Isolation of Autologous Stromal Vascular Fraction (SVF) from Adipose Tissue (Human Lipoaspirates): A Clinical Protocol

Miguel Garber¹, María Gabriela Villalba², José Joaquín Merino¹,2 *

¹SEMERETEC (Sociedad Española de Medicina Regenerativa y Terapia Celular). Madrid, Spain
²CeluMed (Spain)
*Author for Correspondence: JJM: josem2005[at]yahoo.es
Correspondence can be also directed to MG: mggarber[at]gmail.com

Abstract: The autologous stromal vascular fraction (SVF) isolation from adipose tissue is an alternative to cultured adipose-derived stem cells in the regenerative medicine. However, the bioactivity of autologous SVF is not totally documented under disease conditions in patients. This clinical protocol aimed to describe an easy clinic protocol for SVF isolation from abdominal fat in healthy subjects subjected to lipoasuction / lipoaspiration; the protocol described all steps for aqueous and fat phases isolation by separation with collagenase (figures 1-3). Our original procedure for SVF isolation is useful for patients in the field of regenerative medicine; The autologous injection could prevent some pathological conditions and also here illustrate the potential protective/repairative role of SVF in regenerative medicine field in patients.

Keywords: SVF (Subvascular fraction); Stem cells; Liposuction; fat; clinical medicine; regenerative medicine; chemokines, trophic factors.

1. Introduction

The autologous Stromal Vascular Fraction (SVF) from adipose tissue is an alternative to cultured adipose-derived stem cells in the regenerative medicine field [1]. There is a phase II randomized clinical trial for the treatment of chronic leg ulcers by using adipose tissue containing progenitor cells [2]. However, the bioactivity of autologous SVF is not totally documented under disease conditions in patients. This clinical protocol aimed to describe an easy clinic protocol for SVF isolation from healthy subjects subjected to lipoasuction/lipoaspiration [3].Since researchers from UCLA University (LA, USA) discovered a new Adipose-derived Stem Cells (ASC), many studies reported beneficial effects of these adult stem cells populations in medicine regenerative and medical applications. These isolated new adult stem cells populations from lipoasuctioned adipose tissue are Processed Lipoaspirate Cells (PLA) [4].Current data suggest that the SVF are important source of endothelial progenitors, endothelial cells, and pericytes; thus, contributing to essential mechanism for cell repair like vessel remodeling and growth [5].

Current isolation methods depend on enzyme concentration, lysis buffer, long incubation steps and mechanical stress, resulting in single cell dissociation [6]. The Adipose-derived stem/stromal cells (ADSCs) are isolated as part of the aqueous fraction derived from enzymatic digestion of lipoaspirate (the product of lipoasuction, see figure 1-3), which contains ADSCs, endothelial precursor cells (EPCs), endothelial cells (ECs), macrophages, smooth muscle cells, lymphocytes, pericytes, and pre-adipocytes among others; collectively, all these cell types from the Stromal Vascular Fraction (SVF) could promote beneficial effects in humans [5].

Since SVF fraction is easily acquired and cell separation or culturing conditions are not necessary, the closed system for SVF isolation is viable at the clinical level [2]; In fact, SVF fraction promotes protective effects in cosmetic surgery, osteoarticular lesions or sexual disfunction in men among other pathologies [7].

The most widely used technique for the isolation of SVF from lipoaspirate is the collagenase digestion. Digestion of lipoaspirate is achieved by collagenase; the presence of collagenase in the injectable product has some regulatory approved uses by the US Food and Drug Administration (FDA); the collagenase produces the floating mature adipocytes fraction, as well as the cellular components of interest in the lower aqueous fraction [8-11]. However, collagenase can be inactivated by shaking in the collected fat. This separation can be enhanced by centrifugation and separation can be achieved by gravity-based phase separation also [11].Centrifugation of the aqueous fraction yields a reddish pellet that contains SVF fraction (see figure 2). Erythrocytes, a major contaminant in the SVF pellet is lysed to isolate a pure population of ADSCs and/or SVF cells if intended for “in vitro expansión” (figure 1) [12,13].

