CONFIDENTIAL (DRAFT)

Stem Cell Use in Clinical Therapeutics

Abstract

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Regenerative medicine is an up and coming field that uses laboratory-grown or therapeutically-induced human tissue as a replacement for treating injuries, diseases, or cosmetic applications. As such, there has been astounding new advancements in the ability to repair or replace damaged human tissue with the use of stem cell related treatments and technology. One such aspect of regenerative medicine is revolving around taking advantage of the capability of regenerative cells found within adipose tissues. These adult stem cells (ASC's) and progenitor cells have the ability to renew themselves and develop into mature specialized cells. Studies have shown that adipose tissue can be an excellent source of ASCs since it is relatively simple to harvest in great quantities. Further studies have demonstrated that adipose derived ASCs possess pluripotency. This all clearly demonstrates how adipose tissue presents a model source for ASCs for use in regenerative medicine. However, there aren't many viable methods for harvesting adipose derived ASCs in today's medical practices. Existing methods contain a number of deficiencies in yielding a high number of viable cells and the use of enzymes (collagenases) has been a problem with use in a clinical setting due to the potential risks involved with reinserting active or non-sterile enzymes back into the recipient which the FDA prohibits at this time. In this study we examine different methods in collecting fat aspirate and how they contribute to high viable ASC yielding.

Study Summary

A 12+ patient comparative study that involves cell count and viability analysis of stromal vascular fraction (SVF) cells taken from manual lipoaspiration method used in common practice today commonly referred to as Suction Assisted Liposuction (SAL) and that of Nutantional Infrasonic Liposculpture (NIL) via looking at tumescent SVF cell counts and viability after centrifugation. Additionally, wanted to compare SVF cell viability and concentrations after taking both samples adipose through an isolation protocol using collagenase to conduce whether adipose samples from NIL are more receptive to enzymatic dissociation than that of SAL adipose. We compared these results to the same samples tumescent results. Several methods of analysis were used for cell counts including manual hemocytometry and flow cytometry looking for CD34+ and CD45+ cells. For viability we used the trypan blue assay and flow cytometry. Variables were reduced my taking both methodologies (NIL and SAL) samples from the same patient and using the same instrumentation (cannula, aspirators, etc.) where applicable. We were looking to determine if lipoaspirate acquired via NIL, due to its mechanics, is more conducive for the use of autologous grafting procedures, treatments, and isolation protocols for clinical use to that of current manual aspiration techniques. We expect this is due largely to the apparent in-vivo dissociation process the NIL provides by looking at adipose derived regenerative cell (ADRC) concentrations and viability therein.

Study Guidelines and Notes

- 14 patients underwent a single liposuction procedure where SAL was used to extract lipoaspirate tissue from one side of each patient's abdomen, thigh, arm, or flank followed by the extraction of adipose tissue from the adjacent side of using the NIL, also known as Tickle Lipo.
- The same techniques (respective of SAL and NIL), parameters, and equipment were used on all 14 patients. Vacuum pressure during aspiration was reduced to 15mm Hg for both technologies, consistent with standard fat collection/grafting techniques. NIL nutation setting was set to 3.0. Used standard tumescent waiting 20 minutes before aspiration.

- The amount of lipoaspirate collected per patient was 50cc per technology, Manual and NIL. A sterile closed fat collection system was used for harvest and samples were transported in 50cc sterile syringes.
- Samples were harvested at The Cosmetic Surgery and Laser Institute of Newport Beach, CA
- All samples were processed within 4 hours of aspiration at AQ Skin Solutions private laboratory in Mission Viejo, CA.
- Cell viability assays were done via Trypan Blue Protocol with an inverted microscope using an Improved Neubauer Hemocytometer and Millipore Guava flow cytometer using DNA staining reagents.
- Cell counting was done via: Hemocytometer with inverted microscope and Millipore Guava flow cytometer.
- Fluorescence Activated Cell Sorting (FACS) performed via Becton Dickinson FACS Canto Flow Cytometer for CD34+ and CD45+cells
- Data reflects the mean average of 12 patients with the widest outliers discarded

