Research

From Bedside to Bench: The Effect of Muscular Denervation on Fat Grafting to the Breast by Comparing Take Rate, Quality, and Longevity

Aesthetic Surgery Journal
2018, Vol 38(8) 900–910

DOI: 10.1093/asj/sjy073
www.aestheticsurgeryjournal.com
OXFORD UNIVERSITY PRESS

Patricia E. Engels, MD; Elisabeth A. Kappos, MD; Patricia K. Sieber, MD; Robyn M. Benz, MD; Sarah Thommen, MSc; Dirk J. Schaefer, MD; and Daniel F. Kalbermatten, MD, PhD

Abstract

Background: Autologous fat grafting (AFG) to the breast is a frequent procedure in aesthetic and reconstructive surgery. Despite pure volume gain, questions remain regarding the engraftment rate, quality, and longevity. Little is known about the role of recipient tissue or innervation of the grafted area.

Objectives: The goal of this study was to determine the optimal recipient layer and muscular pretreatment of AFG.

Methods: Fat was grafted to the breast, pectoralis muscle, or adjacent subcutaneous tissue of 42 rats. Nerve treatment included excision of a nerve segment, botulinum toxin (BTX) injection, or no treatment. Magnetic resonance imaging (MRI) and histological workup were carried out after 2 and 6 weeks.

Results: Six weeks after AFG, the proportion of viable fat cells within the grafted fat stayed high (median, [IQR]: 81% [72% to 85%]). The signs of inflammation decreased over time. Intramuscular grafting with intact nerves had a decreasing effect on the viability of the grafted cells compared with subcutaneous treatment (-10.21%; 95% confidence interval [-21.1 to 0.68]).

Conclusions: If utilized on an intact nerve, intramuscular injection may lead to inferior results. If the nerve was cut or treated with BTX; however, intramuscular injection tends to be superior. These findings may prove interesting for future studies and eventual clinical application.

Editorial Decision date: March 13, 2018; online publish-ahead-of-print March 27, 2018.

Autologous fat grafting (AFG) has been practiced routinely for decades since its introduction by Coleman.[1] In plastic surgery of the breast, lipofilling currently represents one of the most common procedures.[2]

Despite the common use of AFG, there has been a surprising lack of scientific groundwork to support clinical findings or guide and optimize application. Results of some experimental studies suggest that AFG could potentially induce cancer, although others have shown the opposite.[3,4] Recently published large clinical trials provide further evidence for the oncological safety of AFG.[5] When utilizing AFG in their clinical routine, surgeons tend to concentrate on pure volume gain and might not focus enough on the individual recipient tissue. Consequently, there is no consensus regarding the advantages and disadvantages of utilizing the subcutaneous or intramuscular layer for AFG. Comparisons have given inconsistent results because, although the blood supply in subcutaneous tissue is inferior to that in muscle, the subcutaneous site has in the past proven to be superior regarding fat cell viability.[6]

The purpose of scientific research on this topic should be to exactly determine the quality and quantity of cells and their viability after performing AFG. Clinically, this could help to better inform patients about future surgeries.

From University Hospital Basel, Basel, Switzerland.

Corresponding Author:
Dr Elisabeth A. Kappos, University Hospital Basel, Department of Plastic, Reconstructive, Aesthetic and Hand Surgery, Spitalstrasse 21, Basel 4031, Switzerland.
E-mail: elisabeth.kappos@usb.ch

The goal of this study was to compare different AFG strategies to establish the technique with the highest adipocyte survival rate and the most durable results. To mimic a clinical "fat grafting to the breast setting," the pectoralis muscle and the adjacent subcutaneous tissue of female rats were compared as recipient sites for AFG. Until now, animal experiments in this field of research have mostly been carried out in clinically less relevant environments (eg, the ear).[7] Moreover, the utilization in earlier studies of different anatomical sites for grafting to different layers may have rendered the results less comparable.[8–10]

Another aim of this study was to investigate whether patients would benefit from a simultaneous denervation of the grafted area. We therefore either denervated muscles by partial pectoral nerve excision, or through botulinum toxin (BTX) injection.

