ABSTRACT

Adipose tissue has been established as a high content, easily-accessible source of mesenchymal stem cells. Moreover, these adipose-derived stem cells (ADSCs) have shown exciting potential for various therapeutic applications including bone and cartilage formation, wound healing, nerve regeneration, and the healing of cardiac tissue. However, translational prospects of ADSCs have been slowed in the U.S. by the need to utilize an enzymatic digestion step for initial isolation and lengthy culture in certified labs for enrichment. These forms of processing are very costly and are beyond the scope of 'minimal manipulation' as outlined by the Food and Drug Administration (FDA). In previous work, we investigated the cellular components within human fat samples obtained by liposuction, which had been mechanically processed by passing at high flow rate between two syringes. We found that the result of mechanical processing, termed nanofat, was highly enriched in ADSCs and endothelial progenitor cells in comparison to collagenase digestion. We even observed enrichment of multilineage differentiating stress-enduring cells, which are pluripotent and strongly correlated with tissue regeneration. While this mechanistic insight was exciting, we questioned what was the source of this enrichment, whether it could be enhanced, and whether this manual processing method could be standardized for clinical practice. We will address each of these questions in this proposal through the development of novel microfluidic devices will fully process human lipoaspirate and maximize stem cell enrichment. This will involve 3 separate devices that will emulsify, filter, and enrich stem/progenitor populations through the precise application of hydrodynamic shear forces. This multi-faceted approach will enable us to tailor hydrodynamic shear forces to the appropriate magnitude and size scale, resulting in gradual and complete complete break down of adipose tissue in a fast, efficient, and gentle manner. These devices will be based on our previous work developing microfluidic devices to process tissues into single cells for molecular diagnostics, and prototypes of each device have already been designed, fabricated, and tested in preliminary studies using human lipoaspirate. We will first develop and optimize each device separately, focusing on cell recovery, viability, and stem/progenitor cell content. Next we will combine all devices into a single platform ideal for clinical settings, and perform a series of *in vitro* phenotypic assays to assess healing potential. The Specific Aims for this 1 year seed project include: (1) enable on-chip emulsification and filtering of human lipoaspirate and (2) enrich stem and progenitor cells and maximize healing properties. Our novel and innovative microfluidic technology platform will provide a systematic and standardized methodology that is critically needed to achieve point-of-care processing of adipose tissue, generating a 'minimally manipulated' stem-cell therapeutic that will be clinically translatable and commercially viable. The enriched and activated stem cell populations could then be rapidly deployed to promote wound healing. A major goal in future work will be to study the healing potential of our cellular therapeutic for the treatment of diabetic foot ulcers, which is the leading cause of non-traumatic lower limb amputation. Extramural funding will be sought to test our device platform and cellular therapeutic *in vivo* using animal models, and eventually, in human patients.