Is Microfragmented Adipose Tissue Biologically Different from Unprocessed Lipoaspirate?

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INTRODUCTION: Over the past several decades, there has been an increasing interest in utilizing microfragmented adipose tissue (MF-AT) therapy to treat symptoms of early osteoarthritis and promote healing in other musculoskeletal injuries. As the literature around this product grows, there is still no conclusive evidence for its mechanism of action. Several companies have advocated for the enrichment of mesenchymal stem/stromal cells (MSCs) in MF-AT as a driver of healing. Another proposed mechanism is the production of anti-inflammatory and regenerative signaling molecules by the cells in MF-AT. The purpose of this study was to assess whether the microfragmentation process resulted in significant changes in adipose tissue MSC content or select immunomodulatory signaling molecule production compared to unprocessed adipose tissue (U-AT) from the same patient.

METHODS: Patients undergoing MF-AT injection (n=28, 10 female and 18 male) were consented prior to the procedure under an IRB-approved study protocol. Samples of unprocessed lipoaspirate (U-AT) and prepared MF-AT were collected from patients, with one patient providing samples from two separate procedures for a total of n=29 sets of samples. Tissue was used for either flow cytometry quantification of MSCs or for tissue explant culture and quantification of secreted signaling molecules. For flow cytometry (n=18), tissue was enzymatically digested, the stromal vascular fraction (SVF) was isolated, fluorophore-conjugated antibody staining was performed, and data was acquired with a BD LSR Fortessa flow cytometer and analyzed with FlowJo v10. For tissue explant culture (n=11), equal volumes of MF-AT and U-AT were cultured in duplicate wells in cell culture inserts submerged in DMEM supplemented with penicillin/streptomycin and heat-inactivated FBS. The media from each well was collected and replaced at 17 hours, 24 hours, and every 24 hours thereafter for 7 days total. A 20-target human cytokine antibody array (Abcam, ab197436) quantified changes over time for three pairs of MF-AT and U-AT. Five proteins of interest (IL6, IL8, CXCL1/GRO, CCL5/RANTES, and VEGF) were quantified via ELISA (R&D Systems) for 10 pairs of MF-AT and U-AT media samples at the overnight and Day 7 time points. Statistical analysis was performed in GraphPad Prism version 10.6.1.

RESULTS SECTION: The cohort for this study was 35.7% female, had a median age of 57.5 (ranged 32-79), and a median BMI of 28.2 (20.1-35.8). Comorbidities included diabetes (n=3), tumor without metastasis (n=4), chronic pulmonary disease (n=2), deep vein thrombosis (n=1), and connective tissue disease (n=1). Flow cytometry quantification of adipose-derived progenitor cells (defined as $CD73^+$, $CD90^+$, $CD105^+$, $CD31^-$, and $CD45^-$) identified a small, variable percentage present in both U-AT (mean 0.175% of total SVF cells, std. dev.0.22) and MF-AT (mean 0.371%, std. dev. 0.53). There were no significant differences in MSC content between U-AT and MF-AT (paired T test, n=18, p=0.098, Fig. 1A). The signaling molecules secreted from U-AT and MF-AT in tissue culture were initially assessed across 4 time points within the 7-day culture period from a limited number of samples to identify targets of interest. Many of the 20 array targets were not detected, but some increased in expression over time from both U-AT and MF-AT, including IL6, IL8, MCP-1, and MMP9, while only CCL5/RANTES decreased over time (2-way ANOVAs with Šidák multiple comparisons, n=3, $p<0.05$). There were no significant differences between U-AT and MF-AT from the array. Selected signaling molecules of interest were then further investigated by ELISA at the overnight and Day 7 time points. There were significant differences based on time point but not between U-AT and MF-AT for all targets (Kruskal Wallis tests with Dunn's multiple comparisons, n=10, $p<0.05$): IL6 (Fig. 1B), IL8, CXCL1/GRO, CCL5/RANTES (Fig. 1C), and VEGF (Fig. 1D). Interestingly, patients with tumor comorbidity yielded some of the higher concentrations of IL6, IL8, and VEGF from the Day 7 U-AT and the higher overnight U-AT RANTES concentrations, but the higher concentrations from the MF-AT samples were not strongly associated with this comorbidity.

DISCUSSION: These data do not show a significant difference between U-AT and MF-AT in the categories of MSC enrichment or a panel of immunomodulatory signaling molecules, two of the proposed mechanisms for MF-AT augmented healing. This does not preclude the possibility that the MSC population or secreted signaling molecules do act to promote musculoskeletal healing, but it does raise the question of whether microfragmentation is necessary or superior to unprocessed lipoaspirate injection. There is, of course, the possibility that microfragmentation results in some other beneficial alteration to adipose tissue such as removal of oil/impurities or increase in surface area of the tissue fragments altering the kinetics or distribution of signaling molecule secretion. Additionally, this study is limited by a small sample size and a limited array of analysis targets. However, this study suggests that further research into adipose microfragmentation is warranted before widespread clinical adoption.

SIGNIFICANCE/CLINICAL RELEVANCE: Microfragmented adipose injection is increasing in popularity, but its proposed mechanisms are understudied. The data presented here directly compare some of the most frequently proposed mechanistic variables to determine whether there is a demonstrable difference between unprocessed and microfragmented adipose tissue.

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IMAGES AND TABLES:

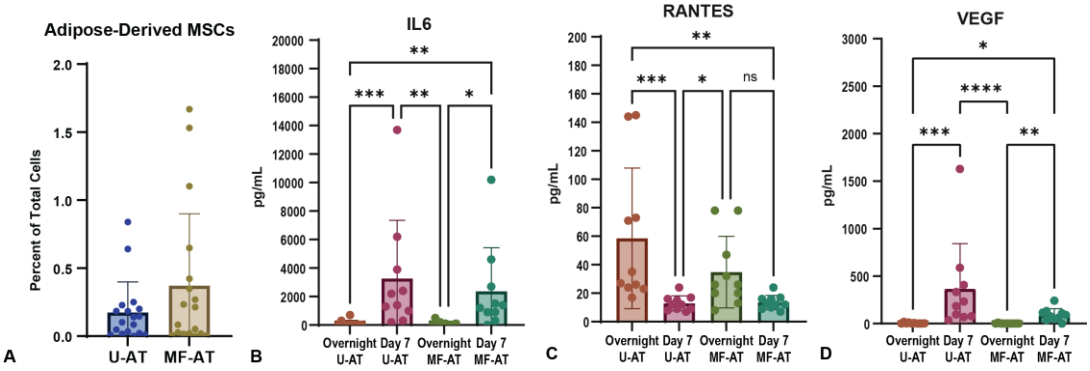

Figure 1. Cell and molecular biology comparison of unprocessed adipose tissue (U-AT) with microfragmented adipose tissue (MF-AT) quantifying the percentage of MSCs in the stromal vascular fraction (A) and production of IL6 (B), CCL5/RANTES (C), and VEGF (D) over time in tissue explant culture.