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# Stromal Vascular Fraction from Lipoaspirate Infranatant: Comparison Between Suction-Assisted Liposuction and Nutational Infrasonic Liposuction

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**Abstract**

*Introduction* Lipoaspirate has shown great promise as a source of progenitor cells for use in regenerative medicine. The stromal vascular fraction (SVF) can be isolated from lipoaspirate using enzyme digestion and centrifugation, but this approach may be limited by the labor-intensive nature of the technique as well as ambiguities in current governmental regulations. An alternative approach to obtain SVF from lipoaspirate was studied.

*Method* Paired (collected from contralateral regions) lipoaspirate specimens were acquired from 30 consenting patients (age 24–62; 22 females, 8 males) by suction-assisted liposuction (SAL) and nutational infrasonic liposuction (NIL). The infranatant from 50 ml of adipose tissue (LAF) was centrifuged at 400*g* × 5 min and the resultant pellet was collected with a pipette. Time = 15–20 min. The respective SVFs cell populations were counted using an optical fluorescent cell counter (Nexcelom A2000) and the fluorescent stains—acridine orange (AO) and propidium iodide (PI).

*Results* The number of nucleated, live cells from SAL infranatant was 97,345 ± 23,435 per ml of adipose tissue and from NIL infranatant was 335,621 ± 81,274 per ml of adipose tissue. The $p$ value is <0.00001, $n = 30$.

*Conclusion* Regenerative cells can be isolated from the lipoaspirate infranatant from either SAL or NIL, although in lower quantities than from enzyme digestion. NIL acquisition yielded 3.5× the number of cells over that acquired from SAL. The time, skill, and cost of producing SVF from infranatant is less than using enzyme digestion, which potentially make these regenerative therapies accessible to more physicians and patients.

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## Introduction

Lipoaspirate has shown great promise as a source of autologous progenitor cells for use in regenerative medicine. A heterogeneous population of such cells can be obtained by the treatment of the adipose portion of lipoaspirate with enzymatic digestion followed by centrifugation. This cell population has been termed the stromal vascular fraction (SVF) [1]. This cell population includes: monocytes, macrophages, pericytes, endothelial precursor cells, pre-adipocytes, and adipose-derived mesenchymal stem cells [2]. These stem cells can be expanded in tissue culture and have shown the capability of differentiating into adipocytes, chondrocytes, osteoblasts, and vascular endothelium [3]. Their potential for use in tissue engineering and regenerative medicine is being extensively studied. In addition, point-of-care applications using the SVF cell mixture have been studied in fat grafting, wound healing, repair of soft tissue defects, and orthopedic applications, among others. A limitation to the clinical application particularly of these point-of-care treatments is the skill, labor-intensive nature, expense, and time required for the isolation process. Another difficulty has been the ambiguity of government regulations in many countries regarding the "manipulation" of the cell with an enzyme.

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## Materials and Methods

Suction-assisted liposuction (SAL) is performed using a cannula attached to a negative pressure pump. Infrasonic nutational liposculpture (NIL) adds an air pressure-driven handpiece (Euromi, Verviers, Belgium) which moves the cannula in three-dimensional pattern at the infrasonic rate of 10–20 Hz. Specimens were obtained using each of these two techniques at −15 inches (−360 mmHg) aspiration pressures. The cannulae used were constructed of stainless steel, 3.5 mm in diameter, and had a total of 10 holes with a diameter of 0.8 mm. Paired specimens from 30 consenting patients (age 24–63; 22 female and 8 male) were acquired using the contralateral anatomical area (for example: right flank vs. left flank or right periumbilical area vs. left periumbilical area). Areas to be sculpted were anesthetized with the tumescent technique using modified Klein solution of 0.04 % lidocaine, 1:1,000,000 epinephrine, and 0.0085 % sodium bicarbonate. The number of cells acquired from NIL infranatant and the number of cells acquired from traditional suction-assisted liposuction (SAL) infranatant were compared. The infranatant from 50 ml of adipose tissue harvested using SAL and NIL was centrifuged at $400g \times 5$ min and the SVF pellets were collected using a pipette. The cell populations were counted using an optical fluorescent cell counter (Nexcelom A2000) and the fluorescent stains—acridine orange (AO) and propidium iodide (PI). The enzyme (collagenase) technique described by Zuk [1] typically requires 45–65 min of incubation at 37°C and passage through a 100 μm filter to remove non-cellular debris. Materials required for this step include: enzyme, shaking incubator, and filters (Fig. 1).

## Results

The number of nucleated, live cells from SAL infranatant was 97,345 ± 23,435 per ml of adipose tissue and from NIL infranatant was 335,621 ± 81,274 per ml of adipose tissue. The $p$ value is <0.00001, $n = 30$.

