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ORIGINAL ARTICLE

Dermal Regeneration with MilliGraft® Kit of Nanofat: the Micrograft of Adipose Tissue. A Clinical Assessment Study

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ABSTRACT

The simple filtration by means of the MilliGraft® Kit of a disaggregated lipoaspirate allows to extract the class of cells defined as progentors with characteristics of adult stem cells present in the nanofat exclusively on the basis of their dimensions. It also allows the elimination of the fibrous branches and cell membranes destroyed by the emulsion phase and obtain a population of cells deprived of the inflammatory component. This method was used in regenerative and aeshetic medicine treatments with excellent and lasting clinical results in the follow-up phase.

Key words: dermal regeneration, nanofat, mesenchymal stromal progenitor cells, microfiltrate, MilliGraft®.

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INTRODUCTION

The physiological turnover of adult tissues loses efficiency after the first third of life through chrono-aging and photo-aging due to the progressive loss of the normal cellular turnover guaranteed by adult stem cells contained in the niches of differentiated tissues. The niches are made up of specialised cells protecting the mesenchymal stromal progenitor cells (MSCs) that are continually attacked by oxygen free radicals and ultraviolet B (UVB) radiation.[1] Through the influence of these highly reactive chemical species, adult stem cells will be carried out in a phase of quiescence that will force them to remain in the Go/G1 phase, influencing and facilitating their transition in the subsequent phase of senescence.[2] The senescence that adult stem cells will meet will prevent the physiological regeneration of tissues of the tissue progenitor population. Previous studies have been conducted on tissue progenitors from the dermis.[3]

In the present clinical study, it was assumed that, in order to improve photo-aging and chrono-aging damage, the dermis could be replenished with a vital micrograft from adipose tissue that contains the progenitors with adult staminal markers and those that best embody the characteristics of adult stem cells. The adipose tissue was chosen because of the simplicity of extraction and its abundance. It was processed and disaggregated through the MilliGraft® kit that allowed to deprive the tissue of cellular debris and fibrous shoots.[4] Such a suspension was injected into the dermis. It was also hypothesised that the triglycerides derived from the fragmentation of adipocytes increased the potential of engraftment[5-7] of the side population that remains suspended in triglycerides after filtration.[8]

It was also assumed that the adult stem cells derived from adipose tissue and their secretomes were able to allow for normalisation of the extracellular matrix with neocollagenogenesis and neovasculogenesis in the dermis without being influenced by hypoxia of the recipient tissue typical for aged tissue.[4] The method adopted is

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the MilliGraft® kit, a method based exclusively on the dimensions of the vital grafts and the triglyceride vehicle[5-7] in which the micrograft is suspended.[9] With the MilliGraft® kit it was able to prepare a sample of adipose tissue according to the technique of cytometry. By means of this method the tissue is disaggregated by special devices and filtered according to the necessary measures. The flow cytometer showed then that the side population, which is the one that best embodies the values of the progenitor, is smaller than the differentiated cells, that they have a greater cytoplasmic complexity and a greater expression of the stem markers. The task of these cells is to precisely maintain the equilibrium, the tissue homeostasis and the regenerative capacity of adult tissues in an incredibly flexible process.[4]

The choice of adipose tissue was also dictated by the biological reasons for the wealth of side population contained in it even after disaggregation.[5,6] In fact, it is possible to isolate 5x103 cells from a gram of it, about 500 times more than from the bone marrow[9] with the surface expression of CD 105, 90, 73 and 44 as markers[8] typical for adult stem cells[10]. Mesenchymal stromal progenitor cells (MSCs) are a population of non-haematopoietic and multipotent cells and are able to self-renew and differentiate into mesodermal cell lines. Under the normal conditions of a cell culture, they adhere to plastics and differentiate into osteoblasts, chondroblasts and adipocytes.[11] MSCs isolated from adipose tissue can function as a source of cells for the repair and dermal regeneration affected by chrono- and photo-ageing.[12] These cells have shown not to lose their vitality during the various cultivation steps up to the passage number eight and without showing typical senescence characteristics of differentiated tissues.[13] They possess a greater proliferative capacity than the bone marrow MSCs.[14]

