Fat Graft Viability in the Subcutaneous Plane versus the Local Fat Pad

Ryan S. Constantine, BA
Bridget Harrison, MD
Kathryn E. Davis, PhD
Rod J. Rohrich, MD, FACS

Background: Fat grafting has been increasingly utilized in both aesthetic and reconstructive surgical procedures, yet the basic scientific understanding of fat grafting has lagged behind the pace of clinical innovation and utilization. This lack of basic scientific understanding has perhaps manifested itself in the wide range of graft viability reported across the literature. This study attempts to further the underlying mechanisms of fat graft take and viability through the comparison of the subcutaneous plane and the local fat pad in athymic rats.

Methods: Lipoaspirate from a consenting patient was grafted into 2 locations in the subcutaneous plane and into the 2 inguinal fat pads in each of 4 athymic rats. Specimens were then collected after 47 days, and immunohistochemistry was utilized to determine angiogenesis in the fat grafts as a measure of fat graft take. Data were analyzed using the Student's t test and analysis of variance followed by multiple comparisons.

Results: There was no statistically significant difference ($P = 0.2913$) between the inguinal fat pad and the subcutaneous plane when measuring neovascularization. Analysis of variance comparing the graft locations also indicated no statistically significant difference when comparing each of the rats.

Conclusions: Investigation into fat graft injection location indicates that there is no statistically significant difference in angiogenesis signals between the subcutaneous plane and the local fat pad in the athymic rat model. Further research should aim to continue to close the gap between clinical practice and basic scientific understanding of fat grafting. *(Plast Reconstr Surg Glob Open 2014;2:e260; doi: 10.1097/GOX.0000000000000228; Published online 3 December 2014)*

Increasingly utilized in aesthetic and reconstructive procedures of the face, hand, buttocks, and breast, autologous fat grafting offers a promising yet little understood avenue for use in plastic and reconstructive surgery.[1–10] As an abundant, easy-to-harvest resource, autogenous fat offers myriad opportunities for clinical use, but the innovative applications of autologous fat grafting have outpaced the basic scientific understanding of how and why autologous fat grafting functions. This lack of understanding is evidenced in the literature by wide variations in reported retention and survival of grafted fat.[11–18] These unpredictable results have been the

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subject of intense study into improving take and maintenance of the fat graft, but there has been little consensus among researchers. Studies aiming to determine critical factors for optimal fat graft response have been difficult to compare with one another as technique and patient demographics are widely inconsistent.[19,20]

The importance of the recipient site represents an area with minimal study and room for greater understanding of the basic scientific mechanisms of fat graft take. Using histologic analysis of α-smooth muscle actin (α-SMA) stains as a measure of graft take, this study analyzes the vascularization of grafted fat in different beds.[21] Through an animal model, this study explores the influence of the recipient site on fat graft take and maintenance.

METHODS

Lipoplasty Adipose Tissue Harvest and Preparation

The surgeon or appropriate member of the study team obtained consent of the human patient utilizing the UT Southwestern Medical Center Institutional Review Board approved consent form. Patients were recruited from those undergoing body contouring procedures by one of the study investigators. The patient was infiltrated with a wetting solution consisting of 1 L of Ringer's lactate and 1 ampoule of epinephrine (1:1000), so that a 1-mL infiltration to aspiration was obtained. Using the standard Coleman technique, 20 mL of lipoaspirate was collected from the consenting patient. This lipoaspirate was then centrifuged at 3000 rpm for 1 minute.

Animals and Graft Implant

This study utilized 4 athymic rats (Rowett Nude [RNU]; Foxn1[rnu]) purchased from Charles River Laboratories (Wilmington, Mass.). The rats were housed in a temperature-controlled sterile environment at 17.7–26.1°C with a 12-hour light cycle. Animals were fed using standard chow (#2916; Harlan-Teklad, Houston, Tex.), and water was available ad libitum. At approximately 10 weeks of age, the athymic rats were anesthetized using a combination of isoflurane gas and oxygen, and the animals were prepared for fat grafting. Fat grafts were placed bilaterally along the rat's dorsum with a 16-gauge cannula, which was tunneled under the epidermis/dermis, to deposit the human fat subcutaneously into the formed tunnels. Additionally, 2 separate human fat grafts were placed bilaterally into the inguinal fat pad of the rat with a 16-gauge needle (Fig. 1). A total volume of 0.2 mL of lipoaspirate for each experimental condition was deposited per location. Sutures were not required to close the skin. Postoperatively, rats were administered 0.01 mg/ kg buprenorphine and given 4.4 mg/kg carprofen wafers. Buprenorphine was further administered every 8–12 hours for the next 48 hours. Tissues were harvested at 47 days from fat graft placement and analyzed for graft survival. Care of all animals and procedures were approved by the Institutional Animal Care and Use Committee at UT Southwestern Medical Center.