## Discussion

In 2001, Zuk and her team at UCLA isolated a population of adult stem cells from adipose tissue which they referred to as adipose-derived stem cells (ADSCs) [3]. These ADSCs and other cells with regenerative potential are found in association with adipose vascular tissue [4]. The potential for these ADSCs to differentiate into cells of all three germ layers [5–7] as well as exhibiting paracrine, anti-inflammatory, and antioxidant effects [8–10] have led to efforts to investigate the use of these cells after culture expansion in tissue engineering and at the point of care in a variety of therapeutic applications including fat grafting, joint, bone, tendon repair, pulmonary arterial hypertension, healing of chronic wounds, renal, and limb ischemia.

Intrinsic barriers to their widespread application include the skill-intensive and cost-intensive nature of the enzyme process. Extrinsic barriers include the ambiguity and diversity of governmental regulations in various jurisdictions around the world with respect to production of autologous cellular products. This study attempts to address these barriers by investigating a method of producing SVF that requires less time, skill, and money and that fulfills the criteria of "minimal manipulation" by the Federal Drug Administration (FDA) in the United States. The time required to complete the enzyme process in our

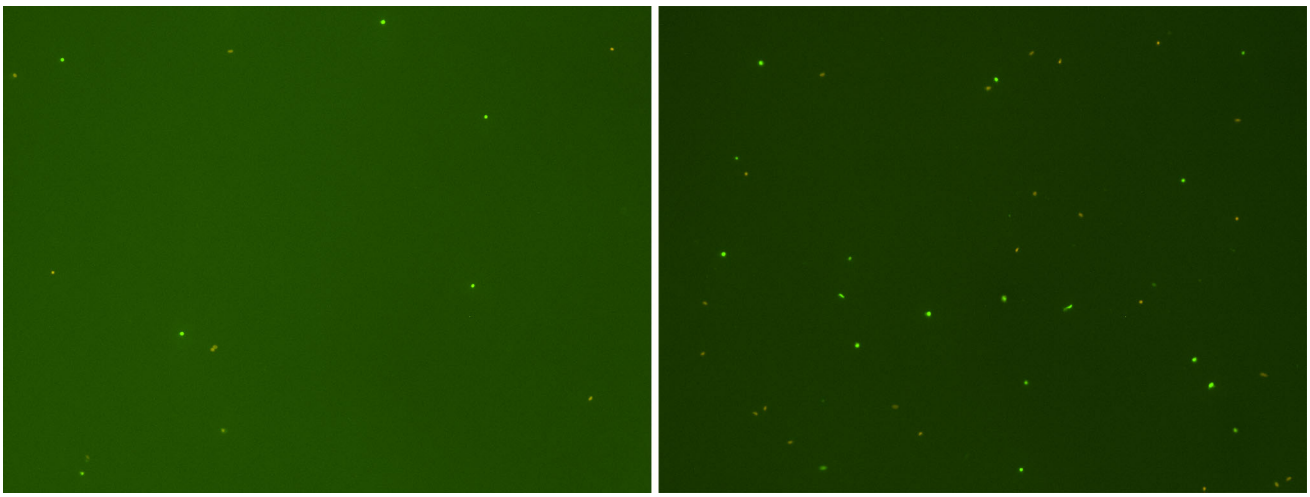

**Fig. 1** NIL versus enzyme paired specimen, Images from AO/PI staining 40:1 dilution

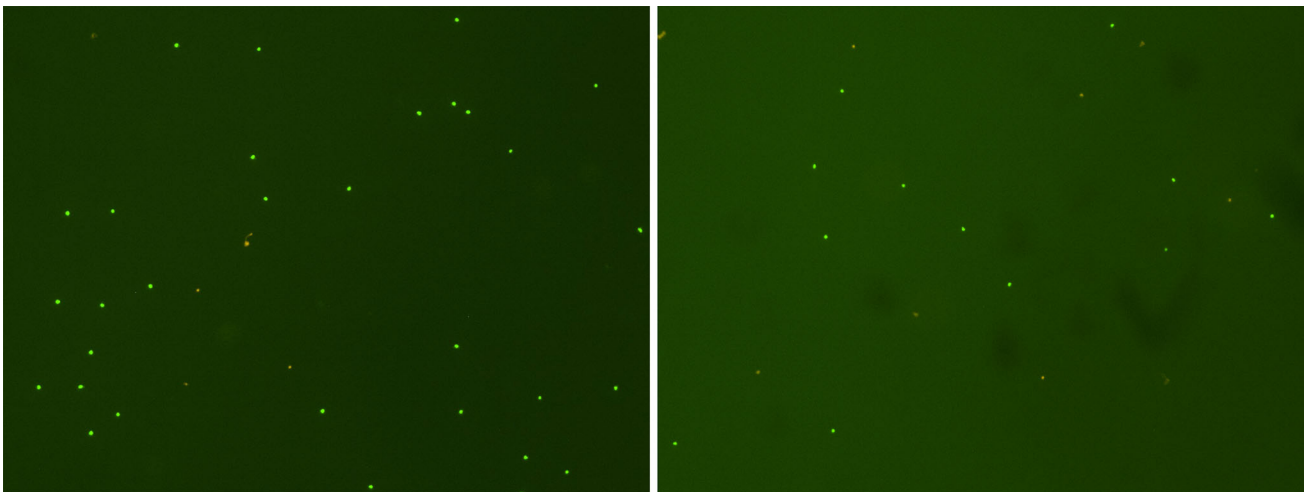

**Fig. 2** NIL versus SAL paired specimen A. Images from AO/PI staining 10:1 dilution

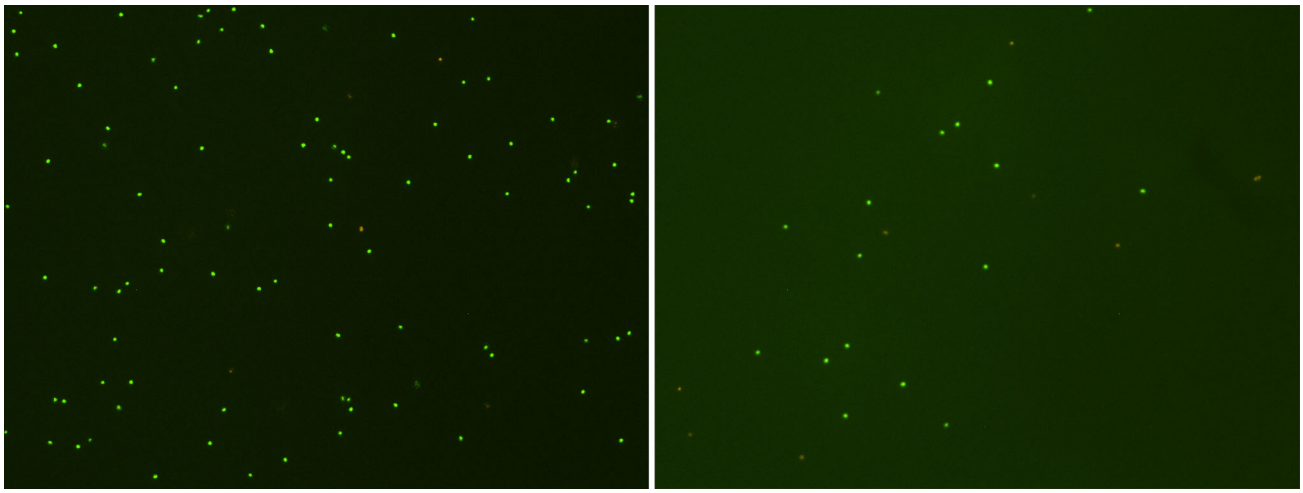

