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The Current State of Fat Grafting: A Review of Harvesting, Processing, and Injection Techniques

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Abstract

Background—Interest in and acceptance of autologous fat grafting for use in contour abnormalities, breast reconstruction, and cosmetic procedures have increased. However, there are many procedural variations that alter the effectiveness of the procedure and may account for the unpredictable resorption rates observed.

Methods—The authors highlighted studies investigating the effects of harvesting procedures, processing techniques, and reinjection methods on the survival of fat grafts. This review focused on the impact different techniques have on outcomes observed in the following: in vitro analyses, in vivo animal experiments, and human studies.

Results—This systemic review revealed the current state of the literature. There was no significant difference in the outcomes of grafted fat obtained from different donor sites, different donor-site preparations, harvest technique, fat harvesting cannula size, or centrifugation speed, when tumescent solution was used. Gauze rolling was found to enhance the volume of grafted fat, and no significant difference in retention was observed following centrifugation, filtration, or sedimentation in animal experiments. In contrast, clinical studies in patients found more favorable outcomes with fat processed by centrifugation compared with sedimentation. In addition, higher retention was observed with slower reinjection speed and when introduced into less mobile areas.

Conclusions—There has been a substantial increase in research interest to identify methodologies for optimizing fat graft survival. Despite some differences in harvest and implantation technique in the laboratory, these findings have not translated into a universal protocol for fat grafting. Therefore, additional human studies are necessary to aid in the development of a universal protocol for clinical practice.

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Autologous fat grafting has become a common technique for treating volume and contour abnormalities in aesthetic and reconstructive surgery. A recent survey showed that approximately 80 percent of plastic surgeons have used fat grafting in their practice.[1] Fat grafting has been used for facial contouring, breast augmentation, radiation damage, breast capsular contracture, posttraumatic deformities, congenital anomalies, and burn injuries.[2–8] Autologous fat grafts have several beneficial characteristics, including lack of immunogenicity, simple surgical procedure, low cost, and easy accessibility. Fat grafts are harvested from a region that is generally more abundant and injected into a secondary site. The initial isolated adipose tissue is composed of adipocytes and stromal vascular fraction cells, which include adipose stem cells, preadipocytes, fibroblasts, vascular endothelial cells, and a variety of immune cells.[9] It has become apparent through extensive research in the past decade that stromal vascular fraction cells and adipose stem cells might improve fat graft survival, largely through their angiogenic properties.[10,11]

Although autologous fat grafting is a common technique used today by many plastic surgeons, fat grafting was not widely accepted until the 1980s. Early reports of fat grafting by Neuber, Czerny, and Holländer demonstrated the positive, natural appearing results achievable with fat grafting for facial and breast reconstruction.[12–14] However, despite these early successes, subsequent reports were met with varying levels of failure, often associated with asymmetry caused by fat resorption.[15,16] Beginning in the early 1980s, several positive reports of fat grafting were published, and subsequently, fat grafting increased in popularity once again.[17–19] However, many who initially reported failure demonstrated the importance of the techniques used to achieve desirable long-term outcomes for the treatment of a multitude of aesthetic and reconstructive conditions. In this review, we summarize the body of literature describing different methods of isolation, processing, and reinjection. These studies have improved our understanding of fat grafting and retention and the cellular elements critical for inclusion in fat grafts to ultimately modify and enhance our fat grafting techniques for improved long-term outcomes.

DONOR SITE

Although identification of the donor site is often based on the location of excess adipose tissue, identifying the optimal donor site will help guide surgical approaches (Table 1).[20–25] Rohrich et al. found no significant difference in cell viability in adipose tissue removed from the abdomen, flank, thigh, and knee with hand-held syringe aspiration.[20] Ullmann et al. injected processed lipoaspirates isolated from the abdomen, breast, and thigh into nude mice and observed no significant difference in the volume of fat grafts.[21] Li and colleagues injected processed lipoaspirates isolated from the flank, upper abdomen, lower abdomen, lateral thigh, and inner thigh into nude mice and observed no significant difference in volume or histological parameters among the grafts even after 12 months posttransplantation.[22]

Studies in humans have also found no significant difference in volume retention of fat grafts formed from abdominal and nonabdominal donor sites. Injected adipose tissue isolated from abdominal and nonabdominal donor sites equally contoured and corrected asymmetry in patients with craniofacial microsomia or Treacher Collins syndrome.[23] Fat transfer for breast

augmentation from the abdomen or thigh demonstrated no significant difference in fat graft volume.[24] Fat grafts formed from the thigh demonstrated greater structural integrity, less cyst formation, less necrosis, and reduced fibrosis, whereas tissue formed from abdominal adipose tissue demonstrated increased vascularity.[21] This increased structural integrity and vascularity could be attributable to greater adipose stem cell viability from the inner thigh and abdomen compared with other regions.[25] Additional analyses are necessary to determine whether these findings translate into differences in long-term retention. Nevertheless, the current literature suggests that there is no significant difference in the volume or weight of the grafted fat obtained from different donor sources.

DONOR-SITE PREPARATION

Tumescent anesthesia was initially developed to perform liposuction procedures with local anesthesia, and many benefits to using tumescent solution have been described, including reduced pain, reduced blood loss, and improved ease of fat removal.[26,27] However, it is unknown whether the infusion of tumescent solution before liposuction adversely impacts fat graft survival (Table 2).[28–32] Agostini and colleagues demonstrated that exposure to tumescent solution enhanced the adipocyte viability in adipose tissue compared with cells isolated by means of dry technique.[28] Exposure to epinephrine in the presence of lidocaine did not permanently alter adipose tissue functions or the metabolic activity of adipocytes.[28,29] Furthermore, the implantation of adipose tissue pretreated with saline or lidocaine and epinephrine demonstrated no significant effect on fat graft volumes or histologic architecture.[30,31] Analysis of different anesthesia drugs demonstrated greater adipose stem cell viability within adipose tissue treated with bupivacaine, mepivacaine, ropivacaine, and lidocaine compared with combined treatment with articaine and epinephrine.[32] Although one would not expect variability between amides, epinephrine may affect the α_1 receptors on the surrounding tissues supporting the implanted cells. Overall, tumescent solution improved cell viability compared with dry technique, and no significant difference was observed between the commonly used anesthesia drugs, with the exception of articaine delivered with epinephrine. It would seem difficult to provide a mechanism for improved fat graft survival resulting from a brief exposure to local anesthetics. However, based on the current studies, it appears that using tumescent solution at the time of fat graft harvest does not have a detrimental effect on fat graft cell viability and may even enhance viability.