Fat Graft Explant

The rats were deeply anesthetized and injected with 0.5 mL of 120 mg/kg pentobarbital barbiturate for ethical euthanization of the rats. Incisions were made along the tailbase of the dorsum from left to right, and the dorsal skin layer was elevated to visualize the grafts. Incisions were also made to elevate and expose the inguinal fat pads. Gross analysis and general observations were noted at this time. Grafts were identified by gross analysis and the uniform presence of capsule around each graft. The grafts were then excised by the surgeon, and connective tissue was removed.
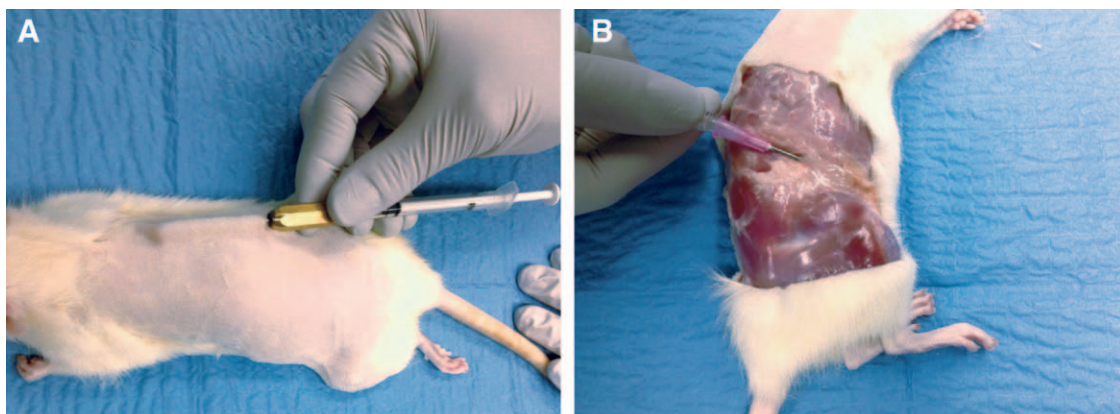

Fig. 1. A, Graft injection location for the subcutaneous plane of a rat utilizing 16-gauge cannula. B, Graft injection location in the inguinal fat pad of a rat utilizing 16-gauge needle.

Fat Graft Histological Analyses

Grafts were cut into 2 sections (cranial and caudal) and each placed in 10% neutral buffered formalin and gently shaken for 48 hours, which allowed the tissue to fix. Each graft sample was then embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin and α-SMA (Sigma-A2547, St. Louis, Mo.) fluorescent dye (for visualization of vascularity, which has been shown to be a measure of graft take).[22] Histological sections were analyzed using an Olympus (Center Valley, Pa.) IX51 epifluorescence microscope equipped with an Optronics (Houston, Tex.) Microfire Color CCD Camera and viewed under fluorescence when appropriate. Images of both hematoxylin and eosin and α-SMA sections were viewed and analyzed with National Institutes of Health (Bethesda, Md.) ImageJ software. These analyses were then utilized for statistical comparison.

Statistical Methods

Data were compiled as a mean ± SEM. Statistical analysis included a 2-way analysis of variance (ANOVA) calculation with multiple comparisons of the graft location means, ANOVA analysis comparing the subcutaneous fat graft with the fat graft in the inguinal fat pad for each rat, and a Student's t test comparing overall graft location. Statistical significance was determined by $P < 0.05$. Data analysis and figures were generated using GraphPad Prism 6.00 for Mac (GraphPad Software, La Jolla, Calif.) statistical software.

RESULTS

Vascular density within the grafts was measured through histological analysis, utilizing the α-SMA stain as strong, positive fluorescent signals indicated angiogenesis. These positive signals were tallied as a total comparing the subcutaneous fat grafts with the grafts in the inguinal fat pad using the Student's t test (Fig. 2); however, there was no statistically significant difference between the 2 graft locations in terms of graft viability ($P = 0.2913$).

In an effort to compare the graft locations while also measuring any appreciable differences between the individual animals, ANOVA analysis followed by multiple comparisons resulted in a statistically significant difference between the inguinal fat pad and the subcutaneous fat graft in animal 2; however, no difference between the 2 fat graft locations in the rest of the rats (Fig. 3).

Further statistical analysis comparing the 4 graft locations to measure any perceptible physiologic difference created by the individual graft site in each of the rats was also conducted. ANOVA analysis followed by multiple comparison tests to measure the difference between each of the 4 locations for fat grafting
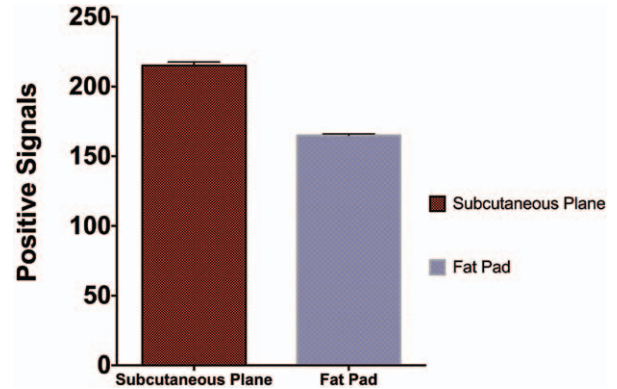

