Title of the study: Effect of Adipose Derived Stem Cells on Survival of Fat as Filler

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Investigators and study group:

Osama Hussein Roshdy MD, PhD

Professor of Dermatology, Dermatology Department, Faculty of Medicine, Alexandria University, Egypt.

Wafaa Ibrahim Abdullah MD, PhD

Professor of Dermatology, Dermatology Department, Faculty of Medicine, Alexandria University, Egypt.

Carmen Ibrahim Farid Mohammed Amin MD, PhD

Professor of Dermatology, Dermatology Department, Faculty of Medicine, Alexandria University, Egypt.

Radwa Ali Mehana, MD, PhD

Assistant Professor of Physiology, Physiology Department, Faculty of Medicine, Alexandria University, Egypt.

Nader Hussein Lotfy Bayoumi. MD, PhD, FRCS (Glasgow)

Professor of Ophthalmology, Ophthalmology Department, Faculty of Medicine, Alexandria University, Egypt.

Aliaa Ismail, MD, PhD

Assistant Lecturer of Dermatology, Dermatology Department, Faculty of Medicine, Alexandria University, Egypt.

INTRODUCTION

Skin aging is a complex biological process that can be categorized into either extrinsic or intrinsic aging. Intrinsic aging is an inherent degenerative process caused by decreased proliferative capacity leading to cellular senescence. Extrinsic aging is caused by factors such as UV radiations, smoking and alcohol consumption.[1,2]

The physiological changes associated with aging of the skin are manifested in xerosis, dramatic loss of skin elasticity due to damage to collagen and elastin fibers; as well as barrier function, modification of rhytides and deficiencies in the regenerative property of the skin. All of which ultimately result in thinning of the skin, malar fat atrophy and pigmentary changes.[3]

Aging skin undergoes thinning of the epidermis that is caused by reduction of vascularity and hydration. On average, the thickness of the epidermis is reduced by about 6.4% during each decade of life.[4]

Skin aging effects can be assessed based on the skin appearance (texture and roughness, fine lines and wrinkles), structure, elasticity, hydration and barrier function. Many new non-invasive or minimally invasive bioengineering advances in recent years have enabled the quantitative analysis of skin properties during the aging process.[5]

There are an increasing number of treatment modalities to improve the appearance of aging skin including dermal fillers, injectable botulinum toxin, laser, chemical peeling and a diverse of topical agents including topical retinoid.[6]

Autologous fat grafting or lipo injection containing stromal vascular fraction (SVF) acts like ideal soft tissue filler for facial filling and rejuvenation.[4] It leads to progressive improvement of the skin texture, elasticity, and color over a few

months, therefore adipose tissue seems to be not only a simple filler but also a dynamic filler with two types of different and supplementary effects, the volumetric effect and the regenerative effect.[7]

Recently, adult stem cells such as mesenchymal stem cells (MSCs) have been used in variable dermatologic conditions due to its regenerative properties such as wound healing, rejuvenation, acne scar and hair fall treatment. Different types of MSCs could be derived from different tissues as for example; bone marrow stem cells (BMSCs), adipose tissue-derived stem cells (ADSCs), and skin-derived stem cells (SDSCs).[8]

Adipose tissue derived stem cells are currently favorable compared to other types of adult stem cells as the procedure is easy, safe with minimal donor site morbidity. The process of obtaining a considerable amount of adipose tissues sufficient to use in skin regeneration is highly appealing due to its relative availability and accessibility. They secrete variable growth factors that affect surrounding environment as vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), insulin like growth factors (IGF) and others.[9]

Cell assisted lipotransfer (CAL) is a technique that combines aspirated fat with concentrated ADSCs in the stromal vascular fraction (SVF) of the lipoaspirate. This technique could enhance the survival rate of the transplanted fat and leads to better cosmetic improvement.[8]

AIM OF THE WORK

The aim of this work is to evaluate the effect of Adipose Derived Stem Cells on Survival of Fat as Filler

PATIENTS

The current study will be carried out on 15 healthy female participants recruited from the Outpatient Clinic of the Dermatology Department of Alexandria Main university hospital.