METHOD OF HARVEST

Studies investigating the impact of hand-held syringe aspiration, suction-assisted lipectomy, and ultrasound-assisted lipectomy have demonstrated differences in cell viability and adipocyte functionality resulting from these different methods of isolation (Table 3).[20,33,35–41] Adipocyte functionality was analyzed by glycerol-3-phosphate dehydrogenase enzyme assay, which assesses the leakage of this lipogenic enzyme through the plasma membrane during cellular damage. Histologically, adipose tissue isolated by suction-assisted lipectomy and ultrasound-assisted lipectomy displayed no evidence of cellular damage, and physiologically, the cells isolated from either techniques were noted to be viable, with normal enzymatic activity.[33–35] Compared with suction-assisted lipectomy, several studies

have shown that hand-held syringe lipoaspirates yielded greater adipocyte count and viability.[36–38] However, when tumescent solution was used to prepare the donor site, no significant difference was observed in cell counts or viability between hand-held syringe aspiration and suction-assisted lipectomy.[39] To further assess the impact of hand-held syringe, suction-assisted lipectomy, and ultrasound-assisted lipectomy, lipoaspirates isolated from these techniques were injected into immunocompromised mice. No significant difference in the volume or weight of the fat grafts isolated from the different methods of isolation was observed.[38,40,41] Based on these, the method used to harvest fat is less important, as adipocyte survival was comparable among the different harvest methods. Although these results provide significant insight into the early engraftment of fat, the short period for which the fat grafts were maintained in mice (4, 6, or 12 weeks) does not allow for long-term assessment of retention. As fat grafting implants remain in patients for years, additional studies investigating time points that extend beyond 12 weeks will provide the necessary evidence to evaluate the impact of different lipoaspirate methods on long-term retention.

LIPOSUCTION CANNULA

Studies investigating differences in liposuction cannula and cannula size have shown that the use of a larger diameter cannula enhances cell viability (Table 4). Erdim et al. demonstrated enhanced adipocyte viability in lipoaspirates isolated with a 6-mm cannula, compared with a 2-mm and 4-mm cannula.[42] Consistent with in vitro reports, Kirkham and colleagues demonstrated that lipoaspirates harvested with a larger cannula size (5-mm diameter) formed larger fat grafts in nude mice after 6 weeks.[43] These fat grafts demonstrated increased histologic integrity, less immune infiltration, and less fibrosis, compared with fat grafts formed with lipoaspirates isolated with a 3-mm cannula.[43] Nevertheless, it should be noted that, in these reports, fat grafts were harvested without tumescent solution. Assessment of cannula size during liposuction in the presence of tumescent solution may be useful, as use of tumescent solution is now considered common practice. In addition to cannula size, reports comparing a multiperforated cannula with the Coleman 3-mm aspiration cannula have shown no significant difference in cell viability or size of the engrafted fat tissue.[44,45]

PROCESSING METHODS

Studies have evaluated the impact on cell viability of various adipose tissue processing techniques, such as centrifugation, gravity separation, washing, and filtration (Table 5).[10,20,38,41,46–54] national consensus survey concluded that of the plastic surgeons that perform fat grafting, 34 percent used centrifugation, 45 percent used gravity separation, 34 percent used filtration, 11 percent used gauze rolling, 3 percent did not process the tissue before engraftment, and 7 percent performed some other techniques not specified.[1] Identification of an optimal processing method will increase the number of viable cells and ultimately increase fat engraftment and retention over time.

Studies have also shown that processing techniques that maintain greater concentrations of stromal vascular fraction cells and adipose stem cells may enhance fat engraftment and retention; therefore, processing methods should be optimized to increase the number of

viable stromal vascular fraction cells and adipose stem cells. Recently, studies have shown that cotton gauze processing of lipoaspirates, compared with centrifugation, filtration, and washing, increased viable cells and fat graft size.[41] These stromal vascular fraction cells and adipose stem cells were shown to increase the volume of fat grafts by enhancing angiogenesis and adipogenic differentiation.[46] In vivo animal studies have also shown that cotton gauze processing resulted in the largest fat grafts and that the engrafted fat maintained the greatest structural architecture, compared with centrifugation.[41] Nevertheless, the method by which cotton gauze processing of lipoaspirates enhances stromal vascular fraction cell or adipose stem cell count, compared with other methods of processing, remains to be determined.

Processing of lipoaspirates by filtration and centrifugation resulted in smaller fat grafts in animal studies, which correlate with studies demonstrating reduced cell proliferation, reduced nucleated adipocytes, and poorer architectural integrity following centrifugation and washing.[20,47,48] However, it is also possible that the filtration method or centrifugation speed could account for the amount of damage in the adipose tissue. Centrifugation speed and the density of cells may account for the reduced size of fat grafts and is discussed in the following sections. Comparing centrifugation, filtration, and sedimentation methods, no significant difference in the weight or architecture of the fat grafts was observed in animal experiments.[10,38,49,50]

In contrast, studies conducted in patients demonstrated more favorable outcomes with centrifugation compared with gravity separation. Butterwick demonstrated more satisfactory results following injection of centrifuged lipoaspirates with increased fullness and smoothness of the hand, compared with gravity-separated lipoaspirates.[51] Comparative studies investigating the effects of fat processing with centrifugation, washing, and filtration have showed no significant difference in fat retention; however, filtration resulted in nodule formation, whereas centrifugation did not.[52,53] In summary, despite the rapidly growing body of literature, there is still a high degree of discordance attributable to the inconsistent results from animal studies and human experiments. One technique is clearly not superior to any other technique when all the data are evaluated. Additional studies are necessary to identify the optimal technique for adipose tissue processing.

CENTRIFUGATION SPEED/FORCES

Centrifugation produces a centripetal force that is represented as the relative centrifugal forces and is comparable between different centrifuges. Therefore, our discussion will focus on the effects of relative centrifugal forces on fat grafting.

