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Selection of Donor Site for Fat Grafting and Cell Isolation

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Abstract
Background Autologous fat grafting has rapidly become an important treatment for soft tissue defects in cosmetic and reconstructive surgery. However, consensus is lacking on the ideal donor site for harvesting and isolating stromal vascular fraction (SVF) cells to improve survival of fat grafts. We aimed to determine the best donor site for tissue harvesting and isolation of SVF cells for fat graft survival.
Methods Adipose tissue samples were harvested from six women who underwent an aesthetic procedure. The samples were harvested by needle aspiration of five commonly used donor sites: flank, upper and lower abdomen, and lateral and inner thigh. The adipose tissue was injected subcutaneously into nude mice and grafts were harvested at 12 weeks. We evaluated graft volume, weight, and histologic parameters of the grafts: integrity, cysts/vacuoles, inflammation, fibrosis, and neovascularization. SVF cells isolated from donor sites were counted and assayed by flow cytometry.
Results At 12 weeks post-transplantation, weight, volume, and histologic parameters did not differ among the grafts from the five tissue donor sites. Also, SVF and levels of cell surface markers did not differ by donor site.
Conclusions This study revealed no ideal tissue donor site for fat grafting and SVF isolation. Choosing a site should be based on ease and safety of access and the preference and request of the patient.

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Keywords Autologous fat graft · Stromal vascular fraction (SVF) · Donor site · Long-term survival · Cell immunophenotype

Introduction

THE concept of fat grafting has existed since 1893 [1] and became popular for cosmetic and reconstructive surgery over the last 26 years with the development of liposuction techniques. Autologous fat is an ideal augmentation material for facial rejuvenation and soft-tissue augmentation because of its availability, low antigenicity, minimal donor morbidity, and comparability with foreign materials. However, the unpredictable absorption rate of autologous fat grafts may be the greatest obstacle to the use of fat as the best cosmetic filler. Recent publications show a resorption rate of 20–90 % [2–4].

Many attempts have been made to improve the long-term survival of fat tissue, including processing by washing, centrifugation after harvesting, and injection techniques. One technique involves centrifugation and atraumatic transfer of fat during surgery [5]. Recent attempts to improve graft survival have involved adding platelet-rich plasma [6], erythropoietin, and stromal vascular fraction (SVF) cells [7]. SVF is a heterogeneous cell mixture containing mostly stromal cells, vascular endothelial cells, and mural cells, which can be extracted from adipose tissue as a cell pellet through collagenase digestion. Some SVF cells

have been identified as adipose-derived stem cells (ADSCs) [8]. ADSCs can improve long-term survival of transplanted adipose by secreting angiogenic factors such as vascular endothelial growth factor (VEGF) and hepatocyte growth factor under hypoxic conditions. VEGF has been shown to enhance revascularization of ischemic tissues [9, 10]. ADSCs can also differentiate toward the osteogenic, chondrogenic, adipogenic, myogenic, neurogenic, and angiogenic lineages [11–13].

Many factors play a role in the success of autologous fat grafting. One factor is the selection of the donor site. Consensus is lacking on the best tissue donor site for fat grafting and isolation of SVFs. We aimed to analyze the role of the donor site in assessing the long-term survival of autologous fat grafts by examining the weight and volume of human grafted tissue transplanted in mice. Fat grafts harvested from the flank, upper and lower abdomen, and lateral and inner thigh areas of six women were implanted in mice and assessed for weight and histologic variables as well as viability and number of SVFs isolated to assess the optimal donor site for isolation of SVFs.

Materials and Methods

Fat Harvesting

Fat samples were obtained from six female donors aged 26–37 years while they underwent cosmetic surgery. From each patient we harvested fat from the flank, upper and lower abdomen, and lateral and inner thigh. The women gave their informed consent for removal of the tissue and the study was approved by the local ethics committee. The donor sites were injected with a tumescent solution containing 0.08 % lidocaine and 1:500,000 epinephrine. Harvesting involved use of a blunt cannula with 3 mm suction and two holes in the tip attached to a 20 ml syringe with the plunger held back with 7–8 ml in order to minimize trauma to the fat grafts. After aspiration, the fat grafts were centrifuged at 1,000 rpm for 3 min to remove the lidocaine, oil, and erythrocytes.

