Characterization of Structure and Cellular Components of Aspirated and Excised Adipose Tissue

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Background: Adipose tissue is an easily accessible tissue for use as a soft-tissue filler and source of adult multipotent cells, called adipose-derived stem/stromal/progenitor cells. However, many aspects of its anatomy remain unclear because of the fragile structure of adipocytes and adipose tissue.
Methods: Aspirated ($n = 15$) or intact ($n = 9$) subcutaneous adipose tissue samples were evaluated by (1) whole-mount histology with triple-fluorescence staining for three-dimensional visualization of living adipose tissue, (2) glycerol-3-phosphate dehydrogenase assay, (3) multicolor flow cytometry (CD34, CD31, and CD45), and (4) adherent cell culture of stromal vascular fraction cells for viable adipose-derived stromal cell yield.
Results: Whole-mount histology revealed the presence of a capillary network running alongside adipocytes, which was partly disrupted in aspirated adipose tissues. Aspirated adipose tissue also lacked large vasculature structures and showed significantly larger numbers of small lipid droplets (ruptured adipocytes) ($p = 0.00016$) and dead cells ($p = 0.0038$) compared with excised adipose tissue. Adipocyte number was less than 20 percent of total cellularity, and vasculature-associated cells, including endothelial cells and adipose-derived stromal cells, constituted over one-half of total cells in both intact and aspirated adipose tissue. Glycerol-3-phosphate dehydrogenase assay suggested that greater than 30 percent and 5 percent of adipocytes were ruptured in aspirated and excised adipose tissue, respectively ($p = 0.032$). Multicolor flow cytometric analysis indicated a much higher percentage of blood-derived cells ($CD45^+$) contaminated in aspirated adipose tissue ($p = 0.0038$), and adipose-derived stromal cell yield in aspirated adipose tissue was approximately one-half that in excised tissue ($p = 0.011$).
Conclusion: The authors' results indicate the differential structure and cellular composition of the two tissues, and significant tissue damage and progenitor yield deficiency in aspirated adipose tissue. (*Plast. Reconstr. Surg.* 124: 1087, 2009.)

Adipose tissue is not a simple organ specific for energy storage but also functions as the largest endocrine organ of the body, releasing various adipokines such as leptin and adiponectin.[1] It can also be used as a valuable soft-tissue filler and is a promising source of adult stem cells, called adipose-derived stem/stromal/progenitor cells. Adipose tissue has an abundance of progenitor cells,[2,3] some of which can differentiate into diverse lineages,[4] have potential similar to that of bone marrow–derived mesenchymal stem cells, are expected to become a valuable tool for a wide range of cell-based therapies,[5,6] and are already used in some clinical trials.[7–11]

Although adipose tissue has become a target of a variety of research and therapeutic applications, its anatomy is not well established because of difficulties in assessment, attributable in part to structural fragility of the tissue. It is thought that adipose tissue contains numerous cell types, including adipocytes, adipose-derived stromal cells, endothelial cells, mural cells, fibroblasts, and blood cells. Although dissociation with enzymes

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has been performed to isolate cellular components, many adipocytes are disrupted, and not all of the other cell components are isolated, with a substantial number of cells remaining in the undissociated tissues.[12,13] Conventional histologic methods require tissue fixation to avoid deterioration of the fragile tissue, and sectioning of the tissue leads to leakage of lipid contained in adipocytes, both of which deform the original structure of adipose tissue. Adipocytes, which constitute more than 90 percent of tissue volume, are extremely larger than other cells in adipose tissue, which may overlap on multiple sections and result in inaccurate counting on histologic assessment; the number and percentage of adipocytes are still controversial.[1,13–18]

Aspirated adipose tissue has become widely used as a valuable soft-tissue filler, but clinical results have been variable and unsolved issues remain, mainly related to partial necrosis of grafted tissue. Aspirated adipose tissue has been suggested to be significantly distinct in structure from intact adipose tissue, probably through processes such as infiltration, aspiration, accompanying bleeding, and storage, which may affect engraftment of aspirated adipose tissue.[19–25] Although we need further improvements in efficiency and reliability of lipografting, we lack knowledge of the detailed morphology of aspirated and intact adipose tissue.

In this study, we used a novel technique to investigate nonfixed adipose tissue without dissociating and sectioning[26,27]; the method enables three-dimensional visualization of living adipose tissue stained with triple-fluorescence. We characterized the structure and cellular components of both aspirated and excised adipose tissues using whole-mount staining, glycerol-3-phosphate dehydrogenase assay,[13,22,24] multicolor flow cytometry,[12,13] and adherent culture for adipose-derived stromal cell yield.[12,19]

PATIENTS AND METHODS

Human Tissue Sampling

We obtained liposuction aspirates alone (n = 11), excised fat tissue alone (n = 5), or both (n = 4) from 20 healthy female donors who underwent plastic surgery; liposuction aspirate alone was harvested from the abdomen and/or thighs, excised tissue alone was harvested from the back or abdomen, and both aspirated and excised fat tissues were harvested from the abdomen of four patients who underwent abdominoplasty. For liposuction, the fatty layer was first infiltrated with a saline solution containing diluted epinephrine (0.001%) under general anesthesia, and adipose tissue was suctioned using a 2.5-mm (inner diameter) cannula at –500 to –700 mmHg with a conventional liposuction machine (Medikan Corp., Seoul, Korea). Participants provided informed consent using a protocol approved by the appropriate institutional review boards.