One already established clinical application of AFG to denervated muscles is the lipofilling to the latissimus dorsi or gracilis flap.[11,12] Unfortunately, in breast reconstruction, the deficient volume and the long-term volume loss caused by muscle atrophy result in a great need for revisional surgery.[13] We also sought to scientifically prove the influence of AFG in maintaining muscle flap volume following denervation.

Following AFG, patients are usually advised to refrain from physical activity for a variable period. Denervation of the specific area for a defined time frame of approximately 3 months would avoid muscle contractions and reduce/eliminate pressure on the freshly grafted tissue. Our hypothesis was that AFG survival, quality, and longevity are optimized in denervated muscle.

METHODS

Approval of Ethical Commission

This experimental animal study was approved by the institutional ethics and animal care committee of the University of Basel, Switzerland (No. 23354).

Study Design and Animals

Forty-two (42) female Sprague-Dawley rats (8 weeks old, 200–250 g; Harlan, The Netherlands) received AFG to the intramuscular layer on their left and the subcutaneous layer on their right pectoral region. Nerve treatment was comprised of either injection of BTX, cutting out a 3 mm nerve segment, or none of the above. Nerve treatment was the same on both sides. The resulting six treatment groups were evaluated 2 or 6 weeks postoperatively (21 animals at each time point). Hence, 84 AFGs were performed in total. Immediately after euthanasia, magnetic resonance imaging (MRI) was carried out, followed by explantation and paraffin fixation of the breast tissue for histological workup. Outcome assessors for MRI and histological data were blinded for the injection layer and nerve treatment.

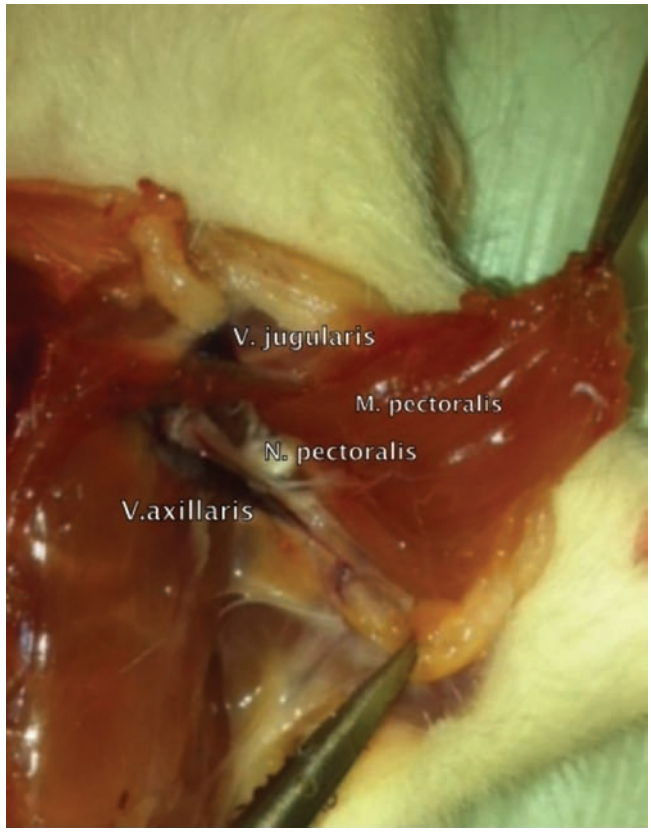

Figure 1. The cranial pectoral nerves are clearly trackable between the external and internal jugular vein and the axillary vein. The pectoral muscle is folded back so that its innervation can be seen.

Surgical Procedure

Anesthesia and analgesia were applied according to the hospital's veterinary protocol. All surgical procedures were performed under an operating microscope (Carl Zeiss, Germany).

Fat from the inguinal fat pad was explanted. It was minced with two razor blades and shuffled between two 1 mL syringes connected by a three-way cock. The skin above the chest was opened in a median manner, starting from the height of the most cranial nipples at a length of 2 to 2.5 cm. The cranial pectoral nerves were identified (Figure 1).

In the denervated group, a 3 mm nerve segment was excised.

