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Research Article

A Protocol of A New Regenerative Treatment of Chrono-Aging and Photo-Aging with Progenitors Cells from Adipose Micrograft Obtained from MilliGraft® Kit

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Abstract

During chrono and photo-aging, in tissues MSCa undergo a decrease due to the stress to which cells go. In aesthetic and regenerative medicine, the supplementation of autologous MSCa from adipose tissue extracted can be a valid alternative to counteract the effects of aging for tissue rejuvenation. An innovative method for extracting MSCa from adipose tissue, in its simplicity, allows the aesthetic doctor to have available a new highly effective approach for his patients. For this clinical study we were inspired by the principle of cellular extraction in cytometry. In this method the tissues are disaggregated by appropriate devices and filtered according to the necessary measures. The cytometry showed that in each differentiated tissue there is a population of progenitors which is smaller than the differentiated cells. These cells have greater cytoplasmic complexity and greater expression of stem markers. Collection, processing, selection and infusion of progenitors with stem markers of adipose tissue it was done with MilliGraft®, the CE surgical procedural kit done of purpose.

Keywords: Adipose Tissue; MSCa; Chrono-Aging; Photo-Aging; Regenerative Medicine; Aesthetic Medicine; Tissutal Progenitors; MilliGraft® Kit

Introduction

The presence of native MSCa in the tissues of the face, neck and décolleté decreases in relation to cronoaging but above all to photoaging [1-3]. In fact, during the aging process the adult stem cells tend to be exhausted also due to exogenous and endogenous stresses and the aged cells produce inflammatory factors that increase the process of exhaustion of the stem cells themselves [1]. Parallel to the decrease of MSCa, during chrono-aging and in general during aging, there is an increase in the connective tissue of dendridic cells and macrophages which, through cytokines produced by them, increase NF-Kb activity, the main factor of inflammatory transcription [2]. There is also an increase in abnormal proteins such as AGEs, mitochondrial mutations, programmed gene-related aging [3] and a decrease in HSP or Heat Shock Protein [4]. Autologous MSCa can be used to counteract chrono-aging [5], are able to interact with photo-aged fibroblasts [6] and are multipotent ie they can replicate. If stimulated appropriately, they can differentiate into specific and different cell populations [bone, cartilage, tendon, muscle, etc.]. They are able to self-replicate and proliferate indefinitely, when placed in a culture area where the physiological conditions of implantation are respected and survive for a long time also in the tissues in which they are implanted [7]. The ability of MSCa to direct replication in a different lineage of the tissue of origin and to enrich its cell population is called plasticity and is the cornerstone of their use in regenerative medicine associated with aesthetic correction of facial wrinkles [8]. With the techniques of using Adult Mesenchymal Stem Cells we identify the whole of the therapies that, in pursuing the goal of regeneration, use or at least exploit the potential of those cells in order to improve the receivent tissue. After the transfer of autologous MSCa, this mechanism can

be done in two ways: in the first, the adult stem cells are grafted and differentiated into the damaged tissue; in the second through the paracrine signals from these cells transmitted [9] to the other adult stem cells still present in the donor tissues that allow self-regeneration [10]. This second way of activating the regeneration proves to be very advantageous for the patient, since even if the cells have a short duration after the transfer, the paracrine signals activated by them persist for a long time in the receiving tissue [9]. Cells that have a paracrine signaling also include those derived from adipose tissue [11] and are attracted to tissues damaged by the inflammatory cytokines produced by them. The advantage on the use of autologous MSCa is the fact that through the secretions of the regeneration factors a physiological modulation will be performed that responds exclusively to the needs of the damaged tissue [12]. Adult stem cells derived from adipose tissue possess multipotent differentiation capacity including the ability to repair and regenerate damaged tissues [13], with a similarity for adult stem cells derived from bone marrow [14]. In fact there is no significant difference with respect to the morphology and immune phenotype of MSCa derived from bone marrow, umbilical cord or adipose tissue concerning morphology, success rate of MSCa isolation, colony frequency, expansion potential, the capacity for multiple differentiation and the immune phenotype [15]. Therefore, an alternative source of adult autologous stem cells that is obtainable in large quantities is that obtained from a classical lipoaspirate [16]. In aesthetic medicine they can be used for the rejuvenation of the dermis and tissues of the face, neck and décolleté for their anti-wrinkle effect [17] and for their ability to remodel damaged tissues through a cross-talk between fibroblasts [18]. Adult Stem Cells reduce fibrosis and scarring processes, restore correct expressions by fibroblasts producing type I and III collagen, participating in a physiological neo-collagenogenesis and normalizing the dermis [19,20]. With the injection of adipose micrografts there is the immediate activation of IL-10, which is responsible for the inhibition of an excess of the inflammatory state and its negative events, typical of fibrosis [20]. Through the use of autologous progenitors, we also obtain an increase in the values of TGFbeta 1 and 3 [19] involved in the control and resolution of inflammatory states, the increase of non-fibrotic matrix [21] of EGF [epidermal growth factor] and of FGF2 [Fibroblast Growth Factor] [22], but not of TGFbeta 2, cytokine present in the inflammatory state [23,24].

