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Adipocytes Viability After Suction-Assisted Lipoplasty: Does the Technique Matter?

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Abstract

Background Suction-assisted lipoplasty (SAL; liposuction) is an established aesthetic procedure in plastic surgery. The main parameters differentiating one method of lipoplasty from another are safety, consistency of results, and other more technical parameters. Due to the recent popularity of lipotransfer, the quality of extracted fat has become a relevant parameter. We compare the viability of extracted adipocytes after dry SAL, hyper-tumescent PAL (power-assisted lipoplasty), and water-assisted lipoplasty (WAL).

Methods We used fluorescent microscopy to differentiate viable from necrotic/apoptotic cells after liposuction using each of the mentioned methods.

Results The ratio of living cells between the three methods was significantly different with dry liposuction yielding inferior ratios ($p = 0.011$). When omitting extreme results, we found that the body-jet technique (WAL) yielded higher ratios of living cells than the hyper-tumescent technique ($p < 0.001$). The total number of cells was highest in the hyper-tumescent method ($p = 0.013$).

Conclusions Our results indicate that the hyper-tumescent technique yields the highest number of cells, whereas the body-jet technique yields the highest living cells ratio. The dry technique is clearly inferior to both.

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Keywords Liposuction · Adipocyte viability · Fat transfer

This study was presented in the "Oncoplastic & Reconstructive Surgery of the Breast" meeting in Milan, Italy/December 2011.

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Background

Suction-assisted lipoplasty (SAL; liposuction) is an established aesthetic procedure in plastic surgery. First attempted by surgeons in the 1920s–1960s and abandoned, it was re-introduced by Illouz in the late 1970s and early 1980s [1, 2]. At first, the procedure was marked by blood loss, electrolyte imbalance, and early disappointing results [3]. However, later refinements of the technique and development of new techniques [i.e., power-assisted lipoplasty (PAL), water-assisted lipoplasty (WAL)] [3–9] have mostly rendered these concerns obsolete [10, 11].

Traditionally, the main parameters differentiating one method of lipoplasty from another are safety (i.e., blood loss, fluid and electrolyte imbalance, etc.), consistency of results, and other more technical parameters such as ease of performance, learning curve, duration of hospitalization, and recovery time. The quality or viability of the extracted fat has never been a relevant parameter. However, in the previous

decade, lipotransfer (the use of the extracted fat as a graft in other receiving areas) has been introduced to both reconstructive [12, 13] and aesthetic [14, 15] plastic surgery. This development has made the viability of extracted fat a major determining factor [16] in the take of the grafted fat. However, to date, this issue has not been resolved.

Objective

To compare the viability rates of extracted adipocytes after dry SAL, hyper-tumescent PAL, and water-assisted lipoplasty.

Patients and Methods

Patients

Sixteen female patients (age range 35–76 years) undergoing routine SAL as part of a reconstructive procedure were included. Patient medical history and personal demographic and characteristics were collected. All patients signed an informed consent form as required by the medical center's internal review board.

Lipoplasty

Before commencement of the procedure each patient underwent dry SAL, hyper-tumescent PAL, and body-jet WAL. Twenty cubic centimeters of lipoaspirate were extracted in each method, all from the flanks, and transferred for further processing.

Dry SAL: As previously described [17, 18] after general anesthesia, fat was extracted using a blunt tip, 26-cm-long cannula, outer diameter of 4 mm (Byron[TM], MENTOR, Santa Barbara, California, MER426L) and a 20-cc luer lock syringe.

Body-Jet WAL: Following dry SAL patients underwent WAL using the body-jet system (Body-Jet; Human Med, Eclipse Ltd., Dallas, TX, USA) with the LipoCollector[TM] 3 and a standard 3.8 mm, 25 cm long cannula (REF 500092). The procedure has been previously described [19]. In the first phase, minimal volumes of standard tumescent solution are infiltrated in a pulsatile fashion to the treated area. In the second phase, fat is extracted simultaneously with the continued infiltration, collected, and separated from the fluids in a LipoCollector[TM]. Adipocytes were sampled into a 20 cc lure lock syringe. In the final stage, suction is performed without infiltration to dry the treated area. It has to be emphasized that the 20 cc of lipoaspirate that were used to evaluate the viability of the cells were extracted from a different area than the dry SAL.