Cell Isolation and Culture

Stromal vascular fraction was isolated from the fatty portion of liposuction aspirates as described previously.[12] Briefly, the aspirated fat tissue was washed with phosphate-buffered saline and digested on a shaker at 37°C in phosphate-buffered saline containing 0.075% collagenase (Wako Pure Chemical Industries, Osaka, Japan) for 30 minutes. Mature adipocytes and connective tissue were separated from pellets by centrifugation at 800 *g* for 10 minutes. Stromal vascular fraction was obtained through resuspending the pellets and filtering the resuspension with 100-μm mesh (Millipore, Billerica, Mass.). The stromal vascular fraction cells were plated (30,000 cells/cm^2) on gelatin-coated dishes and cultured at 37°C. The culture medium was M-199 medium containing 10% fetal bovine serum, 100 IU penicillin, 100 mg/ml streptomycin, 5 μg/ml heparin, and 2 ng/ml acidic fibroblast growth factor. After 7 days, attached cells were counted, passaged by trypsinization, and subcultured in the same medium. Medium was replaced every third day. The excised fat tissue was first minced with scissors into 2-mm pieces and then processed in the same manner as aspirated fat tissue.

Whole-Mount Staining of Living Adipose Tissue

Visualization of nonsectioned living adipose tissue was performed using the procedure of Nishimura et al.[26] Briefly, the adipose tissue was cut into 3-mm pieces within 2 hours after sampling and incubated with the following reagents for 30 minutes: BODIPY 558/568 or BODIPY-FL (both from Molecular Probes, Eugene, Ore.) to stain adipocytes, Alexa Fluor 488-conjugated isolectin GS-IB$_4$ (Molecular Probes) to stain endothelial cells, Hoechst 33342 (Dojindo, Kumamoto, Japan) to stain all nuclei, or propidium iodide (Sigma-Aldrich, St. Louis, Mo.) to stain nuclei of necrotic cells. The sample was then washed and observed directly with a confocal microscope system (Leica TCS SP2; Leica Microsystems GmbH, Wetzlar, Germany). Ten images acquired with every 3-μm interval were used for reconstructing 30-μm-thick three-dimensional images. Nuclei of adipocytes,

vasculature-associated cells (e.g., endothelial cells, adipose-derived stromal cells, and mural cells), and other components (e.g., blood-derived cells and fibroblasts) were counted, and the number of each cell population per unit volume was calculated. Next, the number of small lipid droplets presumably resulting from mechanical damage of adipocytes was counted. Furthermore, number of dead cells (propidium iodide–positive nuclei) and viable cells (propidium iodide–negative/Hoechst-positive nuclei) were counted. We analyzed four field images of each sample.

Glycerol-3-Phosphate Dehydrogenase Assay

For quantitative measurement of glycerol-3-phosphate dehydrogenase, a glycerol-3-phosphate dehydrogenase Assay Kit (Cell Garage, Tokyo, Japan) was used according to the manufacturer's instructions. Aspirated and excised fat samples (1 g of each) were washed with phosphate-buffered saline and centrifuged to obtain an extracellular solution. The remaining adipose tissue sample was mixed with 1 ml of 0.25 M sucrose solution, homogenized, and centrifuged to obtain an intracellular solution. Both solutions were diluted 10 times with an enzyme-extracting reagent, and the optical absorption was measured at 340 nm for 10 minutes after addition of twice the volume of a substrate reagent. Glycerol-3-phosphate dehydrogenase activity was calculated based on the following formula: glycerol-3-phosphate dehydrogenase activity (U/ml) = $\Delta OD \times 0.482 \times 10$, where ΔOD is the change in optical density per minute.

Flow Cytometry

The following monoclonal antibodies conjugated to fluorochromes were used: anti–CD31-phycoerythrin, anti–CD34-phycoerythrin-Cy7, and anti–CD45–fluorescein isothiocyanate (BD Biosciences, San Jose, Calif.). Multicolor flow cytometry was performed with an LSR II (BD Biosciences), and cell composition percentages were calculated according to data of surface marker expression profiles.

Statistical Analysis

Results are expressed as mean ± SEM. To evaluate the variability of the data, the SD can be obtained by multiplying the SEM by the square root of the sample size. The statistical significance was determined using a *t* test for all variables other than comparison of adipose-derived stromal cell yield from the same individuals, for which the paired *t* test was used. As for the count data (lipid droplets), the square root of the data instead of the original raw data was used for analyzing the statistical significance. Values of $p < 0.05$ were considered significant.

RESULTS

Whole-Mount Staining of Living Tissues

This method analyzed all cells localized in the adipose tissue with a number of advantages compared with conventional histology; the limitation of this method is that it cannot measure cells outside of tissue, such as ruptured adipocytes and blood-derived cells released from tissue as hemorrhage.

Whole-mount staining images revealed the presence of capillaries running alongside adipocytes forming a well-organized network in excised adipose tissues, whereas in aspirated adipose tissue the capillary network was disrupted. There were large vessels with a diameter of over 50 μm in excised adipose tissue, whereas aspirated adipose tissue showed only scarce large-diameter blood vessels (Fig. 1).

The number of nuclei was counted and classified into three cell populations according to localization: a cell was regarded as an adipocyte when the nucleus was localized within the BODIPY-positive lipid area, as a vasculature-associated cell (e.g., adipose-derived stromal cells, endothelial cells, pericytes) when the nucleus was in or attached to capillaries, and as other cell type (e.g., blood cells, fibroblasts) when localization of the nucleus did not meet either of these two conditions (Fig. 2). Aspirated adipose tissue ($n = 9$) contained 706 ± 114 adipocytes (11.8 percent), 3635 ± 408 vasculature-associated cells (60.7 percent), and 1649 ± 178 other cells (27.5 percent) per cubic millimeter, whereas excised adipose tissue ($n = 7$) contained 860 ± 128 adipocytes (14.6 percent), 4273 ± 721 vasculature-associated cells (72.5 percent), and 758 ± 122 other cells (12.9 percent) (Fig. 3).

As an evaluation for tissue damage, the numbers of small lipid droplets and ratio of dead cells were measured. The number of small lipid droplets per optical field was 20.6 ± 2.39 and 6.17 ± 1.53 in aspirated and excised adipose tissues, respectively (Fig. 4, *left*). Propidium iodide–positive dead cells were 54.1 ± 2.86 percent of total cells in aspirated adipose tissue and 33.5 ± 6.16 percent in excised adipose tissue (Fig. 4, *right* and Fig. 5). Both small lipid droplets ($p = 0.00016$) and dead cells ($p = 0.0038$) were present in significantly larger numbers in aspirated adipose tissue compared with excised tissue.

