

ADIPOSE TISSUE AS A SOURCE OF STEM CELLS FOR REGENERATIVE MEDICINE



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Plasticity of human adipose tissue

OBJECTIVES
Recently a strong emerging body of evidence has shown that adipose tissue contains cells with extensive and even unrecognized developmental plasticity within the stromal-vascular fraction (SVF). The immunophenotype variation of the plated-SVF cells is not completely clear. Our objective is to perform a clear and exhaustive FACS analysis of plated SVF and to verify the immunophenotypic changing of typical mesenchymal antigens. SVF-plated cells were induced to osteogenic and adipogenic differentiation.

MATERIALS AND METHODS
Adipose tissue samples (N=15) from different anatomical sites were processed and analyzed for the presence of Mesenchymal Stem Cells (MSCs) markers, as described by Dominici et al. (Cytotherapy, 2006). Five-colors FACS analysis was performed on the plated SVF; SVF-cells recovery and viability after enzymatic digestion were evaluated. Mesenchymal tissues *in vitro* induction and CFU-F assays were performed.

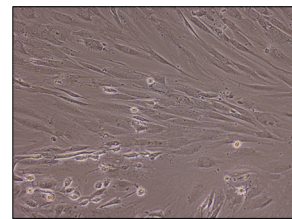


Fig. 1: Cultured SVF purified by adipose tissue from a liposuction aspirate (100X magnification).

Five-Colors immunophenotypic analysis of SVF

Adipose tissue is composed of mature adipocytes and of the so-called Stromal Vascular Fraction (SVF). SVF is obtained from liposyrates after enzymatic digestion of the tissue and subsequent elimination of the lipophilic fraction. This stromal compartment of mesenchymal tissue is thought to harbor stem cells that display extensive proliferative capacity and multilineage potential. Stromal mesenchymal stem cells share key characteristics , including the ability to adhere to plastic to form fibroblastic-like colonies (called CFU-F), extensive proliferative capacity, the differentiation capacity into mesodermal lineages (bone, muscle, cartilage, fat) and the expression of common surface antigens. Because of these key characteristics, stromal cells from adipose tissue may potentially be useful in the field of regenerative medicine. SVF from adipose tissue is composed of a complex and heterogeneous population of cells. The characterization and relative amount of cellular populations in human adipose tissue SVF is controversial in literature. To start our preliminary study on adipose tissue stromal cells, our first aim was to immunophenotypically characterize this complex cellular population by flow cytometry. As to obtain a more complete analysis of the purified cell population, we decided to apply a 5-C Flow Cytometry analysis (Cytomics FC 500 Beckman Coulter) of the entire SVF. We started analyzing the SVF cells immediately after purification (T₀) and later, after long-term culture (T₁₀). At T₀ the vast majority of the stem cells purified (mean value=80%) from different anatomical sites appeared to be CD45- and positive for typical mesenchymal stem cells markers (e.g. CD105, CD29, CD90, CD166). A limited number of cells were CD34+/CD45+ (mean value~15%) and all of the CD34+ cells we isolated were in general negative for CD133 antigen. We kept the SVF in culture and we realized that they were definitely mesenchymal-like cells and this statement was confirmed by our fine immunophenotypic analysis.

CFU-assays

A limited fraction of the SVF is composed of CD45+/34+ cells. CD45 is a pan-leukocyte marker; CD34 marks primitive hematopoietic progenitors and endothelial cells. The ability to support hematopoiesis is another property of mesenchymal stromal cells that may be important in clinical applications. A recent study has claimed to demonstrate that adipose tissue contains a population of cells with hematopoietic stem cell activity. Typically in bone marrow aspirates and cord blood samples such a cell population is considered to possess hematopoietic characteristics and is capable of hematopoietic rescuing. It is puzzling that a low percentage of SVF in adipose tissue is characterized by these hematopoietic stem cells markers. Therefore we decided to test the entire cellular fraction purified for hematopoietic clonal CFU-assays (CFU-E, CFU-G, CFU-M, CFU-GM) in methylcellulose. We visualized the emerging clones by using a cord blood sample purified buffy-coat as a positive control, at the same cellular concentration as adipose purified SVF (1X10⁴ cells/plate and 2X10⁴ cells/plate). After 12 days in culture, there were no colonies in the plated entire SVF compared to the cord blood sample. This preliminary result indicates that there should not be putative hematopoietic stem cells in this fraction. The next step in this study will be the selection by mini-MACS of the CD34+ population within the entire SVF as to enrich this population and test this selected fraction on methylcellulose clonal assays. A preliminary CFU-F staining by May-Grunwald-Giemsa shows that the cultured SVF forms fibroblastic-like colonies, a typical characteristic of mesenchymal stem cells.

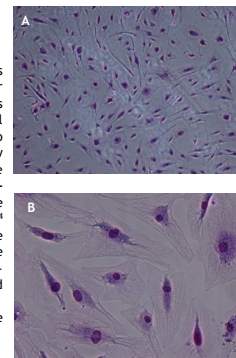


Fig. 5: A preliminary staining by May-Grunwald-Giemsa for CFU-F assay shows that the cultured SVF is composed of fibroblast-like cells, a typical characteristic of mesenchymal stem cells. Nuclei look red-violet-stained and cytoplasm red-blue. The CFU-F clonogenic assay has typically been used to quantify MSCs in marrow. In this assay (colony-forming units, CFU-F) cells are plated at about 1000/cm² and grown for two-three weeks. Colonies of more than 50 cells are then quantified. Using this assay, the number of MSCs in bone marrow is generally found to be approximately 1 in 25,000 to 1 in 100,000. The yield of CFU-F in adipose tissue is estimated to be approximately 5,000 CFU-F/g of adipose tissue. This compares with estimates of approximately 100-1000 CFU-F/ml of bone marrow.

