Mechanical Processing of Emulsified Lipoaspirate Results in a Dose-Dependent Upregulation of Stem Cell Markers and Populations

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INTRODUCTION: Mechanical processing of lipoaspirate (LA) is a commonly employed technique prior to reinjection for the purposes of lipofilling and skin rejuvenation. Our group has previously demonstrated that one form of mechanical processing, ‘nanofat grafting,’ results in a significant upregulation of multipotent mesenchymal stem cell (MSC) markers, adipose-derived stem (ADSCs) and endothelial progenitor cell populations (EPCs).1 Recently, a pluripotent population termed multilineage stress-enduring (Muse) cells was described after subjecting lipoaspirate to various extreme stress conditions.2 Based on these findings, we hypothesized that modulation of shear-stress alone would result in a correlative induction of markers associated with multipotency and/or pluripotency.

METHODS: Two microfluidic devices were created from acrylcs and methacrylic ester using laser etching and 3D printing. Each multichannel construct consists of expansion and constriction regions with minimal widths of 500 µm (v4) or 250 µm (v2) where the narrower the channel, the greater shear force generated. Standard LA (n = 7) was set aside as a control or processed as nanofat.3 Subsequently, two nanofat samples were processed via microfluidic devices regulated by a syringe pump (12.5 ml/min for 10 passes). Finally, each sample was subjected to collagenase digestion and the resulting stromal vascular fraction (SVF) pellets were subjected to automated cell count and multicolor flow cytometry panels.

RESULTS: On average, nanofat processing with or without microfluidic device yielded a four-fold decrease in nucleated cells when compared to control SVF. A dose-dependent pattern of stress-to-phenotype induction was observed for markers CD34 and CD13, as well as the subpopulations of MSCs, Muse cells, EPCs and ADSCs. The induction of MSCs (p < 0.003), Muse cells (p < 0.002), EPCs (p < 0.04) and ADSCs (p < 0.05) was much greater in all mechanically emulsified groups when compared to control, with v2 stress resulting in the largest populations.

CONCLUSION: Mechanical shear stress results in a dose-dependent induction of mesenchymal stem cell markers as well as multipotent/pluripotent populations. More detailed in vitro and in vivo studies are currently being explored to elucidate the mechanisms at play and examine the clinical significance of these findings. Additionally, we are working to determine the optimal stress needed to produce a potent progenitor mix for various clinical applications.

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The Negative Effect of Tumescent Lidocaine on Lipoaspirate Stem Cell Survival

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INTRODUCTION: Previous, in vitro study has shown that lidocaine at clinical concentrations has a significant impact on adipose-derived stem cell (ASC) survival.1 For large-volume liposuction, patients are often sedated. Lidocaine subcutaneous anesthesia is then unnecessary, and local anesthetic can be administered after performing liposuction. We hypothesized that removing lidocaine from tumescent solution would improve in vivo ASC survival.
**MATERIALS AND METHODS:** Adults undergoing liposuction on bilateral body areas were included (n=12). Under sedation, liposuction on one side was conducted with standard tumescent (1L of LR with 30ml of 1% lidocaine and 1 mcg/ml epinephrine). Tumescent without lidocaine was infiltrated on the contralateral side. Five milliliter lipoaspirate samples were processed for isolation of the stromal vascular fraction (SVF). Apoptosis and necrosis of SVF were examined by Annexin V-FITC/PI staining and analyzed by flow cytometry. ASC’s were also cultured and, after 24h, adherent, viable ASC’s were counted. The effect of ropivacaine on ASC survival was compared to PBS control and lidocaine in a cell culture, dose-response model.

**RESULTS:** From Annexin V-FITC/PI flow cytometry, the lidocaine group showed an average percentage of live ASC’s of 68.0±4.0% (28.5±3.8% of apoptosis and 3.4±1.0% of necrosis) as compared to 86.7±3.7% (11.5±3.1% of apoptosis and 1.8±0.7% of necrosis) in the no-lidocaine group (p = 0.002). In cell culture, the average number of viable ASC’s was also lower in the lidocaine group (367,000 ± 107) as compared to the no-lidocaine group (500,000 ± 152), a 26.6% decrease (p = 0.04).

In our dose-response study, ASC survival was significantly lower (p<0.01), in a dose-dependent manner, when treated by lidocaine and ropivacaine as compared to the corresponding PBS control. No significant difference was found between lidocaine and ropivacaine.

Conclusions: Excluding lidocaine from tumescent solution significantly improves ASC survival and decreases the apoptotic response. This increased survival may affect fat graft take by increasing angiogenesis and enhancing the healing cytokine milieu,\(^2,3\) and it will affect expansion and differentiation of cells for use in research or regenerative medicine by optimizing stem cell harvest.\(^4\)

Ropivacaine, despite having a decreased side effect profile, does not represent an alternative to lidocaine in terms of ASC survival.

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