



ELSEVIER



# The effects of the size of liposuction cannula on adipocyte survival and the optimum temperature for fat graft storage: an experimental study

Melike Erdim <sup>a,\*</sup>, Erdem Tezel <sup>a</sup>, Ayhan Numanoglu <sup>a</sup>, Aydin Sav <sup>b</sup>

<sup>a</sup> Department of Plastic and Reconstructive Surgery, Marmara University School of Medicine, 34349 Istanbul, Turkey

<sup>b</sup> Department of Pathology, Marmara University School of Medicine, Istanbul, Turkey

Received 26 July 2007; accepted 4 March 2008

## KEYWORDS

Fat grafts;  
Liposuction cannula  
size;  
Temperature of storage

**Summary** *Background:* Determining the most advantageous size of liposuction cannula and injection needles in terms of adipocyte viability could help to increase fat graft survival. When recurrent injections are necessary, storing fat tissue which is harvested during the first operation could be a practical solution if it is stored at an appropriate temperature providing the highest amount of viable fat cells.

*Methods:* Fat tissue was removed from the abdomen of 10 consecutive female patients by 6-, 4- and 2-mm-diameter liposuction cannulas. Fat tissue harvested with the 6 mm cannula was injected through 14, 16 and 20 g needles and collected in separate tubes. An additional three tubes of fat samples were prepared from fat tissue obtained with the 6 mm cannula to be stored at +4, -20 and -80 °C for 2 weeks. Viability of the fat grafts was evaluated by fat cell isolation with collagenase digestion and staining with supravital dye and counting adipocytes with a haemocytometer.

*Results:* The viability of fat grafts harvested with the 6 mm cannula was higher than grafts obtained with smaller cannulas. The viability of fat grafts injected through 14, 16 and 20 g needles were similar to each other. The viability of fat grafts stored at +4 °C was similar to fresh tissue whereas freezing fat grafts caused significant loss of viable adipocytes compared to fresh tissue.

*Conclusions:* The use of larger liposuction cannulas for fat tissue harvesting provides more viable fat grafts. A temperature of +4 °C could be proposed as an effective and easily available way of storing fat grafts for at least 2 weeks.

© 2008 British Association of Plastic, Reconstructive and Aesthetic Surgeons. Published by Elsevier Ltd. All rights reserved.

\* Corresponding author. Tel.: + 90 216 327 10 10.  
E-mail address: [ermelike@yahoo.com](mailto:ermelike@yahoo.com) (M. Erdim).

Autologous fat grafting is a commonly performed procedure in both reconstructive and aesthetic surgery. It is easier to obtain, cheaper and non-allergenic compared to artificial

tissue substitutes like collagen, hydroxyapatite or hyaluronic acid.<sup>1</sup> The first use of fat transplantation was performed by Neuber in 1893 and later it was used by several authors for different indications.<sup>2–4</sup> After Peer<sup>4</sup> showed that 45% of fat grafts are resorbed by the end of the first postoperative year there was relatively little call for the use of fat grafts in the 1960s and 1970s. The introduction of liposuction by Illouz<sup>5</sup> was a milestone and autogenous fat grafting became repopularised in the 1980s.<sup>6–8</sup> Although this new source of fat, liposuction aspirate, is easy to obtain and use, the most important drawback is still resorption. Resorption rates have been reported in the range of 20 to 90% in different studies.<sup>3,9</sup> Illouz<sup>6</sup> defined the factors which play a role in fat graft survival as the choice of donor and recipient area and the method of harvesting and transplantation of fat grafts. According to the commonly accepted cell survival theory, the success rate of fat grafting is higher if the transplanted fat contains higher amounts of viable adipocytes.<sup>4,10</sup>

The effects of the techniques of fat graft harvesting have been investigated by several authors but quantitative analysis of adipocyte viability among fat grafts obtained by different suction cannulas has not been detected.<sup>3,6,11–15</sup> To overcome the problem of resorption, overcorrection or repeated fat injections were proposed.<sup>1,2,6</sup> Storage of fatty tissue for repeated lipofilling procedures has been attempted both experimentally and clinically but the number of viable adipocytes among the stored fatty tissue has not been clearly identified.<sup>16–18</sup> In this study we have investigated: (1) How the diameter of the liposuction cannula affects the numbers of viable adipocytes in the fatty aspirate; (2) how the size of the injection needles affects the numbers of live adipocytes in the fat grafts; and (3) how the temperature of storage affects the numbers of live adipocytes in the fatty tissue. The purpose of this study, therefore, is to determine the optimum liposuction cannula size, injection needle size and temperature for the storage of the fat grafts that will give the highest numbers of live fat cells with the hope of maximum graft survival.

