Does Water-Jet Force Make a Difference in Fat Grafting? In Vitro and In Vivo Evidence of Improved Lipoaspirate Viability and Fat Graft Survival

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Background: Recent literature has revealed that water-jet–assisted liposuction offers a new method of conventional liposuction techniques by using the gentle spray of fluid. However, there has not yet been a systematic, randomized, controlled study to demonstrate its effect on the vitality and postoperative fat survival of fresh lipoaspirates. In this study, the authors compared liposuction with or without water-jet assistance in a blinded fashion.

Methods: Human lipoaspirates were obtained from healthy Chinese female volunteers for body shaping. Lipoaspirates were harvested by a single surgeon using the same material and machine; water-jet assistance was the only variance in this study. At the beginning of surgery, the authors randomly performed conventional manual liposuction without water-jet assistance for one side to obtain 50 ml of lipoaspirate (group B). At the corresponding area of the other side, the authors used water-jet–assisted liposuction to obtain another 50 ml of lipoaspirate (group A). All of the harvested lipoaspirates were used in the in vitro and in vivo experiments to evaluate the effect of water-jet force on the vitality and postoperative fat survival of fresh lipoaspirates.

Results: Fresh lipoaspirates from group A had greater viability and a higher percentage of $CD34^{+}/CD45^{-}$ cells than group B. Grafted lipoaspirates in group A had better weight retention, less apoptosis, and greater angiogenesis.

Conclusions: The fate of grafted lipoaspirates was affected by water-jet force. With the assistance of water-jet force during the harvesting procedure, the authors could obtain more viable lipoaspirates and achieve better fat survival. (*Plast. Reconstr. Surg.* 135: 127, 2015.)

Autologous fat grafts have been used to treat tissue deficiencies for over 100 years.[1] They have been considered the ideal soft-tissue filler by most investigators, because they are inexpensive, abundant, and easy to obtain.[2] However, the potential high absorption rate (25 to 90 percent) of grafted fat, which may be attributable to damage to the grafts caused by tissue harvesting and grafting procedures, often leads to unpredictable outcomes.[3–7] Investigators continue attempting to find the optimal harvesting method that will reduce damage to fat grafts. As in the Coleman technique, minimized suction pressure and gentle movements are recommended to protect fat grafts.[8–10]

Water-jet–assisted liposuction is a new method for fat harvesting that relies on a fan-shaped water jet to assist liposuction. In this method, the liposuction tube is connected to a negative-pressure pump and the water pump so that the fan-shaped water can jet at a specified frequency during liposuction (Fig. 1). With the assistance of water jet force, adipocytes can be gently detached from the tissue and the mechanical injury to lipoaspirates thereby reduced.[11,12] However, there has not yet been a systematic, randomized, controlled study of the water-jet–assisted liposuction procedure in terms of its

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Received for publication May 17, 2014; accepted June 12, 2014.
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DOI: 10.1097/PRS.0000000000000780

Disclosure: *The authors have no conflicts of interest to disclose.*

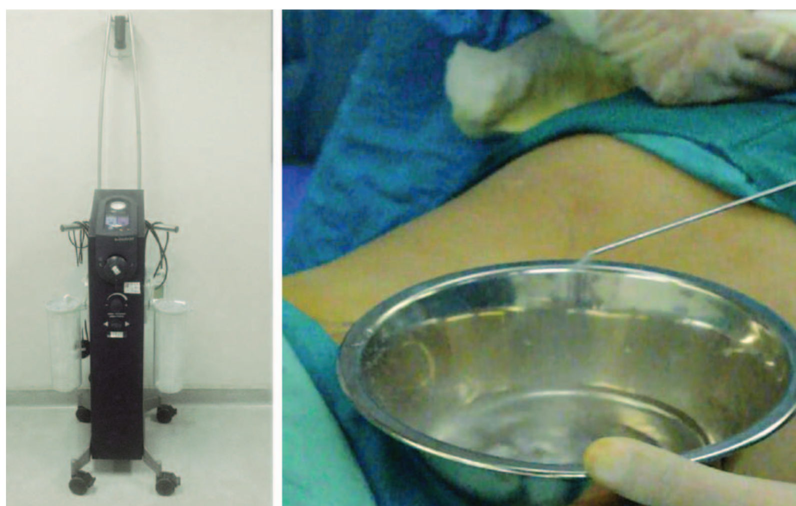

Fig. 1. (*Left*) Water-jet liposuction system, which has a water pump and a negative-pressure pump. (*Right*) Fan-shaped water spray jetting out through the cannula; this method can gently rush fat particles down during harvesting procedure.

effect on the viability and postoperative fat survival of fresh lipoaspirates. In this study, we compared the harvesting procedure with or without the assistance of water-jet force in a blinded fashion.

PATIENTS AND METHODS

Fat Source and Patient Information

Human lipoaspirates were obtained from 20 healthy Chinese female volunteers who underwent water-jet–assisted liposuction of the waist or thighs for body shaping. All volunteers signed informed consent. The study was approved by the Ethics Committee of Peking Union Medical College. Patient information is listed in Table 1.

Table 1. Patient Information*

Patient	Age (yr)	BMI	Donor Site
1	39	21.77	Waist
2	22	17.26	Thighs
3	25	22.99	Waist
4	34	28.10	Thighs
5	35	23.44	Thighs
6	26	23.92	Thighs
7	33	20.86	Thighs
8	25	21.88	Waist
9	38	21.63	Thighs
10	30	24.91	Waist
11	30	22.87	Waist
12	40	25.41	Waist
13	37	20.7	Waist
14	49	22.03	Thighs
15	35	28.28	Waist
16	40	22.21	Thighs
17	49	22.27	Waist
18	36	20.20	Waist
19	24	22.86	Waist
20	48	22.49	Thighs

BMI, body mass index.
*For the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium test, glucose transport test, flow cytometry analysis, cell culture, and fat grafting.

