Adipose and dermis tissue-derived stem cells in regenerative and aesthetic medicine: certainties and perspective after seven working years of personal practice

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SUMMARY

Under the pure biological profile it is unthinkable to use adult stem cells or better cells with adult MSCa staminal characteristics, derived from the mesenchymal tissue, together with methods that negatively affect their function or their correct engraftment. The use of adult mesenchymal stem cells (MSCa) is today one of the areas of greatest interest in regenerative medicine but respecting the biological characteristics of the cells themselves. Adult mesenchymal stem cells, MSCa, are characterized by a morphology similar to that of fibroblasts and are multipotent cells. They can be extracted with outpatient methods, both for the dermis and for the adipose tissue and used even in the nasal lacrimal grooves or in the nasal sulcus.

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INTRODUCTION

Mesenchima is a tissue with support function. It is the embryonic connective tissue of mesodermic derivation, is devoid of its own form and fills the spaces of the differentiating organs. It has a very fluid intercellular substance and multipotent, stellate or irregular stem cells, with extensions. Cells have high mitotic activity and are able to differentiate in all cell types of connective tissues and smooth muscle fibrocells [1].

Under the pure biological profile, it is unthinkable to use adult stem cells or better with adult stem cells derived from mesenchymal, MSCa, together with methods that negatively affect their function or their proper intaking [2].

The use of adult mesenchymal stem cells (MSCa) today is one of the areas of major interest in regenerative medicine but respecting the biological characteristics of the cells themselves. I remember that regenerative medicine is the branch that has the purpose of replacing with the same or potentially similar cells, the damaged or modified tissue of the aging process, and the objective is essentially to restore the function of these organs / tissues with a tissue that is not a repair tissue. If MSCa were used with methods that have the intrinsic property of inducing fibrosis, we could biologically obtain their apoptosis by increasing ROS due to paracrine action of inflammatory cytokines initially produced to repair the damage [2, 3]. The use of MSCa therefore identifies the set of therapies that, in pursuit of the goal of regeneration, use or otherwise exploit the potential of stem cells or better cells with adult stem characteristics. In this case the cells themselves become part of the improvement and regenerative evolution of tissues: cellular and non-pharmacological therapies. Adult mesenchymal stem cells have demonstrated a great ability to regenerate tissues, remodel the immune system and also possess a number of other qualities from which tissues can benefit. In fact, during the aging process the stem pool tends to run out. Aged cells produce inflammatory factors by increasing and increasing the exhaustion process of the stem, just like an epidermis or dermis injury [4]. Recent research on adult stem cells has made it possible to define that these cells are multipotent, that is, they can replicate and stimulate appropriately, differentiate into specific and different cellular populations (bones, cartilages, tendons, muscles etc) but also select a subpopulation of fibroblasts , fibrotic fibroblasts, fibrotic collagen producers and very active in repair processes [5, 6, 7]. They are capable of self-replicating and proliferating unlimitedly when placed in the soil of culture or in our organs, but only if the physiological conditions of the implant are respected. This ability of MSCa is defined as plasticity and is the pillar of their use in regenerative medicine but also and above in aesthetic treatment.

But it is not fair to talk about adult stem cells and the regenerative capacity of these as if it were a unique thing. First in the embryo and then within our adult body, many types of stem cells perform continuous training and regeneration functions of the tissues. Adult mesenchymal stem cells are the only type of cell that can be used in regenerative medicine through the process of *tissue engineering*, taken, immediately processed and re-implanted [1, 2]. These cells are available in many tissues of our body and have the capacity, once increased by number and differentiated, possibly directed towards a specific tissue, to regenerate it partially or entirely. This can also happen at a distance from target tissue. Adult mesenchymal stem cells, MSCa, are characterized by morphology similar of fibroblasts and are multipotent cells [8].

These features have given rise to growing interest in those therapeutic perspectives based on the regenerative and repairing capacity of organs and tissues that stem cells offer provided that they are used according to a vision of *not everything and immediately* on the patient but of maintaining and improving gradual and physiological. Some studies have shown that MSCa are endowed with a strong regenerative power and can be obtained from different tissues such as bone marrow, peripheral blood, umbilical cord, adipose tissue and dermis [8, 9, 10]. The bone marrow contains mesenchymal or stromal stem cells (MSCa) and hematopoietic stem cells (HSCs). MSCs are present in the marrow in a smaller amount than HSCs and are responsible for the genesis of all non-hepopoetic cells that reside in the bone marrow. For many years, bone marrow has been considered to be the primary source of mesenchymal stem cells capable of responding to distant stimuli and being recalled by damaged tissues. However, due to the invasiveness of the withdrawal procedures and the progressive reduction with the age of the number of isolated cells and their potential differentiation, we have been directed towards the search for new sources of MSCa [8].

