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REVIEW BREAST

Autologous Fat Grafting in the Breast: Critical Points and Technique Improvements

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Abstract

Background Breast augmentation or reconstruction is a major challenge in esthetic and reconstructive surgery. While autologous fat grafting (AFG) provides a natural filler and seems easy to harvest, AFG in breast surgery is still problematic especially due to the high resorption rate associated with megavolume transfer. Despite this pending issue, there is growing interest in this method, which is becoming more and more widespread, as can be seen by the recent increase in the number of clinical studies. This review aims to highlight recent knowledge in the technique of AFG to the breast and recent refined procedures to improve fat viability and long-term success of the graft.

Methods Clinical publications and trials of AFG to the breast from the past 5 years were examined. Attention was focused on the different AFG steps and the clinical outcomes, in order to highlight the strengths and weaknesses of the available protocols.

Results Recent studies have concentrated on new techniques to improve fat viability and graft intake. However, all of these studies use different protocols at each step of the procedure. Furthermore, results may vary depending on the technique used for fat harvesting and processing.

Conclusion This review points out the recent advances in breast AFG techniques and their associated outcomes and complications. The bibliography has been carefully examined to reach a consensus so that recommendations could be made for each step of the technique with the aim of improving graft viability and long-term volume maintenance.

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Keywords Lipotransfer · Autologous fat grafting · Breast surgery · Protocols improvement

Introduction

According to the International Society of Aesthetic Plastic Surgery (ISAPS) [1] breast augmentation and reconstruction represented around 20 % of plastic surgery procedures carried out in 2011. Surgical implants are predominantly used. However, prosthetic breast reconstruction is not without risk, since many cases of leakage or rupture, displacement, infection, and capsular contracture have been reviewed [2, 3]. Moreover, even if implants may last for many years, some of them need to be revised or replaced and they might present sometime complications such as capsular contracture.

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An alternative is to use natural fillers for breast reconstruction. By utilizing natural fillers, surgeons use tissue from the patient, such as a flap taken from somewhere in the body and transferred to the breast. The flap can be musculocutaneous (dorsal, abdominal, or transversal) or adipo-cutaneous (DIEP). Finally, subcutaneous adipose tissue alone can be harvested via lipoaspiration and used as a natural filler in autologous fat grafting (AFG), alone or in combination with a flap.

AFG is a safe and easily adjustable technique that results in natural filling of the breast. Even if AFG use is rapidly expanding, the question about its legitimacy continues to be raised because of its main limits: the low rate of long-term graft maintenance [4] and the formation of oil cysts.

It is a fact that the results obtained with the AFG procedure are highly dependent upon the protocol and the experience of the surgeon [5], with these two parameters influencing cell death and resorption. Fortunately, an increasing number of studies and clinical trials are aiming to develop new techniques that limit graft resorption, leading to improvements in AFG efficiency.

The aim of this review is to discuss the pros and cons of AFG to the breast and to outline the different studies and procedures available, in order that recommendations can be made so that the procedures can be optimized to obtain long-lasting results.

Autologous Fat Grafting to the Breast: « The Pros and Cons »

Subcutaneous white adipose tissue is composed of two cell fractions: the adipocyte fraction providing fat volume [6] and the stromal vascular fraction (SVF) that contains numerous cell types (endothelial cells, pericytes, stromal cells, and hematopoietic cells) [7]. It is now widely accepted that these two fractions are critical for graft survival, with the efficacy of AFG being dependent upon their viability.

One of the major strengths of AFG to the breast compared to implant-based reconstruction is the natural aspect and palpation that it gives to the breast. AFG combined with liposuction can provide an esthetically pleasing result for the patient and is easy to perform by surgeons with minimal invasiveness. Another important point is that AFG to the breast gives an unlimited time effect, whereas a prosthesis usually needs to be replaced and does not age with the patient. Finally, the SVF of adipose tissue is a great source of mesenchymal stem cells (MSC), the reason for the increased interest in this tissue over the last decade. With advances in our knowledge of the SVF and cell-assisted lipotransfer, the trophic effect of stromal/stem cells on scars and irradiated zones, as well as the beneficial effects on angiogenesis and skin rejuvenation have been demonstrated. These effects can probably be linked to growth factor secretion by adipose stromal cells (ASC) [8] as well as their ability to differentiate into multiple lineages [9].

Nevertheless, AFG to the breast is also associated with limits and complications. Complications will depend upon the experience of the surgeon and the AFG protocol that is used. In 2013 Leopardi et al. [4] and Largo et al. [10] reviewed previous studies on breast AFG, focusing on the complications that were encountered. With regard to fat injection, the possible complications are: fat necrosis, oil cyst formation, and calcification. These are frequently reported and are not dependent upon the protocol used. Liponecrosis is believed to be caused when an excess of fat is injected, inhibiting the complete vascularization of the graft and causing cell death due to ischemia and nutrient deprivation. Cyst formation is often minor and can be easily detected by palpation or imaging. Of the minor complications, inflammation, striae, bruising, or superficial infections can occur.

One limitation of AFG to the breast comes with the patient morphology. For some patients, large volumes of fat for grafting cannot be harvested (at least not in a single operation without risking deformities of the donor site) especially for Cell-Assisted Lipotransfer (see the dedicated paragraph).