Lipoaspirates were harvested by a single surgeon using the same material and machine; water-jet assistance was the only variance in this study. First, we used a 2-mm cannula for tumescent infiltration (1000 ml of saline solution plus 1 ml of 1:200,000 adrenaline plus 600 mg of lidocaine) in the subcutaneous fat of each donor side at range 3 (range 1 is the lowest jet speed). Then, we used a 3.8-mm cannula with effective suction openings of 0.9 mm to aspirate fat particles under −0.5 bar (1 bar = 100 kPa) negative pressure. At the beginning of surgery, we randomly performed conventional manual liposuction without water-jet assistance for one side to obtain 50 ml of lipoaspirate. At the corresponding area of the other side, we used water-jet–assisted liposuction to obtain another 50 ml lipoaspirate for the experiment. Then, water-jet–assisted liposuction using the LipoCollector (human med AG, Schwerin, Germany) was used for the rest of the operative site (Fig. 2). After 20 minutes of gravity separation, the adipose tissue layers were collected. A blinded method was adopted in our experiment. Lipoaspirates harvested with water-jet assistance were marked as group A, and lipoaspirates harvested without water-jet assistance were marked as group B. The fat source for each mark was unknown by the experimenter.

Histologic Analysis

Fresh lipoaspirates (1 ml) and fat samples harvested at every time point were embedded in

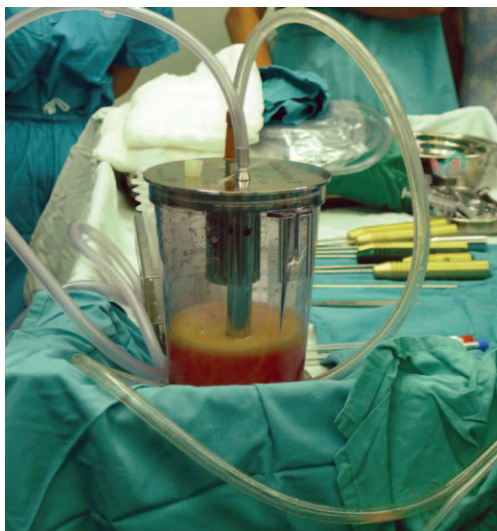

Fig. 2. LipoCollector water-jet system. The LipoCollector is connected to the liposuction cannula and negative-pressure pump during the harvesting procedure and has a prefilter to eliminate the fibrous materials. Lipoaspirates were separated from fluid in the LipoCollector during the liposuction period.

paraffin and sectioned at 5-μm thickness (three sections per sample), stained with hematoxylin and eosin, and observed under a light microscope by a pathologist in a blinded fashion (Carl Zeiss, Oberkochen, Germany).

Glucose Transport Test for Lipoaspirates

Fresh lipoaspirates were washed with Dulbecco's Phosphate-Buffered Saline (HyClone Laboratories, Inc., Logan, Utah) three times. Every 5-ml lipoaspirate was transferred into a 100-mm culture dish (three dishes for each sample) that contained 10 ml of low-glucose Dulbecco's Modified Eagle Medium (HyClone) and 1 unit of human insulin (Sigma Chemical Co., St. Louis, Mo.). A blank dish without lipoaspirates served as a negative control. Then, all dishes were incubated at 37°C with 5% carbon dioxide for 1 hour. After that, the glucose concentration in every dish was measured by a biochemical analyzer (AU640; Olympus Corp., Tokyo, Japan). The transported amount of glucose was calculated by subtraction: glucose concentration of the test dish minus glucose concentration of the blank dish.[13,14]

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide Assay for Lipoaspirates

Every 0.3-ml lipoaspirate in each group was injected into a 1.5-ml Eppendorf tube, mixed with 0.6 ml of 0.1% collagenase I (Sigma), and incubated at 37°C for 45 minutes. Forty microliters of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (Sigma) was added into each tube, and samples were then incubated at 37°C for 3 hours. After incubation, 100 μl of isopropanol was added and spun at 600 rpm for 30 seconds. One hundred microliters of sunflower seed oil was then added to adjust the absorbent range of the upper purple layer to a microplate reader (EnSpire 2300; PerkinElmer, Inc., Waltham, Mass.). Purple solution was transferred into a 96-well plate (100 μl per well). Triple measurements were obtained at 492 nm, and the mean absorbance was calculated.[13]

Human Stromal Vascular Fraction Cell Isolation, Culture, and Surface Antigen Analysis

Lipoaspirates were first digested with 0.075% collagenase I (Sigma). After centrifugation (1000 rpm for 5 minutes), cell pellets at the bottom (stromal vascular fractions) were resuspended, and cultured in low-glucose Dulbecco's Modified Eagle Medium (HyClone) containing 10% fetal bovine serum (Gibco, Carlsbad, Calif.).

For surface antigen analysis, uncultured stromal vascular fraction cells were washed with Dulbecco's Phosphate-Buffered Saline containing 0.1% bovine serum albumin (Sigma), incubated with anti-human CD34-APC (eBioscience, Inc., San Diego, Calif.) and anti-human CD45-FITC (eBioscience), and analyzed on a FACSCalibur flow cytometer (BD Biosciences, Franklin Lakes, N. J.).

Differentiation Test of Cultured Stromal Vascular Fraction Cells and Real-Time Polymerase Chain Reaction Assay for Related Gene Expression

Cells at passages 3 were then cultured in different differentiation medium (Table 2) at the endpoint, and stained with alizarin red solution, oil red-O solution, and human-anti–von Willebrand factor (Abcam, Cambridge, Mass.) to detect osteogenic differentiation, adipogenic differentiation, and endothelial differentiation, respectively. Cellular RNA from adipogenic and endothelial differentiated cells were isolated and reverse transcribed; then, the expression levels of mRNA for von Willebrand factor, CD31, peroxisome proliferator-activated receptor-γ, CCAAT-enhancer-binding protein α, and β-actin were tested by a real-time polymerase chain reaction system (LightCycler 480; Roche, Switzerland). All experiments were performed in triplicate. Primer sequences are listed in Table 3.

Table 2. Composition of Different Differentiation Media

Differentiation Direction	Medium*	Time (days)
Osteogenesis	Low DMEM, 10% fetal bovine serum, 10 M β-glycerophosphate, 1×10^{-8} M dexamethasone, and 50 mg/liter vitamin C	21
Adipogenesis	Low DMEM, 10% fetal bovine serum, 1×10^{-6} M dexamethasone, 0.5 M isobutyl methylxanthine, 10 μM insulin, and 200 μM indomethacin	14
Endothelial differentiation	Medium 199, 20 ng/ml VEGF, 10 ng/ml b-FGF, and 3% fetal bovine serum	14

DMEM, Dulbecco's Modified Eagle Medium; VEGF, vascular endothelial growth factor; b-FGF, basic fibroblast growth factor.
*Low DMEM (HyClone), fetal bovine serum (Gibco), VEGF (PeproTech, Rocky Hill, N.J.), b-FGF (PeproTech), other reagents (all from Sigma).