## Materials and methods

Liposuction specimens were obtained from 10 consecutive female patients who were admitted to the Marmara University Hospital for abdominal liposuction after study approval was obtained from the institutional review board of the university. Patients who have systemic disorders or a history of chronic drug usage were not included in the study. The patients' ages ranged from 30 to 57 years (mean = 37 years).

Patients were operated on under general anaesthesia. Local anaesthetic solution infiltration was not performed until the study samples were collected. Liposuction procedures were performed with 2, 4 and 6 mm aspiration cannulas with pyramid tip via a 5 mm incision at the inferior part of the umbilicus. The abdomen was divided into three imaginary parts: left, middle and right and care was taken to ensure that suction with each of the cannulas was in a part of the abdomen that was not traumatised with the previous cannulas. Aspiration was performed with 50 cc syringes (specimens from each of these cannulas were

collected in three separate syringes). Approximately 20 cc fat aspiration was performed with each of the cannulas. Following the collection of study samples, tumescent solution was infiltrated and ordinary liposuction was performed. After taking the samples, syringes were returned to room temperature for 30 min and serum was separated from fatty tissue by gravity. Then separated serum was poured and 1 cc samples of fatty aspirate were taken from each syringe and collected in separate tubes for the adipocyte count in the fresh aspirate.

To investigate the effect of injection needle size, fatty aspirate from the 6 mm cannula was injected through 14, 16 and 20 g needles to separate tubes and three more samples of 1 cc fatty tissue were prepared.

Moreover, three additional samples of fatty aspirate from the 6 mm cannula were prepared. These were stored at temperatures of +4, –20 and –80 °C for 2 weeks. They were prepared as 1 cc of fatty tissue. After 2 weeks they were allowed to thaw at room temperature for 1 h and investigated for viable adipocyte count using the same method described below.

## Method of viable adipocyte count

Each of the 1 cc samples of fatty aspirate obtained with 2, 4 and 6 mm cannulas, injected from 14, 16 and 20 g needles and stored at +4, –20 and –80 °C temperatures was processed with the addition of 1 ml of collagenase buffer solution, previously described by Moore et al.<sup>19</sup>

1 ml of collagenase buffer solution contains:

1 mg/ml collagenase type 1 enzyme (Sigma-Aldrich, St. Louis, USA);  
10 mg/ml Bovine serum Albumin (Sigma-Aldrich, St. Louis, USA);  
500 µl HBSS (Hank's balanced salts solution; Biological Industries, Israel);  
500 µl DMEM (Dulbecco's modified Eagles medium; Biological Industries, Israel).

After the digestion of collagenase, specimens were centrifuged at 150 g for 2 min. Following the centrifugation, three layers were observed clearly in each tube. A yellow-coloured upper layer contained free fatty acids released from ruptured adipocytes, the pale yellow-white-coloured middle layer contained adipocytes and the red-coloured bottom layer contained blood and the other connective tissue elements.<sup>11,14,20</sup>

Samples (100 µl) were taken from the middle layer containing adipocytes and stained with 100 µl brilliant cresyl blue supravital dye (Merck, Germany) and the number of viable adipocytes was counted with a haemocytometer under 400× magnification.

## Statistical analyses

Statistical analysis of the samples collected with 2, 4 and 6 mm cannulas was completed by using the ANOVA (analysis of variance) test. Repeated measures ANOVA test was used for the statistical analyses of injected and stored samples of fat aspirate. Analysis included the comparison of the

viable adipocytes in fresh samples of fatty tissue obtained with the 6 mm cannula and the stored and reinjected samples which were also prepared from fatty aspirate from the 6 mm cannula. The differences were considered significant at  $P < 0.05$ .