In recent years, a major concern as far as AFG to the breast is concerned, was the possible mammographic interference with cancer diagnosis due to tissue calcification [11]. However, in the 2013 study by Rubin et al, mammographic changes after AFG were less important than those following breast reduction, a commonly accepted procedure [12]. Therefore, care should be taken when analyzing mammographic images following AFG, with a high level of experience required to discern benign calcification from oncological dangers.

The two main remaining issues concerning AFG to the breast are the questions of the possible pro-cancerous role of injected adipose tissue (discussed in the safety paragraph) and the high resorption rate in the months following injection.

The principal limit of this technique addressed in this review is the low maintenance volume due to the use of large volumes of fat [13–15]. The efficacy is estimated to be approximately 50 % with nearly all techniques (Table 1). Most of the resorption occurs in the 3–6 months following adipose tissue injection. Nevertheless, it should be noted that many protocols have been developed since Coleman's LipoStructure®, enhancing our knowledge concerning fat processing prior to grafting.

To summarize, AFG to the breast is an extremely promising technique with few complications, but protocol improvements are still required in order to offer the best possible outcome with enhanced fat maintenance.

Table 1 Recent studies concerning Autologous Fat Grafting to the breast, showing the protocols used and the mid/long-term outcomes following surgery

Study	Purpose/context	Number of patients	Breast preparation	Infiltration method	Cannula	Aspiration method	Processing of the fat	Reinjection	Outcomes	Complications associated with AFG
Yoshimura et al. [25]	Cell-assisted lipotransfer	40	–	Saline solution with epinephrine 0.001 %	Inner diameter 2.5 mm	Liposuction device	Group A & B: wash + decantation Group C: 700 g 3 min Half of the collected tissue is used for SVF isolation	Group A&C: SVF is mixed with fat Group B: SVF is injected separately	Gradual resorption in the first 2 months More natural than implant	At 6 months 1 case in group B:fibrous breast tissue and fibrosis on the sternum At 24 months Cysts (2) Microcalcification (2)
Zocchi et al. [26]	Breast AFG protocol	181	BRAVA	Saline solution with adrenaline 2 mg/L	Teflon-coated 2 mm	60-cc syringe with controlled depression	Decantation on a vibration table	2-mm cannula Manual reshaping	Average persistent volume at 1 year: 55 %	At 8 years Less than 4 % of liponecrosis, microcyst and microcalcifications
Delay et al. [27]	Author's experience	880	3D morphological study in some cases	–	Blunt 4 mm	10-cc syringe	Centrifugation 3200 rpm for 3 min	Overcorrection (140 %)	Gradual resorption in the first 3-4 months (30-40 %) Volume stable at least 5-6 years	Oily cysts 15 % 1 infection Fat necrosis in 3 %
Illouz et al. [28]	Author's experience	820	–	Saline solution with adrenaline 1:500 000	–	Syringe	Decantation	2.5-mm cannula	–	Ecchymosis (76) Striae (36) Hematomas (12) Infections (5)
Panettiere et al. [29]	AFG in irradiated reconstructed breast	61	–	Saline solution with lidocaine 0.5 % and epinephrine 1:200,000	1 hole bullet tip 3 mm	10-cc syringe	Wash with saline solution and decantation	14G needle and overcorrection (10-15 %) Multiple injection sessions	Best esthetic outcome with lipotransfer at 3 months Functional improvement after 3 months with lipotransfer	No complications at 3 months
Rigotti et al. [18]	Compare incidence of local and regional recurrence of breast cancer before and after AFG	137	–	Cold saline solution with epinephrine 1:400,000 and 0.02 % lidocaine	Coleman's instrumentation		Centrifugation at 3000 rpm	2-cc syringe	Statistically not more recurrence since the utilization of lipotransfer	–