Biochemical markers of SVF fraction [12, 14, 15, 16]
These are specific clinical biomarkers of several cell types from the SVF fraction, like stromal tissue compounds (collagen, glycosaminoglycans, fibroblasts), capillaries and vessel structures (CD31+), smooth muscle, actin positive cells. A broad range of cell types have been identified by surface-marker characterization, including mesenchymal, haematopoietic, pericytic, blood and lymphatic vascular [16] as well as epithelial cells markers by flow cytometry [5].

2. Aim

The aim is to describe a clinic protocol for SVF isolation from abdominal fat in patients with overweight (liposuction or lipoaspirate); these cells can be injected in the own patient in order to improve possible physiological alterations or promote beneficial effects in case of pathological conditions. The present protocol for SVF isolation has been implemented in overweight women. She is 65 years old and she got a good healthy state. She has a tendency to metabolic syndrome without pathology.

3. Results (Clinical methodology for SVF isolation)

The FDA had approved a draft guideline late in 2014 [17,18], related to SVF and therapies in patients; the common practices of enzymatic and mechanical disruption of adipose tissue for isolating SVF are explicitly mentioned in the FDA document as “more than minimal manipulation” [19]. As and when the guidelines must be implemented and regulated are important consideration at the clinical levels.SVF fraction is isolated by current protocols (enzymatic digestion), which means this is a “drug/biologic” product (Category 351) that need a “complete” FDA regulation [20]; this situation is also applicable for SVF isolation from abdominal fat in patients. The REGROW Act aims to hasten the “conditional approval” of certain cell and tissue for therapeutic products with “reasonable expectation of effectiveness” along with other criteria in humans [17]. Nevertheless, more clear consensus must be considered for translational use of stem cells and other cell-based therapeutics in patients. The present clinical protocol described a basic enzymatic isolation by collagenase for Subvascular Fraction isolation after abdominoplasties/lipoaspiration in patients; the present figure 1-3 shown all step for aqueous/lipic fraction separations from lipoaspirates (see figure 1-3).

3.1. Methodology (clinical SVF isolation from human fat)

The clinical procedure for Stromal Vascular Fraction (SVF) isolation followed these steps (fig 1-3).

3.1.1. Figure-1: Tools for SVF isolation from abdominal fat (liposuction) in patients.

1-3. The stell tools and syringes (with tops and bottons) are necessary for SVF isolation from abdominal fat (lipoaspirates).
4. The extracted fat from liposuction is shaked during 30 minutes.
5. The fat is centrifugated at 3000 rpm during 30 minutes by shaking.
6. The obtained aqueous as well as fat fractions after centrifugation were isolated from fat. The oil is at the top, and fat in the middle as well as the aqueous fraction contains PRP fraction (Plateed rich plasma) and SVF (Subvascular fraction) are present at the botton.

3.1.2. Figure-2: blood removal and SVF isolation from fat by using several syringes.

7. The fat from lipoaspirated is in the siringe
8. The collagenase is added at 50,000 unit/50 ml of fat lipoaspirated by usinganother syringe at the sailed botton.
9. After centrifugation, the aqueous and fat fractions are perfectly separated within the syringe.
10. The tool allow to seal and remove the oil at the top by aspiration with another syringe from the botton.
11. The oil has been removed now.
12. The SVF fraction appears at the end of botton.
13-14. The aqueous and fat fractions are centrifuged again to improve the rendiment.
15. After centrifugation, the aqueous and fat phases are better separated now.

3.1.3. Figure-3: PRP and SVF fractions are separated together collagenase inactivation before injecting these cell types in patients.

16. The aspiration with another syringe on the stopper serves to release blood.
17. Blood can be released by making pressure on the bottom with another syringe.
18. The fat (yellow) and the aqueous fraction are separated now. The SVF fraction is closed to the bottom.
19-21. The aspiration with another syringe allow us to extract and separate a PRP enriched fraction (Platelet Rich Plasma) from fat fraction (yellow color).
22. The PRP fraction is now separated from fat.
23. The aqueous fraction has been aspirated by using another syringe at the bottom.
24. The collagenase is inactivated by successive passes through both syringes before injecting these cells in patients.
27. Finally, the SVF plus PRP fractions (autologous) are directly injected to patients.