Materials and Methods

The aim of the filtration of disaggregated adipose is suitable to safeguard the size of side population of progenitors cells and injected them into the dermis on patients affected by chrono and photo-aging. To exploit the potentials of fatty MSCa and to know the safety of the extraction, implantation and positive effects of adult stem cells derived from the dermis [8] and of the regeneration of tissues induced by the fat implant known as lipofilling [25] and knowing that in a disaggregated adipose the adult vital stem cells contained in it are the same as a classic lipoaspirate not showing differences in the quantity of viable cells [26] I decided to use this tissue, which is abundantly represented in our body as storage material (Figure 1 and 2), performing treatment with 30 g needles and 1 ml syringes (Figure 3) for rejuvenation of face's dermis (Figure 4 and 5), in each of its areas, neck and décolleté (Figure 6 and 7) adoptable and reproducible in the medical office. For the procedure I used MilliGraft® Kit. The study was performed following the standards of the local ethics committee and in accordance with the Declaration of Helsinki (2000). All patients were female and had no specific pathologies. A total of 45 patients from 29 to 71 years old (median age 45) were included in upon signing an informed consent for the use of the lipoaspirate for experimental procedures. I used: 10 cc of classic lipoaspirate obtained by anaesthetizing the donor part with 20 cc of Klein solution and extracted with 10ml syringe with luer lock (*) through a 14G needle placing (*) the needle cap between plunger and syringe to have sufficient negative pressure suitable for extraction (Figure 1) [27]. I placed the syringes to decant, to remove the anesthesia fluids (Figure 2) and obtained the disaggregate after several steps in a three-way valve (*) (Figure 8). I filtered the lipoaspirate at 50 micron value (*) that allows to separate the Side Population of Progenitors from cell membranes and fibrous shoots [8] (Figure 9) for simple negative aspiration, obtaining a suspension of cells measurable only afterwards (Figure 10). The suspension obtained was injected into the dermis (Figure 6 and 7) through a 1 ml syringe and a 30 G needle [28] (*)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 All patients expressed a positive assessment of the results obtained by providing the operator with complete satisfaction, in line with the stated expectations.

(* included in the MilliGraft® surgical procedural Kit).

Results

Evaluations were carried out *In vivo* on the efficacy of the 30-day with a follow-up visit after three months remote treatment (Graphic 1). Before treatment, we evaluated skin color (vascularity), pliability and surface texture (pigmentation) using the modified Vancouver Scar Scale and and we have adapted the Vancouver scale to our patients such us table 1. As seen in pictures 9 and