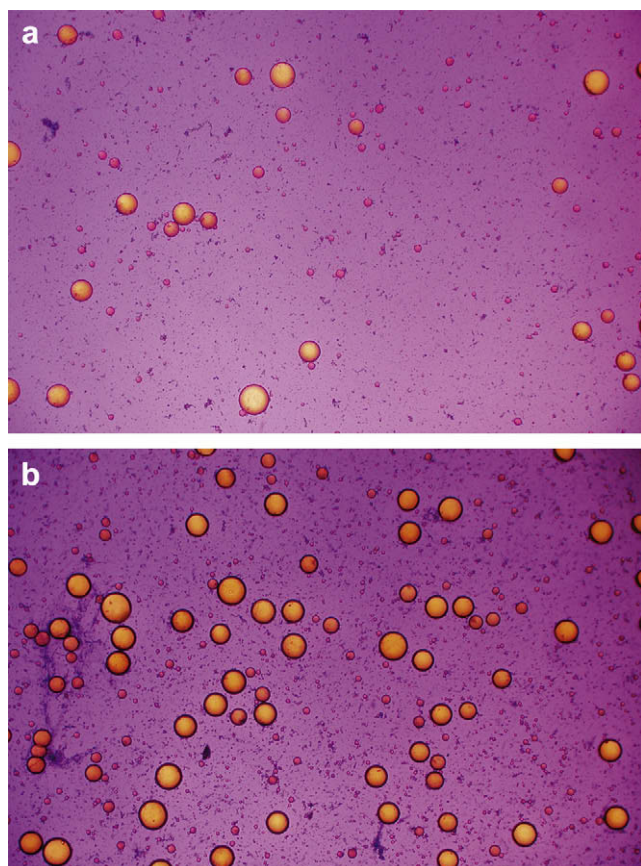
## Results

### Harvesting techniques

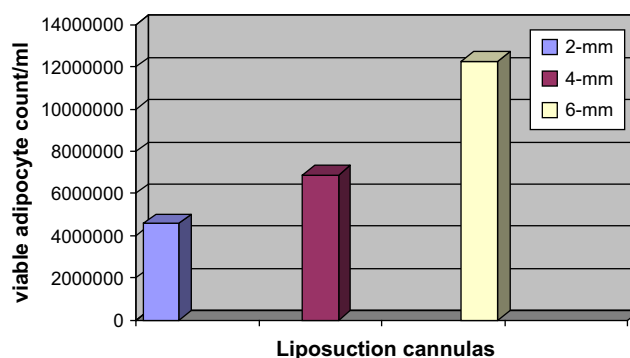
The number of viable adipocytes of the fatty aspirate obtained with the 6 mm cannulas was significantly higher than fatty aspirate obtained with 2 or 4 mm cannulas ( $P < 0.001$  and  $P < 0.01$ , respectively). No statistically significant difference was detected in adipocyte viability of the fatty tissue harvested with 2 and 4 mm cannulas ( $P > 0.05$ ) (Figures 1 and 2).

### Injection techniques

Statistically significant difference was not detected among fatty aspirates injected from 14, 16 and 20 g needles in terms of viable adipocyte content ( $P > 0.05$ ). Compared with fresh fatty tissue obtained with 6 mm cannulas, all of the injected specimens, regardless of needle sizes,



**Figure 1** (a) Microscopic appearance of viable adipocytes harvested with 2 mm cannula under 40 $\times$  magnification. (b) Microscopic appearance of viable adipocytes harvested with 6 mm cannula under 40 $\times$  magnification.



**Figure 2** Amounts of viable adipocytes/ml of fatty tissue obtained with different sizes of liposuction cannulas.

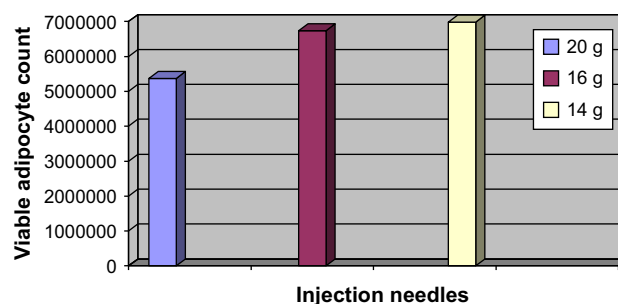
contained fewer viable adipocytes (statistically significant;  $P < 0.001$ ) (Figure 3).