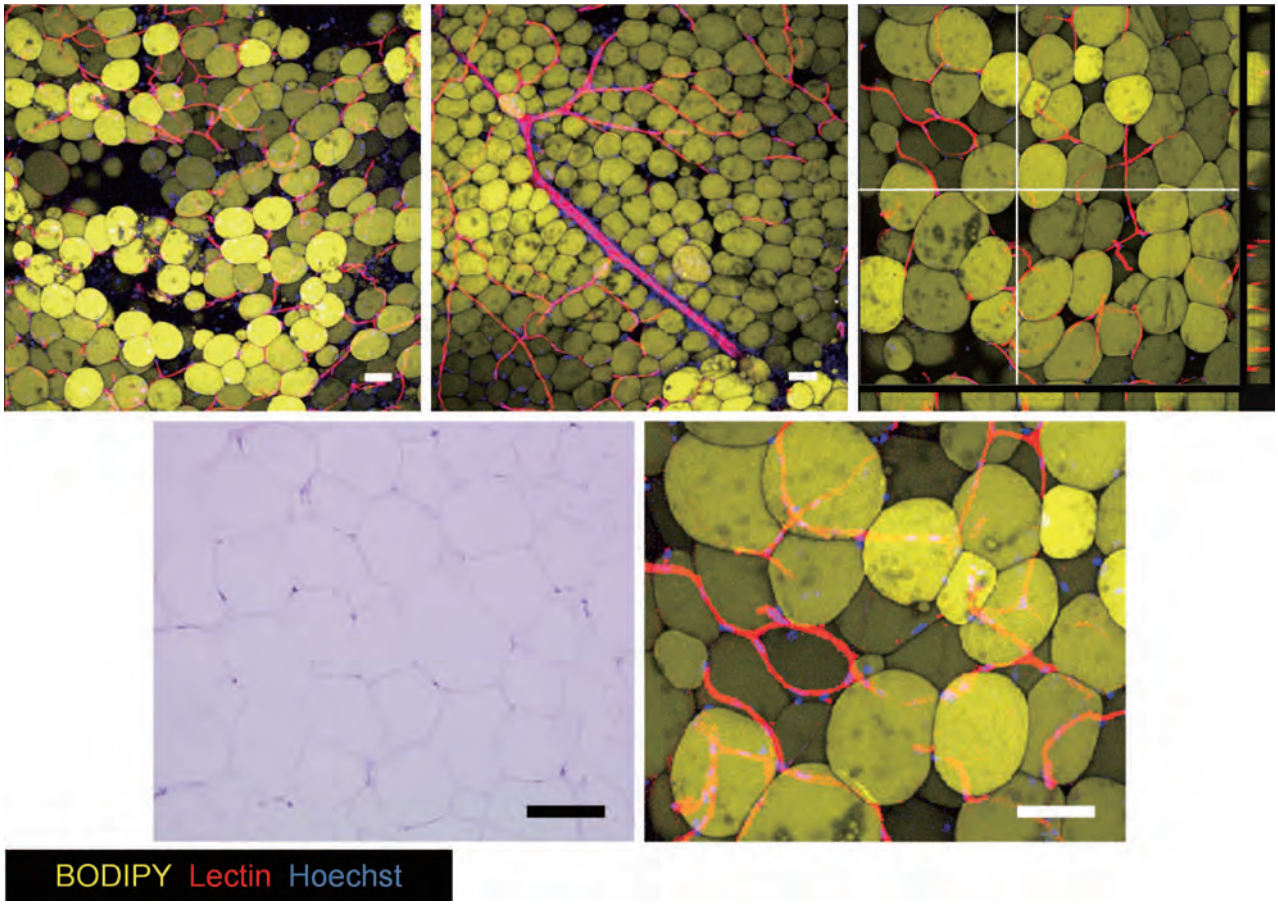

Fig. 1. Structure of living adipose tissue in whole-mount triple-stained images. Nonfixed aspirated and excised adipose tissues were stained with BODIPY (adipocytes; *yellow*), lectin (endothelial cells; *red*), and Hoechst 33342 (nuclei; *blue*) and investigated using a confocal microscope. There were many capillaries running alongside adipocytes in both aspirated and excised adipose tissue. Adipocytes, capillaries, and vessels were arranged in an organized manner in excised adipose tissue, whereas the disrupted tissue contained dead spaces, some adipocytes, and disrupted capillaries, with most of the large vessels lost in aspirated adipose tissue. (*Above*) Merged images of aspirated (*above, left*) and excised (*above, center*) adipose tissue, respectively. Scale bars = 100 μm. (*Above, right*) Dissected image example (excised adipose tissue). Images dissected horizontally and longitudinally at levels of *white lines* are shown on the sides. (*Below*) Conventional histologic image (*below*, *left*) and whole-mount triple-stained confocal image (*below*, *right*). Intact adipose tissue was processed with fixation, paraffin embedding, and sectioning (*below*, *left*). In the conventional histologic examination, adipocytes appear hexagonal and there is little interstitial space between them. On the contrary, in whole-mount triple-stained confocal micrographs of nonfixed nonsectioned adipose tissue, adipocytes are spherical, with a substantial amount of interstitial space. Scale bars = 100 μm.

Glycerol-3-Phosphate Dehydrogenase Assay

Glycerol-3-phosphate dehydrogenase is a cytosolic enzyme found in adipocytes, and extracellular glycerol-3-phosphate dehydrogenase is considered proportional to the amount of adipocyte destruction.[13,22,24] This method analyzes only adipocytes; the method cannot measure an absolute number of adipocytes but rather a relative amount of adipocytes. It can separately measure the amount of nonruptured adipocytes (intracellular glycerol-3-phosphate dehydrogenase activity) and ruptured adipocytes (extracellular glycerol-3-phosphate dehydrogenase activity).