Isolation and Counting of SVFs

Adipose tissue samples from the five donor sites were digested with 0.1 % collagenase in phosphate-buffered saline (PBS) for 30 min on a shaker at 37 °C. An equal volume of Dulbecco's modified Eagle medium (DMEM) with 10 % fetal bovine serum was added to neutralize the collagenase. Mature adipocytes and connective tissues were separated from pellets by centrifugation (1,200×g for 5 min). The pellets were resuspended in erythrocyte lysis buffer (155 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA) and incubated for 5 min at room temperature. After centrifugation at 1,200×g for 5 min, the cell pellet was resuspended with PBS and number of nucleated cells was counted.

Flow Cytometry

Freshly isolated SVF cells were examined for surface molecule expression by flow cytometry. At least 1,000,000 cells were incubated with the antibodies anti-CD90 fluorescein isothiocyanate (FITC), anti-CD44 allophycocyanin (APC), anti-CD31 phycoerythrin (PE), anti-CD34 PE, and anti-CD45 PE for 30 min and washed twice in PBS. Mouse isotype antibodies served as controls. Cells were analyzed using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA).

Fat Transplantation to Nude Mice

Thirty 6-week-old BALB/c-nu nude mice were divided into five groups ($n = 6$ each) for injection with fat from one of the five donor sites. All animal procedures were performed in accordance with the guidelines of the Southern Medical School Animal Care and Use Committee. The backs of nude mice were the recipient sites for fat injection. Each mouse underwent subcutaneous injection in two locations, with 0.5 ml fat injected per site by use of a 14-gauge needle.

Weight and Volume of Transplanted Fat and Histology

Animals were killed 12 weeks after fat transplantation (Fig. 1). The grafted fat tissues were excised and measured for weight and volume. Harvested samples were fixed and processed for histology. Each sample was embedded in paraffin, cut into sections, and stained with hematoxylin and eosin. In total, 12 slides were randomly chosen from each group, with five fields per slide randomly chosen for microscopy evaluation by two blinded reviewers. Each slide was evaluated for the presence of intact and nucleated fat cells; cysts and vacuoles; inflammation, as evidenced by infiltration of lymphocytes and macrophages; fibrosis; and neovascularization by capillary density. The presence of each variable was graded on a scale from 0 to 5: 0, absent; 1, minimal; 2, minimal to moderate; 3, moderate; 4, moderate to extensive; and 5, extensive [14].

Statistical Analysis

Results are expressed as mean ± SD. Statistical analyses involved one-way ANOVA, with post-hoc least significant difference tests in cases of nonhomogeneous variances across groups. A value of $p < 0.05$ was considered statistically significant.