Table 1 continued

Study	Purpose/context	Number of patients	Breast preparation	Infiltration method	Cannula	Aspiration method	Processing of the fat	Reinjection	Outcomes	Complications associated with AFG
Ueberreiter et al. [30]	AFG protocol	85	–	Saline solution with lidocaine 0.05 %, adrenaline 1 mg/L and sodium bicarbonate 0.105 % Infiltration and aspiration are simultaneous	3.8 mm with 0.9 mm apertures	Continuous water jet and 0.5 bar negative pressure	Fat trap and decantation in injection syringe	10-cc syringe	Approximately 50 % of injected volume at 6 months	2 subcutaneous granuloma
Yoshimura et al. [31]	Cell-Assisted Lipotransfer	15	–	Saline solution with epinephrine 0.0001 %	Inner diameter 2.5 mm	Liposuction device	Centrifugation 700 g for 3 min Half of the collected tissue is used for SVF isolation	10- or 20-cc syringe with 16 or 18G needle	Graft survival ranged from 40 to 80 %	At 1 year no cyst formation (> 5 mm) or microcalcifications were detected ($n = 8$)
Caviggioli et al. [32]	AFG in post-mastectomy pain syndrome (PMPS)	63 + 35	Skin expander and silicone prosthesis	Coleman's technique			Centrifugation 3000 rpm for 5 min	18G needle	Pain decrease in AFG group compared to control group	–
Del Vecchio et al. [33]	Use of BRAVA and breast AFG	25	BRAVA	–	–	–	Centrifugation from 20 to 40 g	BRAVA for 2–4 weeks	64 % tissue yield at 6-month for 12 patients	At 6 months no oil cysts, fat necrosis, or breast masses
Kamakura et al. [34]	Cell-Assisted Lipotransfer	20	–	Saline solution with 1 % lidocaine and epinephrine	3 mm three-hole blunt	–	Half of the collected tissue is washed and gravity filtered The other half is used for SVF isolation	Thumb-brush syringe adaptater	Physician satisfaction (69 %) and patient satisfaction (75 %) at 9 months	At 9 months liponecrotic cyst (11 %)
Salgarello et al. [35]	AFG with Platelet-Rich Plasma	10 + 25	–	Saline solution with Xylocaine 2 % and epi nephrine 1:500,000	Inner diameter 3 mm 2 holes blunt	10-cc syringe	Centrifugation 3000 rpm for 3 min 1- or 3-cc syringes with 2.7-cc fat/0.3-cc PRR 10 % in group A and with 0.9-cc fat/0.09-cc saline solution in group B	Injection with a 17G single-hole blunt cannula	No significant differences between the AFG with or without PRP in terms of outcomes, complications or session number	Fat necrosis (7 per group): Oil cyst (4 per group) Complex cystic images (4 per group)
Cigna et al. [36]	Secondary AFG after implant reconstruction	20	Breast implant	Anesthetic solution	3 mm	10-cc syringe	Centrifugation at 3000 rpm for 3 min	1 and 2.5-cc syringes overcorrection 20-25 %	Improvement of cosmetic outcomes	At 1-year Fat necrosis (1)

Table 1 continued

Study	Purpose/context	Number of patients	Breast preparation	Infiltration method	Cannula	Aspiration method	Processing of the fat	Reinjection	Outcomes	Complications associated with AFG
Gentile et al. [37]	AFG enhanced by stromal vascular fraction (CAL) and PRP	10 + 13 + 10	–	Cold saline solution with adrenaline 0.2 %	3 mm	60-cc syringe	Centrifugation at 3000 rpm Group PRP: 1-cc fat with 0.4-cc PRP Group eSVF: half of the collected tissue is used for SVF isolation Group control: Coleman's procedure	1–2-mm cannulas Specific microcannulas for eSVF group	Volume maintenance at 18 months: 65 % for PRP group, 61 % for SVF group and 30 % for control group	Small cystic formation and microcalcification (1)
Khouri et al. [38]	Use of BRAVA and breast AFG	81	BRAVA	–	2.7 mm 12 holes	Constant 300 mmHg syringe pulling	Centrifugation in bags 15 g for 3 min	2–5-cc syringe and 2.4 mm single-sidehole Wear of BRAVA after operation	90–78 % graft survival depending on the injected volume	At 1-year Calcifications (16 %)
Pérez-Cano et al. [39]	Cell-Assisted Lipotransfer	71	–	Tumescent solution	–	Syringe	Wash with saline solution and decantation Half of the collected tissue is used for SVF isolation	Thumb-controlled syringe adapter	Statistical improvement in the LENT-SOMA score	Injection site cysts (14.9 %) At 12-month Small cysts (46 patients)
Petit et al. [16]	Locoregional recurrence after AFG	321	–	Different protocols					No difference in locoregional recurrence between AFG and control except for patient with intraepithelial neoplasia	–
Rubin et al. [12]	Compare mammographic changes after AFG versus Breast reduction	27	–	Saline solution with epinephrine 0.001 %	Inner diameter 2.5 mm	Liposuction device	Half of the collected tissue is used for SVF isolation	–	No difference in abnormality rates for cyst and calcification More scarring and masses requiring biopsy in the breast reduction group	At 12 months Oil cysts 55 (25.5 %) Scarring 38 (17.6 %) Calcifications, benign/fat necrosis 37 (17.1 %) Calcifications warranting biopsy 10 (4.6 %) Mass or distortion warranting biopsy 6 (2.8 %)