Inclusion criteria:

1. Clinically diagnosed facial skin aging.
2. Glogau photoaging score II and III.
3. Body mass index ≥20 with adequate abdominal or other subcutaneous adipose tissue accessible for lipoaspiration.

Exclusion criteria:

1. History of keloid formation.
2. Any coincidental chronic illness (e.g. metabolic, autoimmune or endocrinal) or malignancy.
3. Any bleeding or coagulation disorder or recent use of anticoagulant therapy.
4. Active infection.
5. History of any previous aesthetic procedure on the face within the past 6 months.
6. History of intake of anti-aging systemic or topical medications within the previous 3 months.

An informed consent for both participations in the study and photography will be taken from all participants prior to the onset of the study after full explanation of the procedure and its follow up steps. Patients will have to sign to committing to the study protocol, using sun protection, and abstaining from using or doing any anti-ageing steps during the study period.

METHODS

All participants will be subjected to:

1. Full history taking including personal, family, medical and drug history.
2. History of any previous aesthetic procedures on the face.
3. BMI calculation.[10]
4. Baseline assessment of facial skin ageing will be done using the following techniques:
    - Base line standardized digital photography of the face before the procedure and in each follow up visit.
    - Assessment of degree of improvement using Hollowness severity rating scale: 0: no visible hollowness, 1: mild Hollowness, 2: moderate H, 3: severe Hollowness on serial photography.
    - Assessment of trans-epidermal water loss (TEWL) and skin hydration using noninvasive probe (Dermal measurement system EDS12, UK) on both temple regions of the head.[13]
    - Measurement of epidermal, dermal and hypodermal thickness using UBM (Ultrasound Bio microscopy) on both temple regions of the head.[14]

Lipoaspiration for preparation of ADSC will be done according to the following technique: [7]

Under local anesthesia using strict aseptic technique, a small incision will be done in the lateral aspect of the thigh or lower abdomen, through which the infiltration cannula will be introduced to inject the local anesthetic solution using the wet technique. This will be followed 15 minutes later by lipo-aspiration of (50 ml fat) using a blunt tipped cannula under the negative suction pressure of a 60 ml syringe.

- Autologous adipose tissue derived stem cells (At-ADSCs) will be separated from the lipo-aspirate using enzymatic digestion and

differential centrifugation in the Center of Excellence for Research in Regenerative Medicine and its Application (CERRMA), Alexandria Faculty of Medicine.

- Viability of collected cells will be checked using trypan blue stain.[15] Characterization of the isolated ADSCs population will be performed by flow cytometry to check for specific cell surface CD antigens (CD 90 and CD 34) in three independent cases in triplet.[7]

Aspiration of 75 ml of fat followed by preparation of At-ADSCs from 25 ml.

- One temporal region is randomly selected as control to receive the required volume of fat via subcutaneous injection using blunt cannula. The other side receive equal volume of fat enriched with ADSCs in SVF.

Follow up and evaluation:

- Early assessment of trans-epidermal water loss (TEWL) and skin hydration will be carried out a week after the procedure.
- All participants will be followed up six months after the procedure. Follow up will entail using the same base line assessment steps to detect the effects of treatment. Side effects reported by the participants will be recorded.
- Assessment of the participants and the photos will be carried out by a blinded investigator to the half treated and the before versus after photos.
- A visual analog scale, [16] will be used to assess participants' satisfaction by the treatment

ETHICS OF RESEARCH

Research on human or human products:

☐ Prospective study: informed consent will be taken from patients. In case of incompetent patients the informed consent will be taken from the guardians.

☐ Retrospective study: confidentiality of records will be considered.

☐ DNA/genomic material: informed consent for DNA / genomic test and for research will be taken from patients. No further test will be carried out except with further approval of committee and patients. If the samples will travel outside Egypt the researcher will be responsible for transportation and security approval.

☐ All drugs used in the research are approved by the Egyptian Ministry of Health.

Research on animal:

☐ The animal species are appropriate for the test.

☐ After test, if animal will suffer, it will be euthanized and properly disposed.

☐ After operation, it will have a proper postoperative care.

RESULTS

The results obtained will be tabulated, statistically analyzed and findings will be illustrated using tables and figures.

DISCUSSION

The results obtained will be discussed in view of achievement of the aim of the work and compared with other findings in literature.

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