### Storage temperatures

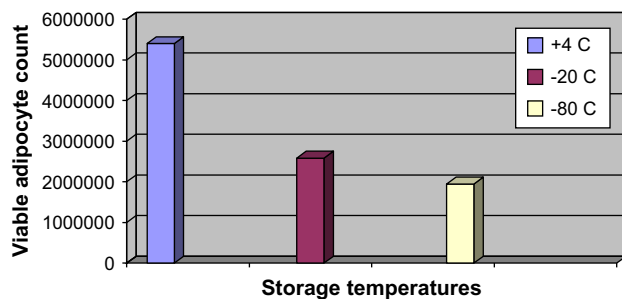
Compared to fresh fatty aspirate, frozen specimens (both  $-20$  and  $-80$  °C) contained significantly lower numbers of viable adipocytes ( $P < 0.01$  and  $P < 0.001$ , respectively). Storage of fatty tissue at  $+4$  °C decreased the live fat cells compared to fresh aspirate but it did not reach statistical significance ( $P > 0.05$ ) (Figure 4).

## Discussion

Fat grafts have been popular as soft tissue fillers for more than 100 years since they were first described by Neuber.<sup>2</sup> Especially since the introduction of liposuction, autogenous fat grafting has been in regular use.<sup>5,8</sup> The main problem with fat grafting procedures is resorption, which is reported to be in the range of 20 to 90%.<sup>3,9</sup> To decrease resorption rates and increase the longevity of the clinical results; various fat graft harvesting, preparation and transfer methods have been suggested. According to the Peer's cell survival theory; if the fat graft contains higher numbers of viable adipocytes, graft survival will increase.<sup>4</sup> Based on this theory we have investigated the viability of adipocytes in fatty tissues obtained with different sizes of liposuction cannulas to find the most advantageous size in terms of adipocyte viability. Although it was stated that larger cannulas are



**Figure 3** Amounts of viable adipocytes/ml of fatty tissue injected with different sizes of needles.



**Figure 4** Amounts of viable adipocytes/ml of fatty tissue stored at different temperatures.

better for fat graft survival, we could not detect a quantitative study showing the numbers of viable adipocytes aspirated with different sizes of suction cannulas.<sup>21,22</sup> In our study we showed that a 6 mm suction cannula provided more viable fatty tissue compared to smaller sizes of cannulas. All of the aspirations were performed with a 50 cc syringe and approximately 20 cc of fat aspiration was done with each of the cannulas to prevent possible differences in aspiration pressures which would have an effect on fat cell viability. In order to prevent possible effects of donor areas, all of the samples were obtained from abdomen. Hudson and Lambert<sup>23</sup> compared adipose tissue from the abdomen, gluteal-femoral region, breast and face and advocated the gluteal-femoral region as a donor region, because this region contains larger adipocytes and more lipogenic activity. However, Chajchir<sup>1</sup> detected no difference in fat graft survival in terms of fat graft donor areas in 319 lipofilling cases. Moreover, Rochrich et al.<sup>10</sup> have shown quantitatively that adipocyte viability does not differ according to the anatomic regions.

In liposuction procedures, adrenalin-containing local anesthetic solution infiltration is frequently used in order to prevent blood loss and to help postoperative analgesia.<sup>24</sup> We did not infiltrate any solution until the study samples were collected in order to prevent the dilution effect of the possible unequal infiltration of the solution in different parts of the abdomen. In clinical practice if the patient is operated on under general anaesthesia and lipofilling is done as an additional operation, fat graft harvesting could be performed without local anaesthetic infiltration to prevent the shown negative effects of lidocaine and adrenaline on adipocytes.<sup>6,19</sup>

In this study, following the aspiration of fatty tissue, syringes were turned back for gravity settling and after pouring the separated serum fatty samples were prepared directly without washing or centrifugation. The centrifugation, described in the methods section, was part of the laboratory investigation into adipocyte viability not fat graft preparation. There are different ideas in the literature related to centrifugation and washing. While Nguyen<sup>3</sup> and Lewis<sup>13</sup> have proposed the washing of the fat grafts, Chajchir<sup>1</sup> has stated that washing removes the fibrin content of the aspirate which is necessary for fat graft adherence that is why he has been opposed to washing. Rochrich et al. have shown quantitatively that neither washing nor centrifugation has any effect on fat cell viability.<sup>10</sup> Moreover, Smith and Adams have shown that, with regard to

the harvesting or preparation techniques (different combinations of centrifugation and/or washing the cells), fat cell viability does not differ as assessed by graft weight maintenance or histological evaluations.<sup>25</sup>