The results demonstrated that excised adipose tissue contained an approximately threefold larger amount of nonruptured adipocytes per gram than aspirated adipose tissue (Fig. 6, *left*). Total (extracellular and intracellular) glycerol-3-phosphate dehydrogenase activity was significantly lower (0.069 ± 0.0074 U/g, $n = 10$) in aspirated adipose tissue than in excised adipose tissue (0.153 ± 0.060 U/g, $n = 4$) ($p = 0.042$). As for adipocyte damage, extracellular glycerol-3-phosphate dehydrogenase activity constituted a significantly higher percentage in aspirated adipose tissue (31.1 ± 6.39 percent) than in excised

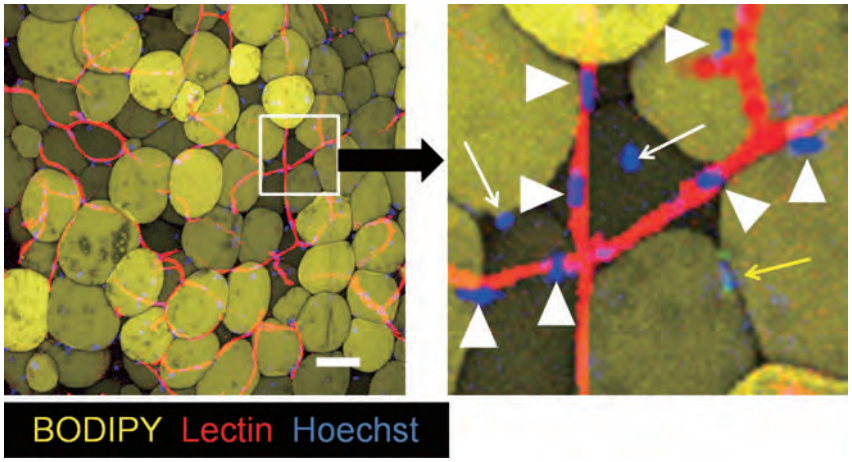

Fig. 2. Numerical measurement of cellular components in whole-mount stained images of adipose tissue. Representative whole-mount images used for counting each cellular component of adipose tissue are shown. Nuclei were classified into three cell components by their localization. A *yellow arrow* indicates the nucleus of an adipocyte. *White arrowheads* indicate nuclei of vasculature-associated cells (e.g., endothelial cells, adipose-derived stromal cells, and mural cells). *White arrows* indicate nuclei of other cells (such as fibroblasts and leukocytes). Scale bar = 100 μm.

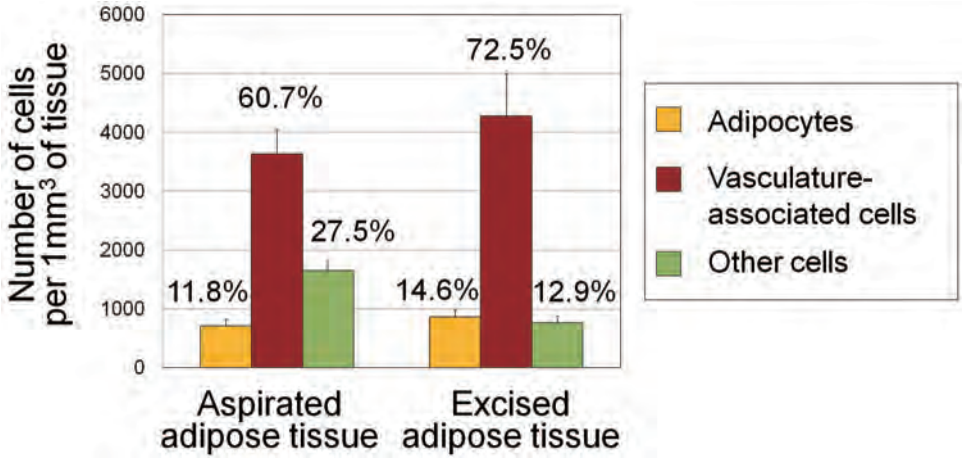

Fig. 3. Numbers of cellular components per cubic millimeter of adipose tissue are shown. Adipocytes, vasculature-associated cells, and other components observed in 1 mm^3 of adipose tissue ($n = 7$ in aspirated adipose tissue, $n = 5$ in excised adipose tissue). Values are mean ± SEM.

adipose tissue (4.98 ± 1.59 percent) ($p = 0.032$) (Fig. 6, *right*).

Flow Cytometry

This method analyzed only nonadipocyte cells (stromal vascular fraction cells) isolated through collagenase digestion; the possible limitation of this method is that not all nonadipocyte cells are completely isolated from the tissue (some cells are thought to remain in the tissue or die in the dissociation process). Characterization and classification of cells can be performed easily by analyzing multiple cell surface antigens (Fig. 7).

Flow cytometric analysis revealed that the stromal vascular fraction from aspirated adipose tissue contained $CD45^+$ cells (blood-derived cells) at 48.8 ± 5.5 percent, adipose-derived stromal cells ($CD45^-CD31^-CD34^+$) at 23.2 ± 3.6 percent, and endothelial cells ($CD45^-CD31^+CD34^+$) at 13.3 ± 2.2 percent ($n = 9$). In contrast, there were blood-derived cells at 11.0 ± 4.2 percent ($p = 0.0038$), adipose-derived stromal cells at 28.5 ± 14.0 percent ($p = 0.59$), and endothelial cells at 5.5 ± 1.3 percent ($p = 0.076$) in the stromal vascular frac-