Similar to the amount of negative pressure applied during suction-assisted lipectomy, the positive pressure applied during centrifugation may significantly impact the lipoaspirates (Table 6).[40,55–58] Ferraro et al. demonstrated that centrifugation with a force greater than 50 *g* resulted in damage to the structural integrity of adipose tissue, increased necrosis and apoptosis of cells, decreased adipogenic differentiation capacity, and decreased tubule formation.[55] Tubule formation during angiogenesis provides blood supply and nutrients to adipose tissue and ultimately sustains the fat graft for long-term retention.[59] Higher

centrifugation speeds have also been correlated with increased fluid proportion, reduced injectable tissue volume, and increased oil portion, which are associated with damage to adipocytes.[56] However, centrifugation had no effect on the number of viable stromal vascular fraction cells or the weight of the fat grafts 4 weeks after injection into nude mice with forces as high as 4200 *g*.[56] Lee et al. showed that lipoaspirates processed by centrifugation at 10,000 *g* resulted in the largest fat grafts after 4 weeks after injection into nude mice.[40] Variability between the studies may be attributed to differences in the centrifuge machine used and the time the adipose tissue was centrifuged. Furthermore, suction-assisted lipectomy may increase the vulnerability of the adipose tissue, such that additional manipulation by centrifugation may result in significant damage.

In contrast, the studies investigating the impact of centrifugation following hand-held syringe liposuction demonstrated no significant effect of centrifugation speed (92 to 20,627 *g*) on cell viability or the weight of fat grafts.[57,58] In fact, higher centrifugation forces resulted in less debris and red blood cell contaminants in the injectable layer.[57,58] These findings suggest that centrifugation following hand-held syringe liposuction may be necessary to remove tissue debris and prevent fibrosis in fat grafts.[57,58] These results further suggest that hand-held syringe lipoaspiration, compared with suction-assisted lipectomy, may expose adipose tissue to less trauma, which may in turn allow the adipose tissue to withstand higher centrifugation speeds without experiencing cellular damage.

DENSITY OF PROCESSED TISSUE

Studies have also shown that centrifugation resulted in different densities of cells within the injectable layer (Table 7).[36,60–62] Previous work has shown that the lower layer of the injectable layer yielded the greatest density of viable cells (high-density fat), whereas the upper portion of this layer yielded the least number of viable cells (low-density fat) (Fig. 1).[36,60] Furthermore, injection of high-density fat into FVB/NJ immunocompetent mice resulted in the largest fat graft 2 and 10 weeks after transplantation.[61] Histologic assessment of the fat graft formed with high-density fat demonstrated an increased number endothelial cells, a reduced number of collagen bands, and decreased fibrosis. These results correlated with increased expression of angiogenic factors such as vascular endothelial growth factor, stromal derived factor 1-alpha, platelet-derived growth factor, and adiponectin. To enhance engraftment and retention of low-density fat, Butala and colleagues[62] exposed low-density fat to plerixafor (AMD3100), an immunostimulant that has previously been shown to mobilize hematopoi-etic stem cells and aid in angiogenesis. Low-density fat grafts treated with AMD3100 were comparable in size to high-density fat grafts, suggesting that low-density fat may generate equally large fat grafts, provided that mobilization of mesenchymal stem cells into the adipose tissue occurs. These studies also suggest the importance of the host response to healing and maintaining the fat grafts.

REINJECTION

Delivery of processed lipoaspirates into the recipient site requires significant care to generate desirable outcomes, particularly for cases involving facial contouring (Table 8).[23,40,63–66] Coleman described the placement of processed lipoaspi-rates with a Luer-Lok

syringe connected to a 17-gauge blunt cannula.[4,17] As described by Cole-man, fatty tissue should only be injected as the cannula is withdrawn to allow the fatty tissue to fall into the natural tissue planes as the cannula is removed.[4] A slow injection speed of 0.5 to 1.0 ml/second resulted in larger fat grafts compared with a fast injection speed of 3.0 to 5.0 ml/second.[40] Increasing injection speeds can result in cellular damage caused by sheer stress and greater collagen deposition and immune infiltration within the fat grafts.

RECIPIENT-SITE VARIABLES

Studies investigating the effect of the recipient site on fat grafting and retention have been inconclusive. Early reports of processed lipoaspi-rates injected into the muscle of rabbits resulted in better outcomes compared with the dermis, likely because of the increased vascularization.[63] However, Rieck and Schlaak demonstrated reduced fat retention following fat grafting to the muscle, which was attributed to increased mobilization.[64] Mobile areas of the face, such as the glabella and lips, were also less amenable to correction, compared with less mobile areas, such as the malar and lateral cheek.[67] Other variables including the age of the patient, trauma to the overlying skin, and the severity of the structural defect may influence fat grafting and the longevity of the graft. With advancing age and progressive loss of facial fullness, the subcutaneous fat deposits and underlying soft-tissue and skeletal structures become more prominent.[68] Rohrich et al. demonstrated that restoration of selective fat compartments with fat grafts to precisely control facial contouring generated a more natural and youthful appearance.[69] Aging is also associated with poor revascularization, which may be associated with lower volume retention from fat grafts. Patients with severe burns or underlying structural deficits may also require serial transplantation to overcome the scarred, fibrotic, and compromised recipient site.[65,70,71] Severe structural defects caused by progressive hemifacial atrophy have also been shown to improve with autologous fat grafting. However, poorer engraftment was noted in progressive hemifacial atrophy patients, suggesting that these more difficult cases may require the use of serial injections to achieve the desired volume.[72,73]

In addition to understanding the potential differences between recipient sites, work reported by del Vecchio and Bucky demonstrated the effectiveness of recipient-site preparation or preconditioning on fat grafting.[66,74] Patients underwent preoperative nonsurgical breast expansion with Brava domes (Brava, LLC, Miami, Fla.) for 3 weeks before implantation of processed lipoaspirates and 3 weeks postoperatively. Application of Brava domes increased parenchymal space, reduced interstitial pressure in the breast, and reduced contouring irregularities. In a 6-year prospective multicenter study, patients who underwent preoperative expansion with Brava domes had larger breast augmentations, minimal fat graft necrosis and complications, and higher graft survival rates.[75] Additional studies are necessary to determine whether this process is applicable to fat grafting to other recipient sites.