**Fig. 3** NIL versus SAL paired specimen B. Images from AO/PI staining 10:1 dilution

laboratory is 80–105 min after acquisition of lipoaspirate. The time for centrifugation of infranatant and collection of the SVF pellet is 15–20 min. A previous pilot study in our lab of paired lipoaspirate specimens demonstrated the presence of live nucleated cells in NIL infranatant at a ratio of 1:4 to that of enzyme digestion and that both NIL and SAL were superior to manual liposuction with a syringe in acquiring regenerative cells. The current investigation was undertaken to compare the cell counts of lipoaspirate infranatant obtained from NIL from those obtained from SAL (Figs. 2, 3, 4).

Previous efforts to characterize the cellular content of lipoaspirate fluid (LAF) began with the work of Yoshimura et al. who compared the cellular character of LAF to enzyme-processed lipoaspirate (PLA). They found that cells from LAF were similar in: plastic adherence (a marker for stem cells), growth in cell culture, growth kinetics, and cell surface markers, although in lower numbers than those derived from enzyme digestion [11]. Baptista et al. described a method used to isolate SVF from LAF as applied to cryopreservation and cell culture. They also noted that the cells demonstrated similar adipogenic, osteogenic, and chondrogenic potential as mesenchymal stem cells from other sources [12]. Francis et al. also described a method of nonenzymatic isolation of ADSCs for tissue engineering using centrifugation of LAF and quantitated the number of ADSC's as approximately 100,000 cells in 100 ML's of infranatant [13]. Gimble et al. also reported isolating SVF from lipoaspirate by manual shaking followed by centrifugation without enzyme digestion [14]. Raposio et al. isolated ADSCs using a vibrating shaker to mechanically agitate lipoaspirate