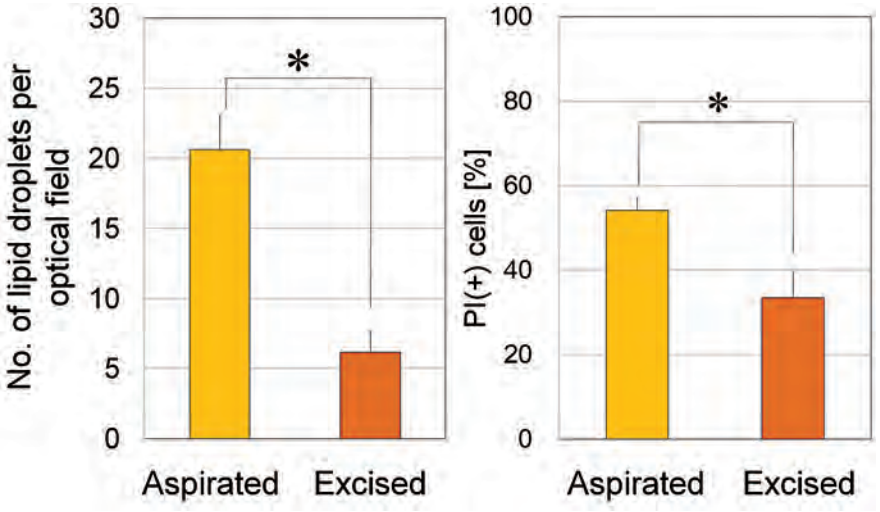

Fig. 4. Evaluation of tissue damage in aspirated ($n = 7$) and excised ($n = 5$) adipose tissue. (*Left*) Number of small lipid droplets. Small lipid droplets, presumably resulting from adipocytes rupture, were seen significantly more frequently in aspirated than in excised adipose tissue. Values are mean ± SEM (*$p < 0.05$). (*Right*) Ratio of propidium iodide–positive cells to all cells in each adipose tissue. Propidium iodide–positive nuclei in cyan (merger of red and blue) and propidium iodide–negative nuclei in blue were counted in whole-mount images. Dead cells with cyan nuclei, which were distinguished from viable cells with blue nuclei, were 54.1 ± 2.9 percent of total cells in aspirated adipose tissue and 33.5 ± 6.2 percent in excised adipose tissue. Values are mean ± SEM (*$p < 0.05$).

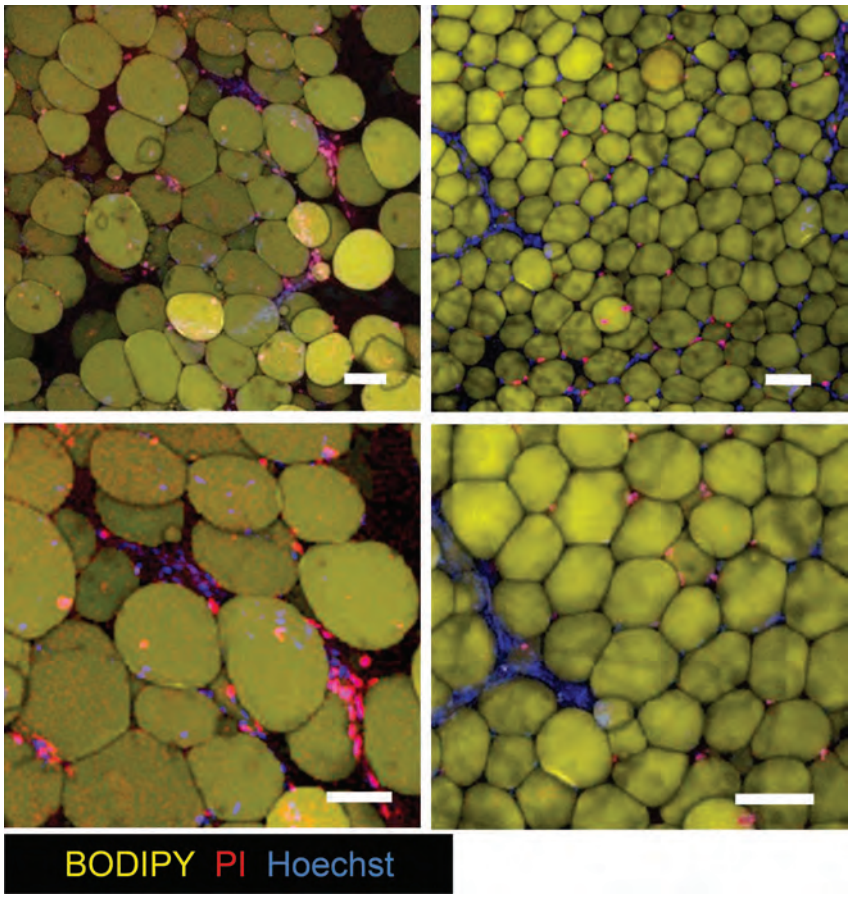

Fig. 5. Whole-mount tissue staining with propidium iodide for detecting dead cells is shown (aspirated tissue in the panels on the *left*, excised tissue in the panels on the *right*). Nonfixed adipose tissue was stained with BODIPY (adipocytes; *yellow*), propidium iodide (nuclei of dead cells; *red*), and Hoechst 33342 (all nuclei; *blue*). Scale bars = 100 μm.