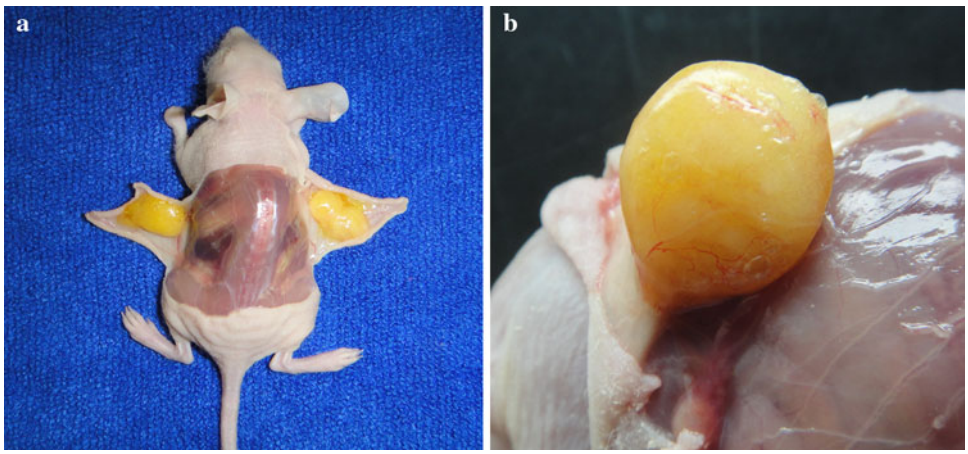

Fig. 1 Fat grafts 12 weeks after transplantation in mice

Table 1 Volume and weight of mouse grafts with fat tissue from five human donor sites

Donor site	No. of samples	Volume (ml)	Weight (g)
Flank	12	0.23 ± 0.04	0.21 ± 0.04
Upper abdomen	12	0.23 ± 0.03	0.22 ± 0.03
Lower abdomen	12	0.23 ± 0.04	0.22 ± 0.04
Inner thigh	12	0.23 ± 0.03	0.22 ± 0.03
Lateral thigh	12	0.24 ± 0.04	0.23 ± 0.05
p value		0.979	0.556

Data are mean ± SD

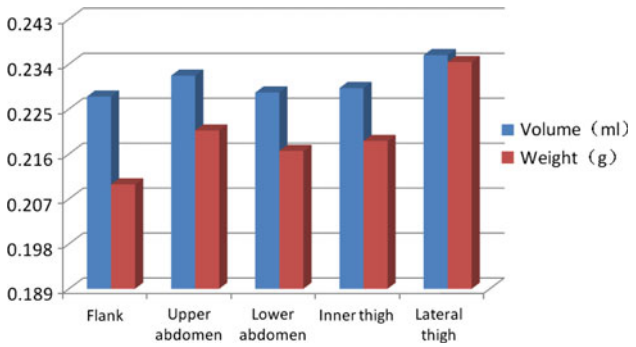

Fig. 2 Volume and weight of mouse grafts with fat tissue from five human donor sites

Results

Long-term Survival of Fat Grafts

No animals died during the study. All grafted adipose tissue was surrounded by a thin envelope that isolated the donor adipose tissue from the recipient tissue (Fig. 1). Graft weight and volume did not differ among the five tissue donor-site grafts ($p = 0.556$ and $p = 0.979$, respectively; Table 1; Fig. 2). Tissue from the lateral thigh showed the best weight and volume but not significantly. As compared with pretransplantation fat volume, the average maintenance volume of all fat grafts was 46 %.

Histology

At 12 weeks post transplantation, mouse grafts with fat tissue from the five donor sites did not differ in histologic variables, including tissue integrity, cyst/vacuole formation, inflammation, fibrosis, and neovascularization (Table 2; Fig. 3). All of the grafts were surrounded by a collagen and fibrous capsule. The peripheral zone was composed of viable mature adipocytes. Also, a few cysts and inflammatory cell infiltration and fibrosis were observed. The central zone showed an inflammatory process with fatty cysts and collagen condensation (Fig. 3).

Table 2 Grading of integrity, cyst/vacuoles, fibrosis, and inflammation in mouse grafts with fat tissue from five human donor sites

Donor site	Integrity	Cyst/vacuoles	Inflammation	Fibrosis	Neovascularization
Flank	2.92 ± 1.24	1.75 ± 0.87	2.50 ± 1.00	3.17 ± 0.58	2.17 ± 0.72
Upper abdomen	2.83 ± 1.12	2.25 ± 0.97	2.33 ± 1.07	2.33 ± 1.07	2.67 ± 0.99
Lower abdomen	2.50 ± 0.91	1.83 ± 0.72	2.33 ± 1.23	2.58 ± 1.17	2.33 ± 0.99
Inner thigh	3.17 ± 1.03	1.92 ± 0.79	2.42 ± 1.24	2.42 ± 0.90	2.42 ± 1.24
Lateral thigh	2.75 ± 1.22	1.75 ± 0.75	2.67 ± 1.23	2.33 ± 0.99	2.75 ± 0.75
p value	0.68	0.555	0.557	0.216	0.759