Therefore, the preparation of adipose tissue obtained by means of the MilliGraft® kit allowed to obtain the side population and the vascular stromal fraction exclusively according to the principle of cell size.[3] The dimensional characteristics of the MilliGraft® kit allowed for the exclusion of the inflammatory fibrous shoots and cellular debris in a reproducible way without the use of enzymes.[12] Despite of the fact that a part of these cells can be lost during the disaggregation,[15] the filtration has shown that more colonies can be obtained in culture than in the unfiltered nanofat.[16] The vascular stromal fraction is rich in pericytes and active mesenchymal cells that are able to accelerate the process of tissue regeneration1.[7, 18]

The disaggregation of adipose tissue allows for the release of lipoprotein lipase and adiponectin. The function of lipoprotein lipase is to hydrolyse triglycerides in fatty acids and glycerol and is a key enzyme for the metabolism of lipoproteins and triglycerides. Lipoprotein lipase induces an increase in retention and absorption of all classes of lipoproteins.[19,20] Consequence of this induction is an increase in energy production in cells and greater protein synthesis. Adiponectin enables the catabolism of fatty acids and inhibition of inflammatory processes by improving metabolic energy in tissues.[6] Through the mesotherapeutic supplementation of adipose-derived MSCs the formation of inflammasomes is inhibited and the regulation of the population of macrophages M1 is activated through the prostaglandin E.[27]

It should be considered that fibroblasts are the most represented cells in the dermis. They are able to accumulate triglycerides[21] and to degrade them together with cholesterol esters in two different catabolic ways.[22] Injection of a microfiltered adipose tissue through MilliGraft® kit in the dermis also enables the regulation of EGFR and ERBB[3] and to normalization of sebaceous lipogenesis. In fact, triglycerides are important compounds for the skin, produced by the sebaceous glands. It was also hypothesised that the triglycerides that carry the MSCs decrease the release of LDH and reactive oxygen species (ROS) generation.[23] According to this hypothesis, there is a greater cellular vitality, a higher metabolic activity of the cells and a higher level of ATP.[24]

Triglycerides as a vehicle for suspended MSCs also cause vasodilation and increased local blood flow.[25] For this reason, it was also hypothesised that a temporary increase in blood flow in the dermis with consequent physiological normalization of the tissue increases the replication and the plastic potential of MSCs.[26] Triglycerides used as a vehicle for MSCs promote cell membrane phospholipids in human fibroblasts.[27]

The study was aimed at evaluating the clinical results of the improvement of the skin condition and to clinically verify the therapeutic response by using a microfiltrate of the population of the adipose tissue.[3]

METHODS

A total of 124 female patients were studied and their age ranged between 28 and 72 (average age 48). They signed informed consent for the use of lipoaspirate for experimental procedures. The study was performed following the standards of the local ethics committee and in accordance with the Helsinki Declaration (2000). None of the patients presented any specific pathology other than the dermal-epidermal aging process. Once the donor area was identified, the Klein solution was injected to anaesthetise the collection site. The adipose tissue was extracted under a tumescent anesthesia that allows the extraction of a greater number of viable grafts than a local anesthesia.[28] The adipocytes were extracted through a 10 ml syringe with Luer lock and a 16-gauge needle (Figure 1).