OTHER CONSIDERATIONS

Although these studies provide an overview of the current state of the literature, several other factors deserve mentioning. It is important to consider each patient independently, as differences in underlying disease process or patient variability may significant impact

engraftment. Patient variability presents as a challenge when deciding the appropriate volume of lipoaspirate to inject. Studies have shown significant differences in the number of stromal vascular fraction cells and adipose stem cells in lipoaspirates between patients, which may account for the differences observed between patients.

Stromal vascular fraction cells and adipose stem cells have recently gained significant attention because of their increased angiogenic and wound healing capacity. Whether supplementing lipoaspirates with stromal vascular fraction cells or adipose stem cells or enriching these cells through centrifugation, both of these methods have yielded larger fat grafts and longer retention of these grafts.[54,76–80] Gentile et al. demonstrated that patients treated with stromal vascular fraction-enhanced autologous fat grafts maintained 63 percent of the fat graft volume after one year compared to the 39 percent maintained in the control group.[77] Comparable results have been achieved with adipose stem cell supplementation.[78] Kølle et al. found that adipose stem cell supplementation of lipoaspirates enhanced the formation of new connective tissue and reduced the amount of necrotic tissue of the fat graft.[80] Zhu et al. demonstrated that lipoaspirates supplemented with adipose stem cells also improved long-term graft retention and induced angiogenesis through the expression of key angiogenic factors, including vascular endothelial growth factor-α, hepatocyte growth factor, and insulin-like growth factor-1.[54] Although studies have extensively characterized the expression of angiogenic factors secreted by adipose stem cells,[81] few studies have examined the composition of stromal vascular fraction cells and the impact of a mixed population of cells on angiogenesis. Additional comparative studies in both basic science and clinical settings are necessary to determine whether stromal vascular fraction or adipose stem cell supplementation results in larger fat grafts with longer retention.

Patients with severe burns or underlying structural deficits may require serial transplantation to overcome the scarred, fibrotic, and compromised recipient site. Serial transplantation may be more beneficial in some cases to reduce the burden of the underlying tissue to undergo angiogenesis and enhance long-term survival of fat grafts.[23,67] Additional human studies will provide a foundation for the future of fat grafting and further increase the use of fat grafting in clinical practice.

CONCLUSIONS

Fat grafting has provided plastic and reconstructive surgeons with an exciting tool with which to address challenging asymmetries and contour irregularities. There has been a substantial increase in research interest to identify methodologies for optimizing fat graft survival by targeting both the adipocytes and stromal vascular fraction cells. Despite some differences in harvest and implantation technique in the laboratory, these findings have not translated into a universal protocol for fat grafting. Furthermore, no Level I or Level II data exist to warrant a consensus recommendation for clinical practice. Therefore, additional human studies are necessary to aid in the development of a universal protocol for clinical practice.

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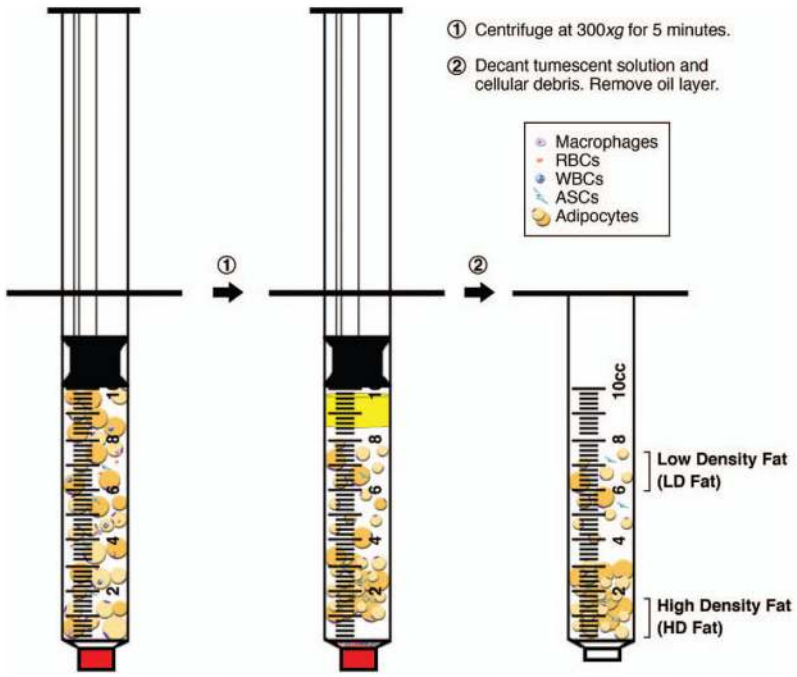

Fig. 1.

Representative schematic of syringe after centrifugation demonstrating the varying densities of processed lipoaspirates.

Table 1

Studies Investigating the Effects of Harvest Site

Reference	Analysis Type	Primary Objective	Detailed Methods	Results
Rohrich et al., 2004[20]	In vitro	Assess different harvest sites: abdomen, flank, thigh, and medial knee	Dry technique; hand-held syringe aspiration; processed by centrifugation at 500 *g* for 2 min	No difference in cell viability between harvest sites
Padoin et al., 2008[25]	In vitro	Assess different harvest sites: upper abdomen, lower abdomen, trochanteric region, inner thigh, knee, and flank	Tumescent solution; SAL; processed by centrifugation at 450 *g* for 5 min; SVF cell isolation was performed with collagenase for 1 hr at 37°C	Greatest number of viable cells was harvested from the lower abdomen > thigh > knee
Ullmann et al., 2005[21]	In vivo animal experiments	Assess different harvest sites: abdomen, breast, and lateral thigh	Tumescent solution; hand-held syringe aspiration; processed by centrifugation at 1500 rpm for 5 min; processed lipoaspirates were injected into CD-1 nude mice and assessed after 16 wk	Thigh tissue resulted in the best overall fat graft in mice, with greatest structural integrity, least cyst formation, least necrosis, and least inflammation and fibrosis; abdominal tissue formed xenografts with increased vascularity; breast tissue formed the least ideal grafts, with increased cyst formation, necrosis, fibrosis, and inflammation and fibrosis
Li et al., 2013[22]	In vivo animal experiments	Assess different harvest sites: upper abdomen, lower abdomen, flank, lateral thigh, and inner thigh	Tumescent solution; hand-held syringe aspiration; processed by centrifugation at 1000 rpm for 3 min; processed lipoaspirates were injected into BALB/c-nu nude mice and assessed after 12 wk	No statistically significant difference between the five donor sites with respect to graft weight and volume after 12 wk
Lim et al., 2012[23]	Human studies	Analysis of different harvest sites: abdomen, other regions	Tumescent solution; hand-held syringe aspiration; processed by centrifugation at 1200 J for 3 min; processed lipoaspirates were injected into patients with craniofacial microsomia or Treacher Collins syndrome	Adipose tissue harvested from abdominal or non- abdominal sources equally corrected asymmetry
Small et al., 2014[24]	Human studies	Analysis of different harvest sites: abdomen and thigh	Tumescent solution; suction- assisted liposuction; processed by centrifugation at 3000 rpm for 3 min; processed lipoaspirates were injected into patients for breast augmentation	No difference in volume retention in fat grafts formed from different donor sites

SAL, suction-assisted lipectomy; SVF, stromal vascular fraction.