Data are mean ± SD; $n = 12$ slides

Scale: 0 = absent, 1 = minimal, 2 = minimal to moderate, 3 = moderate, 4 = moderate to extensive, 5 = extensive

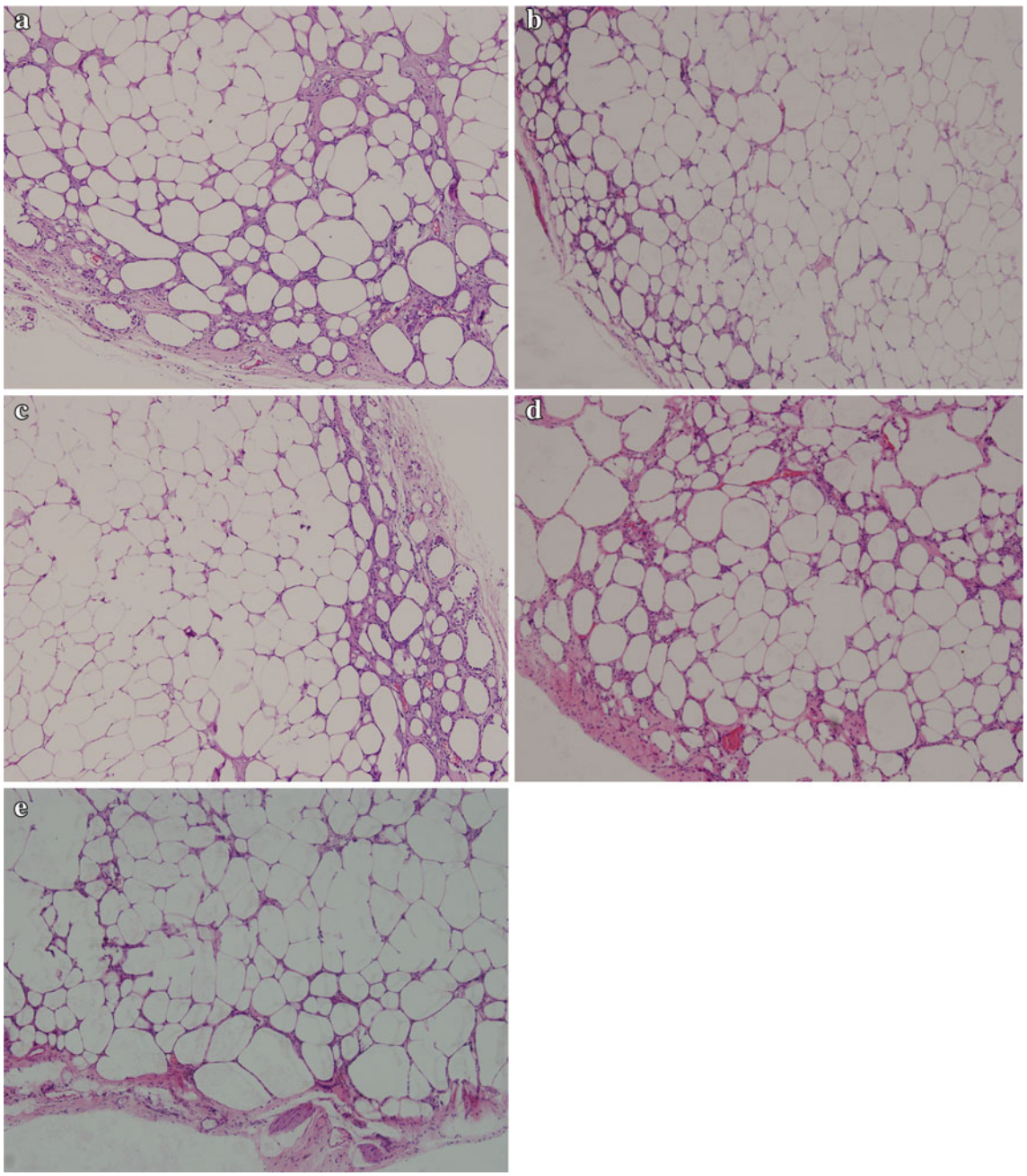

Fig. 3 Histology of mouse tissue injected with fat tissue from five human donor sites 12 weeks after transplantation (hematoxylin and eosin staining, original magnification ×200). A few cysts, inflammatory cell infiltration, and fibrosis were observed. Donor sites are **a** flank, **b** upper abdomen, **c** lower abdomen, **d** inner thigh, and **e** lateral thigh

Number of SVFs and Characterization of SVFs by Flow Cytometry

Fat tissue harvested from the five donor sites did not differ in number of SVF cells isolated ($p = 0.608$). The mean number of nucleated cells in the SVFs was 1.31×10^6 per ml adipose tissue (Table 3). Fat tissue harvested from the five donor sites did not differ in the proportion of cells positive for CD31, CD34, CD44, CD90, or CD45 (Table 4; Fig. 4). The mean percentages of cells positive for CD31, CD34, CD44, CD90, and CD45 were 19.3, 62.9, 74.2, 55.1, and 6.52 %, respectively.

Discussion

With developments in liposuction techniques, liposuction has become one of the most commonly performed

operations for removing unwanted fat or for autologous fat grafting. Autologous fat grafting to fill soft tissue defects or augment tissue is a common procedure that is safe and effective. However, the absorption rate of the fat grafts is the most contentious issue, and the long-term survival of fat grafts is usually disappointing for both the patient and the surgeon. Thus, surgeons have performed many clinical and experimental studies to find the source and a solution to this problem. Research into autologous fat grafts has focused on optimizing graft viability at each step of the process: choosing