Table 3. Primer Sequences Used for Real-Time Polymerase Chain Reaction*

Gene and Primer	Sequence (5′–3′)
β-actin	
Sense	AATGTCACGCACGATTTCCC
Antisense	GAGACCTTCAACACCCCAGCC
CD31	
Sense	CCAAGCCCGAACTGGAATCT
Antisense	CACTGTCCGACTTTGAGGCT
vWF	
Sense	CCTTGACCTCGGACCCTTATG
Antisense	GATGCCCGTTCACACCACT
PPARγ	
Sense	TGGAATTAGATGACAGCGACTTGG
Antisense	CTGGAGCAGCTTGGCAAACA
C/EBPα	
Sense	TTCACATTGCACAAGGCACT
Antisense	ACGATCAGTCCATCCCAGAG
IL-6	
Sense	AGAGCTGTGCAGATGAGTAC
Antisense	CGCAGAATGAGATGAGTT
TNF-α	
Sense	ACCTCTCTCTAATCAGCCCTC
Antisense	GTTATCTCTCAGCTCCACGCCA

*Species was *Homo* for all primers.

Animal Model and Sample Collection

Forty-eight female BALB/c nude mice (CAnN. Cg-Foxn1nu/CrlVr) with an average age of 8 weeks were purchased from the Chinese Academy of Military Medical Sciences. Animal care and experimental procedures were performed under approval from the Animal Care and Ethics Committee of Peking Union Medical College. Mice were randomly divided into two groups ($n = 24$ in each group) and anesthetized with 1% pentobarbital sodium. The marked human lipoaspirates were assigned into each group respectively and injected subcutaneously using a 1-ml syringe with a 16-gauge needle at four paravertebral points (0.2 ml/point) of each mouse (Fig. 3). Mice were killed at the following time intervals: 1, 7, 14, 21, 28, and 56 days after grafting. Each sample was weighed and subjected to the assays described below.

Immunofluorescence and Immunohistochemical Staining for Grafted Adipose Tissue

Paraffin-embedded samples were sectioned at 3-μm thickness. For immunostaining, rabbit anti-human perilipin (Abcam) and Alexa Fluor 488–conjugated secondary antibody were used. For immunohistochemical staining, sections were incubated with rabbit anti-human CD31 (Abcam), followed by incubation with secondary antibody, horseradish peroxidase streptavidin, and diaminobenzidine, and counterstained with Mayer hematoxylin. Slices were observed under a fluorescence microscope equipped with a camera (Carl Zeiss).

Western Blot Analysis

Anti-human annexin V (Abcam) was used to detect apoptosis of fat samples, and every three samples of 1, 7, 28, and 56 days were lysed in radioimmunoprecipitation buffer (Sigma) and evaluated by Western blot technique. An antibody against β-actin (Santa Cruz Biotechnology, Inc., Dallas, Texas) was used to normalize protein loading. The resultant bands were quantified by densitometry.

Statistical Analysis

Data are expressed as mean ± SEM. Comparisons of the two groups were made using one-way analysis of variance with the t test. Statistical significance was defined by a value of $p < 0.05$. SAS Version 9.3 (SAS Institute, Inc., Cary, N.C.) was used for analysis.

RESULTS

Histologic Findings in Fresh Lipoaspirates

Hematoxylin and eosin staining was used to observe the morphologic difference of fresh lipoaspirates between the two groups. The number of intact adipocytes per optical field was calculated. There were more intact adipocytes in group A (water-jet–assisted liposuction) than in group B (conventional manual liposuction) ($p < 0.05$), and more irregularly shaped adipocytes were observed in group B (Fig. 4).

Viability of Adipocytes in Fresh Lipoaspirates

To maintain normal metabolism, viable adipocytes would transport glucose from the medium

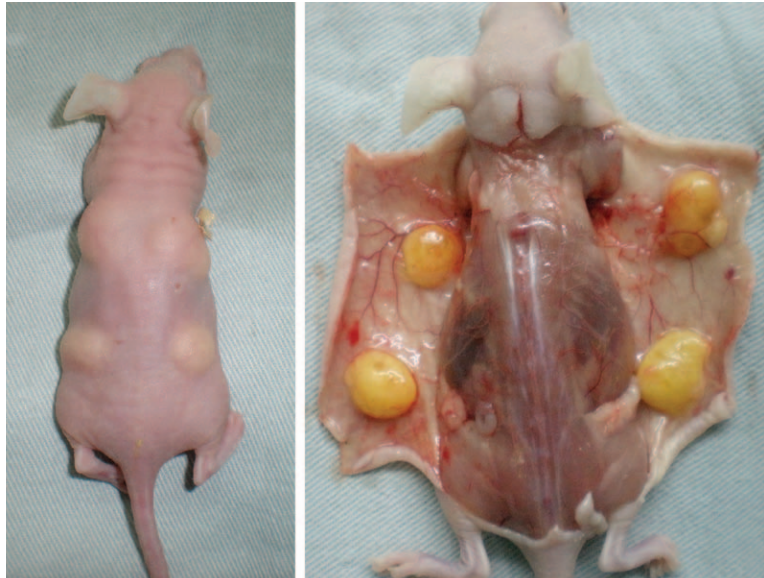

Fig. 3. Forty-eight BALB/C nude mice were divided randomly into two groups (*n* = 24 in each group). Mice in one group were injected with lipoaspirates from group A (water-jet–assisted liposuction), and mice in the other group were injected with lipoaspirates from group B (conventional manual liposuction). Lipoaspirates were injected subcutaneously at four paravertebral points (0.2 ml/point) of each BALB/C nude mouse. Mice were killed at 1, 7, 14, 21, 28, and 56 days after grafting.

into the cell itself. Thus, the transported amount of glucose could reflect the viability of lipoaspirates. Results from the two tests both showed a higher viability of lipoaspirates in group A (water-jet–assisted liposuction). The glucose-transported amount is 1.34 ± 0.05 M in group A and 1.12 ± 0.06 M in group B (conventional manual liposuction) ($p < 0.05$) (Fig. 3). The absorbance amount of MTT assay is 1.579 ± 0.04 in group A and 1.437 ± 0.05 in group B ($p < 0.05$) (Fig. 5).