In the BTX group, rats received intramuscular injections of 1.5 U of Botulinum toxin type A (Botox®, Allergan, Dublin, Ireland) into the pectoral muscles. AFG was performed on the breast of the rats. Because breasts are on

several levels, the ones over the pectoral muscle on both sides were chosen, to come as close as possible to clinical application. Then 0.3 mL of the fat was injected into each recipient area through a two-hole, 1.5 mm diameter Coleman's cannula (Chalybs Medical Devices GmbH, Stein am Rhein, Switzerland). Finally, the skin was sutured.

MRI Measurements and Image Evaluation

Postmortem, the animals were placed in a 16-channel phased-array wrist coil in a 3T static magnetic field strength MRI (MAGNETOM Prisma, Siemens Healthineers, Erlangen, Germany). A wrist coil was utilized (clinically applied for MRI of the hand or fingers) to guarantee high resolution and depiction of even the smallest volume changes. The same method has been previously utilized in a study, and the researchers in that study showed that even nerve regeneration can be monitored successfully by MRI.[14] The images were evaluated by two radiologists (RB and AF). For assessment of the remaining fat graft volume, an open-source imaging software (OsiriX, Pixmeo, Geneva, Switzerland) was utilized. On T1-weighted axial images, the collection was outlined on every consecutive slice with a polygonal region of interest (ROI). The edema was outlined in the same manner on T2-weighted, fat-saturated images. The remaining fat implant volumes were categorized in five arbitrary but predefined groups.

Histological Analysis

Four µm thin paraffin-embedded tissue slices were stained with hematoxylin and eosin (HE). Pictures of longitudinal sections were done utilizing a light microscope (Olympus BX43, Olympus Corporation, Tokyo, Japan) (Figure 2). Analysis was performed with ImageJ 1.46d software (National Institutes of Health, Bethesda, MD, USA). Working with 10x magnification, the area of the fat graft (in μm^2) was measured first, followed by the proportion of viable adipose tissue, oil cysts, and fibrosis. Viable adipose tissue was defined as the area containing adipocytes with a diameter of 50–150 µm. Oil cysts were defined as vacuoles containing remnants of necrotic fat cells >150 µm diameter, because of the disruption of initially viable adipocytes. A distinction was made between the surrounding fibrosis and the fibrosis within the graft.

The proportion of viable fat, oil cysts, and fibrosis was measured in relation to the whole area of the fat graft and divided into a scoring system of 1 to 4 (Table 1). The presence of parameters of inflammation (presence of monocytes, lymphocytes, and macrophages) as well as calcification and surrounding fibrosis was analyzed (without further subclassification; ie, yes/no). Experiments started in January 2013. Histological analysis was completed in December 2015, MRI analysis was completed in June 2016, and statistical analysis was finalized in January 2017.

Statistical Analysis

Linear mixed-effects models were utilized to compare the different treatment groups in terms of quality (percentage of viable fat cells within the graft). The package name of the statistical software package R was utilized for model estimation.[15,16] The model included a random factor for each individual rat to account for the paired data (ie, the two different treatments per rat). The layer of injection, nerve treatment, and time point of measurement were included as fixed factors in the regression model. Interaction terms were included to estimate the effect of the different recipient layers at different time points and for different nerve treatments separately. Continuous and quasi-continuous outcome variables were graphically visualized and compared utilizing boxplots. For all binary outcome variables, the proportion of individuals with signs of damage in each treatment group was calculated and displayed in a summarizing area chart.

RESULTS

Magnetic Resonance Imaging

The volumes of the AFG decreased between two and six weeks after injection (median, [IQR] 35% [20% to 72%] and median, [IQR] 1% [0% to 9%], respectively). Two weeks after injection, the proportion of the grafted fat that had remained was 20% or higher for most of the implants, whereas after 6 weeks, nearly all remaining proportions were lower than 20%.

Two weeks after treatment, the median remaining fat implant (in %) was lower in rats with intramuscular grafting compared with the subcutaneous group (median, [IQR] 32% [20% to 59%] vs 51% [22% to 79%]). However, this trend did not reach the 5% significance level (Figures 3–4).