candidates for surgery and donor sites, fat harvesting, fat processing, and transplantation techniques. Moreover, the effect of adding vitamins, insulin, or growth factors on fat graft survival has been evaluated [15, 16]. SVF cells isolated from liposuction aspirates without any manipulation such as cell sorting or culture has recently become the focus of attention because fat transplantation with SVFs could enhance the survival rate of the graft fat [17]. However, consensus is lacking on the best donor site for isolation of SVFs.

We aimed to determine the best donor site for tissue harvesting and isolation of SVF cells. Adipose tissue samples were harvested by needle aspiration from five commonly used donor sites in six healthy women undergoing an aesthetic procedure, then injected subcutaneously into nude mice. At 12 weeks post transplantation, graft weight, volume, and histologic parameters did not differ by tissue donor site, not only for grafts from a single patient

Table 3 Number of stromal vascular fraction (SVF) cells in fat tissue harvested from five human donor sites

Donor site	No. of samples	No. of SVFs (10^6/ml adipose tissue)
Flank	8	1.20 ± 0.32
Upper abdomen	8	1.36 ± 0.25
Lower abdomen	8	1.32 ± 0.36
Inner thigh	8	1.41 ± 0.34
Lateral thigh	8	1.23 ± 0.24
p value		0.608

Table 4 Surface phenotype characterization of SVF cells isolated in fat tissue harvested from five human donor sites

Donor site	CD31	CD34	CD44	CD90	CD45
Flank	17.3 ± 6.2	64.7 ± 9.2	72.8 ± 12.2	52.0 ± 6.8	7.5 ± 3.0
Upper abdomen	18.2 ± 5.3	65.4 ± 8.2	74.2 ± 9.4	56.3 ± 12.0	6.9 ± 3.2
Lower abdomen	20.5 ± 5.9	61.4 ± 6.8	70.9 ± 12.0	52.8 ± 6.4	6.6 ± 2.4
Inner thigh	21.2 ± 5.5	63.5 ± 7.8	72.0 ± 6.14	54.5 ± 5.3	4.8 ± 1.6
Lateral thigh	19.2 ± 4.1	59.2 ± 7.3	74.2 ± 10.6	60.0 ± 8.5	6.8 ± 2.8
p value	0.601	0.528	0.333	0.350	0.393