Table 2

Studies Investigating the Effects of Harvest-Site Preparation

Reference	Analysis Type	Primary Objective	Detailed Methods	Results
Moore et al., 1995[29]	In vitro	Compare saline, lidocaine, and lidocaine/epinephrine	Saline, lidocaine, or lidocaine/epinephrine exposure; SAL; processed by centrifugation at 450 *g* for 5 min; SVF cell isolation was performed with collagenase for 1 hr at 37°C and exposed to lidocaine	Adipose tissue exposed to saline, lidocaine, and lidocaine/epinephrine exposure demonstrated no difference in cell viability; SVF cells treated with lidocaine demonstrated diminished cell attachment and differentiation, but the effects are reversible with washing
Keck et al., 2010[32]	In vitro	Compare bupivacaine, mepivacaine, ropivacaine, articaine/epinephrine, and lidocaine	Excision of adipose tissue; SVF cell isolation was performed followed by exposure to bupivacaine, mepivacaine, ropivacaine, articaine/epinephrine, or lidocaine	Highest cell viability was bupivacaine > mepivacaine and ropivacaine > lidocaine > articaine/epinephrine; adipogenic differentiation was high among all groups except for articaine/epinephrine
Agostini et al., 2012[28]	In vitro	Compare dry technique with lidocaine/epinephrine	Dry technique or lidocaine/epinephrine exposure; hand-held syringe aspiration	Adipocytes obtained from lidocaine/epinephrine adipose tissue showed greater surface area and viability; no difference in architecture of adipocytes
Shoshani et al., 2005[31]	In vivo animal experiments	Compare saline and lidocaine/epinephrine	Saline or lidocaine/epinephrine exposure; SAL; processed by centrifugation at 1500 rpm (377 *g*) for 5 min; processed lipoaspirates were implanted into CD-1 nude mice and assessed after 15 wk	No difference between saline or lidocaine/epinephrine in weight, volume, or histology of xenografts (e.g., fibrosis, inflammation, cyst, integrity)
Livaoglu et al., 2012[30]	In vivo animal experiments*	Compare saline, lidocaine/epinephrine, and prilocaine	Saline, lidocaine/epinephrine, or prilocaine exposure; excision of adipose tissue; adipose tissue was implanted into rats and assessed after 26 wk	No difference between the groups in weight and volume of grafts

SAL, suction-assisted lipectomy; SVF, stromal vascular fraction. *Indicates source of tissue is from rat.

Table 3

Studies Investigating Adipose Tissue Isolation Technique

Reference	Analysis Type	Primary Objective	Detailed Methods	Results
Rohrich et al., 2000[20]	In vitro	Compare SAL, internal UAL, external UAL, and massage	Tumescent solution; SAL, internal UAL, external UAL, or massage for 7 min	Internal UAL resulted in 70–90% damage to the adipocytes, with significant thermal liquefaction; SAL and external UAL resulted in minimal histologic disruption of adipose tissue
Pu et al., 2005[33]	In vitro	Compare excised adipose tissue and SAL	Tumescent solution; excision or SAL; processed by centrifugation at 50 *g* for 10 min	SAL resulted in minimal damage to adipocyte viability; G3PDH assay demonstrated reduced cellular functionality of adipocytes following liposuction and processing; SAL resulted in slight histologic disruption
Pu et al., 2008[37]	In vitro	Compare hand-held syringe aspiration and SAL	Tumescent solution; hand-held syringe aspiration or SAL; hand-held syringe lipoaspirates were processed by centrifugation at 3000 rpm for 3 min; SAL aspirates were processed by centrifugation at 500 rpm for 10 min	Hand-held syringe lipoaspirates yielded greater adipocyte viability; G3PDH assay demonstrated greater viability with hand-held syringe aspiration; no difference histologically between hand-held syringe aspiration and SAL
Crawford, et al., 2010[36]	In vitro	Compare hand-held syringe aspiration and SAL	Tumescent solution; hand- held syringe aspiration with the Viafill system or SAL; processed by centrifugation at 50 *g* for 2 min	Hand-held syringe aspiration resulted in greater adipocyte count
Schafer et al., 2013[35]	In vitro	Assess UAL	Tumescent solution; UAL applied with 3.7-mm probe at 60% pulsed mode; processed by centrifugation at 400 *g* for 5 min	Histologically intact adipocytes and vasculature following UAL; viability (88%) remained unaffected by UAL
Keck et al., 2014[39]	In vitro	Compare hand-held syringe aspiration and SAL	Dry technique; hand-held syringe aspiration or SAL; processed by centrifugation at 380 *g* for 5 min	No difference in cell counts or cell viability between hand-held syringe aspiration and SAL; hand-held syringe aspiration resulted in slightly greater oil release; cells isolated from SAL differentiated into adipocytes expressed higher levels of adiponectin, GLUT4, and PPAR-γ
Smith et al., 2006[36]	In vivo animal experiments	Compare hand-held syringe aspiration and SAL	Tumescent solution; hand-held syringe aspiration or SAL; nonprocessed tissue and processed tissue centrifuged at 500 *g* for 2 min were injected into SCID mice and assessed after 12 wk	Hand-held liposuction resulted in greater adipocyte viability; no difference in xenograft volumes between hand-held liposuction and SAL, with or without processing
Lee et al., 2013[40]	In vivo animal experiments	Compare SAL set to -15 inHg and -25 inHg	Tumescent solution; SAL at -15 inHg or -25 inHg; injected into the flank of nude mice and assessed after 4 wk	No difference in weight or volume between xenografts formed with lipoaspirates isolated with −15 inHg or −25 inHg
Fisher et al., 2013[41]	In vivo animal experiments	Compare UAL and SAL	Tumescent solution; UAL applied with 2.9-mm probe at 60% pulse mode or SAL at 430 mmHg; lipoaspirates were injected into nude mice and assessed after 6 wk	No difference in SVF cell counts between UAL and SAL; no difference in weight or volume between xenografts formed with lipoaspirates from UAL or SAL

SAL, suction-assisted lipectomy; UAL, ultrasound-assisted lipectomy; G3PDH, glycerol-3-phosphate dehydrogenase; PPAR-γ, peroxisome proliferator-activated receptor gamma; SCID, severe combined immunodeficiency; SVF, stromal vascular fraction.