Table 1 continued

Study	Purpose/context	Number of patients	Breast preparation	Infiltration method	Cannula	Aspiration method	Processing of the fat	Reinjection	Outcomes	Complications associated with AFG
Auclair et al. [40]	Composite breast augmentation	197	Breast implant BRAVA for revision augmentation	Tumescent solution	1.5 mm for fat overlay 12 holes and 3.5–4 mm for submuscular primary augmentation and revision augmentation	Syringe for fat overlay and liposuction device for other	Centrifugation (Coleman for fat overlay or low G-Force)	14G side hole blunt cannula	Average volume maintenance of 57 % in 20 patients at 1 year	At 1-year cystic mass (2)
Bonomi et al. [41]	Immediate and delayed reconstructive surgery	31	Latissimus dorsi (LD) flap and/or implant-based reconstruction	Saline solution with marcaine 0.25 % and adrenaline 1:200,000	3 mm	10-cc syringe	Centrifugation 3000 rpm for 3 min	2-mm cannula 30 % overcorrection	Patient satisfaction (29 patients rated good or above)	Fat necrosis (2) oil cysts (1) cancer recurrence (1 at 4 years)
Costantini et al. [42]	Radiological evaluation of breast AFG	24	–	Saline solution with epinephrine 1:500,000	–	Syringe	Centrifugation 3000 rpm for 3 min	17G blunt cannula	AFG do not interfere with early diagnosis Ultrasounds are best for identification of oil cyst and MRI for fat necrosis	At 1 year, benign calcifications (3) Simple cysts (6) Oil cysts (23) Liponecrosis (8) Benign nodules (2) Cancer recurrence (1)
Fiaschetti et al. [43]	Imaging evaluation after AFG + PRP	15	–	Klein solution	–	–	Centrifugation 3000 rpm for 4 min Mix with PRP	Coleman microcannula	MRI allows a good volume estimate. At 6 months there is a 15.36 % or resorption and 28.23 % at 12 months	At 12 months by ultrasound: oil cyst (45.83 %) cytosteatonecrotic areas (12.5 %) by MRI: oil cyst (4.17 %) cytosteatonecrotic areas (16.67 %) by mammography: microcalcifications (20.83 %)
Hoppe et al. [44]	AFG protocol	28	–	Saline solution with lidocaine 0.05 %, adrenaline 1 mg/L and sodium bicarbonate 0.105 % Infiltration and aspiration are simultaneous	–	Continuous water jet and 0.5 bar negative pressure	Decantation in 50-cc syringes for 5–10 min	10-cc syringes	Generally 4–6 procedures, irradiated patients needed a higher amount of fat. 68 % good or excellent results.	At 6 months, liponecrosis (2.59 %), infection (0.74 %) and granuloma (0.74 %)

Table 1 continued

Study	Purpose/context	Number of patients	Breast preparation	Infiltration method	Cannula	Aspiration method	Processing of the fat	Reinjection	Outcomes	Complications associated with AFG
Peltoniemi et al. [45]	Comparison of CAL versus conventional AFG	10 + 8	–	Saline solution containing 1-cc epinephrine 1:1000, 12.5-cc sodium bicarbonate 8 mval and 500 mg lidocaine each 1000-cc saline 0.9 %	3.8 mm	Continuous water jet and 0.5 bar negative pressure	Decantation in fat trap. For CAL, half the harvested tissue is used for SVF isolation	10-cc syringes and controlled injector	54 % (AFG) and 50 % (AGF + CAL) volume survival measured by MRI. No significant volume benefit with CAL	At 6 months, no complications but late, oil cysts (1 in each group)
Petit et al. [17]	Safety of AFG in patients with intra epithelial neoplasia	59 + 118	–	Coleman's technique					A significant increase of local events after AFG in patients with intraepithelial neoplasia	At 5 years Local event incidence (18 % in AFG group and 3 % in control group)
Khouri et al. [46]	Use of BRAVA and breast AFG	476	BRAVA	–	12 holes, 2.7 mm	300 mmHg syringe	Centrifugation in bags 15×*g* for 2 min	2.4 mm single-hole cannula	After 6 months or more, 79.8 % graft retention	Fat necrosis (19) Small palpable nodules (15 %) Minor infection (7) Pneumothorax (1)
Maione et al. [47]	AFG in post-mastectomy pain syndrome (PMPS)	57 + 35	Conservative breast surgery and radiotherapy	Coleman's technique			Centrifugation 3000 rpm for 5 min	18G angiography needle	AFG significantly decreased pain in patients with PMPS following lumpectomy and radiotherapy	–
Mestak et al. [82]	PureGraft vs Centrifugation for breast AFG	15 + 15	–	Saline solution with 1 % adrenaline	3-mm Mercedes cannula	60-cc syringe	Filtration in Puregraft with two washes in Ringer for one group Centrifugation 3000 rpm, 3 min for the other group	9 cm type III Coleman cannula	After 1 year, patient satisfaction is the same in the two groups	Infection (1) Solitary cysts (3)
Small et al. [48]	Investigate donor site for breast AFG	46 + 27	–	Tumescent solution with lidocaine 4:10 000 and epinephrine 1:1000000	3–4 mm	10-cc syringe	Centrifugation 3000 rpm for 3 min	3-cc syringes	45 % retention at 140 days and no difference between graft from abdomen or from thighs Less retention if more than 100 cc is grafted	–

Table 1 continued

Study	Purpose/context	Number of patients	Breast preparation	Infiltration method	Cannula	Aspiration method	Processing of the fat	Reinjection	Outcomes	Complications associated with AFG
Spear et al. [15]	Breast AFG	10	–	–	3 mm	Liposuction device (500–600 mmHg)	Centrifugation 3000 rpm for 3 min	blunt cannula	Volume retention at 1 year 36 % for the right breast and 39.2 % for the left breast	Fat necrosis
Uda et al. [49]	Use of BRAVA and breast AFG	14	BRAVA	Tumescent solution	3 mm	Liposuction aspirator (<350 mmHg)	Centrifugation 1200×*g* for 3 min	Blunt Coleman's cannula connected to 2.5-cc syringes Wear of BRAVA after operation	Better results in non-irradiated breasts	Fat lysis (1) Cellulitis (1) Cysts (5) Calcification (1)

Safety Concerns About AFG to the Breast

Even though breast autologous fat grafts are used worldwide, there is concern about the interaction between stem cells contained in the grafted adipose tissue and any possible residual cancerous cells from a mastectomy procedure. This debate on the role of stem cells in the fat transfer is of further concern when considering cell-assisted lipotransfer and the supra-physiological concentration of stem cells associated with this procedure.