Influence of Water-Jet Force on Stromal Vascular Fraction Characteristics

Positive results of alizarin red staining (osteogenic differentiation), oil red-O staining (adipogenic differentiation), and immunofluorescence staining for von Willebrand factor (endothelial differentiation) all demonstrated that the stromal vascular fractions we isolated from fresh lipoaspirates had a great potential ability for multidirectional differentiation (Fig. 6). Real-time quantitative polymerase chain reaction was used to compare the differentiation ability of cultured stromal vascular fraction cells in each group. In group A (water-jet–assisted liposuction), the expression levels of von Willebrand factor, CD31 (endothelial differentiation), peroxisome proliferator-activated receptor-γ, and CCAAT-enhancer-binding protein α (adipogenic differentiation) were much higher than the expression levels in group B (conventional manual liposuction) (Table 4). Cell subpopulations of stromal vascular fraction cells were analyzed by flow cytometry. Result exhibits a different ratio of $CD34^{+}CD45^{-}$ cells in stromal vascular fractions between these two groups: stromal vascular fraction cells contained 42.25 ± 6.51 percent $CD34^{+}CD45^{-}$ cells in group A and 28.18 ± 6.41 percent $CD34^{+}CD45^{-}$ cells in group B ($p < 0.05$) (Fig. 7 and Table 5).

Fat Survival Assessment in the Animal Study

Fat survival was evaluated by mass measurement at each time interval. The weight of the grafted fat tissue decreased over time (Table 6). At 0, 1, and 7 days, there was no statistical significance between the two groups ($p > 0.05$). At 14, 21, 28, and 56 days, the survival weights of group A (water-jet–assisted liposuction) were significantly greater than those of group B (conventional manual liposuction) ($p < 0.05$).

Analysis of Adipocyte Survival in Grafted Adipose Tissue

Histologic characteristics of fat grafts at each time interval were evaluated by an experienced pathologist in a double-blinded fashion. The

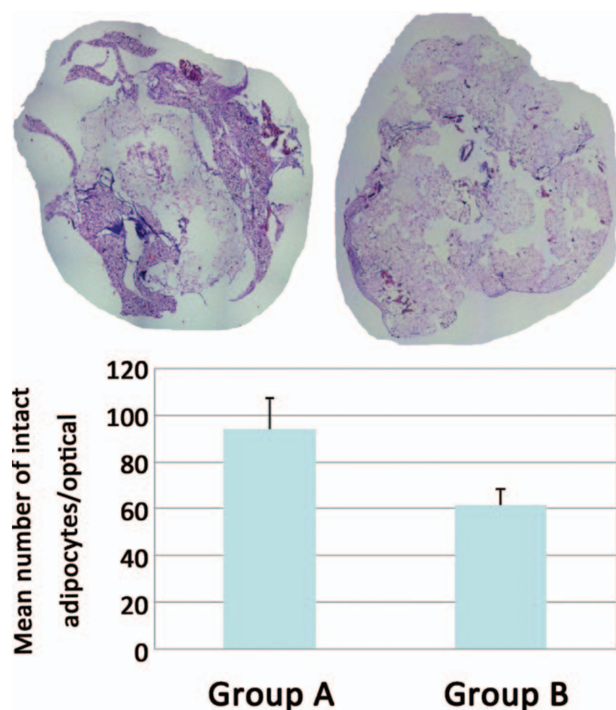

Fig. 4. (*Above*) Hematoxylin and eosin–stained images of freshly harvested lipoaspirates. (*Above*, *left*) Hematoxylin and eosin panoramic photograph of fresh lipoaspirates harvested with water-jet assistance (group A). (*Above*, *right*) Lipoaspirates harvested without water-jet assistance (group B). Groups of small and morphologically intact adipose cells were observed in the water-jet–assisted group, which were very few in group B. Clear membrane could be seen around the small adipocytes. (*Below*) The *bar chart* shows the number of intact adipocytes per optical field; there are 94.27 ± 13.17 intact adipocytes in group A and 61.60 ± 6.92 intact adipocytes in group B ($p < 0.05$).

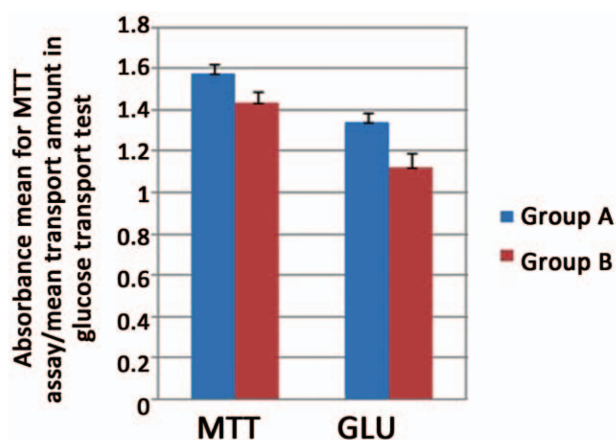

Fig. 5. Results of the MTT test and the glucose transport test (*GLU*). Comparison of the glucose-transported amount showed us a higher viability of lipoaspirates harvested with water-jet assistance (group A). Results from the MTT test showed the same tendency. The height of the *column* is the mean amount of absorbance/glucose transportation, and the *short line* is the standard error. *The star* indicates a significant difference ($p < 0.05$).

percentage area of intact adipocytes in every slide was calculated by means of Adobe Photoshop CS5 (Adobe Systems, Inc., San Jose, Calif.). Ten slides from each time point were observed. Samples from group A (water jet–assisted liposuction) maintained a higher percentage of intact adipocytes at 21, 28, and 56 days ($p < 0.05$) (Fig. 8). The number of perilipin-positive adipocytes per optical field (five fields for every section, four sections for each time point) was counted under a microscope with 100× magnification (Carl Zeiss). The results showed that samples from group A maintained more viable adipocytes than samples from group B ($p < 0.05$) (Fig. 9).