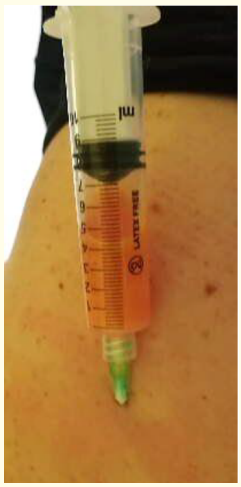


Figure 1

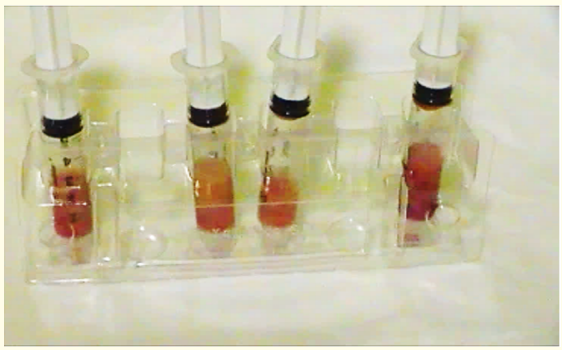


Figure 2

Figure 3

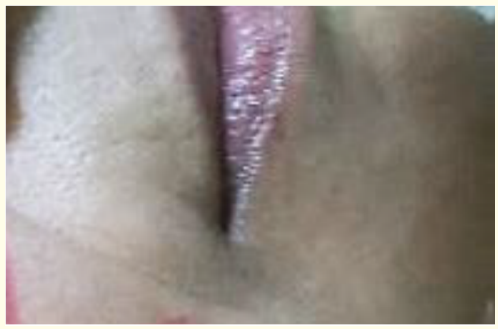


Figure 4: Time 0.

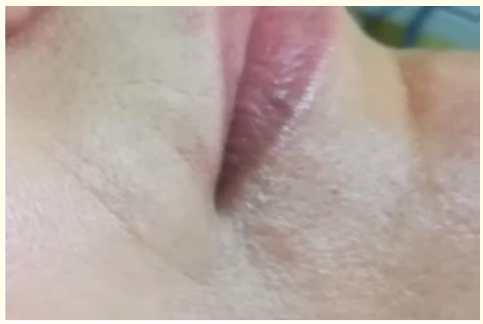


Figure 5: After 30 days.