There are numerous in vitro and in vivo models available to investigate the possible pro-cancerous role of fat injection; however, these models do not accurately simulate clinical results while often giving contradictory results [16–19]. There is currently a lack of large controlled studies with long-term follow-up to study this issue leaving the topic understudied. Several individual cases have been reported in which adipose tissue injection led to increased tumor growth and even cancer relapse [20, 21]. The mechanisms involved in these phenomena remain to be identified, but may be cancer dependent such as patients with intraepithelial neoplasia or other undetermined factors.

Despite these concerns, in 2012, the ASPS Fat Graft Task Force did not find "any reports suggesting an increased risk of malignancy associated with fat grafting" and so far, clinical reviews have not revealed any increased cancerous relapse when using AFG to the breast [18, 22, 23].

Recent Studies

AFG to the breast is of great interest in breast surgery with 12 clinical studies currently registered on the clinicaltrial.gov website (research that contains the key words "fat graft", "fat grafting", "adipose tissue injection") [24].

In addition to these clinical trials, Table 1 resumes clinical studies published on breast AFG in the last 5 years.

This table highlights the main issue with breast AFG, which is the high resorption rate of the graft in the months following injection: 15–40 and 20–55 % resorption at 3 and 6 months, respectively (Table 1).

The table also demonstrates that currently, several different protocols are being used by surgeons. This demonstrates the complexity that surrounds adipose tissue handling in AFG and makes it difficult to compare the outcomes.

Moreover, one of the most striking observations is that despite the deleterious effect of high-speed centrifugation being demonstrated, over the past years, 17 studies have used centrifugation steps equal or above 700×*g*. This underlines the importance of determining a clear protocol for the purification of fat prior to reinjection.

A further piece of information that is highlighted by this table is that it now seems to be commonly accepted that microinjection of small lipid aliquots is employed in order to enhance fat revascularization upon reinjection.

Finally, the most recent breast AFG studies highlight the growing interest in enhancing harvested fat with a graft supplement (with SVF or platelet-rich plasma: PRP).

Processing of Adipose Tissue

Lipotransfer can be divided into four steps: infiltration, lipoaspiration, fat processing, and injection. Although numerous protocols are currently used in clinics for all of these steps, several studies compared the different methods with the common aim of reducing fat resorption [52–57].

Infiltration

Lipoaspiration can be carried out while simultaneously or previously injecting a physiological solution (wet lipoaspiration). Dry lipoaspiration, without infiltration, is no longer used for AFG because of the amount of associated blood loss [50]. The standard ratio for infiltration is commonly 1-cc infiltrated for 1 cc of fat tissue removed, but a higher ratio can be used with tumescent lipoaspiration techniques. The infiltration solution can contain local anesthetic (to avoid peri- and post-operative pain) and vasoconstrictor drugs (adrenaline) to prevent bleeding. Although adrenaline has been proven harmless at low doses, the use of local anesthetic (e.g., lidocaine) is still an issue. Indeed, these drugs have a cytotoxic effect on ASC or can affect their metabolism. Therefore, these drugs must be used at an appropriate concentration to prevent any damage to the tissue [51, 52]. Their use increases the importance of tissue washing prior to reinjection in order to avoid any prolonged contact.

Lipoaspiration

Harvesting Site

As far as fat viability and graft outcome are concerned, several studies have been unable to demonstrate a significant difference between harvesting sites [48, 53, 54]. Furthermore, the most common source for the harvesting of fat grafts is the abdomen [11].

Cannulae

In 2009 Erdim et al. evaluated the influence of cannula size and showed that a cannula that is too small could damage the tissue [55]. The inner diameter of the cannula varies between 2 and 4 mm, with the choice of cannula dependent upon the surgeon carrying out the technique (Table 1). Aside from the cannula size, the number and the size of the holes in the cannula is important. In fact a higher number of holes will enable faster fat harvesting with a concomitant reduction in depression pressure [56, 57]. The diameter of the holes will also affect the size of the harvested lobules, with smaller lobules supposedly being more viable, thus making revascularization easier [56].

Aspiration

The lipoaspiration cannula can either be connected to a syringe, so that aspiration can be carried out manually, or be power assisted. In all cases, the negative pressure caused by aspiration is a critical factor in graft survival [58]. Indeed, in 2001, Shiffman demonstrated that a depression of over 700 mmHg (by manual or by power-assisted liposuction) should not be used for lipotransfer [59]. Cheriyan obtained the same conclusion comparing −250 and −760 mmHg pressures [60]. A questionnaire was sent to the members of the American Society of Plastic Surgeons in 2013, with 55 % of the responders using manual suction instead of other techniques [11].

Pulling of the plunger should be carried out with caution because it will determine the negative pressure inflicted on the tissue [61]. With this in mind, special syringes with a controlled plunger can be used (e.g., Macrofill by Adip'sculpt). In any case, manual or power-assisted systems that enable the control of negative pressure (e.g., K-VAC Syringe by Lipocosm) are preferable.

A further factor that has given contradictory results is the effects of direct exposure to air [62]. Further data should be obtained to determine if non-closed systems should be avoided or not.