Analysis of Fat Graft Vascularization

In vivo angiogenesis of fat grafts was evaluated by immunohistochemical staining for CD31. The number of $CD31^+$ vessels per optical field (five fields for every section, four sections for each time point) was counted under a microscope with 100× magnification. At the early stages after fat grafting (from 1 to 14 days), samples from group B (conventional manual liposuction) exhibited more vessels than samples from group A (water-jet–assisted liposuction). Then, after 21 days, the number of vessels increased obviously in group A, and after 28 days, the number of vessels also increased in group B. Samples from group A exhibited more vessels than samples from group B from 21 days to 56 days ($p < 0.05$) (Figs. 10 and 11).

Detection of Apoptosis in Grafted Adipose Tissues

Annexin V expression was tested by Western blot analysis. Both groups appeared to have obvious apoptosis at day 1. At days 7, 28, and 56, samples from group A showed very low expression levels of annexin V, whereas a high level expression of annexin V was detected in samples from group B (Fig. 12).

DISCUSSION

In 1955, Peer first advanced the theory that the survival of grafted fat depends on the number of viable adipocytes grafted. This theory became one of the most popular theories and was then promoted by many other investigators.[15–18] To achieve better grafting results, many investigators attempted to improve the graft harvesting procedure to reduce damage to adipocytes.[7,19] Water-jet–assisted liposuction was considered a less harmful

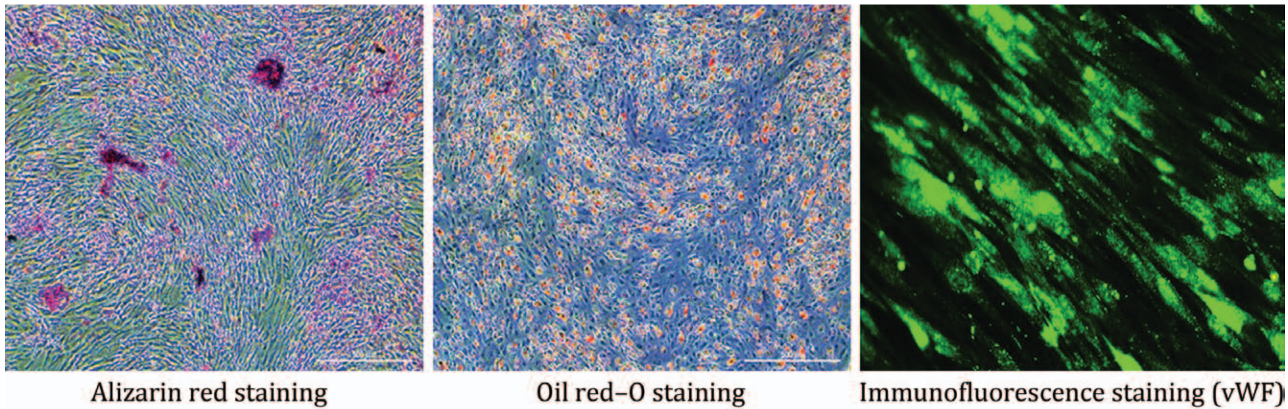

Fig. 6. Stromal vascular fractions were cultured and differentiated into three cell types. (*Left*) Positive results of alizarin red staining to demonstrate osteogenic differentiation capacity. (*Center*) Oil red-O staining to demonstrate adipogenic differentiation capacity. (*Right*) Immunofluorescence staining for von Willebrand factor, which demonstrates endothelial differentiation of stromal vascular fraction cells. All three positive results demonstrate that the stromal vascular fraction cells we isolated have multidifferentiation capacity.

Table 4. Gene Up-Regulation Level of Cultured Stromal Vascular Fraction Cells after Adipogenic and Endothelial Differentiation in Each Group*

	Genes							
	CD31		*vWF*		*PPARγ*		*CEBPα*	
Patient	Group A	Group B	Group A	Group B	Group A	Group B	Group A	Group B
1	3.58	2.93	2.52	2.10	6.06×10^3	4.03×10^3	1.68×10^2	1.07×10^2
2	2.29	1.75	6.32	1.24	7.08×10^3	5.71×10^3	5.51×10^2	2.09×10^2
3	5.05×10^1	9.59	1.31×10^3	9.09×10^1	2.69×10^4	1.48×10^3	9.52×10^2	4.03×10^3

*Group A represents stromal vascular fraction cells from lipoaspirate harvested with water-jet assistance; group B represents stromal vascular fraction cells from lipoaspirate harvested using conventional manual liposuction without water-jet assistance. Numbers represent gene up-regulation level (differentiation capacity), equal to the level of gene transcripts in differentiated stromal vascular fraction cells divided by the level of gene transcripts in the control group.

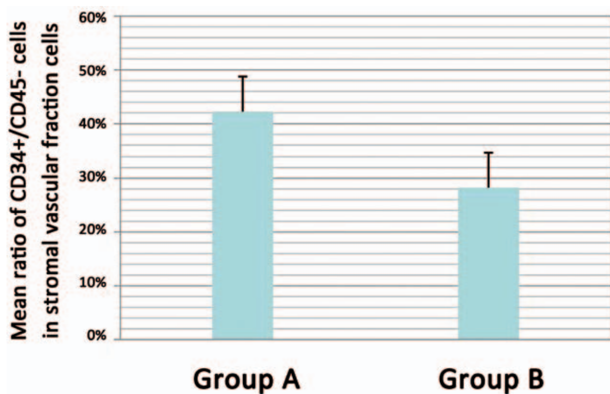

Fig. 7. The bar chart shows the ratio of $CD34^+/CD45^-$ cells of stromal vascular fraction analyzed by flow cytometry. Result exhibits a different ratio of $CD34^+/CD45^-$ cells in stromal vascular fractions between these two groups: stromal vascular fractions contained 42.25 ± 6.51 percent $CD34^+/CD45^-$ cells in group A (water-jet–assisted liposuction) and 28.18 ± 6.41 percent $CD34^+/CD45^-$ cells in group B (conventional manual liposuction) ($p < 0.05$).

method, which uses a gentle spray of fluid to rush fat particles down during the harvesting procedure. In the present study, we proved less damage