Histology

Percentage of Viable Fat

The proportion of viable fat cells within the grafted fat remained high. There was an increase in the percentage of viable fat cells from week 2 to week 6 (median, [IQR] 73% [68% to 80%] vs 81% [72% to 85%]). Note that Figures 5 and 6 do not show absolute values but rather the proportion of viable fat in relation to the total fat graft. After 2 weeks, slightly better results were observed in the intramuscular groups, whereas after 6 weeks, the percentage of viable fat was slightly higher in the subcutaneous groups. Especially in the intact nerve group, the

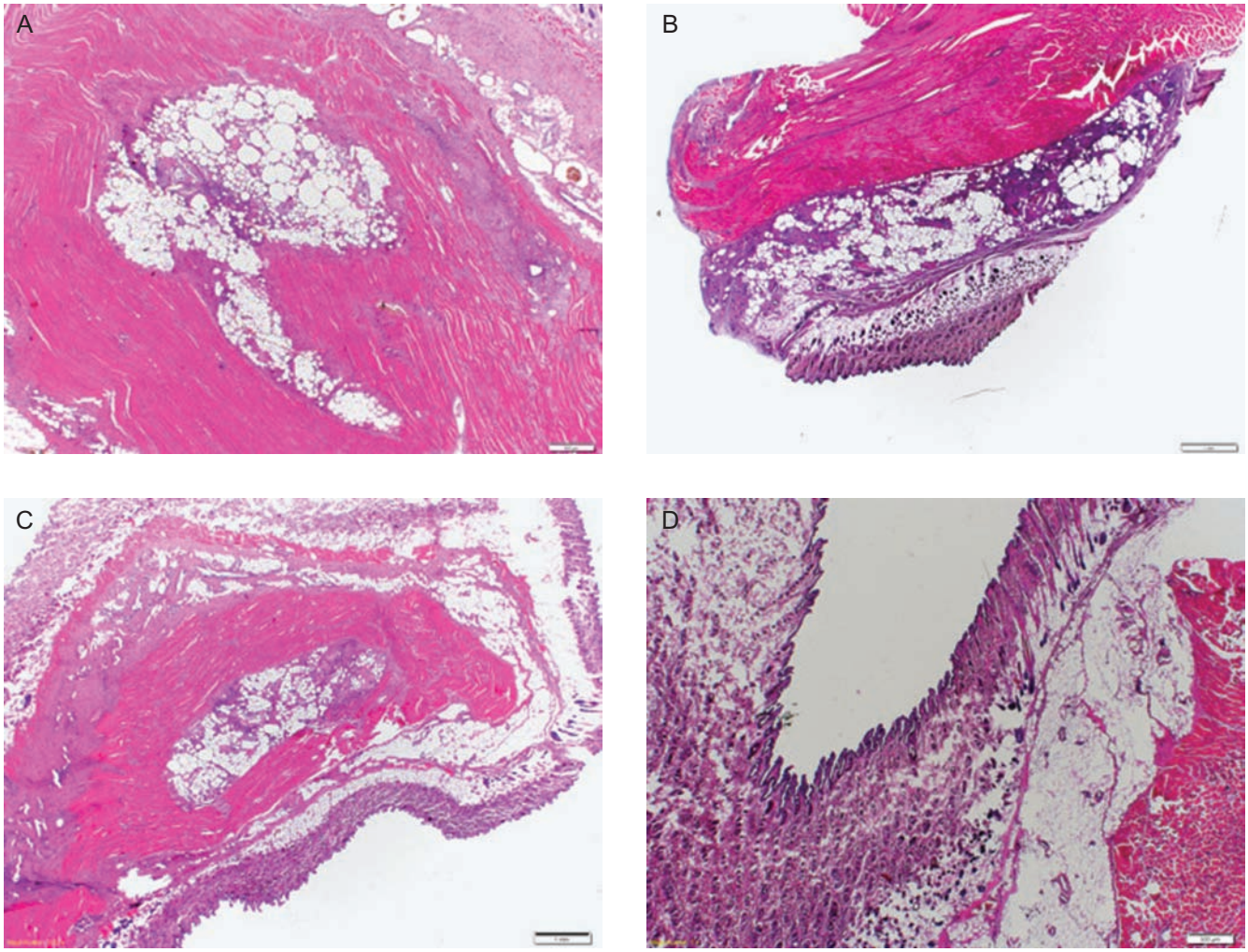

Figure 2. Light microscopy images of 4µm thin longitudinal sections of paraffin-embedded tissue stained with hematoxylin and eosin. (A) Two weeks after autologous fat grafting to the intramuscular layer, (B) 2 weeks after autologous fat grafting to the subcutaneous layer, (C) 6 weeks after autologous fat grafting to the intramuscular layer, (D) 6 weeks after autologous fat grafting to the subcutaneous layer. Scale bar: 500µm.

