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Research Article

Safety of Antria Cell Preparation Process to Enhance Facial Fat Grafting with Adipose Derived Stem Cells - @

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ABSTRACT

The use of adipose tissue transfer in plastic and reconstructive surgery is not new and has been studied for more than a century but problems such as unpredictability in results and a low rate of graft survival due to partial necrosis were always among major concerns [1]. However, emerging information regarding the potential of adipose derived stem cells, new methods of cell extraction, graft preparation and injection techniques have increased the popularity of fat transfer and the efforts toward development of cell based therapies for various diseases from Adipose Derived Stem Cells (ADSC's) and Stromal Vascular Fraction (SVF) of the adipose tissue. Although the mechanism of action of those stem cells is not fully known, their paracrine activities and transformation to various cell types can be responsible for reported clinical outcomes [1,2]. Many clinicians and researchers report better outcomes in fat grafting upon addition of SVF cells [1,2]. This study aims to investigate the long-term (3 years) safety of Antria's cell preparation process utilizing a digestive enzyme in SVF assisted fat grafting. The outcomes of this study was utilized to conduct further safety and efficacy studies to obtain regulatory and marketing approval for a novel SVF extraction method in the US.

BACKGROUND

Facial lipoatrophy has been observed in a variety of patient populations [3]. Lipoatrophy is diagnosed by a progressive loss of tissue in the face over time. Lupus erythematosus profundus and scleroderma en coup de sabre are diseases that give rise to facial lipoatrophy. As a result of aging, the face also loses natural contour, bone, and muscle structure [4]. Treating facial lipoatrophy can be an economically damaging and time constraining process. An alternative therapy can be dermal filler. However the cost, need for repeated injections and adverse events such as skin discolorations, early injection-related events, and immediate and delayed hypersensitivity reactions, unequal distribution of the filler, pain, short-term results, skin necrosis, and granuloma infections demonstrate the need for safer therapies for this indication [5-9].

Facial lipoatrophy can affect underlying bones and can contribute to the malposition of the lower eyelid with lateral bowing and sclera exposure [10]. Soft tissue atrophy in the face occurs due to a depression over the anterior mandibular groove [11]. Neither of these symptoms can be overcome by facelift techniques. Muscles, especially the zygomaticus and orbicularis oculi, begin to continuously relax with aging and can lead to the commonly observed droop in the midface [4]. Thus far there are no medical treatments to completely correct facial lipoatrophy. The applications to pursue should be multi-dimensional and should not only yield to facial lipoatrophy but also cosmetic facial enhancements, traumatic deformities, hemifacial atrophy, HIV-associated lipodystrophy, post liposuction irregularities, and others. Autologous SVF-assisted lipoinjection is an alternative for various augmentation processes including facial fat grafting and its efficacy was demonstrated by Yoshimura, et al. [1]. Non-SVF supplemented facial fat grafting generally entails fat obtained via liposuction in order to subsequently administer lipoinjections to a region requiring soft tissue augmentation. Soft tissue augmentation is frequently used to augment the breast during instances in which the breast tissue portrays inborn or acquired defects [12]. However, traditional fat grafting procedures used to generate facial rejuvenation and breast augmentation produce unpredictable results and are characterized by low graft survival rates due to partial necrosis [13,14].

Our preclinical work demonstrated that Antria's Adipolyx kit and methodology was able to extract SVF cells confirmed by CD marker assessment of CD31, CD34, CD44, CD45, CD90 and CD140 surface markers (table 1). The average nucleated cell count was 6.6×10^5 / ml with an average of 78% viability. We utilized the same kit and methodology to assess the safety of this process under and Food and Drug Administration (FDA) approved Investigational New Drug (IND) application process.

Table 1: Immunophenotyping of SVF cells.

Cell Marker	Mean (%) \pm SDs
CD31	14.25 \pm 2.2
CD34	4.25 \pm 2.1
CD44	68.5 \pm 2.1
CD45	35.25 \pm 12
CD90	91 \pm 2.9
CD140	37.25 \pm 5.2

MATERIALS AND METHODS

Study population

Eligible subjects already scheduled for a liposuction and a facial fat grafting procedure as a standard of care and elect to participate in the aforementioned study.