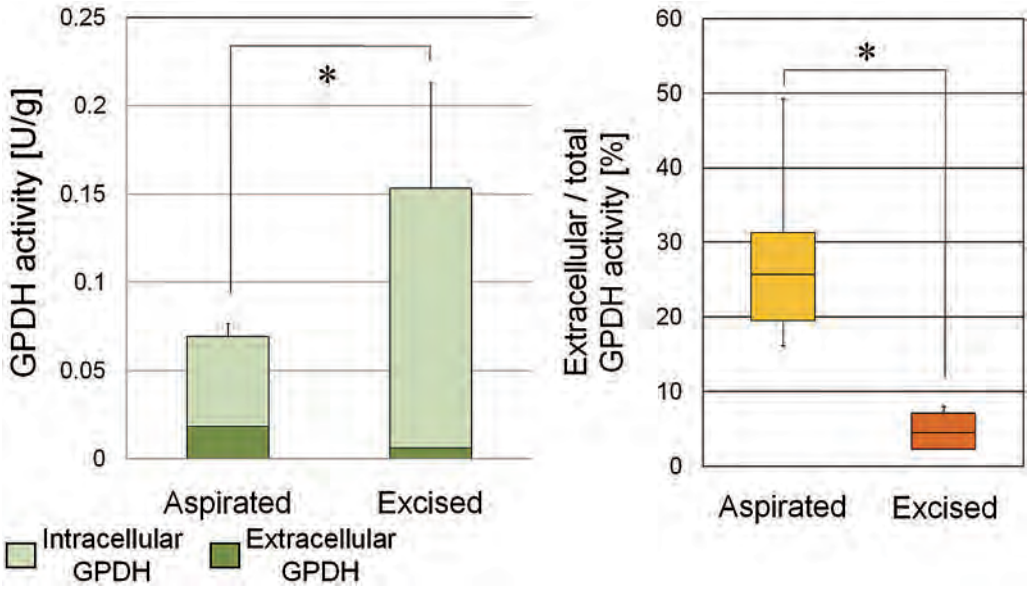

Fig. 6. Glycerol-3-phosphate dehydrogenase (*GPDH*) assay for aspirated ($n = 10$) and excised adipose tissue ($n = 4$). (*Left*) Intracellular and extracellular glycerol-3-phosphate dehydrogenase activity for 1 g of each tissue. Intracellular and extracellular GPDH activity values correspond to nonruptured and ruptured adipocyte amounts, respectively. Total (extracellular and intracellular) GPDH activity of aspirated adipose tissue was significantly lower than that of excised adipose tissue. Values are mean ± SEM. (*Right*) Rate of extracellular GPDH activity to total GPDH activity. A significantly higher level of extracellular GPDH was detected from aspirated adipose tissue than from excised adipose tissue. Values are mean ± SEM (*$p < 0.05$).

tion from excised adipose tissue ($n = 3$) (Fig. 8). The results suggested that aspirated adipose tissue contained a larger amount of contaminating blood cells (leukocytes).

Comparison of Adipose-Derived Stromal Cell Yield between Aspirated Adipose Tissue and Excised Adipose Tissue

This assay analyzed only viable and expandable adipose-derived stromal cells; the possible limitation of this assay is that nonviable adipose-derived stromal cells cannot be measured and not all adipose-derived stromal cells may be isolated through collagenase digestion.

The same weights of aspirated and excised adipose tissue harvested from the abdomen of the same four patients were processed for stromal vascular fraction isolation. The stromal vascular fraction cells were cultured for 1 week with Dulbecco's Modified Eagle Medium to exclude contamination of other cells such as endothelial cells and leukocytes, and the number of adherent adipose-derived stromal cells was counted. The ratio of normalized adipose-derived stromal cell number in aspirated adipose tissue was significantly lower (57 ± 12 percent; paired t test, $n = 4$, $p = 0.011$) than that from excised adipose tissue (Fig. 9).

Data Summary

Data of the composition of cellular components obtained from three-dimensional whole-mount staining of living tissue, flow cytometry of the stromal vascular fraction, glycerol-3-phosphate dehydrogenase assay, and adherent culture of adipose-derived stromal cells are summarized in Table 1. Note that the four assays have individual advantages and limitations as mentioned above and the results do not fully correlate with each other.

DISCUSSION

We used a whole-mount staining method, for the first time, to assess the structure and cellular composition of adipose tissue and to compare liposaspirates (aspirated adipose tissue) with intact (excised) adipose tissue. With this method, specimens are immediately stained without any fixing or sectioning procedures so that we can observe living adipose tissue images, which should reflect the original tissue structures and allow three-dimensional measurement and analysis. Indeed, the images obtained using this method are totally different from conventional histologic images. In conventional histology, adipocytes are hexagonal and lose their lipid content, with little interstitial

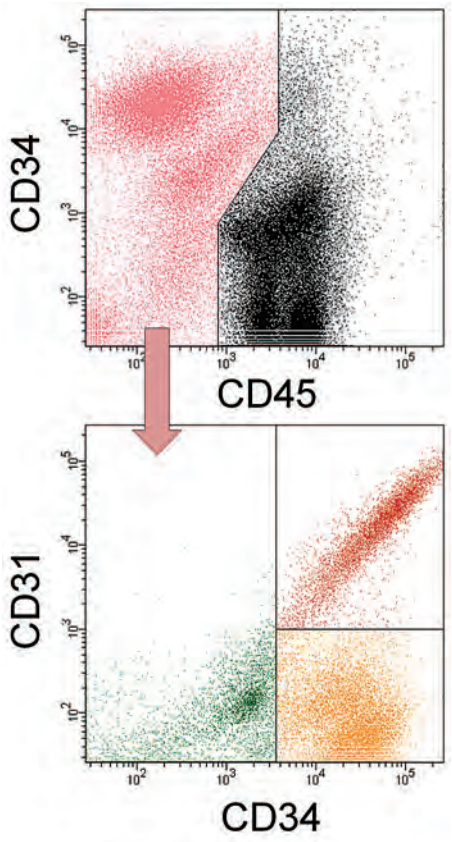

Fig. 7. Multicolor flow cytometric analysis of stromal vascular fraction. Representative plotted data of stromal vascular fraction cells obtained from aspirated adipose tissue. $CD45^+$ cells were regarded as blood-derived cells, whereas $CD45^-$ cells were regarded as adipose-derived stromal cells and processed to the next analysis. $CD45^-CD31^-CD34^+$ cells, $CD45^-CD31^+CD34^+$ cells, and $CD45^-CD31^-CD34^-$ cells were regarded as adipose-derived stromal cells, vascular endothelial cells, and other cells (fibroblasts, mural cells, and others), respectively.