Table 1. Scoring System With Subcategories (1–4) for Qualities (viable fat, oil cysts, fibrosis) of Remnant Fat Implants (percentage of surface)

Code	Viable fat (%)	Oil cysts (%)	Fibrosis (%)
1	>90	≤5	≤5
2	80–90	5–10	5–10
3	70–80	10–20	10–20
4	≤70	>20	>20

percentage of viable fat cells in the subcutaneous layer was higher than in the intramuscular layer (percentage of viable fat cells 6 weeks after AFG subcutaneous median, [IQR] 89% [79% to 91%] vs intramuscular, 73% [68% to 78%]) (Figures 5–6).

At the 5% significance level, no statistically significant effect was detectable for the different treatments. However, at the 10% significance level, a statistically significant effect was observed after 6 weeks: In animals with intact nerves, intramuscular injection had a negative effect on the viability of the grafted fat cells compared with subcutaneous treatment (-10.21%; 95% confidence interval [-21.1 to 0.68], *P* value = .07) (Table 2).

Fibrosis and Oil Cysts

Overall, there was less fibrosis in the histological slides of the 6 weeks groups in comparison to the 2 weeks groups (median, [IQR] 92% [87% to 96%] vs 84% [79% to 88%]). In contrast, there was no relevant difference in the area free of oil cysts between the two time points (median, [IQR] 93% [87% to 97%] 2 weeks after AFG vs a 91% [86% to 95%] difference 6 weeks after AFG). Most oil cysts were detected in the intact-nerve group after 6 weeks (data not shown).

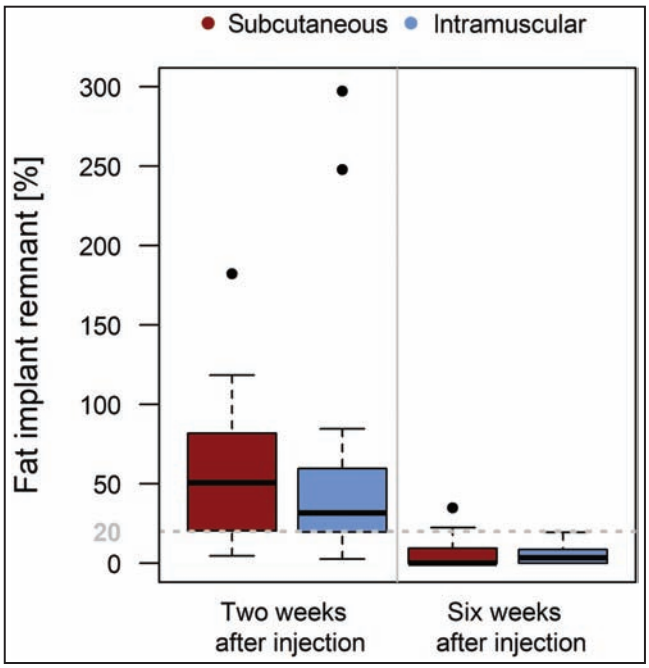

Figure 3. The proportion of the fat implant remnant for all injected fat grafts in the two treatment groups at 2 weeks and at 6 weeks after the intervention. Boxes represent first and third quartiles with the median central. The whiskers represent the 5th and 95th percentiles, and the observed data are represented by the white dots. The treatment is color-coded. The dotted grey line illustrates the 20% mark to facilitate comparison of the two time points (weeks 2 and 6).

Inflammation in MRI and Histology

Different parameters of the MRI and histology images were combined in terms of signs of inflammation: edema on the MRI as well as presence of signs of acute (monocytes, lymphocytes, macrophages) and indirect (fibrosis at the border, calcification) inflammation on the histological images. No significant difference was detectable between the nerve treatments and the recipient tissue. In both MRI and histology images, signs of inflammation decreased between the two time points.