Table 4

Studies Investigating the E_ects of Cannula Size

Reference	Analysis Type	Primary Objective	Detailed Methods	Results
Erdim et al., 2009[42]	In vitro	Compare 2-mm, 4-mm, and 6-mm aspiration cannula size	Dry technique; hand-held syringe aspiration isolation from patients; ex vivo tumescent solution infiltration and liposuction; gravity separation; adipocyte isolation was performed with collagenase	6-mm aspiration cannula resulted in the greatest adipocyte viability
Alharbi et al., 2013[44]	In vitro	Compare the Coleman 3-mm aspiration cannula and the st'RIM multiperforated cannula	Tumescent solution; hand-held syringe aspiration; SVF cell isolation was performed with collagenase for 45 min at 37°C	No difference in SVF cell number
Alharbi et al., 2013[44]	In vivo animal experiments	Compare the Coleman 3-mm aspiration cannula and multiperforated cannula	Dry technique; hand-held syringe aspiration; processed by centrifugation at 3000 rpm for 3 min; processed lipoaspirates were injected into the flank of nude mice with 17- and 20-gauge needles and assessed after 12 wk	No difference in the weight between xenografts formed; no difference histologically in adipocyte architecture or vasculature
Alharbi et al., 2013[44]	In vivo animal experiments	Compare 3-mm and 5-mm aspiration cannula size	Dry technique; SAL at −25 inHg; processed by centrifugation at 200 *g*; processed lipoaspirates were injected into the flank of nude mice and assessed after 6 wk	5-mm aspiration cannula resulted in larger xenografts; xenografts formed with 5-mm aspiration cannula demonstrated histologically greater adipocyte architecture; xenografts formed with 3-mm aspiration cannula demonstrated greater infiltrating immune cells, greater fibrosis, and decreased intact adipocytes

SVF, stromal vascular fraction; SAL, suction-assisted lipectomy.

Table 5

Studies Investigating Processing Techniques

Reference	Analysis Type	Primary Objective	Detailed Methods	Results
Rohrich et al., 2004[20]	In vitro	Compare with and without centrifugation	Dry technique; hand-held syringe aspiration; processed by centrifugation at 500 *g* for 2 min	Centrifugation reduced cell proliferation
Rose et al., 2006[48]	In vitro	Compare gravity separation, centrifugation, and manual washing/centrifugation	Tumescent solution; hand-held syringe aspiration; processed by gravity separation, centrifugation at 3000 rpm (6000 *g*) for 3 min, or manual washing with saline/centrifugation at 3000 rpm (6000 *g*) for 3 min	Centrifugation and washing reduces cell number, nucleated adipocytes, and cross-sectional area of adipocytes
Condé-Green et al., 2010[47]	In vitro	Compare gravity separation and centrifugation	Tumescent solution; SAL; processed by gravity separation or centrifugation at 3000 rpm for 3 min	Centrifugation resulted in damage to the integrity of the adipose tissue; centrifugation reduced immune cells
Zhu et al., 2010[54]	In vitro	Compare gravity separation, centrifugation, and washing/filtering	Tumescent solution; suction-assisted liposuction; processed by gravity separation for 20 min, centrifugation at 3000 rpm (1200 *g*) for 3 min, or machine washing/filtering	Washing/filtrating yielded the least amount of RBCs and WBCs, reduced lipid content, and increased functional adipocytes
Pfaff et al., 2014[46]	In vitro	Compare centrifugation and cotton gauze processing	Tumescent solution; hand-held syringe aspiration; processed by centrifugation at 1500 rpm for 3 min or cotton gauze rolling with large pieces of nonadherent dressing for 30 sec; SVF cell isolation was performed with collagenase for 1 hr at 37°C	Cotton gauze rolling resulted in a greater number of SVF cells isolated from adipose tissue; cotton gauze rolling increased the number of stem cells isolated from lipoaspirates
Minn et al., 2010[49]	In vivo animal experiments	Compare centrifugation, cotton gauze processing, and metal sieve filtering	Tumescent solution; hand-held syringe aspiration; processed by centrifugation at 1800 *g* for 3 min, gauze filtration for 3 min, or metal sieve filtration for 3 min; processed lipoaspirates were injected into BALB/c-nu mice and assessed after 4 and 12 wk	No difference in xenograft weight between processing techniques; metal sieve filtration increased fat necrosis in xenografts; centrifugation enhanced the vascularity of xenografts
Ramon et al., 2005[50]	In vivo animal experiments	Compare centrifugation and towel processing	Tumescent solution; hand-held syringe aspiration; processed by centrifugation at 1500 rpm for 5 min or towel processing by placing lipoaspirates on a towel to remove fluid, oil, and debris; processed lipoaspirates were injected into nude mice and assessed after 16 wk	No difference in xenograft weight between processing techniques; histologic evaluation of grafts revealed less fibrosis with towel processing
Smith et al., 2006[38]	In vivo animal experiments	Compare centrifugation, washing, and washing/centrifugation	Tumescent solution; hand-held syringe aspiration or SAL; processed by centrifugation at 500 *g* for 2 min, washing with lactated Ringer solution, washing with 0.9% saline, washing with lactated Ringer solution and centrifugation, or washing with normal saline and centrifugation; processed lipoaspirates were injected into SCID mice and assessed after 12 wk	Centrifugation reduced adipocyte viability; no difference in xenograft weight between processing techniques; variable amounts of fat necrosis and inflammation were observed among samples
Fisher et al., 2013[41]	In vivo animal experiments	Compare centrifugation, cotton gauze processing, and filtering	Tumescent solution; hand-held syringe aspiration was performed for processing by centrifugation and cotton gauze rolling, while SAL was performed for filtering; processed by centrifugation at 3000 rpm (1200 *g*) for 3 min, cotton gauze processing with large pieces of nonadherent dressing, or filtration; processed lipoaspirates were injected into nude mice and assessed after 6 wk; SVF cell isolation was performed with collagenase for 1 hr at 37°C	Cotton gauze rolling removed the oil and the aqueous fraction most efficiently, followed by filtering and centrifugation; cotton gauze rolling resulted in the highest number of SVF cells isolated from adipose tissue; cotton gauze rolling resulted in the highest fat grafts > filtration > centrifugation; xenografts