Table 5. Ratio of $CD34^+/CD45^-$ Cells in Freshly Isolated Stromal Vascular Fraction Cells from Six Patients*

	Ratio of $CD34^+/CD45^-$ Cells	
Patient	Group A (%)	Group B (%)
1	59.2	51
2	50.6	39.1
3	45.7	31
4	27	9.77
5	52.5	25.5
6	18.5	12.7
Mean	42.25	28.18
SEM	6.51	6.41

*$p = 0.006$ ($p < 0.05$ indicates statistical significance).

and better grafting results by using water-jet assistance (the only variance in our study) in the harvesting procedure. Results from the MTT assay and glucose transport test showed that lipoaspirates harvested with water-jet assistance maintain higher viability, suggesting that water-jet–assisted liposuction may be a more gentle harvesting method compared with the traditional manual liposuction method. The in vivo study also showed

Table 6. Graft Mass at Every Time Point in Each Group*

Time Point (day)	Group A (g)	Group B (g)	p†
0	0.1820 ± 0.0012	0.1790 ± 0.0012	0.6725
1	0.1398 ± 0.0016	0.1368 ± 0.0016	0.1976
7	0.1329 ± 0.0033	0.1229 ± 0.0039	0.1140
14	0.1191 ± 0.0023	0.1324 ± 0.0023	0.0004
21	0.1193 ± 0.0030	0.0928 ± 0.0058	0.0003
28	0.1188 ± 0.0030	0.0916 ± 0.0057	<0.0001
56	0.0911 ± 0.0067	0.0705 ± 0.0069	0.0378

*Group A, lipoaspirate harvested with water-jet assistance; group B, lipoaspirate harvested using conventional manual liposuction without water-jet assistance. Day 0 represents the mass of lipoaspirate before grafting. Data are expressed as the mean ± SEM ($n = 16$ in each group).
†A value of $p < 0.05$ is considered significant.

better weight retention of grafted lipoaspirates harvested with water-jet assistance.

Previous studies have demonstrated that apoptosis is related to long-term survival of grafted lipoaspirates. In the early stage after grafting, ischemia was the main reason for graft damage. It caused adipocyte death and apoptosis in the early stage, which would lead to graft absorption. In the late stage, when angiogenesis increased, apoptosis became the main factor to cause cell death and led to graft absorption.[20] Because adipocytes in the early apoptotic stage maintain membrane integrity and partial viability, it is hard for us to detect apoptotic conditions on histologic slides.[2,21,22] Annexin V is a sensitive detector for observing early apoptosis of adipocytes, which could help us to know more about the actual survival circumstances of grafted lipoaspirates.[23] In our study, both groups appeared to be in a state of obvious apoptosis at day 1, whereas only grafted lipoaspirates in group B (conventional manual liposuction) showed high-level expression of annexin V

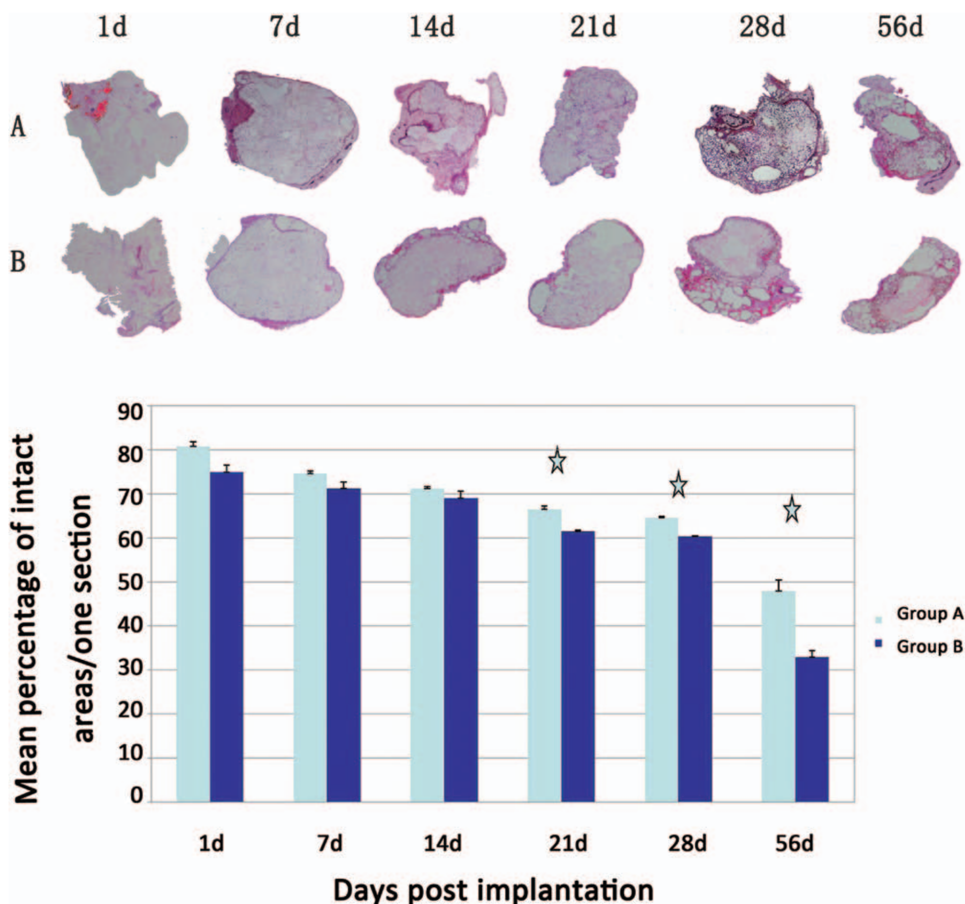

Fig. 8. (*Above*) Hematoxylin and eosin-stained panoramic photographs from group A (water-force–assisted liposuction) and group B (conventional manual liposuction) at every time point. (*Below*) Bar chart showing the percentage of intact fat area; hematoxylin and eosin–stained photographs from one section were first assembled into a panoramic photograph and then intact fat areas of the sample were calculated using Photoshop software. The height of the *column* is the mean percentage of intact area per section, and the *short line* is the standard error. The *star* indicates a significant difference ($p < 0.05$).