Recently it has been shown that peripheral venous blood can be considered an alternative source of mesenchymal stem cells, this in line also with PRP treatments [11]. Thanks to their particular characteristics, MSCa can be used in regenerative medici-

ne, in cellular and in tissue engineering alone or in combination with non-fibrogenic biomaterials, which work from scaffolding exclusively to allow proliferation at a given point. The high in vitro proliferative potential, trophism, anti-inflammatory capacity, the ability to have off-the-self cells and in particular the ability to differentiate and trans differentiate to specialized cells, implanted in the right context and a welcoming micro environment, ensure MSCa can be a tool for regenerating and not repairing tissues [12, 13, 14]. Under appropriate conditions, specific stimuli may result in these cells producing different differentiated cytotypes in the tissues that host them and their properties can be subdivided into static and dynamic. The static properties are: being undifferentiated, having proliferative capacity, having the possibility of self-defense, having the ability to generate differentiated progenitors while the dynamic properties are proliferation and self-sufficiency, along with flexibility in their differentiation. The term "adult stem cells" stem cells are derived from indicated tissues of adult organisms, to distinguish them from those taken from embryos. They are multipotent, able to differentiate into a number of cell types limited to the tissue in which they reside. They are non-differentiated cells capable of self-replication, that is to divide symmetrically by giving rise to two cells equal to the starting cell, or under the influence of particular stimuli, to differentiate, giving rise to specialized cells of the tissue in which they are located or in the case of aesthetic and regenerative medicine where they are transferred. Generally, differentiation does not occur directly, but through the generation of well-defined intermediates and orientation cells that are called progenitors / precursors or trans amplifying cells. This process takes place by asymmetric division or by symmetric division followed by a de-differentiation of the progenitor's stem cell: this ensures the continued presence of a stem pool in the niche of belonging, provided that the specific niche is not attacked and exhausted for example by ROS or from localized inflammatory stimuli [15, 16]. The main role of stem cells is to ensure the physiological replacement of "aging" cells (*tissue renewing*) and to restore the cells possibly damaged as a result of aging. In particular, adult human stem cells do not exhibit the ethical and safety problems arising from the use of fetal embryos because they do not require the sacrifice of an entire organism and are not tumorigenic. In fact, adult stem cells are isolated from body tissues and can be used in autologous settings by eluding the problem of immunological response and rejection.

MSCa are the adult stem cells that have been studied since they have their own characteristics, in addition to stem cells derived from other tissues / organs. All cells are defined in function of surface molecules, mostly glycoproteins expressed on their cell membrane. These molecules are commonly used as cellular markers and are called "Cluster of Differentiation" (CD). More than 320 CDs have been identified today. Generally, a combination of markers can characterize only one cell type, which is then described as having these markers. Cellular classification by the use of CD has also been applied to adult stem cells. Phenotypically MSCa express a number of markers which, unfortunately, are not specific to each MSCa. Adult MSCa do not express CD4, CD34, CD14, CD11 hematopoietic markers. They also do not express the co-stimulatory molecules CD80, CD86, CD40 or adhesion molecules CD31, CD18, or CD56. They can express CD105 (SH2), CD73 (SH3 / a), CD44, CD90 (Thy-1), CD71, Stro-1, CD106 binding molecules, CD166, intracellular adhesion molecules (ICAM) -1 and CD29 markers [8, 17, 18].

In particular, it has been observed that MSCa are:

- easily isolable thanks to their adhesive capacity;
- easily separable from other cell types by expressing a set of specific membrane markers (CD44 +, CD90 +, CD105 + 166 + 73 +, CD34-, CD45-31-14-) [19, 22];
- easily expandable in vitro as they have a high potential for replication;
- capable of immunosuppressive and immunomodulatory functions [22];
- able to migrate spontaneously into the origin tissues and selectively into damaged tissues (multiorgan homing capacity / trophism) [22].

In damage, they promote the regeneration of compromised tissue by both differentiation and secretion of anti-inflammatory factors [22]. They also have a distinct functional plasticity and a multilieneage differentiation potential [20].

Obviously, there are differences between tissue and tissue and during picking for subsequent extraction it is necessary to preserve in the tissues the present pool so as not to overstate it excessively. The cells being harvested and re-planted must maintain and possess a high proliferative, differentiating capacity and with the potential for interactivity with biomaterials.