Hyper-Tumescent PAL: After fat extraction using the dry and body-jet methods, the patients underwent hyper-

tumescent PAL as previously described [20]. In short, the treated area was infiltrated with high volumes (three times or more of the expected aspiration volume. Actual infiltration volume ranged 1–8 L per patient depending on the amount of expected fat harvest) of tumescent solution (prilocaine 2 %, 30 mL/3 L, lidocaine 2 %, 30 mL/3 L, NaHCO$_3$ 8.4 %, 20 mL/3 L), followed by fat extraction using a MicroAire PAL®-600E (Power Assisted Liposuction) LipoSculptor[TM], with reciprocating 4 mm cannulas 22 and 30 cm in length (4000 oscillations/min) into a negative pressure sterile collector, and adipocytes were sampled into a 20-cc Lure Lock syringe. As with WAL, hyper-tumescent PAL was performed in a previously unsuctioned site.

Lipoaspirate Processing

As previously described by Son [21], adipocytes were separated from blood cells, debris, and supernatant by centrifugation at 3000 rpm for 10 min (Eppendorf 5810R, 1800G) at room temperature (Fig. 1). One milliliter of the adipocyte layer was digested with 5 mL of 0.1 % collagenase 1A (Crude Type IA C2674-100MG from Clostridium histolyticum) in phosphate-buffered saline at 37 °C for 40 min, the collagen degradation reaction was stopped with 6 mL of DMEM (DMEM With 4.5 g/L D-Glucose Without L-Glutamine, Biological Industries Kibbutz BeitHaemek). Mature adipocytes were separated from the pellets by centrifugation at 1000 rpm for 10 min (Eppendorf 5810R,
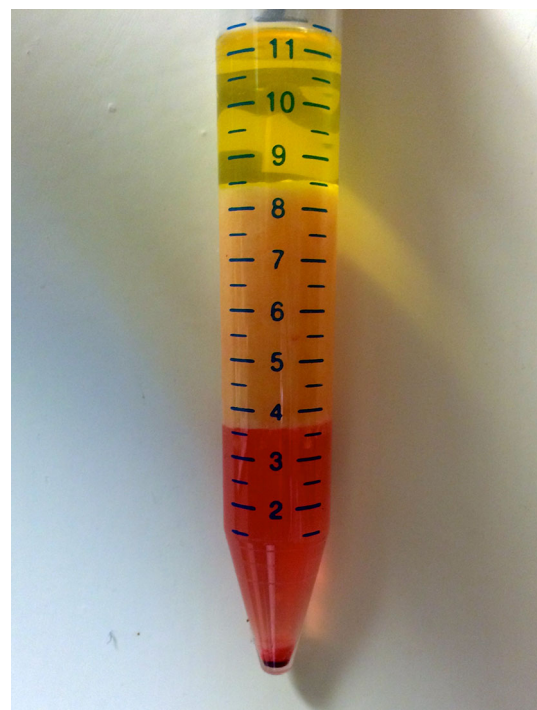

Fig. 1 Adipocytes separated from oil, blood cells, and debris by centrifugation, *scale* in millimeters

200G). Specimens were divided into four layers: oil, mature adipocytes, blood, and pre-adipocytes. Then 400 μL was taken from the middle of the mature adipocyte layer. Twelve micromolar of fluorescein diacetate (Fluorescein Diacetate, Sigma 100 μL of 0.02 mg/μL) and 3.75 μM of propidium iodide (propidium iodide, Sigma 50 μL, 1 mg/μL) were added together with 250 μL of PBS, mixed, and inverted. After 5 min, 10 μL of the adipocytes was placed on a disposable hemocytometer (C-Chip Disposable Hemocytometer, Digital Bio).

Viability Evaluation

Mature adipocytes were extracted and purified from each lipoaspirate specimen harvested by one of the three different aspiration methods. Two sets of hemocytometers were prepared from each sample. The hemocytometers were examined under a fluorescence microscope (Olympus IX81) and ultraviolet illumination. Viable adipocytes fluoresced bright green (particularly around the edges; Fig. 2), whereas the nuclei of dead (necrotic/apoptotic) cells fluoresced red (propidium iodide; Fig. 3). Five high power fields (magnification ×20) were evaluated in every hemocytometer, cells were counted, and the number of stained cells alive or dead was recorded (Fig. 4).

