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Purified Adipose Derived Stromal Cells Provide Superior Fat Graft Retention Compared to Unenriched Stromal Vascular Fraction

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Abstract

Cell-assisted lipotransfer (CAL) has shown much promise as a technique to improve fat graft retention in both mouse and human studies. However, the literature varies as to whether fresh stromal vascular fraction (SVF) or culture-expanded adipose derived stromal cells (ASCs) are used to augment volume retention. Our study sought to determine whether there was a significant advantage to using ASCs over unpurified SVF cells in a mouse model of CAL.

Introduction

CAL, the addition of supplemental cells to autologous fat, has been promoted as a technique to enhance fat graft volume retention (1-4). A recent meta-analysis of 17 clinical CAL studies concluded that it was more efficacious than autologous fat grafting alone (5), though systematic reviews have highlighted the need for further studies, particularly given variability in methodologies reported, and more detailed investigations to define safety (6, 7). As it stands, the current body of literature consists of a mixture of two different CAL techniques: supplementation of fat with autologous SVF cells, or supplementation with *in vitro* expanded ASCs.

While the use of fresh SVF is the most easily accomplished in the clinical setting, it means using a notoriously heterogenous cell population. That is, SVF consists of fibroblasts, endothelial cells, pericytes, preadipocytes, and various immune cells, in addition to ASCs (3, 8-10). Furthermore, the exact proportion/quality of ASCs present in SVF varies between both individual patients and the body sites that lipoaspirate is obtained from (11-13). This is an important consideration even in non-CAL fat grafting, as Philips et al. have shown a

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correlation between graft retention and the number of CD34+ cells present within a patient's adipose tissue (14). Though ASCs can be identified within SVF as the CD34+/CD31-/CD45- proportion of cells by flow cytometry, they can also be defined as the adherent portion of SVF *in vitro* (10). Thus, the rationale for the use of *in vitro*-expanded cells in CAL is the ability to obtain a potentially larger number of more homogenous cells. However, use of an *in vitro* culture system represents a significant hurdle to be overcome before CAL is approved for clinical use, and based on studies from our own lab, large numbers of supplemental cells may not be needed for enhancement of fat graft retention (15). We therefore compared whether use of ASCs isolated through flow cytometry would better enhance fat graft volume with CAL, or whether freshly obtained heterogeneous SVF would provide equivalent results, obviating the need for additional steps to isolate ASCs.

Materials and Methods

Cell Harvest and Flow Cytometry

Fresh human lipoaspirate was obtained from three female patients, ages 45-55, BMI 24-30, using suction-assisted liposuction. Donors had no medical comorbidities, and informed consent was obtained in accordance with a Stanford Institutional Review Board approved protocol (no. 2188). Lipoaspirate was processed for SVF as previously described (16). Briefly, the tissue was washed with phosphate buffered saline (PBS) and digested with 0.075% type II collagenase (Sigma-Aldrich Co. LLC., St. Louis, MO). Red blood cell lysis buffer was used for removal of erythrocytes. Cells were counted, and a portion set aside for combination with lipoaspirate for SVF-supplemented fat grafts.

The remaining cells were washed in staining buffer (1% fetal bovine serum, 1% P188, 1% penicillin-streptomycin) prior to resuspension in a solution containing anti-CD45, anti-CD34, and anti-CD31 antibodies (eBioscience, Inc., San Diego, CA). A BD FACS Aria II (BD Biosciences, San Jose, CA) was used for flow cytometry, with live CD34+/CD31-/CD45- cells (ASCs) isolated after gating to exclude debris.

Fat Processing and Grafting

Lipoaspirate was centrifuged for three minutes at 1200 rcf in order to separate the oil and aqueous components for removal. Either SVF cells or ASCs were gently mixed with lipoaspirate to a concentration of 5×10^4 cells per milliliter of fat. This concentration was used based on our previous study determining the optimum concentration of cells to be used in CAL for maximum fat graft retention (15). Patient cells and fat were matched for all experiments (n=4 animals per patient for SVF and ASCs). Processed fat was then loaded into 1cc syringes, and 200 μ l grafts placed beneath the scalps of immunocompromised mice, using a 14-gauge cannula.

Imaging Analysis

Micro-CT imaging was performed during week zero following grafting for baseline volume measurements, and subsequently at eight weeks (17). A MicroCAT-II *in vivo* CT scanner (Imtek, Inc./Siemens, Munich, Bavaria) was used, with fat distinguished from skin and bone by pixel intensity. As previously described, adipose tissue volume at each timepoint was

calculated utilizing Inveon Acquisition Workplace software (Siemens Medical Solutions, Malvern, PA) for interpolation of voxels based on pixel intensity (18). Fat graft volume retentions were calculated as percent change from baseline.

Results

Flow cytometry showed that CD45-/CD34+ cells make up approximately $37.8 \pm 8.5\%$ of SVF cells, with ASCs (CD45-/CD34+/CD31-) and CD45-/CD34+/CD31+ (includes endothelial cells) accounting for $21.9 \pm 2.4\%$ and $15.9\% \pm 7.4\%$ of cells, respectively (Figure 1). SVF-supplemented grafts had final volume retentions of $53.13 \pm 24.59\%$, whereas the ASC-supplemented grafts retained $67.77 \pm 7.007\%$ of the original volume at eight weeks. An F test to compare the variances of each group found them to be significantly different (** $p < 0.01$). Thus, an unpaired t test with Welch's correction was used for analysis, determining that the differences in volume retentions between the groups were statistically significant (* $p < 0.05$) (Figure 2).

Discussion

While SVF is undoubtedly efficacious in enhancing fat graft take, the large standard deviation of the volume retention speaks to the varied effects of using a heterogeneous cell population. In contrast, use of purified ASCs (a “standardized” cell population) allows for more predictable outcomes, and ultimately facilitates superior volume retention. As there is not yet a way to predetermine the quality of a patient's fat, the potential costs, both monetary and in terms of patient morbidity/dissatisfaction, of suboptimal outcomes must be weighed against the cost of adding a cell enrichment step to the CAL procedure.

That said, with the abundance of SVF processing systems currently in clinical trials, the addition of a simplified procedure for immediate enrichment for CD34+ cells is conceivable. In fact, an FDA-approved device that positively selects for hematopoietic stem cells from peripheral blood samples, based on CD34 expression, is currently on the market. While the CliniMACS® CD34 Reagent System (Miltenyi Biotec Inc., San Diego, CA) is currently only approved for bone marrow transplants in cases of acute myeloid leukemia, the principles behind such a device could easily be extrapolated to human SVF samples. Thus, with continued developments in automated cell processing systems, the benefits of improved and predictable volume retention seen with the use of ASCs in CAL could be realized without the need for additional time and inherent complications associated with *in vitro* culture.

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Figure 1. After gating for exclusion of debris and isolation of single cells, CD45-/CD34+/CD31- cells were isolated ($21.9 \pm 2.4\%$). Flow cytometry gates were drawn based on unstained control ASCs.

Figure 2.

Fat grafts enriched with ASCs retain significantly higher percentages of their original volumes (* $p < 0.05$) at eight weeks in comparison to fat grafts enriched with SVF cells (a). These differences were visualized via 3D volume reconstructions of micro-CT scans, with ASC-enriched grafts maintaining relatively uniform volumes (left panel), in contrast to SVF-enriched grafts, some of which demonstrated very low retentions (right panel).