It has been shown that in a differentiated tissue, mesenchymal progenitors have different characteristics than differentiators: they have a reduced size with high cytoplasmic complexity. The stem is transcriptio-

nal, that is, transcribes all the genes, but will only translate those typical proteins of the tissue where it has been transferred, so it is important not to transfer them to tissues under such acute inflammatory stimuli. These cells, then, are able to neovascularize and turn to the tissue cytotype in which they are injected. They potentially produce a number of autocrine factors (affecting the cell itself) and paracrine (affecting nearby cells) through the integrin system, which represent the mode of interconnecting with neighboring cells by recognizing the tissue in which they are implanted. MSCa is thought to be able to avoid the typical differentiation process of cells during embryonic development and to colonize appropriate niches; the latter would have the function of both maintaining cellular potential in adulthood and limiting differentiation processes [21, 22].

Materials and methods

1. Dermis

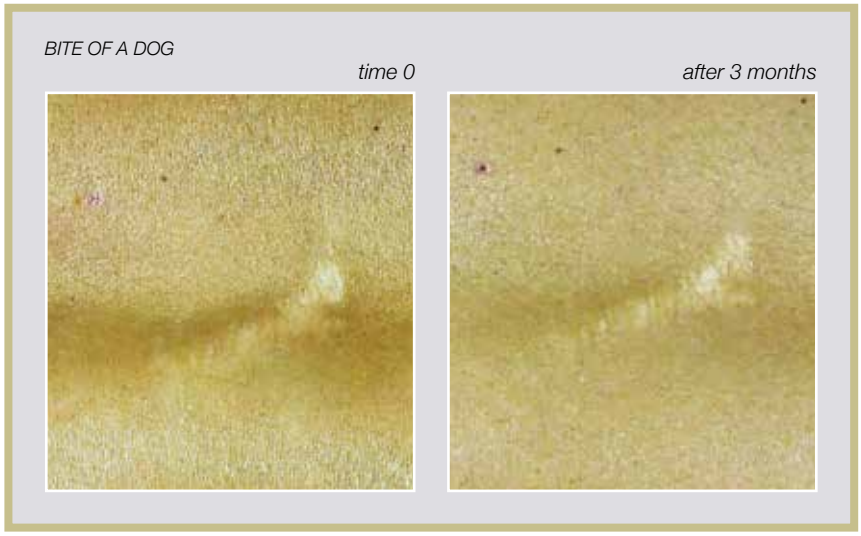
The study and procedure related to treatment with cells with adult staminal characteristics derived from the dermis, began at the end of 2013 and through a predefined protocol I obtained the results that are exposed below and the indications of this method are those typical of regenerative medicine in aesthetic medicine: wrinkles, tissue atonia, chelloids, scars, stretch marks, vitiligo, burns, sores, etc. [19]. Nowadays, the methods of extracting stem from the dermis for use in aesthetic medicine allow us to perform the surgery in the patient without risk to the patient and in compliance with the law. In fact, by simply picking up a 1 mm of dermis tissue, it is inserted into a special tool that disrupts it **(fig. 1)**. Disaggregating and filtering it releases a selected active stem suspension according to the measurement **(fig. 2)**.

This suspension contains 50.00 to 100.000 multipotent cells (MSCa) that can differentiate when inserted into the tissue we intend to regenerate by introducing an MSC.

Figure 1.

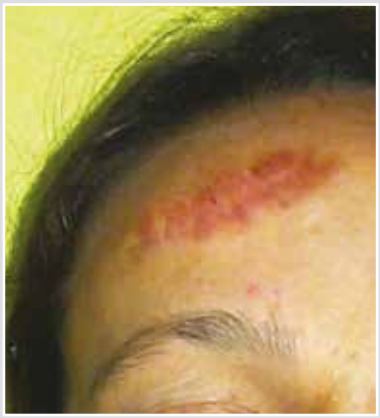
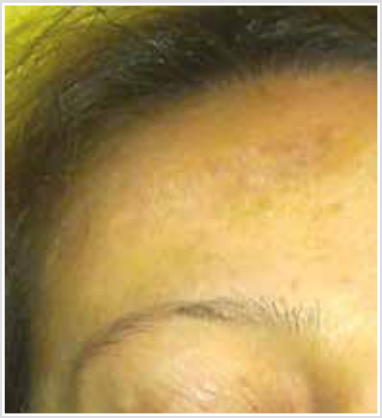
Figure 2.