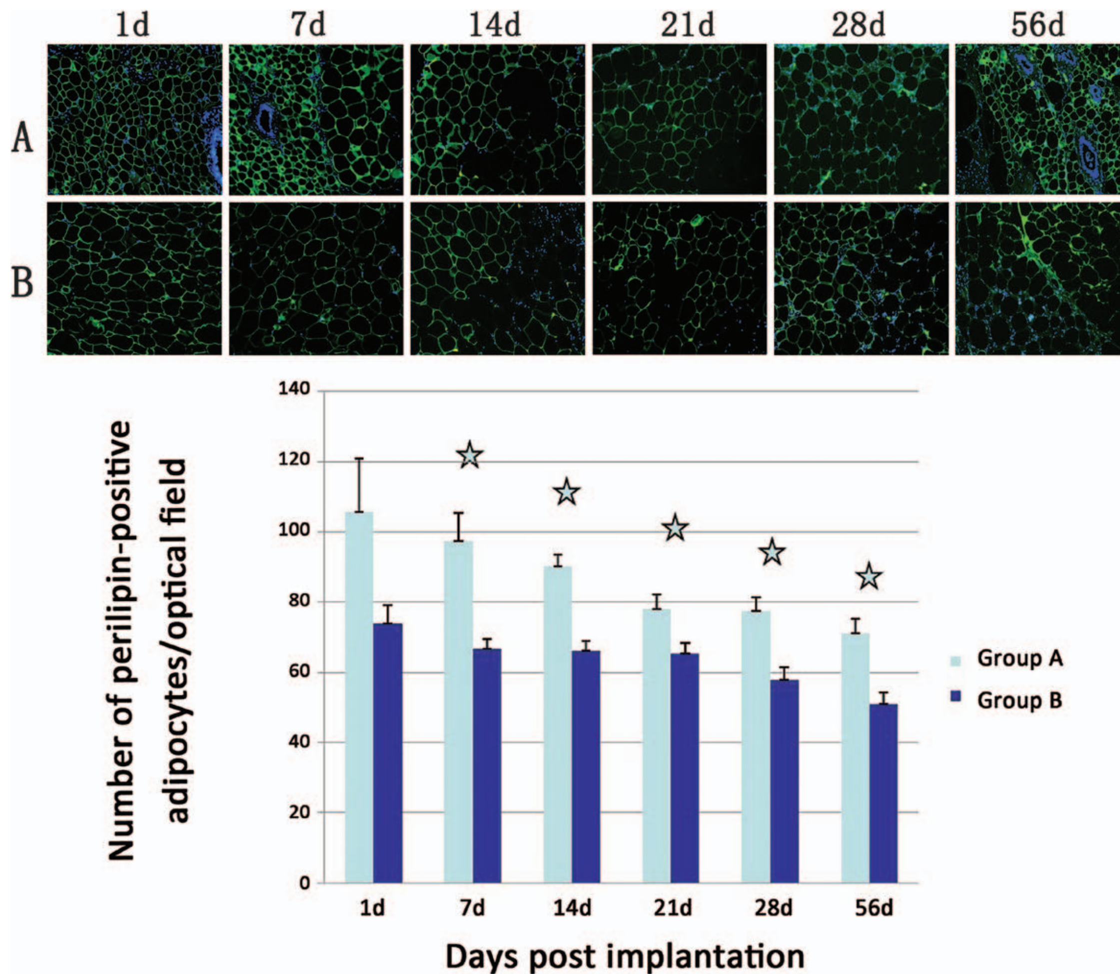

Fig. 9. (*Above*) Samples from group A (water-force–assisted liposuction) and group B (conventional manual liposuction) stained for perilipin at every time point. (*Below*) Bar chart showing the counting results for perilipin-positive adipocytes; five fields (original magnification, × 100) for every section, four sections for each time points were counted. The height of the *column* is the mean number of perilipin-positive adipocytes, and the *short line* is the standard error. The *star* indicates a significant difference ($p < 0.05$).

from 7 days to 56 days. These results indicated better long-term survival of grafted lipoaspirates harvested with water-jet assistance.

Because water-jet assistance was proved to improve the survival of grafted lipoaspirates, its mechanisms are quite important for investigators. Adipocytes and stromal vascular fraction cells, which have effects on volume preservation, were the main components of the fat grafts. In our study, we found that the composition of adipocytes and stromal vascular fraction cells in lipoaspirates was changed just by adding the influence of water-jet force to the harvesting procedure. In samples of fresh lipoaspirates, there are more intact round adipocytes in group A (water-jet–assisted liposuction). A large number of small intact adipocytes (diameter, 20 to 40 μm) were clearly observed. They have a clear membrane and stain strongly for perilipin, which suggests that they are viable adipocytes. These small adipocytes were hardly found in lipoaspirates harvested without water-jet assistance. Because these small adipocytes increased the total number of viable adipocytes in lipoaspirates under the same volume, it is possible to explain why lipoaspirates harvested with water-jet assistance have greater viability and better survival after grafting. These small adipocytes have also been observed in graft samples at each time point in group A. They were located at the peripheral region of the graft sample and stained strongly for perilipin. However, the real characteristics of those small adipocytes were not clear; they may have been either the surviving adipocytes or newborn adipocytes.

Besides Peer's theory, many investigators have proved that multidifferentiated cells in

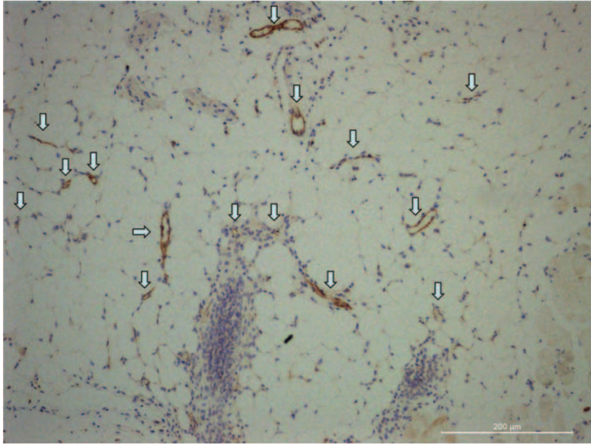

Fig. 10. Angiogenesis was evaluated by CD31 staining; *arrows* indicate vessels in graft samples.