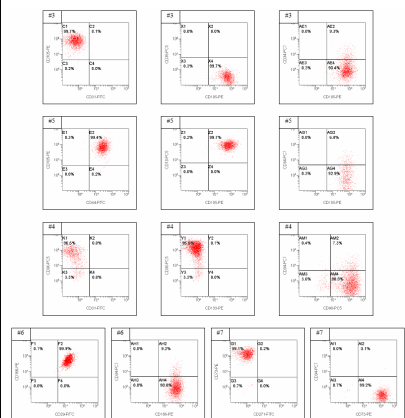


Fig. 2: Flow cytometer analysis of SVF from a cultured abdominal liposuction aspirate (T3). Mesenchymal CD marker antigens are tested. Cells are cultured in DMEM, 10% FBS, 1% antibiotic and antimycotic solution at 37°C and 5% CO₂.

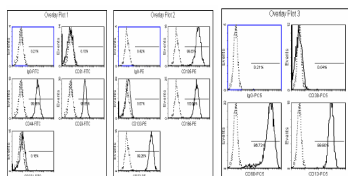


Fig. 3: Monoparametric comparative analysis of mesenchymal CD antigens of SVF cultured cells purified by abdominal liposuction (T3 passage). Confluent SVF cultures (approximately 80% confluence) were passaged at a ratio of 1:3 in trypsin-EDTA. Passaging (N) is indicated by T_N in culture.

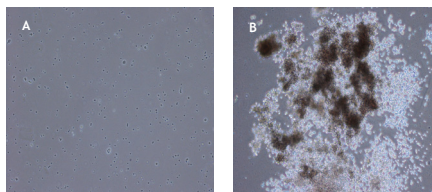
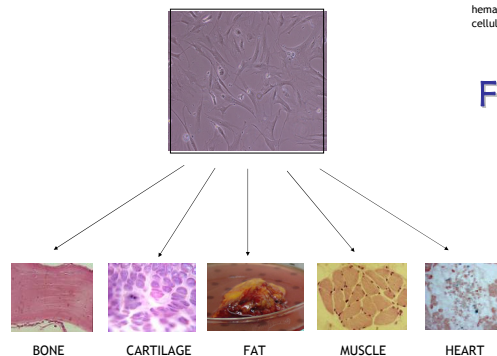


Fig. 4: Comparison of hematopoietic CFU activity of SVF purified by human adipose tissue (A) and cord-blood purified buffy coat (B). Cells of both samples were counted and plated at the same density in methylcellulose inductive medium containing 30% FBS, 1% BSA, 10 ng/ml GM-CSF, 10 ng/ml IL-3, 3U/ml Erythropoietin (Methocult medium, Stem Cells technologies Inc). Cells were kept in culture until the first clones began to appear in the buffy-coat sample. After 20 days in culture we could not appreciate any hematopoietic clone in the methylcellulose plates containing the entire adipose stromal cellular fraction.

Future applications in regenerative medicine

In the recent years, interest has rapidly grown in the developmental plasticity and therapeutic potential of stromal cells isolated from adipose tissue. Adipose tissue represents an abundant, practical, and appealing source of multipotent mesenchymal stromal cells (MSCs) for autologous cell replacement. Mesenchymal stem cells isolated from bone marrow stroma have been shown to possess adipogenic, osteogenic, chondrogenic, myogenic and neurogenic potential *in vitro*. However, bone marrow procurement is severely painful for donors and often requires general anesthesia. Moreover, only small numbers of cells can be harvested. Like bone marrow, adipose tissue is a mesodermally-derived organ that contains a stromal population easy to obtain by enzymatic digestion and separation from buoyant adipocytes. A more homogeneous cell population emerges in culture under conditions supportive of MSCs growth. This population shares many of the characteristics of its counterpart in marrow including extensive proliferative potential and putative ability to undergo multilineage differentiation. By a detailed immunofluorescence analysis, our study confirms that the stromal cell population of adipose tissue from different sources consists of cells of mesenchymal origin. Since human adipose tissue is plentiful, easily harvested in large quantity under local anesthesia with little patient discomfort, it may be an alternative stem cells source for mesenchymal tissue regeneration and engineering.

The use of stem cells including embryonic stem cells (ESCs) and adult stem cells is promising. Adult stem cells especially are potentially the best candidates, as postnatal stem cells are by nature immunocompatible and there are no ethical issues related to their use. The harvesting of adipose tissue to obtain SVF is technically easy through liposuction procedures. Therefore, we believe that human adipose tissue may be an ideal source of MSCs which can be applied for future tissue regeneration and tissue engineering through a multidisciplinary approach.



Induction of mesenchymal phenotypes

As we learned so far, the key characteristic of the adherent fraction of the SVF purified by liposyrates is that is principally composed of mesenchymal stromal cells. Mesenchymal stromal cells are interesting because they represent an extremely versatile population of cells, as they can potentially differentiate into many mesodermal lineages. To further characterize and exploit this complex cellular population that we purified by adipose tissue we are now involved in the *in-vitro* differentiation of this adherent fraction into different mesenchymal lineages. Spontaneous differentiation without the need of a lineage-specific differential medium was not appreciated, in contrast to what is described in previous literature. The *in-vitro* differentiation of SVF into myogenic, adipogenic and osteogenic lineages with supplemented media is now under study.

References

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