Statistical Analysis

Adipocyte viability is presented as a live to total adipocyte (dead and alive) ratio. Viability ratios were compared between the different harvesting methods and analyzed
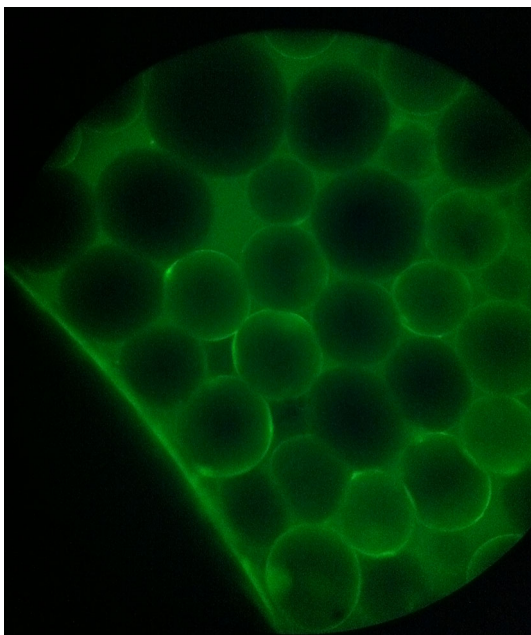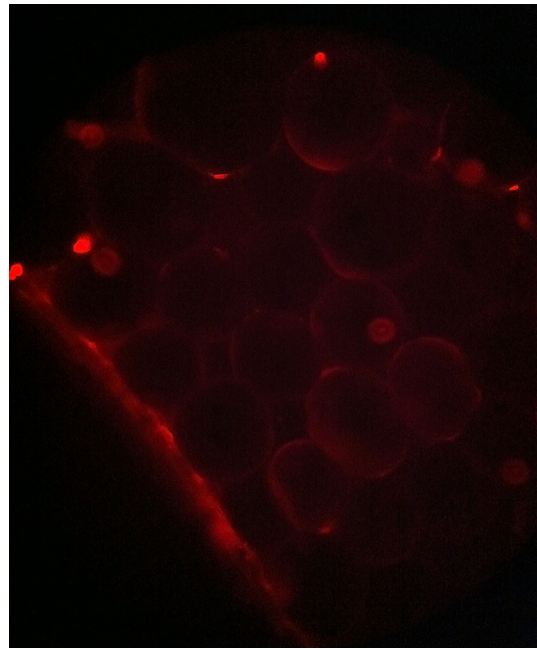

Fig. 3 Dead adipocytes display *red* nuclear fluorescence
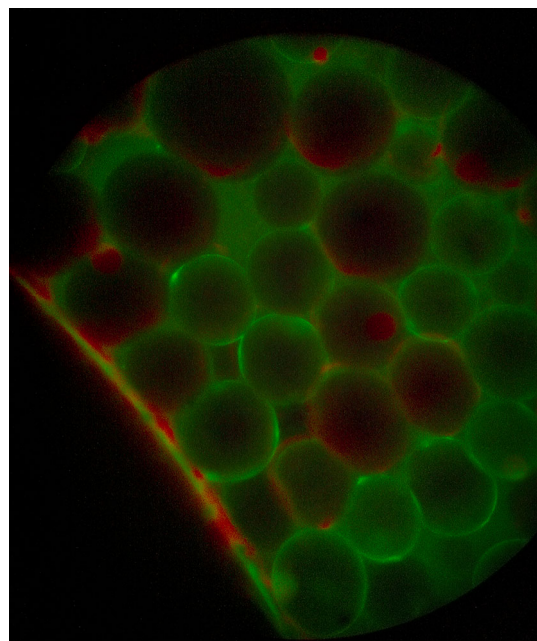
Fig. 4 Digitally overlap of Figs. 1 and 2, demonstrating the complementary illumination of the adipocytes fluorescent with FDA (*in green*) and PI (*in red*) cells

using the ANOVA test repeated measures. The mean value was calculated and reported with standard deviation. Comparison of the viability ratios and total cell counts (dead + alive) within the same harvesting method between patients with normal BMI and normal medical history, and patients with elevated BMI and systemic illnesses was performed using the unpaired *t* test. Comparison of the

Fig. 2 Viable adipocytes display *green* peripheral fluorescence

total cell counts between the different harvesting methods was performed using the ANOVA test in repeated measures.