In addition to assisted aspiration, several other technological advances have been developed in an attempt to improve the quantity and the quality of harvested fat. Water-assisted lipoaspiration (WAL) uses continuous infiltration that enables "pre-dissection" of the tissue prior to its aspiration (e.g., BodyJet by human med) [30, 44] but this also results in a greater residual interstitial liquid phase.

Some devices also improve the speed of aspiration by making the cannula vibrate during the aspiration process (e.g., PAL by microaire). This technique allows easier and faster aspiration with less effort. However, even if it is possible to regulate the intensity of the cannula vibration, this technique might be more suited to performing high volume liposuction rather than lipotransfer due to the damage done to the harvested tissue with the vibrating cannula.

Purification

The purification step aims to remove the interstitial liquid whilst at the same time ensuring the viability of the graft cells. The two main purification protocols that are mostly used nowadays are decantation and centrifugation. In 2013, 45 % of surgeons used a decantation process and 34 % used centrifugation to purify the graft before AFG to the breast [11].

Decantation

The decantation process is easy to perform and does not necessarily require any additional devices. It can be performed directly in syringes or in a special device designed to trap the fat tissue and to isolate the liquid fraction (e.g., Lipocollector by human med; Tissu-Trans Filtron by Shippert Medical). Even if it is probably the cheapest and easiest to use for surgeons, the main limit of this process is that a significant fraction of liquid will still be trapped in the adipose fraction and will be the first to be reabsorbed after reinjection [30]. The remaining infiltration liquid can also be responsible for a higher concentration of pro-inflammatory cytokines secreted by the adipose tissue during aspiration. This may trigger inflammation at the recipient site, with recruitment of immune cells that can eventually lead to increased graft resorption [63].

Centrifugation

Due to this limitation in the decantation process, since the 1990s the lipoaspirate can also be centrifuged to remove the liquid fraction and improve graft uptake [11, 62, 64, 65]. Moreover, in 2010, Condé-Green found a greater concentration of ASC in the centrifuged fat than with the decantation method alone [61]. However, this step requires a centrifuge and probably more time to carry out than simple decantation. Since Coleman described the purification step with 3 min of centrifugation at 3000 rpm (rarely mentioned but corresponding to 900–1200 g, depending upon the rotor used), many studies have investigated the role of centrifugation (speed and time) on the viability and the maintenance of the reinjected tissue [61, 63, 65–67]. Caution must be taken with this centrifugation step to preserve the integrity of the adipose tissue. Indeed, the centrifugation speed is correlated with fat tissue compaction but also with the release of oil due to the death of adipocytes. A balance must then be found between interstitial liquid removal and adipose tissue damage. Tissue damage can lead to an increase in the secretion of pro-inflammatory cytokines, such as Monocyte Chimoattractant Protein 1 (MCP1) or Interleukin 6 (IL6) [63]. Hoareau and Kurita have both shown a decrease in survival with centrifugation forces higher than $400\times g$ for 1 min [63, 67]. However, there are some protocols at the moment that still use a centrifugation step of 3 min or more at 3000 rpm. On the other hand, some protocols use one or several low speed/time centrifugation(s) [66, 68]. There are some new protocols that use a similar approach to reduce or limit damage to the tissue through the use of manual centrifugation ($15\times g$). There is however little data in the literature to make conclusions regarding such a method.

Alternative Processes

The aim of the filtration method is the same as that of the decantation or centrifugation methods: removal of the liquid fraction to purify the fat before reinjection. It is accomplished by passing the lipoaspirate through a filter (e.g., PureGraft by Cytori or Lipivage by Genesis Biosystem) to remove particles below the chosen size. In 2013, Zhu found less oil and greater viability with this system compared to high-speed centrifugation [69].

Some protocols also integrate additional washing steps (e.g., Macrofill by Adip'sculpt; PureGraft by Cytori) that aim to remove the remaining infiltration solution [61, 69, 70], to get rid of most of the local anesthetic, vasoconstrictors, inflammatory molecules, and death factors (released during liposuction) that may damage the tissue and limit graft success [51, 63]. In 2013, only 28 % of surgeons were using a washing step with breast AFG [11].

Reinjection

The final step of AFG is the reinjection of the adipose tissue. This step usually requires the use of different sized syringes (from 1 cc to 50 cc) depending on the quantity of adipose tissue to graft. The injection is carried out with a retrograde movement that deposes a small amount of fat tissue in multiple directions via microtunneling at the recipient site. If the graft deposit is too large it will take longer for vascularization to reach the center of the graft, resulting in fat necrosis (Fig. 1).

Therefore, the injection of small aliquots of fat is now commonly accepted, with a cannula of 2 mm being preferred for fat reinjection (Table 1). Surgeons usually graft in all tissue planes (except glandular areas) [56]. No consensus is available however on whether a particular grafting zone is preferable even though muscular zones are more vascularized and could thus prove to be good recipients.