DISCUSSION

Bedside

Lipofilling has become increasingly popular in cosmetic and reconstructive surgery[12,17] and can be utilized in breast augmentation, total breast reconstruction following mastectomy, or correction of deformities after breast-conserving surgery.[12,18–21] Even more often, AFG is combined with other reconstructive procedures.[12,22–24] AFG can serve as a salvage procedure following a failed implant reconstruction.[19,25] Lipofilling also plays a role in purely aesthetic surgery, in breast augmentation or as part of the surgical treatment of tuberous breast deformity, and breast asymmetry in general.[26,27]

The procedure is safely repeatable and minimally invasive, including low donor-site morbidity, which results in less hospitalization and operating time. Despite very low postoperative complication rates,[22,28] disadvantages for patients undergoing AFG remain, including the unpredictable resorption rate and the risk of fat necrosis, oil cyst development, and calcifications.[28–33]

This study indicates that, given an intact nerve, intramuscular injection results in unfavorable results compared with subcutaneous injection. If the nerve was cut or BTX treated; however, intramuscular injection appeared to be superior to subcutaneous injection. The previously mentioned findings have potentially interesting clinical implications. If this study's findings can be repeated in future experiments, one possible clinical scenario for further study would be to temporarily weaken the injected muscle (eg, the pectoralis muscle in the breast) to optimize fat take rates and thereby reduce the need for repeat AFG surgery.

Bench

In contrast to its manifold clinical applications, AFG has many parameters such as processing technique, recipient tissue/plane, and the role of pressure that to date been poorly investigated. Thus, the long-term viability of AFG remains highly variable and cannot be reliably predicted, which in turn affects clinical outcomes and may lead to a greater need for additional procedures.[34]

In the previously mentioned experiments, the observed ratio of viable fat cells within the grafts remained stable 6 weeks after AFG at approximately 81%. Signs of inflammation diminished between weeks 2 and 6. Future studies could focus on how to counteract inflammation after AFG, which might result in better take rates.

Processing

Processing of the fat is required to provide the highest concentration of intact adipocytes for AFG.[9] Volume retention and viability of the resulting graft are important aspects.[35] Unfortunately, research to date has not resulted in compelling evidence that one technique is superior to the others. The lack of high-quality data continues to limit the clinician's ability to determine the optimal method for purifying harvested adipose tissue.[34]

Dissociation of the inguinal fat pad in smaller rodents is a well-established proxy technique for liposuction; however, it may increase interstitial cell susceptibility to cell death.[36] The influence of quick mechanical processing through two