All of the statistical analyses were performed using InStat, Version 3.1a; GraphPad Software Inc and http://vassarstats.net.

Results

Sixteen female patients, 35–76 years old, underwent routine SAL. The patient BMIs ranged from 21.6 to 34.8. Ten patients had a normal BMI whereas the rest were either overweight or obese. None of the patients were smokers, and all of the patients had a past medical history involving Ca of Breast. Other medical conditions were asthma, dyslipidemia, and hypothyroidism and hypertension. Table 1 summarizes patient characteristics.

Lipoaspirate volume ranged between 100 and 1500 cc, with a mean volume of 450 cc (not including fat harvested in the various methods for the current study). In all patients, fat was harvested from the lower abdomen and flanks. No adverse events or complications were noted in any of the patients. Table 2 summarizes the volume of fat harvested in each patient.

Table 3 summarizes the cell count for each patient in each harvest method. In all three methods, the lowest total number of cells per high power field was obtained from patient number 1. This finding is coupled with the lowest ratio of living to dead cells in all three methods. In fact, patient 1 is the only patient displaying a lower than 1 ratio (more dead than living cells), and does so in all three

Table 2 Aspiration volumes

Patient	Total lipoaspirate volume (cc)
1	450
2	150
3	100
4	1500
5	150
6	1300
7	300
8	160
9	700
10	200
11	150
12	240
13	100
14	200
15	700
16	800

methods of fat harvest. In contrast, the highest ratio of living cells was observed in specimens harvested from patient 6. In the dry SAL specimens, patients with normal BMI and normal medical history the ratios of living cells were similar to those found in the elevated BMI group (1.96 ± 0.65 vs. 1.4 ± 0.5; $p = 0.13$) as well as their total cell count (145.1 ± 23.5 vs. 134.2 ± 56.6; $p = 0.67$). In the WAL specimens, patients with normal BMI and normal medical history had higher ratios of living cells (3.4 ± 0.8 vs. 2.1 ± 1; $p = 0.03$), but their total cell count was not

Table 1 Patients' characteristics

Patient	age	Medical history	BMI	Smoking	Previous surgeries
1	54	Asthma, dyslipidemia, HTN, osteoporosis, S/P Ca of breast	32.9	No	
2	51	Asthma, HTN, Hypothyroidism, S/P Ca of Breast	34.8	No	BSO
3	47	HTN, S/P Ca of breast, adjustment disorder	32.3	No	
4	76	HTN, S/P Ca of breast	27.2	No	
5	46	S/P Ca of breast	22.7	No	
6	54	S/P Ca of breast	23.7	No	Hysterectomy BSO
7	73	S/P Ca of breast	24	No	
8	57	S/P Ca of breast	20.6	No	
9	54	Hypothyroidism, dyslipidemia S/P Ca of breast	33.6	No	
10	42	Hypothyroidism, S/P Ca of Breast	24.3	No	CS X2
11	47	S/P Ca of breast	22.3	No	BSO
12	73	S/P Ca of breast	24.6	No	
13	42	Hypothyroidism, S/P Ca of breast	24.3	No	CS X2
14	35	S/P Ca of breast	22	No	
15	51	Hypothyroidism, S/P Ca of breast	25.7	No	
16	35	S/P Ca of breast	21.6	No	

BSO bilateral salpingo-oophorectomy, *CS* caesarean section

Table 3 Cell count results (average cell number per high power field; magnification ×20)

Patient	Dry	Body-jet	Hyper-tumescent
1	47	13	31
2	132	122	223
3	123	90	228
4	131	131	106
5	153	263	342
6	115	127	147
7	117	137	163
8	186	198	181
9	213	349	308
10	145	143	116
11	130	128	111
12	79	117	112
13	125	131	161
14	81	75	122
15	159	225	221
16	190	114	260
Mean	132.88	147.69	177.00
SD	42.78	78.88	82.05