The technique is very simple, since it is a matter of infiltrating the micro-pompous tissues of our attention to these results:



BURNING IN ADOLESCENT AGE

time 0 after 4 months

BURNING IN ADOLESCENT AGE

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BURNING IN ADOLESCENT AGE

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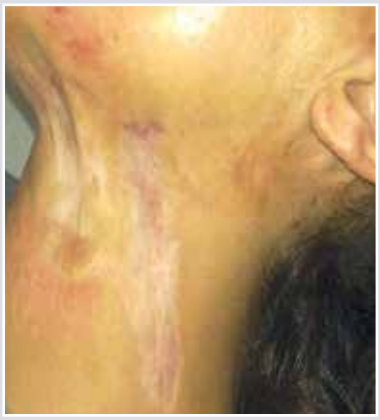
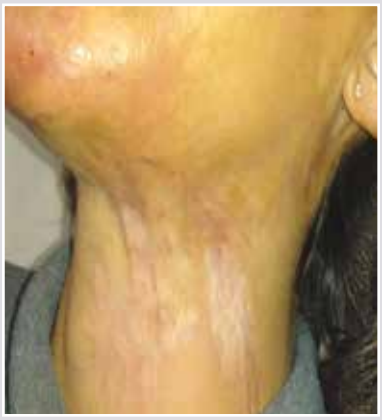



2. Adipose tissue

The study and procedure related to treatment with cells with adult staminal characteristics derived from adipose tissue, began at the end of the year 2010 and through a predefined protocol I obtained the results that are exposed below and another approach to regeneration and stimulus on adult stem cells, following the findings of the literature, is to use the adipose tissue, which is abundantly represented in our body as storage and storage material. As we know, adipose tissue, which is a highly specialized backing tissue, contains many adult mesenchymal stem cells, which are multipotent cells [23] capable of giving rise to different cell lines for their plasticity, exploited in aesthetic and reconstructive medicine. Adult stem cells reduce fibrosis and healing processes, restoring the correct expression by type I and III collagen fibroblasts [21]. Not only that, adipose tissue is able to perform autocrine, paracrine and endocrine action when present in abundance in certain areas of the body. The intention is to exploit autocrine and paracrine action but also the lipid content of each cell extracted from the donor site.

In fact, through a microfat, aggregate microstrip of stem cells and adipose material, it is possible to exploit the activation of IL-6 which produces an acute phase response locally, which allows tissue remodeling and to exploit in the immediate future the properties of IL -10, a typical anti-inflammatory cytokine that inhibits excess inflammatory status and of adverse events. With microfat biostimulation also results in an increase in the values of TGFbeta 1 and 3 (not the TGFbeta 2 typical of the inflammatory state), including those implicated in the control and "shutdown" of inflammatory, non-fibrotic ma-

trix growth [25] of EGF (epidermal growth factor) and FGF2.

Adipose tissue MSCs (ADMSCs) have the same differentiating potential of bone marrow MSCs [10]. This tissue offers the same possibilities of the marrow since the latter and the fatty tissue have the same ontogenic origin, mesoderm. The presence in a polar and non polar lipid microfat allows optimization and stabilization of cell membranes, stimulation of cells through various kinase pathways and support for the reintegration of cell separation systems through suspensions in intercellular fluids [26]. I remember that the only cells in addition to hepatocytes to exploit the presence of lipid material, in our case an inert microfat, are the smooth muscle cells for tone enhancement, and the fibroblasts that possess a LDL receptor, which activates some signal transduction pathways through specific receptors [27].

Regarding MSCa derived from adipose tissue, the manipulation technique is as follows. Once removed with a simple 14G needle, 10 cc of adipose material **(fig. 3)** and disassembled it **(fig. 4)** can be implanted without major risks, however, which should be evaluated case by case on the same patient even in difficult access or treatment points such as nasal- tear through 22G or 25G micro-cannulas **(fig. 5)**. The method described above allows us to be considerably more effective than any filler, maintaining a very high level of professionalism, the latter being of a certain membership to the aesthetic M.D.