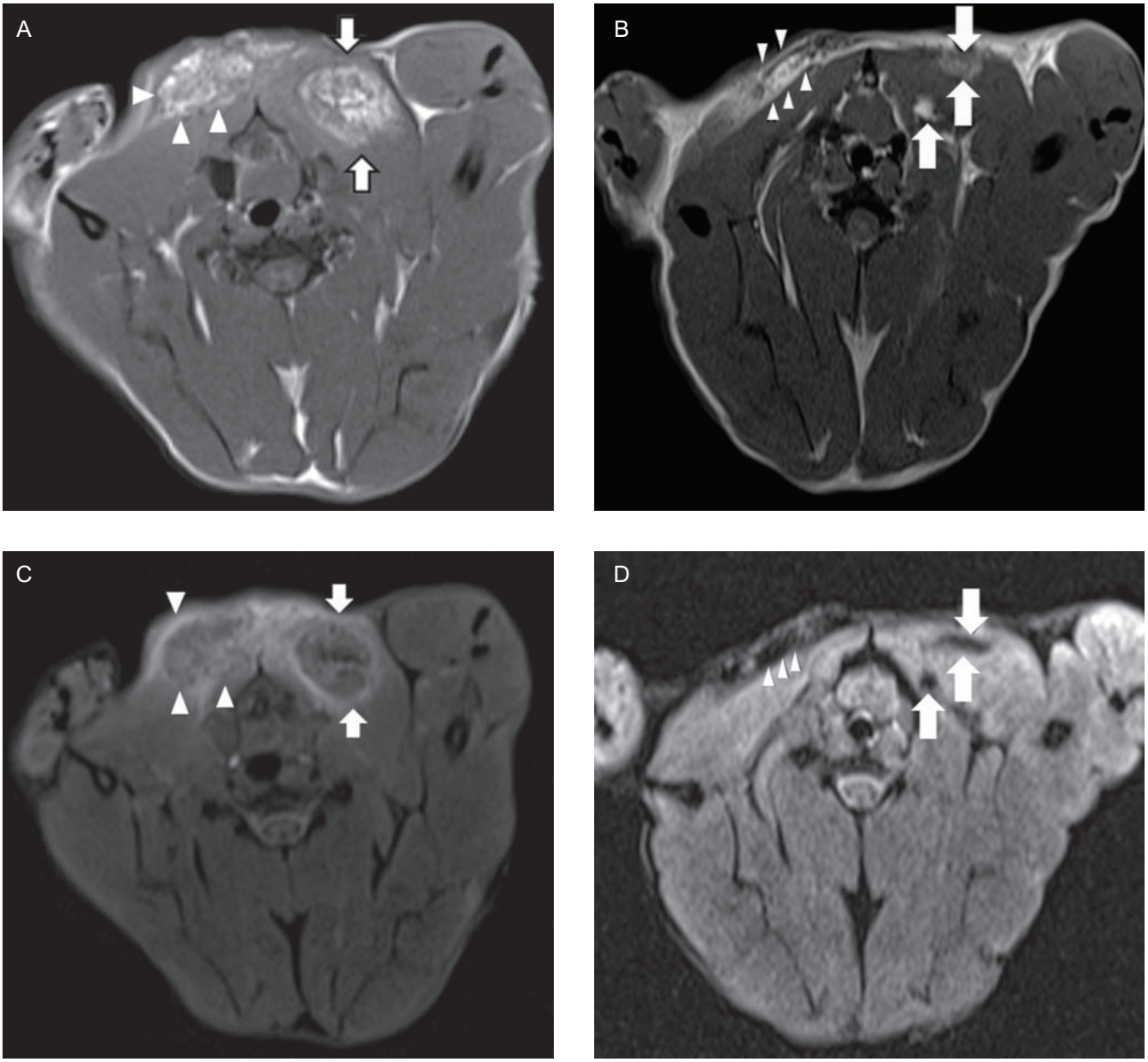

Figure 4. T1-weighted magnetic resonance imaging of two animals at (A) 2 and (B) 6 weeks post-implantation. The intramuscular fat graft (arrows) as well as the subcutaneous fat graft (arrowheads) are clearly visible in the T1-weighted image at 2 weeks (A). At 6 weeks, (B) both the intramuscular (arrows) and the subcutaneous fat grafts (arrowheads) decreased in size. The subcutaneous graft (arrowhead) is merely distinguishable from the surrounding subcutaneous fat. Images of the same animals utilizing Turbo Inversion Recovery Magnitude (TIRM). (C) Image displays edematous changes around the implanted tissue, indicating an inflammatory reaction clearly visible at 2 weeks for both the intramuscular (arrows) as well as the subcutaneous graft (arrowheads). (D) At 6 weeks, the inflammatory changes around the muscles have almost completely vanished. The increased signal around the subcutaneous graft likely represents an artifact at the tissue-air border. However, corresponding to the T1-weighted image, the volume of the intramuscular and subcutaneous grafts also decreased in size.

interconnected small-diameter syringes ("shuffling") on fat tissue was analyzed by Osinga et al,[37] who found that this process did not change the microscopic fat structure. Thus, shuffling can produce the right texture of fat to be administered through a small diameter Coleman cannulae, as needed in our trial to allow it to be precisely grafted to the tiny layers of the rats' tissue. Our results showed a very satisfactory percentage of viable fat cells within the graft (approximately 81%). In contrast, volume retention was quite low.