Table 4 Average living cells' ratio

Patient	Dry	Body-jet	Hyper-tumescent
1	0.74	0.86	0.63
2	1.49	1.71	1.48
3	1.08	2.10	1.65
4	1.73	1.73	1.00
5	2.83	2.02	1.48
6	3.79	6.06	11.25
7	1.09	3.42	1.96
8	2.80	3.71	2.77
9	2.09	3.26	3.46
10	2.22	4.11	3.64
11	1.55	1.98	1.27
12	1.26	2.90	2.39
13	1.31	3.09	2.43
14	1.45	3.41	2.13
15	1.27	3.09	2.30
16	1.26	3.22	2.61
Mean	1.75	2.92	2.65
SD	0.81	1.21	2.44

higher than that of the elevated BMI patients (182.8 ± 59.3 vs. 155 ± 122.9; $p = 0.62$). In the hyper-tumescent PAL specimens, patients with normal BMI and normal medical history had higher ratios of living cells, but this difference did not reach statistical significance (3.2 ± 2.1 vs. 1.75 ± 1.1; $p = 0.18$). Their total cell count was not higher than that of the elevated BMI patients (208 ± 65 vs. 186 ± 105; $p = 0.67$).

The total number of cells was highest in the hyper-tumescent PAL method (Table 3; $p = 0.013$). When comparing the average ratio of living cells between the three harvesting methods, we found a statistically significant difference in which dry SAL yielded ratios that were inferior to those of the two other methods (Table 4; $p = 0.011$). We did not find a statistically significant difference between the hyper-tumescent PAL technique and the body-jet WAL technique ($p = 0.49$). However, when omitting extremely high ratios (patient 6) and extremely low ratios (patient 1), the body-jet PAL technique had a statistically significant advantage (2.84 vs. 2.18; $p < 0.001$).

Discussion

The popularity of fat transfer has been rising in recent years. The techniques of harvesting and grafting vary widely, with equally varying results. However, standardization is lacking and an agreed upon set of guidelines is not available. This study aims to clarify whether or not the method of adipose tissue harvest plays a role in the final result.

Free fat graft viability evaluation presents a challenging target. Current methods include [22] glucose uptake, trypan blue staining, GPDH uptake assays, FDA-PI staining, and lately MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasodium bromide] colorimetric assay. Most of the above methods require in vivo isolation of the adipocyte from the surrounding stromal components. The use of FDA-PI [23] staining to determine cell viability is well described, commonly used, and reliable, and we found this method easy to implement.

In a recent study by Agostini et al. [24], the viability of fat cells was compared following the dry and wet techniques. In that study, no difference was found between the methods.

Our results, in contrast, show clear and significant differences between the methods. We found lower cell counts and viability ratios in the dry technique. The hyper-tumescent PAL technique yielded higher total cell counts, whereas the body-jet WAL technique yielded higher living cells ratios. Because adipocytes and pre-adipocytes are considered more vulnerable to mechanical trauma, as well as to changes in the chemical environment (i.e., the infiltration solution), the authors carefully raise the hypothesis that other, more resilient cells, namely mesenchymal stem and progenitor cells, may follow the same pattern [25, 26], thus rendering the harvest method critical to the final result of more complex procedures such as mesenchymal cell extraction and enrichment of fat grafts. However, this study

did not aim to examine this question, and the design of the study does not allow the authors to draw a conclusion on this subject. Further research is needed to validate this hypothesis.

Although different cannula diameters were used in each lipoaspirate method and may influence the adipocyte viability, the overall change in cannula diameter was not prominent enough to solely justify our findings.

Another notable finding was that the ratio of living cells was higher in patients with normal BMI and unremarkable medical history only in the body-jet WAL specimens compared to patients with elevated BMI and other illnesses, but not in other harvest methods. The finding of a higher ratio of living cells in the body-jet WAL specimens in patients with normal BMI was to be expected; however, this is the first study to prove it. More surprising is the lack of statistically significant difference (between normal and elevated BMI patients) in other methods. Whether this is a result of the small group or not is a matter for further research.

Implications and Future Research

As mentioned before, our results indicate that the harvesting methods influence the quantities of cells per extraction volume, and their viability ratios. Thus, the authors recommend using either the body-jet WAL or hyper-tumescent PAL techniques for fat harvesting procedures. The dry technique should be avoided in this setup.

When the body-jet technique WAL is used, normal BMI/medical history patients have a better prognosis for a successful procedure.

Compliance with Ethical Standards

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