Reference	Analysis Type	Primary Objective	Detailed Methods	Results
				demonstrated similar histologic architecture
Condé-Green et al., 2013[10]	In vivo animal experiments	Compare gravity separation, centrifugation, washing, and cell supplementation	Tumescent solution; hand-held syringe aspiration; processed by gravity separation for 15 min, centrifugation at 1256 *g* for 3 min, washing with saline, and supplementation with SVF cells; SVF cell isolation was performed with collagenase for 45 min at 37°C; processed lipoaspirates were injected into nude rats and assessed after 12 wk	No difference in xenograft weight between processing techniques; no difference in histologic evaluation of xenografts; cell supplementation enhanced the weight of xenografts
Butterwick, 2002[51]	Human studies	Compare gravity separation and centrifugation	Tumescent solution; hand-held syringe aspiration; processed by gravity separation or centrifugation at 3600 rpm for 3 min; processed lipoaspirates were injected into the dorsum of the hand	Centrifuged lipoaspirates yielded more satisfactory results; centrifuged lipoaspirates injected into the hand increased the fullness and smoothness of the hand, compared with gravity separated
Khater et al., 2009[53]	Human studies	Compare centrifugation and washing	Dry technique; hand-held syringe aspiration; processed by centrifugation at 3400 rpm for 3 min or washed with saline; processed lipoaspirates were injected into the face	No difference was in outcomes between centrifuged and noncentrifuged according to the surgeon; nonprocessed or washed lipoaspirates yielded more satisfactory results according to the patients
Botti et al., 2011[52]	Human studies	Compare centrifugation and washing/filtering	Tumescent solution; hand-held syringe aspiration; processed by centrifugation at 3000 rpm for 3 min or washing/ filtering; processed lipoaspirates were injected into the face	No difference in centrifugation or washing/ filtering; filtration and washing resulted in nodule formation, whereas centrifugation did not

SAL, suction-assisted lipectomy; RBCs, red blood cells; WBCs, white blood cells; SVF, stromal vascular fraction; SCID, severe combined immunodeficiency.

Table 6

Studies Investigating the Impact of Centrifugation Speed

Reference	Type of Analysis	Primary Objective	Detailed Methods	Results
Ferraro et al., 2010[55]	In vitro	Compare centrifugal forces 50 *g*, 250 g, and 1500 *g*	Tumescent solution; suction- assisted liposuction; processed by centrifugation at 500 rpm (50 *g*) for 10 min, 1300 rpm (250 *g*) for 5 min, or 3000 rpm (1500 *g*) for 3 min; SVF cell isolation was performed with collagenase and Dispase for 1 hr at 37°C	Centrifugation speed > 500 rpm (50 *g*) resulted in damage to the structural integrity of adipose tissue; higher centrifugation speed resulted in increased necrotic and apoptotic cells, decreased ability to form tubules, and differentiate into mature adipocytes
Pulsfort et al., 2011[58]	In vitro	Compare centrifugal forces 92 *g*, 206 *g*, 825 *g*, 2292 *g*, 5157 *g*, 9168 *g*, and 20,627 *g*	Tumescent solution; hand-held syringe aspiration; processed by centrifugation at 50 *g* for 5 min; semiprocessed lipoaspirates were further centrifuged at 1000 rpm (92 *g*), 1500 rpm (206 *g*), 3000 rpm (825 *g*), 5000 rpm (2292 *g*), 7500 rpm (5157 *g*), 10,000 rpm (9168 *g*), or 15,000 rpm (20,627 *g*)	No differences in viable cells after centrifugation; higher centrifugal forces resulted in less debris and blood content in the fibrous fatty layer
Kurita et al., 2008[56]	In vitro and in vivo	Compare centrifugal forces 0 *g*, 400 *g*, 800 *g*, 1200 *g*, 3000 *g*, and 4200 *g*	Tumescent solution; SAL: processed by centrifugation at 0 *g*, 400 *g*, 800 *g*, 1200 *g*, 3000 *g*, 4200 *g* for 3 min; SVF cell isolation was performed with collagenase for 30 min at 37°C; processed lipoaspirates were injected into nude mice and assessed after 4 wk	Increased fluid portion, reduced fibrous fatty layer, and increased oil portion with centrifugation; centrifugation increased the number of RBCs in the fluid layer; centrifugation had no effect on the number of SVF cells in the layers; lipoaspirates centrifuged > 3000 *g* demonstrated decreased SVF cell number; largest xenografts were formed with processed lipoaspirates centrifuged at 3000 *g*
Hoareau et al., 2013[57]	In vivo animal experiments	Compare centrifugation forces 100 *g*, 400 *g*, 900 *g*, and 1800 *g*	Tumescent solution; hand-held syringe aspiration; processed by centrifugation at 100 *g* for 1 sec, 100 *g* for 1 min, 400 *g* for 1 min, 900 *g* for 1 min, 900 *g* for 3 min, and 1800 *g* for 10 min; processed lipoaspirates were injected into SCID/ beige mice and assessed after 4 wk	Higher centrifugation speed and duration resulted in increased percentage of oil; no significant difference in xenograft weights; low-speed centrifugation resulted in a significant collagen band or fibrosis
Lee et al., 2013[40]	In vivo animal experiments	Compare centrifugal forces: 50 *g*, 1200 *g*, 5000 *g*, 10,000 *g*, and 23,000 *g*	Tumescent solution; suction- assisted liposuction; processed by centrifugation at 50 *g*, 1200 *g*, 5000 *g*, 10,000 *g*, or 23,000 *g* for 3 min; processed lipoaspirates were injected into the flank of nude mice and assessed after 4 wk	Centrifugation enhanced the weight of xenografts; largest xenografts were formed with processed lipoaspirates centrifuged at 10,000 *g*.