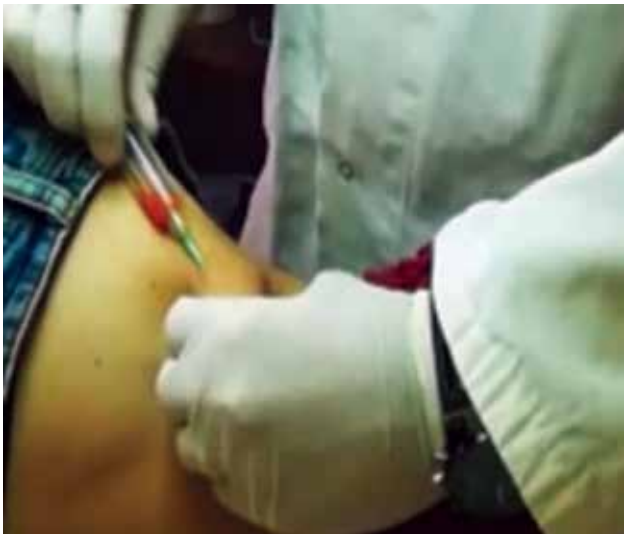

Figure 1: Tissue estraction

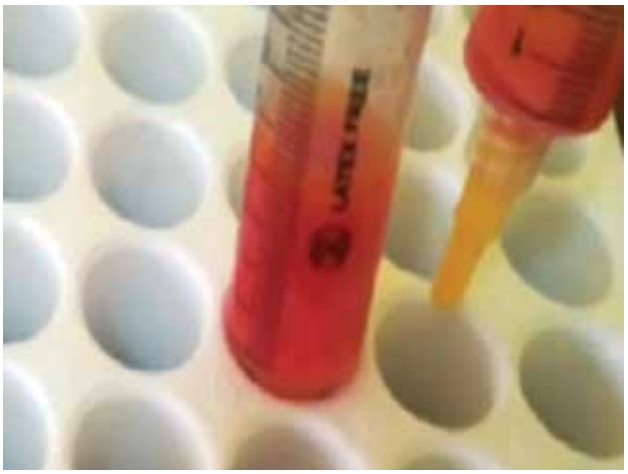

Figure 2: Decantation of fat

Figure 3: Microfiltrate

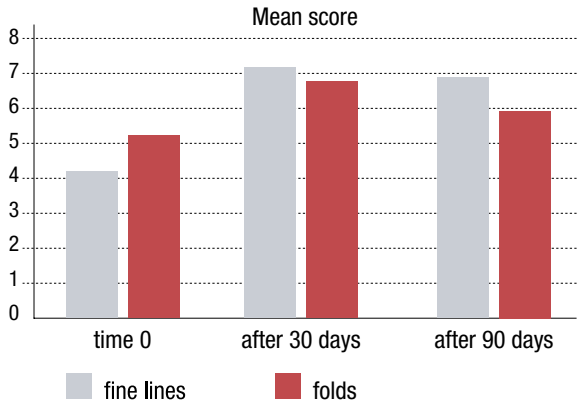

Figure 4: Clinical Mean score of treatment

An aliquot of 6/7 ml of tissue that has been decanted to eliminate anesthesia fluids was extracted (Figure 2) to obtain 5 ml of adipose tissue that was disaggregated through the MilliGraft® kit and filtered to 50 microns in order to obtain the vital side population[3,9] (Figure 3) and MicroGraft® according to the principles of cytometry. In this way the fibrous shoots and cellular debris were eliminated.[4,16] The microfiltrate was used for face, neck and décolleté treatments with 1 ml Luer lock syringes and 30 gauge needles. All the patients have shown an improvement with the treatment they have undergone and they have expressed satisfaction with the improvements achieved. Inspection visits and evaluations were made on the first day (D1, baseline) and after 30 (D30) days of treatment, with a follow-up visit after three months (Figure 4).

DISCUSION

This *in vivo* study of the MilliGraft® kit technique enabled the evaluation of a new method of dermal regeneration inspired by the cytometry method applied to a nanofat on our patients. The cytometry counts the cells in a suspension and identifies them through the markers. Because MSCs are the same as a classic lipoaspirate in the disaggregated adipose tissue, it was separated from the fibrous shoots and cell membranes of the destroyed adipocytes to make sure that a less polluted side population is achieved. The presence of MSCs is provided by the size of the filter used and the size of tissue progenitors.[3,4]

In vivo results we obtained with the MilliGraft® kit show that tissue progenitors can be isolated from the examined samples. This technique is suitable for preserving the side population thanks to the filtration measurement to which the nanofat is subjected. In this way, it was injected into the dermis of our patients through the mesotherapeutic technique, a considerable amount of viable MicroGraft deprived of the potentially inflammatory component. These MicroGrafts are able to normalise a physiological production of collagen type I and type III.[29]

Through filtration we have overcome and attenuated the biological events caused by the production of inflammatory cytokines produced by macrophages and dendritic cells present in the dermis to an increasing extent at the age[1] and triggered by fibrous shoots and by the cell membranes of the destroyed adipocytes.

The fibrous shoots and cell membranes that are injected through the nanofat technique are recognized by the TOLL-LIKE system to trigger, within that class of cells and other cells, the pro-inflammatory NF-Kb1 program interfering with the rejuvenation treatment.

The initiation of the inflammatory phenomenon through the injection of nanofat has been demonstrated[30,31] and the inflammatory state related to the dermis selects fibrotic fibroblasts with production of fibrotic collagen.[32] Through this simple procedure it will be possible to inject a nanofat deprived of the inflammatory component.

CONCLUSION

The treatment was well tolerated and the clinical results confirmed the working hypothesis on dermal-epidermal regeneration with the MilliGraft® kit procedure.

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None.

CONFLICT OF INTEREST

None.

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