For the determination of the viable adipocyte count in 1 ml of the fatty aspirate, fat cell isolation was done by tissue digestion with collagenase type 1 enzyme as it was first described by Rodbell<sup>26</sup> and later modified by Moore.<sup>19</sup> Other kinds of enzymatic digestion techniques have been described in the literature in which collagenase type 2 or trypsin have been used.<sup>15,27</sup> Adipocytes were stained with brilliant cresyl supravital dye which is a basic dye that stains deoxyribonucleic acid of the viable cells.<sup>28</sup>

Our results demonstrated that fat grafts contain lower amounts of viable adipocytes when they are injected from needles. But no statistically significant difference was detected between the sizes of needles with regard to fat cell viability. Sommer and Sattler also have demonstrated that there is no relationship between the needle sizes and the longevity of the fat grafts.<sup>29</sup> On the other hand, Chajchir and Benzaquen<sup>30</sup> have proposed the use of larger sized needles (1.5–2 mm) for lipofilling to prevent damage to the fat cells. But these proposals have not been dependent on quantitative data.

Although a small patient collective is the limitation of our study, it was detected that storing fatty tissue at +4 °C provides highest numbers of viable adipocytes compared to dry frozen specimens (–20 and –80 °C). Lidagoster<sup>17</sup> has shown similar findings and stated that inflammatory reactions and tissue necrosis were more apparent in specimens that are stored at –16 °C than specimens stored at +1 °C. These histological findings increased as the time of storage increased from 1 week to 2 weeks. However, Shoshani<sup>16</sup> has demonstrated that there is no difference in histological findings of human fat grafts when they are transplanted to rats as a fresh tissue or a stored tissue at –18 °C for 2 weeks. Neither of these studies has quantitatively shown adipocyte viability in stored fatty tissue. They have determined the effect of the temperature of storage based on the histological findings of the fat grafts after transplantation. However viability of the fat grafts is affected by several factors including some differences in transplantation methods or recipient area properties. That is why it would be more specific to investigate adipocyte viability directly in fresh and stored fatty tissue samples.

Some practitioners in aesthetic surgery see a major advantage in the storage of frozen fatty tissue for future augmentations. However our study results showed that frozen specimens had the fewest vital adipocytes. This could be explained by adipocyte damage either during freezing or thawing procedures. It might be expected that, if proper cryoprotective agents are added, increased numbers of vital adipocytes could be stored. We believe that additional studies are necessary to see how the effects of slow cooling and further improvements in cryopreservation techniques may allow more viable adipocytes in stored fat grafts. Until then, +4 °C temperature storage of fatty tissue could be proposed since it is more practical and available compared to liquid nitrogen and more effective than freezing in terms of fat cell viability.

In conclusion, according to our study results, a 6-mm-diameter suction cannula provides the fatty tissue that

contains the highest numbers of viable adipocytes compared to smaller-diameter suction cannulas. Therefore, in order to increase the success of fat grafts, we propose the use of larger liposuction cannulas for fat graft harvesting. We have no suggestion regarding the size of injection cannulas for fat graft transplantation. For fat graft storage a temperature of  $+4^{\circ}\text{C}$  could be advised because it is more favourable for adipocyte viability compared to freezing for at least a 2-week period and it is more readily available and cheaper than liquid nitrogen.