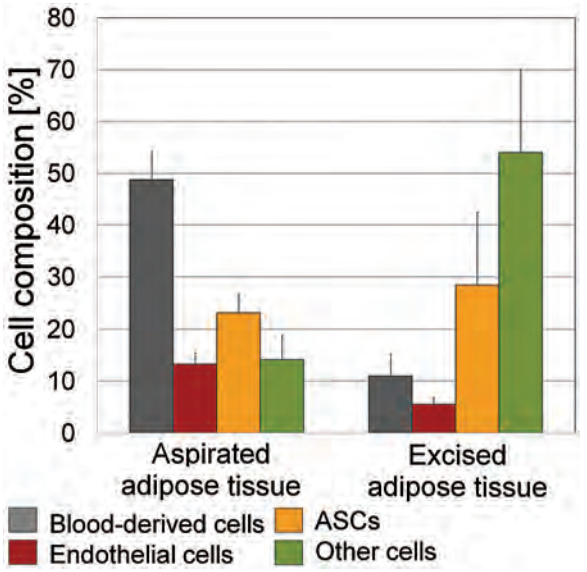

Fig. 8. Summarized data of cell composition obtained from stromal vascular fraction analyses (aspirated adipose tissue, $n = 9$; excised adipose tissue, $n = 3$). Values are mean ± SEM. *ASCs*, adipose-derived stromal cells.

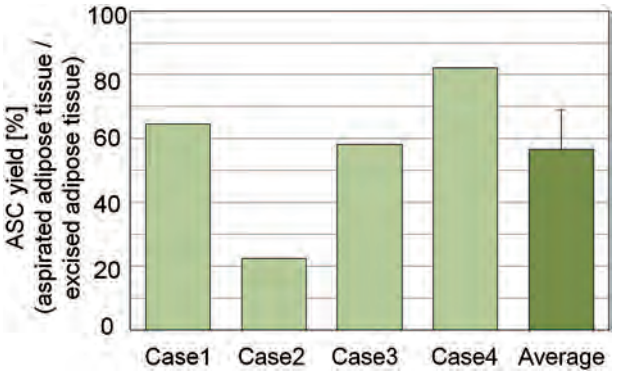

Fig. 9. Comparison of adipose-derived stromal cell (*ASC*) yields between aspirated and excised adipose tissue obtained from a single site of the same patients. Both tissues were processed for isolation of stromal vascular fraction cells, which were then cultured for 1 week. Ratios of adipose-derived stromal cell yield per volume from aspirated adipose tissue to that from excised adipose tissue were calculated; data from four patients and their average value are demonstrated. The adipose-derived stromal cell yield from aspirated adipose tissue was significantly less (57 ± 12 percent, mean ± SEM) than that from excised adipose tissue.

space between adipocytes. In whole-mount staining, adipocytes are filled with lipid and are spherical, with a substantial amount of interstitial space. Staining with triple-fluorescence demonstrated that adipose tissue is rich in microvasculature, supporting an unproven notion[28] that capillaries directly feed every adipocyte. It was also shown that the capillary network was generally disrupted, with loss of most large vessels (>50 μm) in aspirated adipose tissue.

Whole-mount staining images showed that, in intact adipose tissue, adipocytes were 14.6 percent of the cell population, a value much lower than in previous reports.[1,13–15] We also showed that vasculature-associated cells constitute more than half of the cells in both aspirated and intact adipose tissue. This finding indicates a richness of microvasculature in adipose tissue as far as cell numbers are concerned; however, the microvasculature is not really rich per tissue volume because capillaries run separately, with an interval of adipocyte size between them that is extremely large (50 to 130

Table 1. Summarized Results of Cellular Components in Adipose Tissue*

			Adipocytes		(Stromal vascular fraction: SVF)					
								Other cells (in whole mount assay)		
							Other cells (in flow cytometry)			
					Vasculature-associated cells			Fibroblasts and others	Blood cells (Leukocytes)	
			Ruptured	Non-ruptured	ASCs	Endothelial cells	Mural cells		Localized in the tissue	Released from the tissue
Aspirated adipose tissue	Cell number [cells/mm^3] (By whole-mount staining)	n=7		706 (±114) [11.8%]	3635 (±408) [60.7%]			1649 (±178) [27.5%]		
	Cell composition of SVF (By flow cytometry)	n=9			23.2 (±3.6) %	13.3 (±2.2) %	14.2 (±4.7) %		48.8 (±5.5) %	
	GPDH activity [U/g] (=Amount of adipocytes)	n=10	0.021 (±0.003)	0.048 (±0.007)						
			0.069 (±0.007)							
	Relative number of viable ASCs	n=4			57 (±12)					
Excised (intact) adipose tissue	Cell number [cells/mm^3] (By whole-mount staining)	n=5		860 (±128) [14.6%]	4274 (±721) [72.5%]			758 (±122) [12.9%]		
	Cell composition of SVF (By flow cytometry)	n=3			28.5 (±13.9) %	5.5 (±1.3) %	54.0 (±15.9) %		11.0 (±4.2) %	
	GPDH activity [U/g] (=Amount of adipocytes)	n=4	0.008 (±0.002)	0.145 (±0.059)						
			0.153 (±0.060)							
	Relative number of viable ASCs	n=4			100					

ASCs, adipose-derived stromal cells; SVF, stromal vascular fraction; GPDH, glycerol-3-phosphate dehydrogenase.
*Results obtained from four assays were combined and summarized. As no assay can evaluate all cellular components in the adipose tissue, specific features of each assay must be recognized for accurate interpretation of the data. Whole-mount histology counted only nonruptured adipocytes, whereas the glycerol-3-phosphate dehydrogenase assay measured both ruptured and nonruptured adipocytes, although ruptured adipocytes may be underevaluated because of partial release into waste liposuction fluid. Whole-mount histology cannot discriminate the three vasculature-associated cell types (adipose-derived stromal cells, endothelial cells, and mural cells), whereas flow cytometry could not separate mural cells from fibroblasts. As for blood-derived nucleated cells (leukocytes), flow cytometry of stromal vascular fraction cells counted not only blood cells localized in the tissue but also those released from the tissue into the fluid, whereas whole-mount histology counted only the former. Data are expressed as mean ± SEM. Adherent cell culture assay of adipose-derived stromal cells used aspirated and excised adipose tissue obtained from the identical four patients, whereas other assays used tissues from different patients.