SVF, stromal vascular fraction; SAL, suction-assisted lipectomy; RBCs, red blood cells; SCID, severe combined immunodeficiency.

Table 7

Studies Investigating Differences in Centrifuged Tissue

Reference	Analysis Type	Primary Objective	Detailed Methods	Results
Boschert et al., 2002[60]	In vitro	Compare density of cells within the fibrous fatty layer following centrifugation	Tumescent solution; suction- assisted liposuction; processed by centrifugation at 50 *g* for 2 min; processed lipoaspirates from the top, middle, and bottom layers of the fibrous fatty layer were assessed	Lower layer (of the fibrous fatty layer) yielded the greatest density of viable cells; upper layer (of the fibrous fatty layer) yielded the least number of viable cells
Crawford et al., 2010[36]	In vitro	Compare density of cells within the fibrous fatty layer following centrifugation	Tumescent solution; hand-held syringe aspiration; processed lipoaspirates from the top, middle, and bottom layers of the fibrous fatty layer was assessed; SVF cell isolation was performed with collagenase	Increased number of SVF cells isolated from the lower layer of the fibrous fatty layer
Allen et al., 2013[61]	In vivo animal experiments	Compare density of cells within the fibrous fatty layer following centrifugation	Tumescent solution; hand-held syringe aspiration; processed by centrifugation at 1200 *g* for 3 min; processed lipoaspirates from the top and bottom layer of the fibrous fatty layer were injected into the dorsum of immunocompetent FVB mice and assessed after 2 and 10 wk; SVF cell isolation was performed with collagenase	Xenografts formed with the lower layer (of the fibrous fatty layer) led to the greater retention at 2 and 10 wk; xenografts formed from the lower layer demonstrated greater endothelial cells (CD31), reduced collagen bands, and decreased areas of fibrosis; SVF cells from the lower layer demonstrated increased expression of angiogenic and adipogenic factors (VEGF, SDF-1α, PDGF-β, adiponectin)
Butala et al., 2012[62]	In vivo animal experiments	Compare density of cells within the fibrous fatty layer following injection with AMD3100 and centrifugation	Tumescent solution; hand-held syringe aspiration; processed lipoaspirates from the top and bottom layers of the fibrous fatty layer were injected into the dorsum of immunocompetent FVB mice and assessed after 2 and 10 wk; mice grafted with lipoaspirates from the top layer of the fibrous fatty layer were also injected with AMD3100 (to mobilize stem cells from the bone marrow)	Xenografts formed from the lower layer demonstrated increased fat retention at 2 and 10 wk, compared with xenografts formed from the upper layer; mice injected with AMD3100 that received lipoaspirates from the top layer demonstrated comparable fat grafting and retention potential as those mice receiving lipoaspirates from the upper layer; AMD3100 administration increased SDF-1α and VEGF expression in the mice receiving lipoaspirates from the upper layer

SVF, stromal vascular fraction; VEGF, vascular endothelial growth factor; PDGF-β, platelet-derived growth factor-β; SDF-1α, stromal cell–derived factor 1-alpha.

Table 8

Studies Investigating the Impact of Reinjection Technique

Reference	Analysis Type	Primary Objective	Detailed Methods	Results
Lee et al., 2013[40]	In vivo animal experiment	Compare injection speed: 0.5– 1.0 ml/sec and 3.0–5.0 ml/sec	Tumescent solution; suction- assisted liposuction; processed by centrifugation at 1200 *g* for 3 min; processed lipoaspirates were injected into the flanks of nude mice at a speed of 0.5–1.0 ml/sec or 3.0–5.0 ml/sec and assessed after 4 wk	Slow injection speed resulted in larger xenografts, whereas fast injection speed formed xenografts with greater collagen deposition and immune cell infiltration
Recipient site Nguyen et al., 1990[63]	In vivo animal experiments *	Compare dermis and muscle as recipient sites	Dry technique; hand-held syringe aspiration; processed by washing with saline; processed lipoaspirates were injected into the dermis of the dorsal ear or the rectus muscle of rabbits	Processed lipoaspirates injected into the muscle led to better results, possibly because of increased vascularization of the muscle compared with the dermis
Rieck and Schlaak, 2003[64]	In vivo animal experiments†	Compare fat and muscle as recipient sites	Excision of epididymal adipose tissue; SVF cell isolation was performed with collagenase for 1 hr at 37°C; processed lipoaspirates were injected subcutaneously into the back region, the visceral adipose tissue, the capsule of the left kidney, the subcutaneous adipose tissue, or intramuscularly into the quadriceps muscle	Fat retention of processed lipoaspirates was greatest in the back region > kidney and visceral adipose tissue > subcutaneous adipose tissue and muscle; increased mobilization of muscle may reduce retention
Recipient-site del Vecchio and Bucky, 2010[66]	Human studies	Investigate the outcomes with Brava domes	Preoperative nonsurgical breast expansion with Brava domes for 3 wk; tumescent solution; suction-assisted liposuction; adipose tissue was processed by gravity separation and centrifugation at 20–40 *g*; Brava domes were used for 3 wk	Preexpansion increased parenchymal space, reduced interstitial pressure in the breast, reduced contouring irregularities, and allowed for shape modification
Serial transplantation Mojallal et al., 2009[65]	Human studies	Assess efficacy of serial transplantation	Tumescent solution; hand-held syringe aspiration; processed lipoaspirates by centrifugation at 3000 rpm for 3 min; serial transplantation was performed	Serial transplantation provided satisfactory results, no overgrafting was observed; mobile areas of the face (e.g., glabella and lips) were less amenable to correction, compared with less mobile areas (e.g., molar and lateral cheek)
Lim et al., 2012[23]	Human studies	Assess efficacy of serial transplantation	Tumescent solution; hand-held syringe aspiration; processed lipoaspirates by centrifugation at 1200 J for 3 min; serial transplantation was performed; patients were diagnosed with craniofacial microsomia or Treacher Collins syndrome	Serial transplantation increased symmetry and provided satisfactory results; reduced time interval between serial transplantation (<10.5 mo) improved symmetry

SVF, stromal vascular fraction.

*Indicates source of tissue is from rabbit. †Indicates source of tissue is from rat.