## References

- Chajchir A, Benzaquen I, Wexler E, et al. Fat injection. *Aesthetic Plast Surg* 1990;**14**:127.
- Billings E, May JW. Historical review and present status of free fat graft autotransplantation in plastic and reconstructive surgery. *Plast Reconstr Surg* 1989;**83**:368.
- Nguyen A, Pasky KA, Bouvier TN, et al. Comparative study of survival of autologous adipose tissue taken and transplanted by different techniques. *Plast Reconstr Surg* 1990;**85**:378.
- Peer LA. Loss of weight and volume in human fat grafts: with postulation of a "cell survival theory". *Plast Reconstr Surg* 1950;**5**:217.
- Illouz YG. Body contouring by lipolysis: a 5 year experience with over 3000 cases. *Plast Reconstr Surg* 1983;**72**:591.
- Illouz YG. Present results of fat injection. *Aesthetic Plast Surg* 1988;**12**:175.
- Ellenbogen R. Free autologous pearl fat grafts in the face—a preliminary report of a rediscovered technique. *Ann Plast Surg* 1986;**16**:179.
- Courtiss EH. Suction lipectomy: a retrospective analysis of 100 patients. *Plast Reconstr Surg* 1984;**73**:780.
- Matsudo P, Toledo L. Experience of injected fat grafting. *Aesthetic Plast Surg* 1988;**12**:35.
- Rohrich RJ, Sorokin ES, Brown SA. In search of improved fat transfer viability: a quantitative analysis of the role of centrifugation and harvest site. *Plast Reconstr Surg* 2004;**113**:391.
- Novaes F, Reis N, Baroudi R. Counting method of live fat cells used in lipoinjection procedures. *Aesthetic Plast Surg* 1998;**22**:12.
- Lewis CM. Comparison of the syringe and pump aspiration methods of lipoplasty. *Aesthetic Plast Surg* 1991;**15**:203.
- Lewis CM. Transplantation of autologous fat. *Plast Reconstr Surg* 1991;**88**:1110.
- Har-Shai Y, Lindenbaum ES, Gamliel-Lazarovich A, et al. An integrated approach for increasing the survival of autologous fat grafts in the treatment of contour defects. *Plast Reconstr Surg* 1999;**104**:945.
- Niechajev I, Sevcuk O. Long term results of fat transplantation: clinical and histological studies. *Plast Reconstr Surg* 1994;**94**:496.
- Shoshani O, Ullmann Y, Shupak A, et al. The role of frozen storage in preserving adipose tissue obtained by suction-assisted lipectomy for repeated fat injection procedures. *Dermatol Surg* 2001;**27**:645.
- Lidagoster MI, Cinelli PB, Levee EM, et al. Comparison of autologous fat transfer in fresh, refrigerated and frozen specimens: an animal model. *Ann Plast Surg* 2000;**44**:512.
- Atik B, Ozturk G, Erdogan E, et al. Comparison of techniques for long term storage of fat grafts: an experimental study. *Plast Reconstr Surg* 2006;**118**:1533.
- Moore JH, Kolaczynski JW, Morales LM, et al. Viability of fat obtained by syringe suction lipectomy: effects of local anesthesia with lidocaine. *Aesthetic Plast Surg* 1995;**19**:335.
- Boschert MT, Beckert BW, Puckett CL, et al. Analysis of lipocyte viability after liposuction. *Plast Reconstr Surg* 2002;**109**:761.
- Shiffman MA, Mirrafati S. Fat transfer techniques: the effect of harvest and transfer methods on adipocyte viability and review of the literature. *Dermatol Surg* 2001;**27**:819.
- Asken S. Autologous fat transplantation: micro and macro techniques. *Am J Cosmet Surg* 1987;**4**:111.
- Hudson DA, Lambert EV, Bloch CE. Site selection for fat autotransplantation: some observations. *Aesthetic Plast Surg* 1990;**14**:195.
- Pitman GH. Liposuction and body contouring. In: Aston SJ, Beasley RW, Thorne CHM, editors. *Grabb and Smith's plastic surgery*. 5th Edition. Philadelphia: Lippincott-Raven; 1997. p. 673–5.
- Smith P, Adams Jr WP, Lipschitz AH, et al. Autologous human fat grafting: effect of harvesting and preparation techniques on adipocyte survival. *Plast Reconstr Surg* 2006;**117**:1836.
- Rodbell M. Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. *J Biol Chem* 1964;**239**:375.
- Rieck B, Schlaak S. Measurement in vivo of the survival rate in autologous adipocyte transplantation. *Plast Reconstr Surg* 2003;**111**:2315.
- Wang YT, Zhao FL, Li KA, et al. Molecular spectroscopy study of the reaction of nucleic acids with brilliant cresol blue. *Spectrochim Acta A Mol Biomol Spectrosc* 2000;**56**:1827.
- Sommer B, Sattler G. Current concepts of fat graft survival: histology of aspirated adipose tissue and review of literature. *Dermatol Surg* 2000;**26**:1159.
- Chajchir A, Benzaquen I. Fat grafting injection for soft tissue augmentation. *Plast Reconstr Surg* 1989;**84**:921.