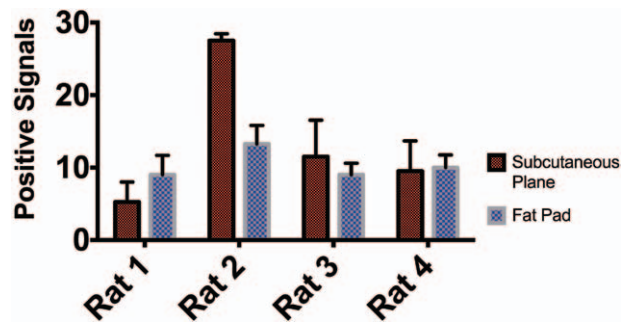**Fig. 2.** Positive fluorescent signals indicating angiogenesis in the subcutaneous plane versus the inguinal fat pad.

Fig. 3. Positive fluorescent signals indicating angiogenesis in the subcutaneous plane versus the inguinal fat pad in each animal.
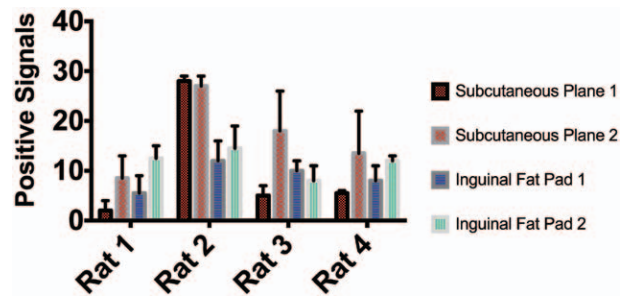

Fig. 4. Positive fluorescent signals indicating angiogenesis in each of the 4 graft locations (2 along the subcutaneous plane and 2 in the inguinal fat pad) in each animal.

indicated that there was no statistically significant difference between any of the locations tested (Fig. 4).

DISCUSSION

Survival, and thereby volume maintenance, of fat grafts has been the goal since first described by Van der Meulen in 1889.[23] In order for this to occur, the fat graft arguably must acquire or maintain a source of tissue perfusion and nutrient supply early after transplantation. Several reports have suggested that survival is optimal when fat is placed intramuscularly, with suggestions that the vascular nature of muscle

increases the survival of the transplant.[24–26] Karacaoglu et al[27] compared submuscular, supramuscular, and subcutaneous fat injections and found improved survival with supramuscular injections. The authors did not include injections into fat pads. Intramuscular injection is not always possible clinically, particularly if larger volumes are used, and injection into the well-described fat compartments of the face is a common practice.[28,29] The current study, therefore, examined alternative placement in the subcutaneous plane versus injection into a local fat pad.

Using the α-SMA stain as a marker for revascularization, no statistically significant difference was observed between these alternative injection sites. Harvest time in current fat grafting studies is highly variable and was performed at 47 days in this study. Fat apoptosis reaches a maximum at 30 days post transfer, so this harvest time should allow for accurate assessment of viable fat.[30] The equivalent results found in this study suggest that fat can be grafted to subcutaneous or local fat compartments with equal success.

A standard technique for fat harvest, consistent with the senior author's routine practice, was utilized for this study. Although arguments have been made for and against different harvesting methods, there is no consensus on one definitive method. Given the reliability of this harvesting method and the lack of information suggesting superiority of alternatives, the authors believe that these results should be applicable to surgeons who may use a slightly different harvest technique.

One limitation of this study is the small number of animals in the sample. Whenever possible, the least number of animals for any experiment are used. A total of 4 rats were examined in this study, with 2 sections placed in each rat, for a total of 8 samples. Although this sample size was thought to be sufficient to account for any variability in specimens, the sample size may have resulted in lower power of the study and increased the possibility of a type II error. α-SMA immunohistochemistry is widely used as a specific marker of vascular smooth muscle cells but does limit results to the accuracy of a secondary indicator of neovascularization.[31,32]

Although neovascularization is thought to be necessary for survival and clinical response to fat grafting,[33–35] variability in graft "take" and the optimal method for harvest, processing, and transfer of fat remain to be determined.[36,37] The results of this study suggest that placement in either subcutaneous or local fat compartments does not affect final results and reinforces the variety of currently described techniques for facial fat grafting.[38–41] Although this evidenced-based conclusion does not provide the answer to the search for the perfect fat graft, it does reduce the number of variables through which the clinician must ferret in the pursuit of safe and reliable fat transplantation.

CONCLUSIONS

Autologous fat presents ample opportunity for clinical use and innovation, and investigation into fat graft injection location indicates that there is no statistically significant difference in neovascularization signals between the subcutaneous plane and the local fat pad in the athymic rat model. As fat grafting continues to grow within the field of plastic and reconstructive surgery, we must continue to investigate the variables that may affect this useful procedure to obtain evidence-based, optimal results.

Rod J. Rohrich, MD
Department of Plastic and Reconstructive Surgery
University of Texas Southwestern Medical Center
1801 Inwood Road, Dallas, TX 75390-9132
E-mail: rod.rohrich@utsouthwestern.edu

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