Figure 3. *Extraction.*

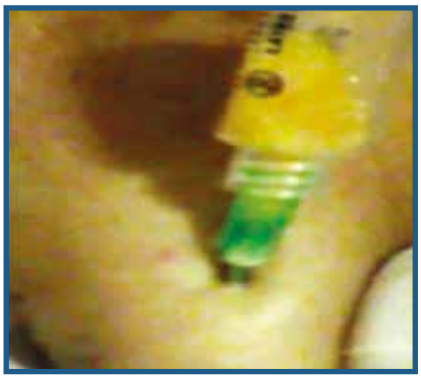


Figure 4. *Cellular disintegration with a three-way valve.*

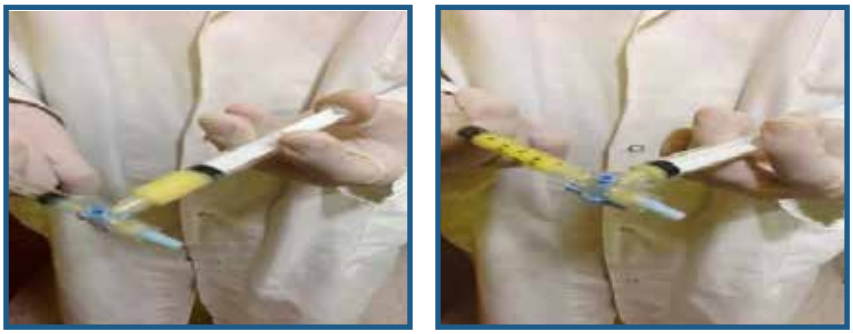
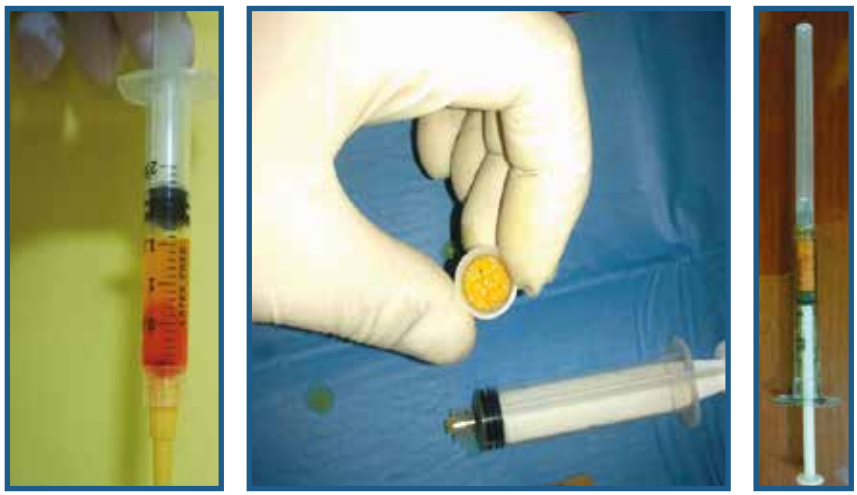
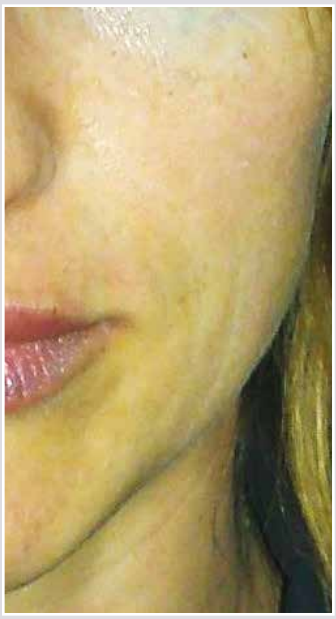


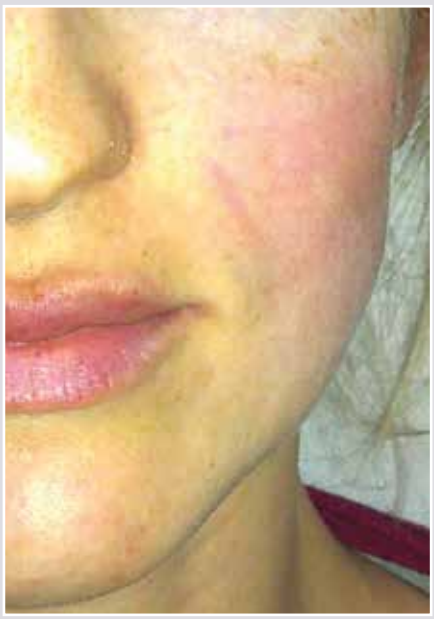
Figure 5. *Collection of cells with adult stamina characteristics through a filter with holes of adequate thickness and injection with 1 ml syringes and 22G cannulae.*



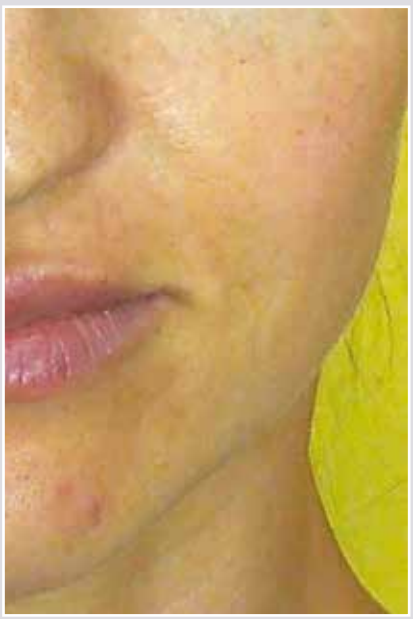
We can use this technique to restore particular volumes, depleted with time such as nasal-lacrimal hairs, or approach a facial rejuvenation using the fan technique on facial wrinkles to achieve these results:



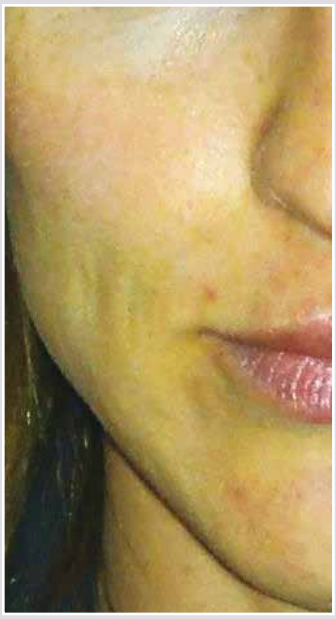
Time 0

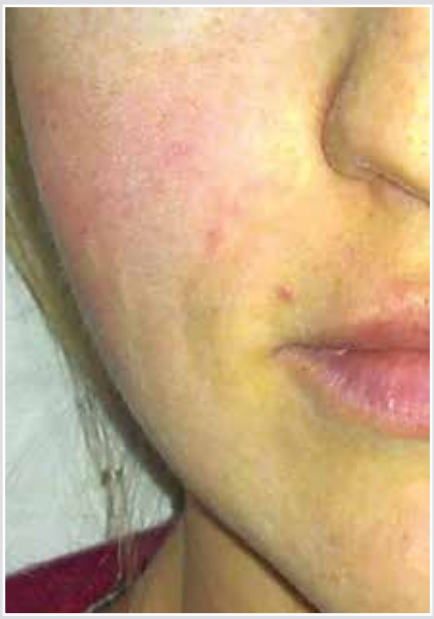


immediatly

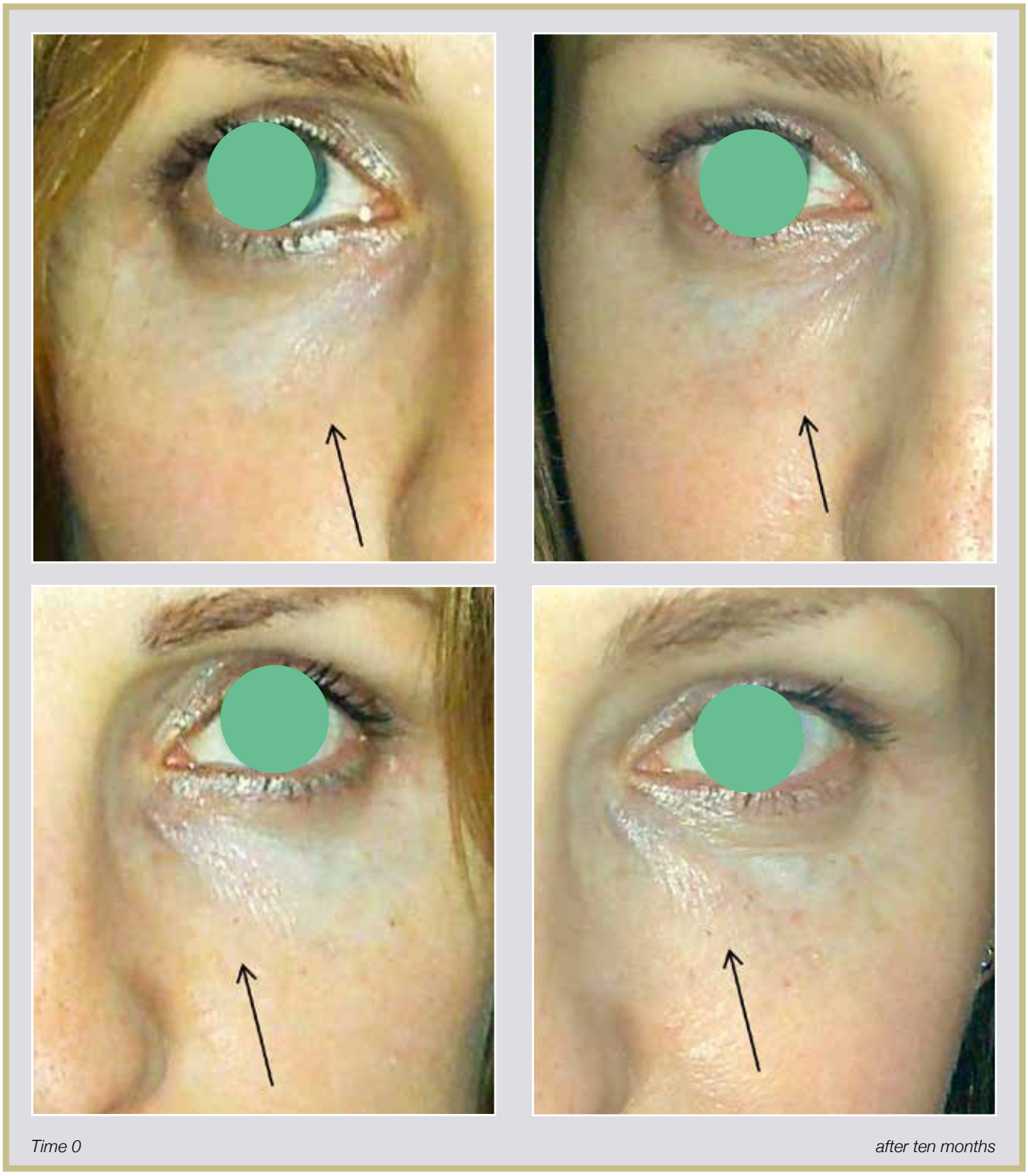


after ten months

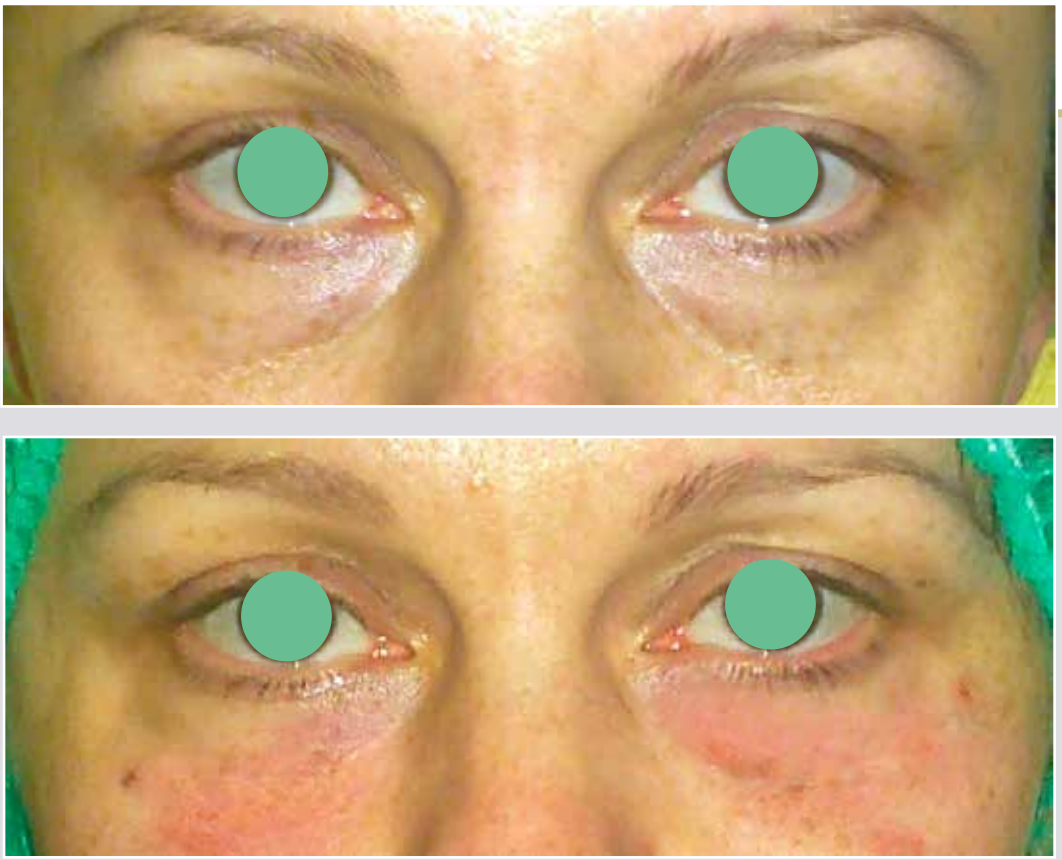




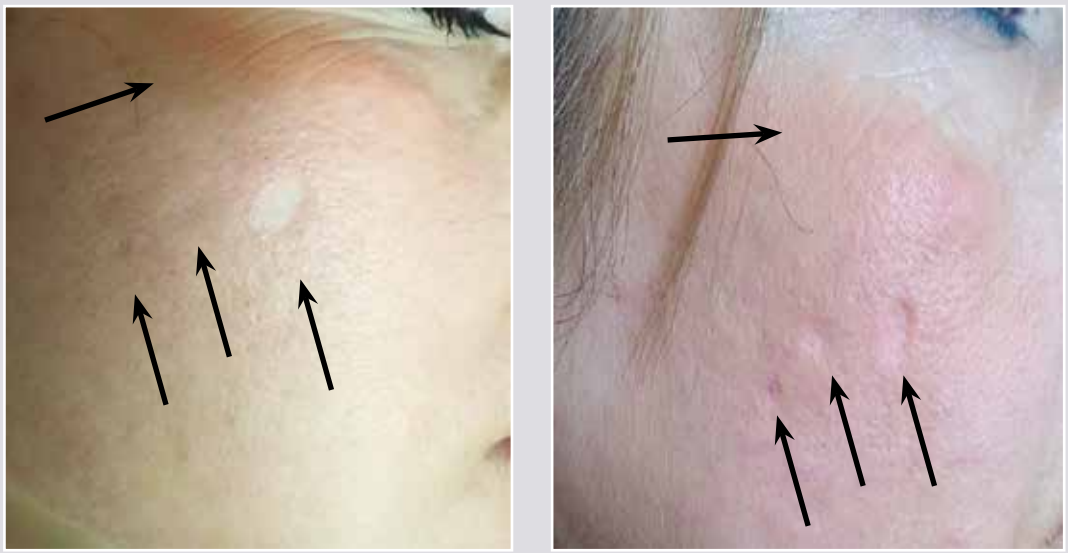
The technique is applicable for the nose furrows lacrimal and standardizable.



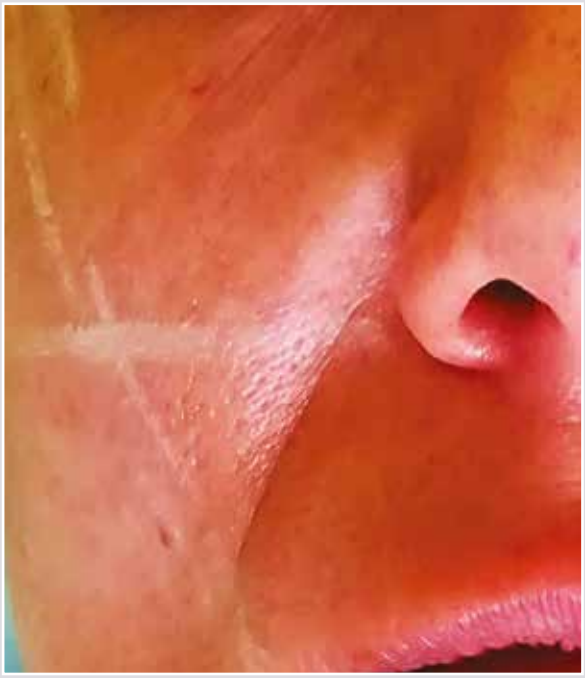
Time 0 *after ten months*

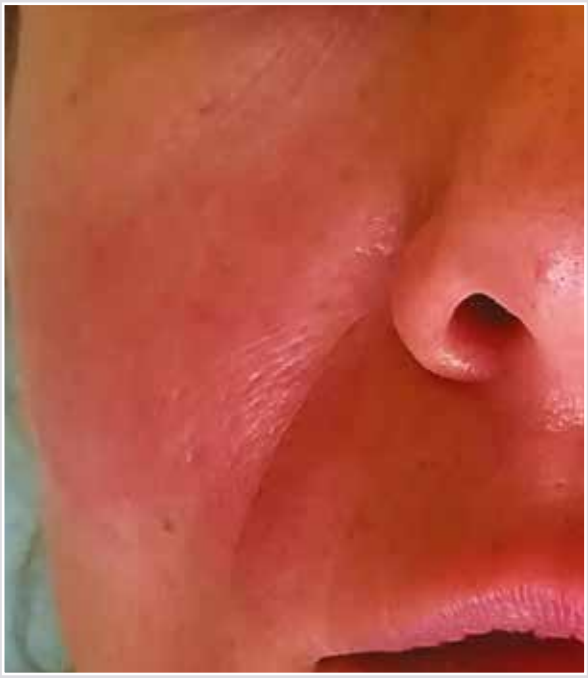


Lacrimal nose. Immediately to the treatment



Time 0 *after two months*





Nasal sulcus. Immediately to the treatment.

Conclusions

As we have shown, with an extremely simple procedure and with regard to the adipose tissue without the aid of particular accessories / machines it has been possible to obtain cells with adult staminate characteristics.

Extraction from the dermis and adipose tissue is extremely simple and the procedure is standardizable.

Unfortunately, in the classic clinical procedures it is not possible to know a priori the number of adult cells contained in the dermal frustulus or in the lipoaspiato, subsequently disrupted. It would be possible retrospect only through the cytofluorimetry. The numerous publications and evidence-based medicine show us their presence as the results reported in this work.

Declaration: For this publication I declare that there is no conflict of interess.

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