**Figures:** Tolos for SVF isolation from human fat (lipoaspiration)
4. Discussion and Future Perspectives

The results in patients are currently limited with research ongoing in multiples centres but without cell therapy consensus. The regenerative capacity of SVF fraction was demonstrated in many pathological contexts by exhibiting immunomodulatory, anti-inflammatory, angiogenic effects [8,21-25]. There are several human studies in which the SVF fraction promote beneficial effects in regenerative medicine field with promise results in terms of clinical
efficiency. The clinical use of SVF have demonstrated protective mechanisms in rodent models of diseases and beneficial effects in patients [1,27-31]. These beneficial effects are associated to several trophic factor released from SVF fraction in patients [2-34]. The Adipose-Derived Stem Cell Treatments and Formulations could prevent certain pathological conditions in patients [35,36].

**Conflict of Interest**

The authors declare no conflict of interest.

**Acknowledgments**

We thank Jesus Garcíá Corcobado (FEMEL, Madrid) as well as Cristina Aguilá-Collantes Reyes (Medical Train, Spain) their collaboration.

**References**


**Author Profile**

**Miguel Garber, MD**

- MD (Cardiologist). National University, Buenos Aires (Argentina)
- Head of SEMERETEC (Spanish Society of Regenerative Medicine and Cell Therapy).
- Cardiology and Regenerative Medicine (Global Health Premium, 2015-2017)
- CARTIER AWARD
- Member of the American Heart Association-
- Hospital Ignacio Pirovano, Dept Internal Medicine (Buenos Aires, Argentina, 1985-1987)
- Fellowship. Dept. Cardiology Palmeto Medical Center de Miami, Florida, USA (1990-1991)
- Máster Health Care Management, Miami University, Florida, USA (1996-1997)
- Publications, conferences and research in the field of regenerative medicine (clinical level).
- Academic from Istituto Medico di Ramon and Cajal Health Science.

**María Gabriela Villalba Samaniego**

- Jur. Ma. University of Colony (Germany) Celumed S.L
- Specialist in international and european law (University of Cologne).
- Research and Project development in stem cell therapies and regenerative medicine.
International Journal of Science and Research (IJSR)  
ISSN: 2319-7064  

- Consultant for international proyect development and commercial efficiency.

Dr. José Joaquín Merino.

- 40 international papers, 115 total impact factor (total score) and 918 internacional citations
- Scientific Assesor of CIROM (Murcia, Spain) in toxicology of heavy metals: inflammatory mediators and oxidative stress in patients.
- Scientific Assesor of Celumed (Regenerative medicine field).
- BsCh and PhD. Universidad Complutense de Madrid (UCM, Spain).
- Conway Institute of Molecular Biology (Ireland).
- Schollar Research (MCP, USA).
- Teaching and Research (Developmental Psychobiology: neurobiology Lab; Psychobiology Dept, UNED, Madrid) and Biology (Science Faculty, UNED).
- Teaching and research neuropharmacology (UCM, Madrid).
- Biomedical Researcher “Ramon and Cajal Research program” (IdiPaz, Madrid).
- Unidad Experimental Hospital Donostia (Basque Country, Spain).
- Researcher: Neuropathology and Neuroimmunology, Neuroscience and Regenerative Medicine field.
- Postdoctoral Researcher (Biochemistry and Molecular Biology Dept; I.U.I.N, UCM. Madrid, Spain).
- Director of several Thesis Doctorals (Cell Biology, Biochemistry and toxicology of heavy metals)
- Director of D.E.A (Role of chemokines in neurodegeneration and/or neural plasticity)
- Several research Awards
- 100 congress (communications) and 39 conferences.
- Research: NCAM and chemokines in neuronal plasticity and neurodegeneration.
- Neural repair field by using Neural progenitor cells and chemokines in CNS diseases.
- Editor of international journals.