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CELL PRODUCTS: COLLECTION, QUALITY ASSURANCE AND MONITORING

Ultrasound-assisted liposuction provides a source for functional adipose-derived stromal cells

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Abstract

Background aims. Regenerative medicine employs human mesenchymal stromal cells (MSCs) for their multi-lineage plasticity and their pro-regenerative cytokine secretome. Adipose-derived mesenchymal stromal cells (ASCs) are concentrated in fat tissue, and the ease of harvest via liposuction makes them a particularly interesting cell source. However, there are various liposuction methods, and few have been assessed regarding their impact on ASC functionality. Here we study the impact of the two most popular ultrasound-assisted liposuction (UAL) devices currently in clinical use, VASER (Solta Medical) and Lysonix 3000 (Mentor) on ASCs. *Methods.* After lipoaspirate harvest and processing, we sorted for ASCs using fluorescent-assisted cell sorting based on an established surface marker profile ($CD34^{+}CD31^{-}CD45^{-}$). ASC yield, viability, osteogenic and adipogenic differentiation capacity and *in vivo* regenerative performance were assessed. *Results.* Both UAL samples demonstrated equivalent ASC yield and viability. VASER UAL ASCs showed higher osteogenic and adipogenic marker expression, but a comparable differentiation capacity was observed. Soft tissue healing and neovascularization were significantly enhanced via both UAL-derived ASCs *in vivo*, and there was no significant difference between the cell therapy groups. *Conclusions.* Taken together, our data suggest that UAL allows safe and efficient harvesting of the mesenchymal stromal cellular fraction of adipose tissue and that cells harvested via this approach are suitable for cell therapy and tissue engineering applications.

Key Words: *adult mesenchymal stromal cells, adipose-derived stromal cells, ASCs, cell therapy, regenerative medicine, ultrasound-assisted liposuction, VASER, Lysonix*

Introduction

Surgeons have been optimizing methods for the removal of fat from different sites in the body for nearly a century. The first documented instance of removal of adipose tissue took place in 1921, when Charles Dujarrier used a uterine curette to remove subcutaneous fat from a patient's calves and knees, unfortunately resulting in injury of the femoral artery and eventual amputation of the limb [1]. En bloc adipose tissue resection was also attempted, although popularity of the technique was limited by the resultant large scars. Further attempts to remove fat via curettage followed, most notably involving a blunt

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cannula with suction pioneered by Arpad and Giorgio Fischer in the 1970s [2].

Currently, more than 205 000 surgeries involving liposuction are performed each year in the United States alone, and it is now considered to be a relatively safe procedure. Since the era of modern liposuction, focus has turned to the fine-tuning of the technique to improve factors such as skin retraction, blood loss and operative time and effort [3]. Along with traditional suction-assisted liposuction (SAL) and other modalities such as power-assisted liposuction, radiofrequency-assisted liposuction and laser-assisted liposuction, the use of ultrasound-assisted liposuction (UAL) has become a major component in many plastic surgery practices today. The basic principle of UAL is to turn electric energy into vibration, causing thermal, cavitational and mechanical effects that lead to fragmentation of fat [4]. The use of ultrasound energy in liposuction was first developed in the late 1980s and 1990s by Scuderi and Zocchi [5], with the rationale that the energy would selectively disintegrate adipose tissue and thereby facilitate removal with decreased bleeding [6].

Concurrently, surgeons and researchers alike have begun to explore the utility of the aspirated adipose tissue, a source that is abundant and otherwise unwanted or discarded. Most prominently, the technique of fat grafting (lipofilling/lipotransfer) has emerged as a method to restore soft tissue deficits [7]. However, the technique is thus far unpredictable in outcomes, often with poor volume retention combined with an incomplete understanding of the underlying physiology of fat grafts and their components. Adipose-derived stromal cells (ASCs), a population of multipotent cells found in relatively high proportions within adipose tissue [7], have been shown to play a crucial role for the efficacy of lipotransfer. However, ASCs have not only been employed to further augment fat grafts to improve retention [8], they can also be applied to wounds to improve healing [9] and used in critical-sized calvarial defects to contribute to bone formation [10]. Evidence is emerging that harvesting methods may affect the quality and regenerative potential of ASCs in aspirated adipose tissue [3]. In previous reports, we have established that SAL lipoaspirates are a functional ASC source compared with excisional fat and that VASER UAL-derived ASCs are not diminished in their regenerative capacities when compared to SAL ASCs [11,12]. However, it is as yet unclear whether the two most popular UAL devices differ in their ability to preserve the stromal cellular components of the aspirated tissue and therefore in the resultant regenerative potential. Here we determine the effect of VASER (Solta Medical) and Lysonix 3000 (Mentor) UAL on ASC regenerative functionality.

Methods

ASC harvest and isolation

Under approval of the Stanford Institutional Review Board (Protocol No. 2188), human lipoaspirate was collected from the abdomen of three adult female patients between the ages of 32 and 50. No patient had major medical comorbidities. Paired specimens were collected using VASER and Lysonix UAL devices sequentially on adjacent areas of the abdomen.

ASCs were isolated from the lipoaspirate specimens, according to previously described methods [13]. Briefly, lipoaspirates were washed with phosphate-buffered saline (PBS) and digested with 0.075% collagenase type II (Sigma-Aldrich) in M199 (Cellgro) for 1 h at 37°C with gentle agitation. The collagenase was neutralized using an equal volume of complete cell culture medium, consisting of Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen) plus GlutaMAX, 10% fetal bovine serum (FBS; Sigma-Aldrich), and 1% penicillin/streptomycin (ThermoFisher Scientific), and then centrifuged at 1500 rpm at 4°C for 20 min (350 rcf/g). The pellet was re-suspended in complete cell culture medium, filtered through a 100-μm cell strainer (Falcon; BD Biosciences), and then re-pelleted at 350 rcf/g for 15 min. The cell pellet was resuspended in red cell lysis buffer and then centrifuged once more at 350 rcf/g for 15 min at room temperature to obtain a stromal vascular fraction (SVF) pellet.

Fluorescence-activated cell sorting

Our group recently demonstrated significant differences in the transcriptional profile between primary ASCs and cultured cells stressing the importance of using primary or very early passage cells in in all translational studies [14]. Therefore, freshly harvested and pelleted SVF cells were directly stained for fluorescence-activated cell sorting (FACS) analysis to identify and isolate the ASC population, defined as the $CD45^-/CD31^-/CD34^+$ cell fraction. Cells were stained using the mouse anti-human monoclonal antibodies (all BD Biosciences) CD31-PE (Clone WM59; catalog no. 560983), CD45-PeCy7 (Clone D058-1283, catalog no. 561294) and CD34-APC (Clone 563, catalog no. 561209), and propidium iodide was used to exclude dead cells. A BD FACSAria (BD Biosciences) machine and FlowJo software (Miltenyi Biotec) were used to perform FACS analysis.

In vitro cell viability

Cell Viability of ASCs harvested via VASER and Lysonix was detected with a Vybrant MTT Cell Assay