lipoaspirates have a positive effect on fat grafts; they can differentiate into adipocytes, promote angiogenesis, and play an important role in volume preservation.[20,24–27] Stromal vascular fraction cells were a group of heterogeneous cells isolated from lipoaspirates that contained multidifferentiated cells. Lipoaspirates enriched with stromal vascular fraction cells would achieve a better grafting result.[15] Previous studies considered at least four subgroups in stromal vascular fraction cells: blood-derived cells ($CD45^{+}$), adipose-derived stem cells ($CD31^{-}CD34^{+}CD45^{-}$), endothelial (progenitor) cells ($CD31^{+}CD34^{+}CD45^{-}$), and pericytes ($CD31^{-}CD34^{-}CD45^{-}$).[16] Functions of each cell subgroup are still poorly understand. In our study, the $CD34^{+}/CD45^{-}$ ratio in the isolated stromal vascular fraction cells was increased when using water-jet force during the harvesting procedure. This cell group was reported to promote angiogenesis by differentiation into endotheliocytes and play a very important role in connecting grafted adipocytes to their supply blood vessels.[16,28] In vitro study revealed that cultured stromal vascular fraction cells in the group with water-jet–assisted liposuction have a greater capacity for adipogenic differentiation and endothelial differentiation. Although some researchers suggested that a higher ratio of $CD34^{+}$ to $CD45^{-}$ cells in fat grafts could bring better volume preservation,[29] there is no systematic evidence to support the positive relationship between fat grafts survival and the quantity of $CD34^{+}/CD45^{-}$ cells. In addition, the mechanisms by which $CD34^{+}/CD45^{-}$ cells work are still not very clear to us.

CONCLUSIONS

We show convincing evidence that the fate of grafted fat was affected by water-jet force. With the assistance of water-jet force during the harvesting procedure, we could obtain lipoaspirate that was more viable and achieve a better survival result.

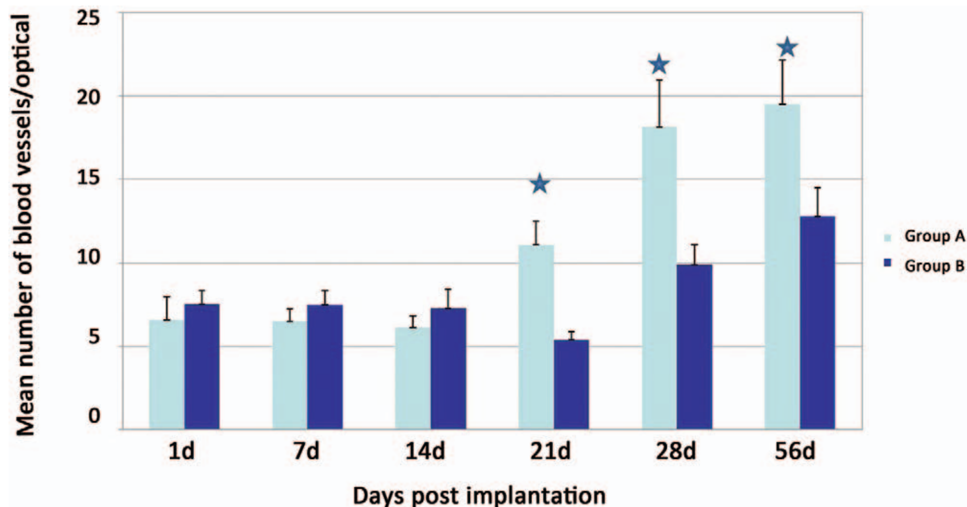

Fig. 11. Bar chart showing the result of vessel counting; vessel numbers did not change much from 1 day to 14 days in either group. Then, at day 21, the number of vessels in group A increased obviously, and kept rising until the endpoint. The number of vessels increased from 28 days to 56 days for both groups, but it is much lower in the group without water-jet assistance (group B) than that in the group with water-jet assistance. The height of the column is the mean number of blood vessels per optical field and the short line is the standard error. The *star* indicates a significant difference ($p < 0.05$).

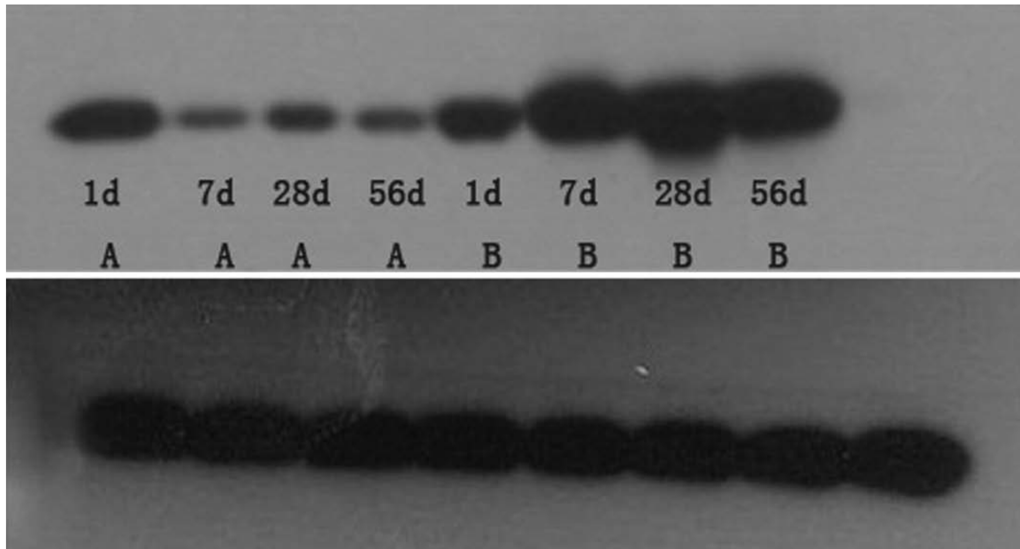

Fig. 12. Apoptosis was tested by Western blot analysis at 1, 7, 28, and 56 days. From the resultant band, we can see that apoptosis was evident for all four time points in samples harvested without water-jet force (group B), whereas apoptosis only obviously showed at 1 day in samples harvested with water-jet force (group A). (*Below*) The band of β-actin that was used to normalize protein loading. The resultant bands were quantified by densitometry.

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