To protect the injected tissue from injection-related shear stress, automated devices have been developed. These devices aim to inject the fat with rigorous continuous pressure. Control of the injection (with an adipose tissue injector) was shown to improve in vivo fat retention in a

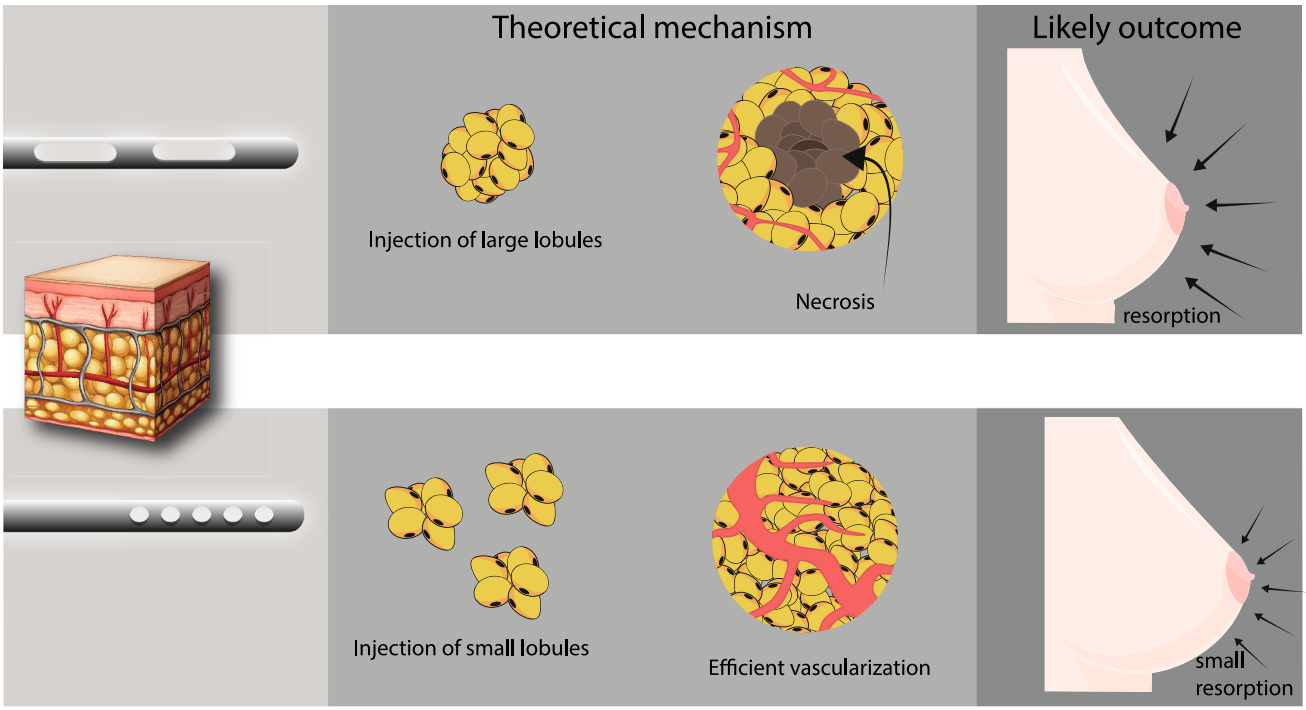

Fig. 1 Injection of small lobules of fat enhances revascularization of the graft and long-term survival

nude mouse model compared to a modified Coleman's method following simple decantation [71].

Finally, care must be taken not to overgraft in the breast. Faced with resorption, many surgeons try to overcome this limit by "overcorrection" of the recipient site. However, exceeding breast volume might lead to an inverse effect with increased graft pressure resulting in tissue damage [27, 56].

More Complex Protocols

In the last five years, a new approach has been developed to improve fat grafting through the addition of stem cells or growth factors [25, 31, 34, 35, 37, 39]. In all of these protocols, whether the addition of PRP, SVF, or ASC is implied, the supra-physiological concentrations of stem cells and growth factors is a sensitive issue in reconstructive breast surgery with a risk of cancer relapse [72].

PRP Enriched Grafts

In surgical fields, great interest has been demonstrated in platelet concentrates, notably in PRP, which is easy to use and only requires a blood sample. This blood derived formula contains a concentrated number of platelets that releases several growth factors (contained in the alpha-granules) such as platelet-derived growth factor (PDGF), transforming growth factor beta (TGFß), vascular endothelial growth factor (VEGF), and epidermal growth factor (EGF). These growth factors are responsible for high angiogenesis and proliferation potential in vitro [73]. Because of its properties, the use of PRP in breast AFG has been considered. There are still limited and conflicting results in the literature. In 2011, Salgarello et al. showed no benefit of using PRP with Coleman AFG, whilst in 2012 Gentile et al. reported a 1-year graft survival of 69 % compared to 39 % for the Coleman technique alone [35, 37]. PRP clearly has a concentration-dependent effect, with standardization of PRP preparation still unavailable. These factors and the numerous available protocols [74] can explain the contradictory results that have been obtained. More studies with a higher number of patients will be required to reach a consensus on the use of PRP at a defined concentration.

Cell-Assisted Lipotransfer (CAL)

Over the past decade, studies have shown a great deal of interest in the SVF mainly because of its ASC content and its ability to secrete pro-angiogenic factors [75, 76]. Thus, new procedures have emerged, consisting of the injection of a stem cell enriched fat graft. The common name for this protocol is Cell-Assisted Lipotransfer (CAL) [25].

