ligand produced by a cell binds to a receptor of another cell, inducing a conformational change of the receptor. The receptor then catalyzes a cascade of phosphorylation reactions that eventually trigger a transcription factor or cytoskeleton proteins, activating the differentiation process of the target cell. [36]

Other important mechanisms fall into the category of the **asymmetric cell divisions**, divisions which give rise to daughter cells with distinct developmental fates. Asymmetric division is a fundamental step for the development of the embryo and also for storage of stem cells. Normally when a cell divides, produces two identical daughter cells but in some cases the daughter cells have different properties. Scientists have found that for the occurrence of the asymmetric division, it is necessary that the mitotic fuse is positioned towards the rear of the cell (not centrally). This positioning of the fuse occurs through the interaction of the microtubules forming the mitotic fuse and the network of actin filaments adhering to the plasma membrane. This leads us to investigate the molecular interactions of cells with other cells based on the accession process. [37]

### 3.4 The cell adhesion

Adhesion is a system of communication between cells based on the interaction of pairs of receptors expressed by cells adhering to each other. This system is an alternative to communication related to the release of cellular soluble messengers (hormones, neurotransmitters, cytokines, etc.). The cell adhesion is involved in a variety of physiological and pathological mechanisms. The adhesion between cells happens when a plasma membrane receptor form a bond with one molecule that is located in the extracellular matrix, or in the neighboring cell. The receptor binding then establishes a connection with the cell cytoskeleton. From this, adult stem cells have a state of differentiation that implies cell junctions. These are a specialization of the membrane strip that enables and controls the processes of adhesion between cells. Among the various types of cell junctions, the junction's members provide to structural support to tissues using binding to actin filaments. We can differentiate groups of **adhesion and focal contacts**.

The adhesion contacts are links established, between a cell and other adjacent, thanks to cadherins. The focal contacts, however, are joints that connect the cell to the matrix, except that instead of cadherins they use integrins, associates with actin filaments via transmembrane proteins such as the alfa-actina, talina, vinculina and filamina. Therefore, this type of cells may express a regulation of inhibition contact with other cells following the accession which induces a block to the anarchic proliferation. In the normal process of contact inhibition is mainly the accumulation of p27Kip1 . protein to trigger the inhibition of Cyclin E/CDK2 complex, which in turn inhibits the phosphorylation of Rb protein, leading to cell cycle block. [38] [39]

We can now reach the ultimate explanation and that the absence risk of neoplastic transformation of adult stem cells.

### 3.5 Carcinogenesis

Cancer is characterized by the uncontrolled reproduction of some body cells that stop responding to physiological mechanisms of cell control after damage to their genetic heritage.

A cell to become cancerous, it must accumulate a series of damage to its system of control of reproduction. To all cancer and precancerous cells changes have occurred, often very large,

in their chromosome structure (karyotype). Underlying the pathogenesis of cancer is therefore the mutation of certain genes

- proto-oncogenes,
- tumor suppressor genes,
- genes involved in DNA repair.

The latter are those that ensure genetic stability because if other genes are mutated by the carcinogens actions, these repair the DNA before the replication, which was before these changes become permanent.

Mutations necessary that a given cell must accumulate to give rise to cancer are as follows, and are common to all types of cancer:

1. acquisition of autonomy multiplicative for incapacity to submit to the regulatory mechanisms of cell proliferation;
2. absence of density-dependent inhibition (the normal cells multiply up to a certain cell density, reached by which they become quiescent);
3. reduced adhesion with other cells or tissue components;
4. absence of extracellular matrix (usually digested by proteases), which promotes the invasion of adjacent normal tissues;
5. angiogenesis: formation of new blood vessels to deliver oxygen and nutritional factors to cancer cells;
6. reduction or loss of ability to differentiate;
7. acquisition of the capacity for unlimited replication effect of the expression of telomerase;
8. reduction or loss of the possibility of getting programmed cell death (apoptosis).
9. loss of so-called contact inhibition.

These events require more than one mutation, in general, the most mutations of certain classes of genes. The loss of proliferation control will take place only in response to mutations in genes that control cell division, cell death and DNA repair processes.

Because the cells begin a uncontrollably division must be damaged the genes that regulate growth. The proto-oncogenes are genes that promote cell growth and mitosis that is the process of cell division, the tumor suppressor genes discourage cell growth or prevent cell division to allow DNA repair. Typically requires a series of several mutations in these genes before a normal cell turns into a cancer cell.

So, various types of gene mutations are required to form cancer. A mutation limited to one oncogene would be removed from the normal control processes of mitosis and tumor suppressor genes. A mutation of a single tumor suppressor gene, would also be insufficient to cause cancer by the presence of numerous copies of "backup" genes that duplicate its function. It is only when a sufficient number of proto-oncogene is mutated in oncogenes and a sufficient quantity of tumor suppressor genes have been turned off that the signals to cell growth are superior to the inhibitors signals that this increases rapidly and out of control. [40] [41] [42] [43] [44] [45] [46] [47] [48]

#### 4. Conclusions

From the above, we can conclude that the use of adult stem cells or transit amplifying cells adipose tissue derived (Liposowing) is devoid of possible side effects and does not require control laws. The proposed treatment, in fact, does not include cell handling. The proliferation of adult stem cells is done by physiological means and the new stem cells



produced are returned to the same tissue (adipose) allowing the multiplication adjustment by contact inhibition.

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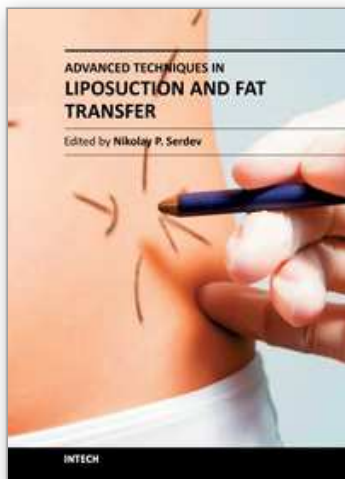
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## **Advanced Techniques in Liposuction and Fat Transfer**

Edited by Prof. Nikolay Serdev

ISBN 978-953-307-668-3

Hard cover, 230 pages

**Publisher** InTech

**Published online** 12, September, 2011

**Published in print edition** September, 2011

Liposuction is the first cosmetic procedure to change beautification surgery from open extensive excision surgery into a more atraumatic closed one. It gave rise to the modern understanding of minimally scarring and minimally invasive surgery and changed the understanding and preferences of both patients and doctors. It also became the most common procedure in cosmetic surgery world-wide, practiced by an increased number of physicians from various specialties. The techniques of fat grafting, closely bound with liposuction, have found widespread application and fat stem cells seem to be changing the future of many areas in medicine. Turning the pages, the reader will find a lot of information about advances, tips and tricks, as well as important milestones in the development of the different methods available, such as classic, power, ultrasound, laser and radio-frequency assisted liposuction etc. Most useful anesthesia techniques are described and discussed, and guidelines have been established for medical indications. Special attention is paid to good patient selection, complications and risks.

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