$n = 8$ samples

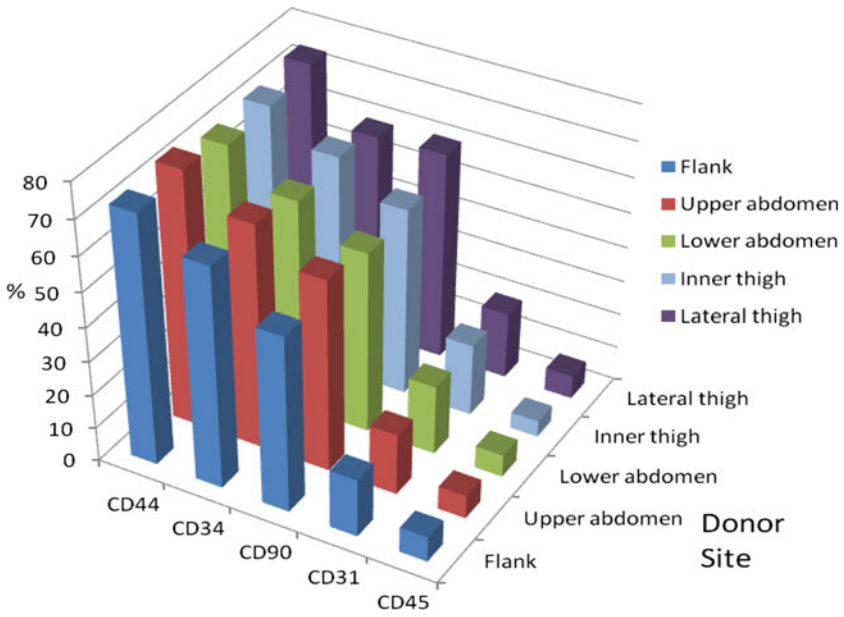

Fig. 4 The immunophenotype of SVF cells isolated from the five donor sites

but also across all six patients. Also, the SVF fraction and cell surface marker levels did not differ by tissue donor site on flow cytometry.

Rohrich et al. [18] first attempted to find the ideal donor site for fat grafting. Quantitative in vitro colorimetric assay of cell proliferation to analyze viability revealed no body part that provided any advantage as a donor site because abdominal, thigh, flank, and knee fat were all equivalent in terms of cell viability [18]. Ullmann et al. [19] used the nude mice model to study the long-term survival of human fat in vivo. At 16 weeks post transplantation in mice, the three donor sites evaluated—thigh, abdomen, and breast—did not differ in vascularization, cyst formation, fibrosis, necrosis, or inflammation [19]. In our study we used the nude mouse model to study the long-term survival of human fat in vivo and examined five commonly used donor sites: flank, upper and lower abdomen, and lateral and inner thigh. We also did not find a statistical difference in volume, weight, and histologic variables by tissue donor site.

In determining the best donor site for SVF isolation, we did not find significant differences in SVF cell number by tissue donor site. Also, the SVFs did not differ in percentages of cells positive for CD31, CD34, CD44, CD90, or CD45 expression. A mean of 62.9 % of the SVFs expressed the stem cell-associated marker CD34, and stromal cell marker expression of CD44 and CD90 was present in 74.2 and 55.1 %, respectively, of the SVF cells. The percentages of cells positive for the endothelial cell-associated marker CD31 was 19.3 %. These results are similar to those of Mitchell et al. [20].

Conclusion

The findings in this study will be encouraging to surgeons in choosing a donor site for fat grafting and SVF isolation, which should be based on ease and safety of access and the preference and request of patients. However, long-term survival for SVF-assisted lipotransfer requires investigation in animal models.

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