Findings

1. Within 4 hours, the lipoaspirate collected by NIL then processed, without collagenase, into SVF yielded a mean average 93% ADRC viability in comparison to that of SAL which was 88% by trypan blue assay.
2. When the lipoaspirate for NIL and SAL were initially processed for flow cytometry, without collagenase into SVF, yielded a mean average 90% ADRC viability in comparison to that of SAL which was 72% by a BD flow cytometer. (see figure 1)
3. When the samples were re-ran 2 months later for CD45+ the lipoaspirate collected by NIL then processed, without collagenase, into SVF yielded a mean average 79.80% ADRC viability in comparison to that of SAL which was 72.93% by Millipore Guava using the ViaCount assay.
4. On average per case, the NIL technology yielded a 3.495x greater nucleated SVF count than that of the SAL sample per Millipore Guava flow cytometer using the ViaCount assay.
5. The lipoaspirate collected by NIL, then processed into SVF without collagenase, yielded a mean average of 59% increase in CD34+ population by BD FACS Canto to that of the SVF of SAL. (see figure 1)
6. Additional findings in the study showed that the average CD 45+ population in the SVF was 6% in the NIL samples in comparison to that of 17% in the SAL samples.

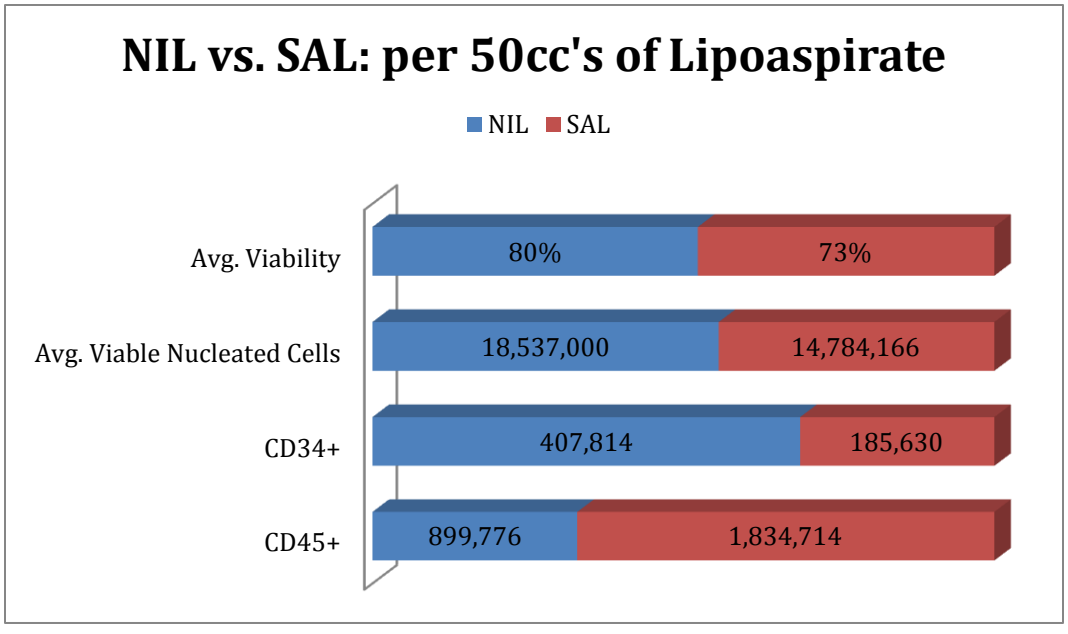


FIGURE 1

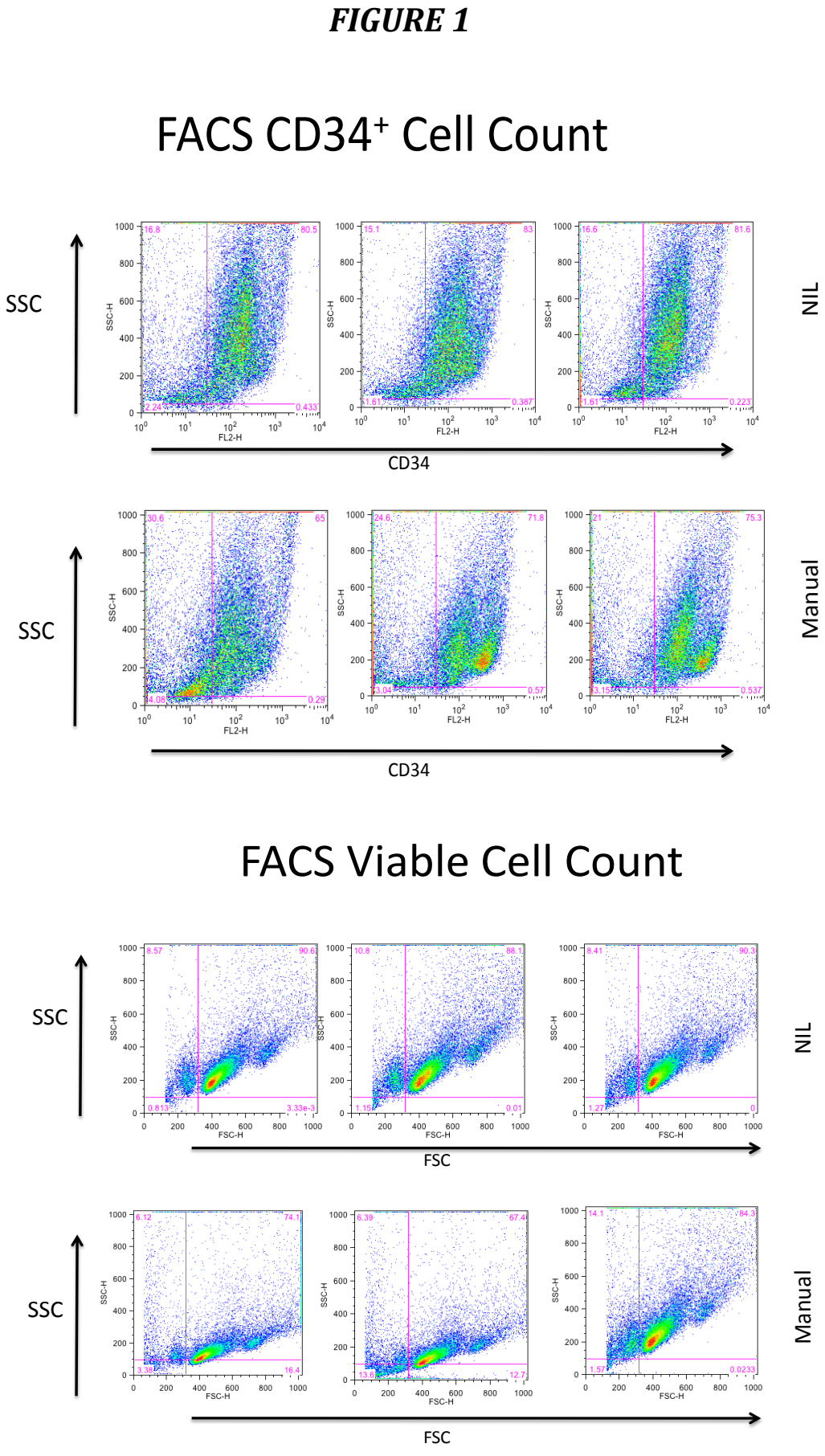
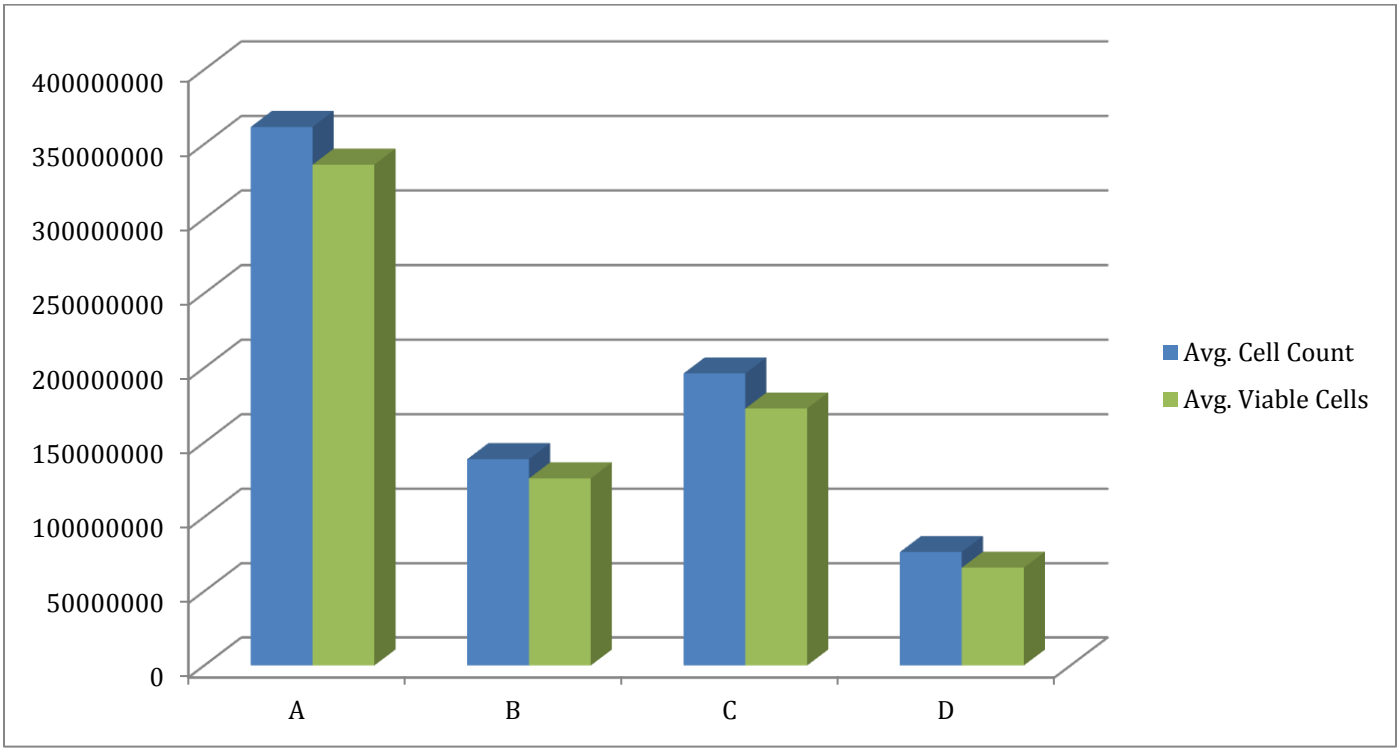

FACS CD34+ Cell Count
SSC
CD34
NIL
Manual
FACS Viable Cell Count
FSC

FIGURE 2

Average Count Per 50cc of Lipoaspirate



Sample Descriptions:

A: NIL 1st SVF pellet

B: NIL 2nd SVF pellet using collagenase dissociation on sample supernatant (adipose)

C: SAL 1st SVF pellet

D: SAL 2nd SVF pellet using collagenase dissociation on sample supernatant (adipose)

Details for samples B and D: saved the supernatant of 1st spin (adipose) and took it through a collagenase process to produce a 2nd SVF pellet in order to get an idea how many cells are in the adipose in comparison to the tumescent on the same 50cc sample using SAL and NIL.

The collagenase samples cell counts were performed via hemocytometry and viability was performed trypan blue, FACS was not run on these samples.

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