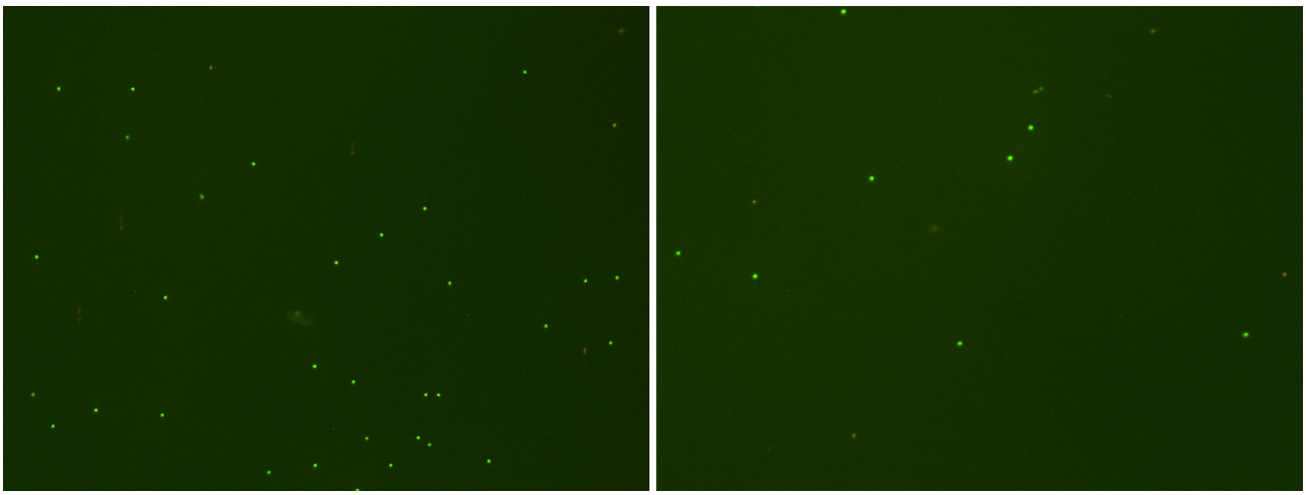

**Fig. 4** NIL versus SAL paired specimen C. Images from AO/PI staining 10:1 dilution


**Fig. 5** Adipose tissue and infranatant collection

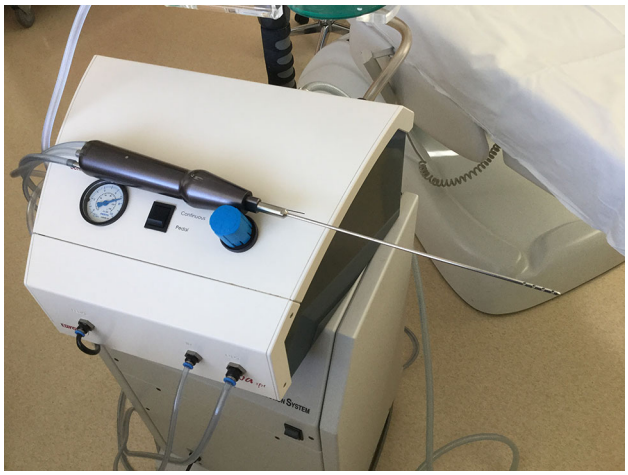

**Fig. 6** NIL device and cannula

**Fig. 7** Lab and Procedure room

followed by centrifugation [15]. These previous studies did not specifically address the use of non-tissue expanded SVF in point-of-care treatments and it is not known if an adequate number of cells could be acquired from a relatively small volume of lipoaspirate. In the present study, the data reveal that a substantial number of cells can be acquired from centrifuging lipoaspirate infranatant (Figs. 5, 6, 7).

The mechanism for the increase of nucleated infranatant cells from NIL over SAL is unknown, but it is possible that

the 3-dimensional movement of the cannula helps to mechanically dislodge these cells from their perivascular niche.

## Conclusion

A substantial cell population can be isolated from the lipoaspirate infranatant from either SAL or NIL. The cell yield is approximately 3.5× greater for cells harvested by NIL than for SAL. The time, skill, and cost of producing SVF from infranatant is less using enzyme digestion, especially when using NIL (secondary to the higher cell yield, the time to acquire the same number of cells is reduced by 3.5×). This potential makes these regenerative therapies accessible to more physicians and patients. This method may be particularly applicable to physicians practicing in jurisdictions in which the use of collagenase is considered more than minimal manipulation. Additionally, practitioners using enzyme-derived SVF in cell-assisted lipotransfer can increase regenerative cell yield by adding the infranatant pellet from NIL or SAL to the cells acquired by enzymatic digestion.

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**Compliance with Ethical Standards**

**Conflict of Interest** The author serves as a consultant to Sciton, a laser manufacturer in Palo Alto, CA, and as a consultant and a stockholder with ThermiAesthetics in Dallas, TX.

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