The goal of this approach is to inject more SVF cells to promote vascularization and survival of the graft thanks to the presence of more endothelial precursors and pro-

Table 2 Guidelines for the different steps of AFG to the breast (according to the literature new data)

Procedure step	Tips
Infiltration	Usually conducted with a 1/1 ratio. While adrenalin can be used without problem, local anesthetics should be avoided. If necessary, local anesthetics should be used with appropriate concentration (maximum 0.4 mg/mL), and the tissue should be washed
Harvesting	Use a low depression (less than 0.5 atm or 375 mmHg), and a small cannula (2–4 mm in diameter) with multiple holes to decrease the pressure in order to preserve the tissue and to harvest small fat lobules. Devices that accelerate fat tissue harvesting may damage the fat graft if they are not used with caution
Processing	Washing steps are important in order to remove infiltration drugs and inflammatory molecules. Low-speed centrifugation (less than 400 g) or equivalent should be used to remove the liquid part so that a sufficient fat density is maintained for reinjection
Reinjection	Neovascularization of the graft is critical for its survival. It is therefore important to inject small adipose aliquots in order to decrease hypoxic stress. Preferably use a small cannula (2 mm diameter or less) with small holes. Additional care of the recipient site (BRAVA) or enhanced lipotransfer (CAL) might be suited for low trophicity breast (e.g., after radiotherapy)

angiogenic factors like the vascular endothelial growth factor (VEGF) secreted by the SVF [8]. Moreover, the enriched grafts contain more ASC that may be able to differentiate in the adipocyte lineage in situ to overcome the death of adipocytes that occurs during AFG. Finally, the immunoregulatory potential of ASC [77] may decrease the inflammation that is responsible for a sub-optimal outcome, causing adipocyte death. In the CAL protocol, the SVF is isolated of half of the harvested fat and then combined with the other half to create a stromal cell enriched fat graft. Several devices are now used in clinical practice to isolate the adipose SVF. These devices often use enzymatic digestion to digest the tissue and allow the isolation of the SVF cells as a pellet following centrifugation of the digested tissue [78].

So far the results obtained with CAL do not allow us to reach any conclusion as far as the superiority of this technique is concerned when compared with conventional fat transfer [45]. There is still a lack of real comparative studies with an objective assessment of graft survival. Moreover, the higher cost and quantity of tissue required for CAL remain limiting factors. It is likely that the CAL technique may not be suited to every AFG application. However, SVF seems to improve scar healing and might be suitable for low trophicity recipient sites [79].

ASC Enriched Graft

Last year, Trojahn Kølle et al. published a study carried out with volunteers in which fat grafts were compared with fat grafts enriched with cultured ASC. Even if this study was conducted on bolus injections of fat, the improvement in fat survival at 121 days (80.9 vs. 16.3 %) has now raised interest in cultured ASC supplemented fat for AFG [80]. We can expect in future breast AFG studies with cultured ASC to assess safety and cost effectiveness of this concept. However, this protocol is not a one-step procedure, with additional costs, time and the possibility of contamination related to the expansion of stromal cells. Furthermore, the intensive expansion of stromal cells increases the mutation risk in these cells (abnormal caryotype).

Breast Expansion System

Focusing on the recipient site, Khouri has developed the BRAVA system. The principle of this device is to extend the breast skin to make it a more favorable recipient for fat injection. The device applies a negative pressure (from 15 to 33 mmHg) to the breast area to (1) untighten the skin, increasing recipient volume, and (2) increasing blood supply via mechanotransduction effects [14]. Several studies and a clinical trial have supported the use of this technique and have demonstrated good results [26, 33, 38, 40, 49]. However, the BRAVA is quite expensive and its use is unwieldy for the patient. Considering its cost and its cumbersome nature it might be better suited for selected patients, for example with a "hostile" recipient breast (post-radiotherapy [49]) or those who want a large augmentation with limited procedures [81]. Nowadays, 8 % of American plastic surgeons are using this device prior to AFG [11].

Guidelines

In Table 2, we resume some tips that, according to the literature, can help to improve outcome for breast AFG. There is still no ideal protocol but we believe that the recent knowledge can be used to avoid deleterious procedures.

Conclusion

AFG to the breast is now a standard practice for breast reconstruction and augmentation procedures. However, there remain serious limitations as far as graft intake is

concerned. To improve outcome, many studies have focused on finding new approaches to treat the fat before reinjection. Here, we summarized current knowledge for each step of the AFG to the breast technique.

We believe it is possible to improve long-term outcomes by respecting both adipocyte and stromal fractions during the entire AFG to the breast process. Graft survival can be improved by controlled aspiration with low negative pressure (<700 mmHg) and with multi-small-holed cannulae. Before reinjection the fat needs to be concentrated while care is taken to preserve cell survival (positive balance between liquid removal and fat damages).

In the past 10 years, many research groups have shown interest in "stem cell enriched" grafts, especially the CAL protocol with SVF. It is still not clear if this protocol is better than conventional fat grafting. It might be promising for hostile hypotrophic recipient sites (an irradiated breast for example) justifying the higher cost, the necessity to harvest more tissue (half of it is digested) and the supplementary operation time that is required (approximately 2 h more). For all other applications high graft maintenance can be achieved by carefully processing the fat with simple and low-cost techniques.

The future of AFG to the breast is yet to be written; but it is clear that since the advent of the optimized Coleman's lipostructure, a great deal of fat grafting knowledge has been gathered and we now have more clues about how to handle fat in order to offer the best esthetic outcomes for patients.

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