SETTING UP A PROCEDURE FOR ACCURATE HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELL COUNTING BY CYTOFLUORIMETRIC ANALYSIS

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Background In recent years human adipose tissue-derived mesenchymal stem cells (AT-MSCs) have been extensively studied to evaluate their potential for differentiation towards several lineages. Unfortunately, to date there is not an immediate and standard assay capable of evaluating the number of these cells upon isolation. Accurate counting of MSCs harvested from human adipose tissue is still challenging despite the fact that this issue is very important to evaluate in order to perform clinical applications with these cells. For this purpose we are developing a cytofluorimetric panel able to estimate the number of MSCs in the freshly-isolated stromal vascular fraction (SVF), using the standard markers CD90, CD105 and CD73 to identify the MSCs and hematopoietic markers CD34, CD45 and CD14 to exclude the presence of this type of cells.
Methods SVF obtained from liposuction aspirates with a clinically-applicable protocol developed by our lab were counted using both hemocytometer and cytofluorimeter protocols to estimate the number of MSCs. Subsequently CFU-F assays using hemocytometer and cytofluorimeter values were performed to compare these two different counting protocols.
Results MSC counting perfomed using hemocytometer is highly overvalued, in fact a very few number of colonies were observed in tissue culture plates where cells are seeded according to hemocytometer values. Conversely, the number of colonies in culture plates where cells are seeded using cytofluorimeter values are more suitable and convincing and correspond to the percentage of triple positive (CD73+, CD90+, CD105+) cells evaluated with our panel.
Conclusion SVF is a heterogeneous cell population, therefore it is of great importance having an assay able to distinguish the different cell types within this fraction. Cytofluorimetric assay with specific markers able to detect MSCs is a powerful way to estimate the accurate number of human MSCs harvested from human adipose tissue.

The MSC count panel used in this study was developed in collaboration with Beckman Coulter International, Switzerland.

COMPARISON OF HUMAN ADIPOSE-DERIVED SVF CELL YIELD AT DIFFERENT SUBCUTANEOUS ANATOMICAL SITES AND WITH DIFFERENT TYPES OF TISSUE – HARVESTING SURGICAL PROCEDURES

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Purpose: Adipose tissue has proven to serve as an abundant, accessible, and rich source of adult stem cells with multipotent properties suitable for tissue engineering and regenerative medicine. The purpose of this study was to compare the yield and growth characteristics of MSCs, which were isolated from adipose tissue harvested through different types of surgical procedures. Additionally, we investigated whether the anatomical location from which the adipose tissue was retrieved did affect the yield and viability of the isolated stromal vascular fraction (SVF) cells.
Methods: Human subcutaneous adipose tissue samples were obtained from healthy donors (N= 30) undergoing liposuction surgery. Adipose tissue was harvested from the abdomen and the hip/thigh region. The average donor age was 37 (ranging from 24-52 years). Tissue was collected using different approaches: 1) manual aspiration into a syringe 2) low pressure pump-assisted mechanical suction 3) high pressure pump-assisted mechanical suction 4) Power-assisted liposuction (PAL or Lipomatic) 5) manual suction followed by mechanical centrifugation; 6) manual suction followed by manual centrifugation 7) natant obtained after centrifugation. The SVF isolation protocol is standardized for all the liposuction procedures.
Results: SVF-cell recovery and viability after digestion of the adipose tissue obtained by the different surgical procedures and from different anatomical sites was evaluated using flow cytometric counting using commonly accepted mesenchymal markers and then assayed for clonogenic assays (CFU-F). Lipomatic, manual suction followed by centrifugation and natant provided significantly greater recovery of SVF cells than liposuction mechanical pump at high and low pressure. Conversely, CFU-F assay performed with the first three methods showed that the number of isolated cells is not proportional with their growth and expansion properties.
Conclusion: According to our final observations, adipose tissue obtained by the Lipomatic and the high pressure pump-assisted mechanical suction constitute the most efficient methods to provide an efficient clonogenic activity while the SVF obtained from the other harvesting methods presented very low clonogenic activity. The anatomical site of harvesting could affect the efficiency in SVF yield and their clonal capacity.