μm). The whole-mount staining method has potential utility in determining the viability and integrity of living fat grafts, a task that remains challenging to date. A reliable method of evaluating the quality of engrafted fat tissue would contribute to collecting practical data for optimizing fat grafting techniques such as harvesting, processing, injection, and preservation of fat tissue.

The whole-mount analyses also indicated significant mechanical damage to adipocytes, such as small lipid droplets in aspirated adipose tissue. This finding was confirmed by glycerol-3-phosphate dehydrogenase assay, which showed that more than 30 percent of adipocytes were disrupted in aspirated adipose tissue but only 5 percent in excised adipose tissue. Another study using human and ferret fat tissue also showed that significantly higher levels of glycerol-3-phosphate dehydrogenase release were detected from aspirated fat tissue than from scraped fat tissue.[22] In addition, significantly more propidium iodide–positive dead cells were detected in aspirated adipose tissue (54.1 percent) than in excised adipose tissue (33.5 percent); the high percentage of dead cells even in excised adipose tissue likely reflects the fragile characteristics of adipose tissue, as it is easily damaged by ischemia and room temperature storage.

Total glycerol-3-phosphate dehydrogenase activity (both ruptured and nonruptured adipocytes) in aspirated tissue was approximately one-half that of excised adipose tissue, although a similar number of adipocytes were detected by whole-mount histology. There are two possible explanations for this discrepancy. First, we used the floating adipose portion of liposuction aspirates as aspirated adipose tissue and discarded the fluid portion. Therefore, glycerol-3-phosphate dehydrogenase released from ruptured adipocytes should be contained in the discarded

suction fluid and thus the ruptured adipocytes (extracellular glycerol-3-phosphate dehydrogenase) may be underestimated. Second, a substantial amount of water contamination in aspirated adipose tissue may decrease the intracellular glycerol-3-phosphate dehydrogenase value, whereas water contamination was not compensated in the whole-mount histology analysis.

Multicolor flow cytometric analysis revealed cellular compositions of the stromal vascular fraction from aspirated adipose tissue that were consistent with our previous findings,[12,13] although stromal vascular fraction may not reflect an original nonadipocyte cellular composition because of possible incomplete dissociation of the tissue. The stromal vascular fraction from aspirated adipose tissue generally contains a higher percentage of blood-derived cells, because of contamination from hemorrhage, than that from intact adipose tissue, whereas the whole-mount histology of aspirated adipose tissue detected a much smaller percentage of other cells, including blood cells; an enormous proportion of leukocytes contaminated in the stromal vascular fraction may be lost in the whole-mount staining procedures, especially in the washing process after staining. Our results also showed that the stromal vascular fraction from intact adipose tissue contained a larger percentage of other cells (e.g., mural cells, fibroblasts), perhaps because aspirated adipose tissue contains less interstitial connective tissue. It is our impression that the ratio of endothelial cells in the stromal vascular fraction from excised adipose tissue (5.5 percent) is too small despite its vasculature-rich structure seen on whole-mount histology; this may be attributable to the dissociation protocol, which was optimized for aspirated adipose tissue but not for excised adipose tissue.

Our results indicate that approximately one-half the number of adipose-derived stromal cells were obtained from aspirated adipose tissue compared with excised adipose tissue. There was a study reporting a controversial result, but the investigators centrifuged the aspirated adipose tissue and removed fibrous structures and visible blood vessels from excised adipose tissue before cell isolation.[29] The relative deficiency of adipose-derived stromal cell yield in aspirated adipose tissue may result from several factors: (1) a substantial number of adipose-derived stromal cells are located around large vessels and left in the donor tissue, as recently suggested in some reports[19,30]; (2) some adipose-derived stromal cells are released into the fluid portion of liposuction aspirates[12]; and (3) some adipose-derived stromal cells are mortally damaged through the lipoaspiration procedure, as suggested by a low composition rate of adipose-derived stromal cells in flow cytometry and a higher percentage of dead cells in the whole-mount staining assay. The relative deficiency of adipose-derived stromal cell yield may be one of the reasons for long-term atrophy of transplanted aspirated adipose tissue,[6,19] and supplementation of adipose-derived stromal cells to the graft materials has been clinically performed with successful results.[9–11]

CONCLUSIONS

We used whole-mount staining of living adipose tissue to assess the structure and cellular composition and presented the comparative results of aspirated and excised adipose tissues together with those assessed by glycerol-3-phosphate dehydrogenase assay, flow cytometry, and adherent cell culture. Based on the quantitative data summarized in Table 1, we estimated that the cellular composition of original intact adipose tissue is as follows: adipocytes, 16 percent; adipose-derived stromal cells, 30 percent; endothelial cells, 15 percent; others (e.g., fibroblasts, smooth muscle cells), 30 percent; and blood-derived cells (leukocytes; erythrocytes not included), 9 percent. The results also indicate differential structure and cellular compositions of the two tissues, and significant tissue damage and progenitor yield deficiency in aspirated adipose tissue. Aspirated adipose tissue contains less connective tissue, less large vasculatures, more blood cells, and more ruptured adipocytes and many dead cells compared with excised adipose tissue.

Based on clinical results of lipoinjection with appropriate techniques, aspirated adipose tissue should have substantial potential for adipogenesis and angiogenesis, which are considered to be derived predominantly from adipose-derived stromal cells. Recently, it was elegantly revealed that human adipose tissue turns over with an average adipocyte lifespan of 10 years.[31] This adipose tissue turnover is managed by tissue-specific progenitor cells (adipose-derived stromal cells),[31] which were recently shown to be localized in perivascular regions and have pericytic characteristics.[2,30,32,33] Further understanding of the anatomy and biology of human adipose tissue (both intact and aspirated tissues) and accumulation of knowledge on adipose tissue–specific progenitor cells will lead to improvement of clinical results of adipose tissue

transplantation and future development of adipose tissue manipulation.

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