Inclusion Criteria

1. Female or Male, Age 30 to 70 years old
2. Subjects eligible for liposuction and facial fat grafting procedures for cosmetic purposes and facial atrophy.
3. Subjects require augmentation to the infra-malar region. Furthermore, facial engraftment to additional, non-study related regions is optional, but not required.
4. Inframalar Atrophy Assessment Scale of 2 to 4
5. Facial volume defect range: 2 to 10 ml
6. BMI between and including 22 and 29
7. Able to understand and provide written and verbal informed consent
8. Fitzpatrick Scale 1 to 6

Exclusion criteria

1. Taking or have taken NSAIDs within last two weeks or corticosteroids within the last six weeks prior to screening
2. Diagnosis of any of the following medical conditions:
 - ◆ Active malignancy (diagnosed within 5 years), except for treated non-melanoma skin cancer or other non-invasive or in-situ neoplasm (e.g. cervical cancer)
 - ◆ Active infection

- ◆ Type I or Type II Diabetes
- ◆ Skin/Bone deformities in the face, including scarring or hyper pigmentation within the graft site.
- 3. Subjects who were unlikely to comply with the protocol (e.g., uncooperative attitude, inability to return for subsequent visits, dementia, and/or otherwise considered by the Investigator to be unlikely to complete the study)
- 4. Subjects with a known drug or alcohol dependence within the past 12 months as judged by the Investigator
- 5. Dermal fillers or facial reconstruction within the past 24 months, Subjects must also refrain from such procedures during the duration of the study.
- 6. Subjects with major illnesses involving the renal, hepatic, cardiovascular, and/or nervous systems.
- 7. Subjects with elevated kidney and/or liver functions
- 8. Any other disease condition or laboratory results that in the opinion of the investigator may be clinically significant and render the subject inappropriate for the study procedure(s), may alter the accuracy of study results, or increase risk for subjects.
- 9. Subjects with life-expectancies less than 9 months
- 10. Subjects with known collagenase allergies
- 11. Subjects with idiopathic or drug-induced coagulopathy
- 12. Pregnant females
- 13. On radiotherapy or chemotherapy agents
- 14. Taking strong CYP450 inhibitors, which cannot be stopped during the screening, such as protease inhibitors (ritonavir, indinavir, nelfinavir, saquinavir), macrolide antibiotics (clarithromycin, telithromycin), chloramphenicol, azole antibiotics (ketoconazole, itraconazole) and nefazodone.
- 15. Subjects with a history of keloids or hypertrophic scar formations
- 16. Previous treatment with any synthetic fillers in the inframalar area

STUDY PROCEDURES

Liposuction

Upon signing the informed consent and completion of screening procedures, eligible subjects underwent a gentle (less than 1 atmosphere) liposuction procedure from Hips and Abdominal area utilizing a standard cannula and a conventional liposuction machine to aspirate fat tissue. Approximately 25 ml - 100 ml of lipoaspirate was extracted for the formulation of a graft-SVF mixture. Prior to liposuction, the abdominal wall was irrigated with a sterile tumescent solution which consists of 1% lidocaine and 1mg / 1000 ml epinephrine in normal saline solution. This is a standard of care to facilitate the aspiration process and to reduce the bleeding and pain after the procedure.

SVF isolation protocol

The isolation protocol utilizes Antria Cell Preparation Process and the Adipolyx kit (see the contents in table 2) to extract the desired SVF from adipose tissue acquired from lipoaspirate. The

protocol consists of three major processes: preparation, incubation, and washing. A portion of the lipoaspirate was collected and subject to the preparation, incubation and washing processes. The isolation protocol approximately requires 60-90 minutes and occurs in a sterile (operating) room.

SVF preparation process

All research and medical staff followed universal precautions including appropriate scrubs, shoe covers, masks, gloves and other standard policies of the operating room to prevent contamination.

The gentle liposuction occurs as the standard of care and the lipoaspirate was directly collected within 50 ml syringes that were appropriately marked and labelled with subject's number. Half of the collected lipoaspirate was utilized for SVF extraction and the other half as the graft. 50 ml syringes containing the lipoaspirate were centrifuged at 3200 rpm for 5 minutes to remove free oils, erythrocytes, small cellular debris, and some of the tumescent solution. Centrifugation yields three distinctive layers: A yellow liquid containing free fat on the top, a white-yellow fat layer in the middle, and red fluid containing erythrocytes, leukocytes, and other tissue cells at the bottom. The top and bottom layers were removed via suction of the top layer and pushing out the bottom part. The middle layer, which contains the SVF and adipose tissue, was subjected to collagenase digestion in order to separate mature adipocytes and SVF cells. The collagenase in a frozen powder formation was reconstituted by adding 5 ml of Antrialyte and 95 ml of saline to reach a total volume of 100ml to reach an enzyme concentration of 0.04 mg / ml. An equal volumetric amount of this solution was transferred to the syringe containing lipoaspirate for enzymatic digestion. Type I and II collagenase dissociate ADSC's from adipocytes and endothelial cells of the vasculature. Trypsin also supports this dissociation, targets the larger fibrous tissue parts and facilitates further processing of the fat through luer-lock syringes and needs.