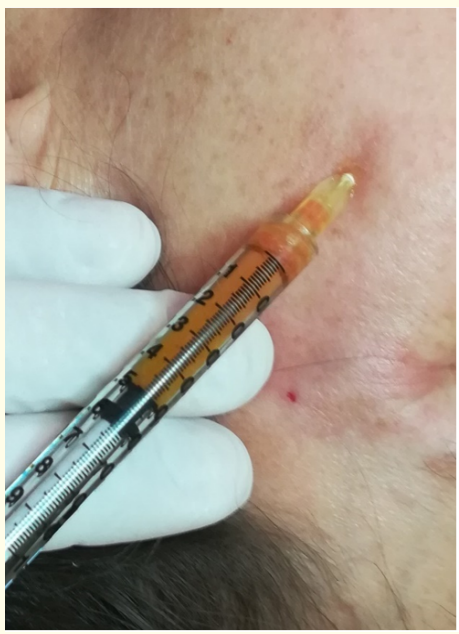


Figure 6

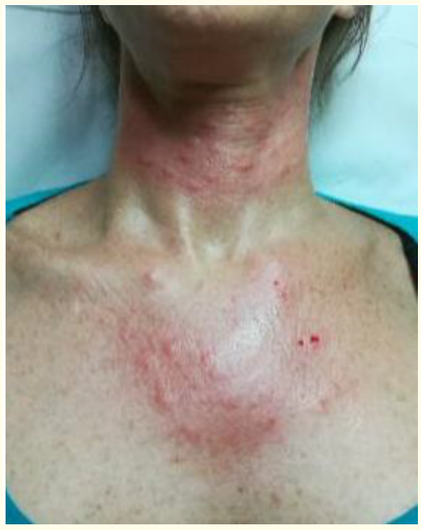


Figure 7

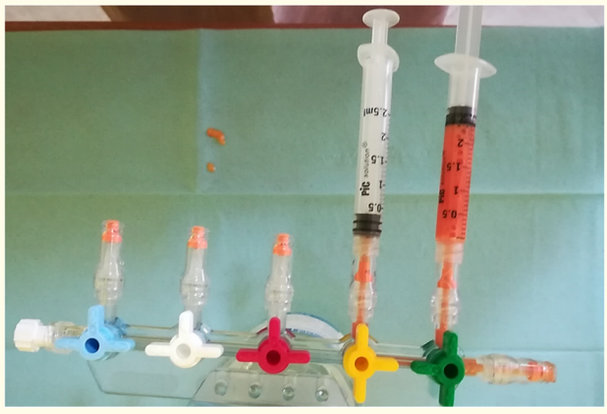


Figure 8

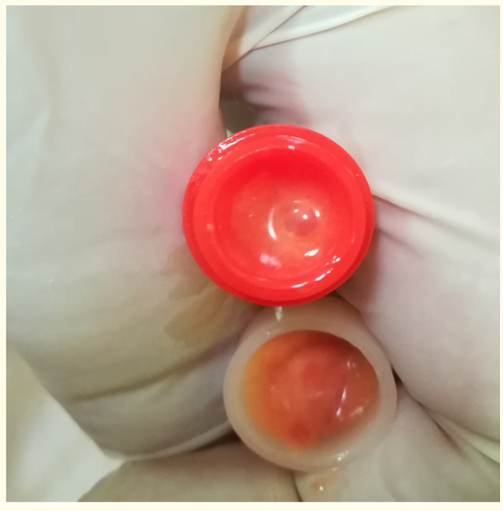
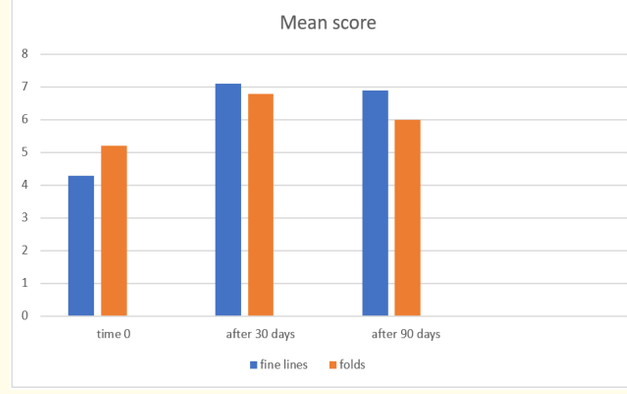


Figure 8

Figure 10

10, it has been shown that the overall appearance of the skin has improved. In line with patient self-assessment. The appearance of fine wrinkles has been greatly reduced and the resulting softness and firmness of the skin have been improved. This interesting overall improvement is shown in photo 10, where a reduction in wrinkles and fine lines is evident. The *In vivo* results are confirmed according to our intuitions described in the introduction, making useful the filtration of adequate measure of a fragmented classic lipoaspirate, as an antiaging remedy and photo-aging in aesthetic medicine. In conclusion, this simple bioregeneration with autologous tissue can be used with great satisfaction for the doctor and the patient.



Graphic 1: Clinical Mean score of treatment.

Baseline	3 Months follow-up
Vascularity: Red/Purple/ Grey	Normal
Pigmentation: Hyperpigmentation/ Hypopigmentation	Normal
Pliability: Firm/ropes	Normal/Supple

Table 1: Modified Vancouver Scale , after 3 months from treatment with MilliGraft® protocol for Patients.

Discussion

This protocol is innovative, reproducible and easy to perform for the rejuvenation of tissues affected by chrono and photoaging. The filtration with certain measures allows us to isolate the Progenitor Cells present in the classic lipoaspirate avoiding as much as possible the presence of fibrous shoots and cellular debris that can activate the inflammatory cascade and to integrate with vital micrografts the tissues of the face, neck and décolleté of our patients. The treatment is well tolerated and free of major risks if performed according to the protocol we have adopted and the simple rules of prudence. The clinical results on our patients have confirmed our working hypotheses. Overall, these data suggest that MSCa can be applied as a potential therapeutic agent to improve aged human skin.

Conclusion

The results obtained through the simple and reproducible method of disaggregation of adipose tissue, its filtration and subsequent implantation in the dermis of tissues aged by chrono and photo-aging lead us to think that although the presence of MSCa is numerically quantifiable only retrospectively they are sufficiently suitable enough to promote tissue regeneration either by contributing directly to formation the new tissue or by the paracrine effect of stimulating endogenous repair. This is in complete agreement with the premises presented in the introduction of this manuscript and the results obtained.

There are no conflicts of interest for the preparation of this manuscript.

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