Incubation process

After manual blending of syringes, enzymatic digestion was performed at 37°C in a shaking incubator for 30 minutes. The tissue suspension was centrifuged for 4 minutes at 200 g and dissociated fat (supernatant) was removed from the top of the mixture. The aforementioned centrifugation allowed the unnecessary mature adipocytes and connective tissue to separate from the SVF [1].

Washing process

The washing process was performed to remove any residual enzyme and RBC's. By adding 20 ml of 5 % dextrose solution and centrifugation for 4 minutes at 200 g. The wash fluid was removed along with remaining adipocytes from the top layer and the same process was repeated 2 more times. The final SVF was collected from the bottom of the syringe.

SVF acceptance criteria

We utilized an automated cell counter (Luna stem) and counted

Content	Details
Type I and II Collagenase	GMP grade, no animal origin
Trypsin	GMP grade
Antrialyte	Sterile mixture of Saline, Mg, Ca and Zn

the total number cells and nucleated cells and their viability in the operating room and before administration of the graft. The acceptance criteria were viability equal to or greater than 70 % and a minimum of 5×10^5 nucleated cells / ml. A sample of each aliquot was sent to an independent laboratory for 14 days sterility testing and Mycoplasma testing after the procedure. The 14 days sterility testing and Mycoplasma testing were regulatory and quality assurance requirements.

Graft preparation process

The extracted SVF (average volume of 2.5 - 3 ml) was evenly mixed with the fat graft via 2 luer-lock connecting sterile syringes.

Fat grafting process

An average Graft Volume of 17.3 ml was administered to the Malar, Inframalar, Oral Commisure, Nasolabial, Lips, and Glabella areas via a Coleman 19 gage cannula during the same surgical operation with liposuction.

STUDY DESIGN

In this phase 1, open label, single arm study; patients with facial atrophy that were eligible for facial fat grafting were enrolled. In order to reduce the risk and to comply with the regulatory requests the BMI was between (and including) 23 and 28 and the graft volume was restricted between 1 - 50 ml.

Safety assessments

- Physical Examinations
- Vital Signs, Weight and Height, Body Mass Index (BMI) measurements
- 12-lead ECGs
- Laboratory testing: Complete blood count, blood chemistry and urinalysis
- Other Evaluations: a 14 days sterility testing and Mycoplasma testing
- Collection of all adverse events, serious adverse events and medical treatments

RESULTS

6 patients (one male and 5 female) were enrolled into the study. The median age was 51.

All subjects returned to the study site for follow up visits and long term follow up was performed via phone calls and reviewing of medical records for 3 years post-op. All patients completed their follow up visits: No serious adverse events were observed.

Only one case of urinary Tract Infection was reported which was assessed as not related to the study.

No significant Post op swelling and pain were observed and no additional medical interventions were required. No positive culture from Mycoplasma or 14 days sterility testing was observed.

Cell count and viability results

All grafts met the acceptance criteria with an average nucleated cell count of 5.38×10^5 (SD: 1.01) and an average cell viability of 74.5 (SD: 2.07).

Clinical outcomes

This study was a safety study only and was not designed to assess efficacy. However, all subjects completed their standard of care clinical evaluation visits with the investigator and no further treatment for their facial atrophy was required.

DISCUSSION

SVF assisted fat grafting is a relatively safe procedure that has the potential to improve fat grafting outcomes and many other regenerative cell therapies [15]. In order to demonstrate its efficacy, more controlled clinical trials are needed. The result of this study was utilized to conduct further safety and efficacy studies by our team at Antria, Inc. More precise equipment such as 3-D camera's and software to measure the subcutaneous fat before and after the procedure and blinded investigators to assess the clinical improvement scales and biopsy and histological evaluations were utilized to demonstrate the efficacy in those studies that were still ongoing as this report was being prepared.

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SUMMARY

A phase one, open label study to demonstrate the safety of Antria cell preparation process in healthy individuals undergoing facial fat grafting enhanced with autologous, Stromal Vascular Fraction (SVF) of the adipose tissue.

Disclosure

Authors have stocks and ownership in Antria, Inc.

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