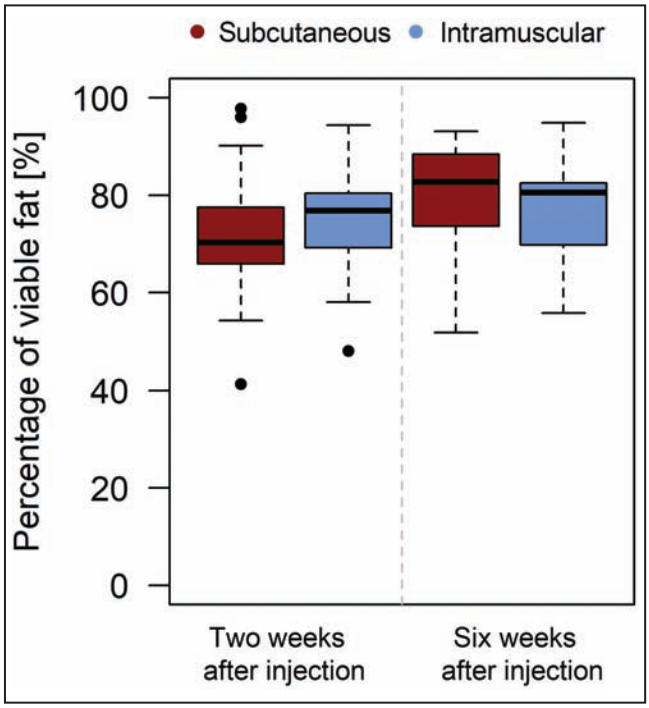

Figure 5. Percentage of viable fat cells within one representative histological section at 2 weeks and at 6 weeks after the intervention stratified for injection layer. Boxes represent first and third quartiles with the median central. The whiskers represent fifth and 95th percentiles, and outliers are represented by white dots. The injection layer is color-coded.

Recipient Tissue Plane and Innervation

In most cases of AFG to the breast, the surgeon can choose whether to graft to the subcutaneous[27] or intramuscular plane.[38,39] Most commonly, a combination of both is carried out.[20,29] Intraglandular grafting has also been described.[40] However, there are cases in which the anatomical situation determines the injection plane (eg, in patients who do not have any subcutaneous fat following radiation).[41] An indication for AFG being applied to the subcutaneous layer exclusively can be Poland's syndrome with (in severe cases) a total absence of the pectoral muscles.[11,24,42] When grafting to the muscle, one might graft to an innervated muscle such as the pectoralis major and serratus.[43] An example of AFG to a denervated muscle is the pedicled transfer of the latissimus dorsi muscle for breast reconstruction.[11,12,38,44] We further examined BTX injection as a form of nonsurgical denervation, because BTX would be an easily applicable way to clinically test the effectiveness of denervation in AFG patients.

Coleman argued that some recipient areas of extreme motion may have a percentage of the infiltrate forced out by motion postoperatively.[45] Comparing AFG with the subcutaneous and intramuscular planes, as well as to the fat pad of mice, Shi et al[10] found that the worst retention rates were detected in the intramuscular plane. Unfortunately, different recipient areas were chosen for the three types of recipient tissue—the dorsal dermis, the biceps femoris, and the inguinal fat pad—rendering these results less significant.

In our trial, one statistically significant effect was observed at the 10% significance level: In animals with intact nerves, after 6 weeks, intramuscular grafting resulted in decreased viability compared with subcutaneous AFG (-10.21%; 95% confidence interval [-21.1 to 0.68], *P*-value = .07). This confirms our hypothesis that muscle contractions diminish the viability of recently transplanted fat.

Engraftment Rate

There is great variability in estimating engraftment rates, and resorption rates ranging from 25% to 90% have been reported.[46–48] Engraftment rates in humans are mostly recorded to be approximately 60%.[11,23,40] Exceptionally, reduced or missing engraftment caused by heavy scarring or radio damage has been noted.[19] Results comparable to ours were observed in the following animal studies: Mikus et al[49] examined the outcome of AFG in the canine vocal fold, where results showed an average volumetric "take" of only approximately 20%. A similar study by Kruschewsky et al[50] revealed a rate of AFG absorption ranging from 68% to 82%. Interestingly, those studies measured the fat volume immediately after injection, and found a marked difference between the volume intended for injection and that detected by MRI: Fat injection resulted in a considerable loss of fat volume (approximately 50%).[50]

Very little is known about the engraftment rates in rodents, but Seaman et al[36] found that duration ex vivo (while processing) affects interstitial cell viability more in murine than human adipose tissue. This may explain in part the difference in results between clinical and animal studies.

Measurement Methods

A further reason for leading to such diverse results among different studies may be that most of them lack quantifiable measurement methods. Common monitoring techniques in humans include 2-dimensional (2D) clinical photography and often unvalidated questionnaires.[19,20,22] Unfortunately, these techniques do not allow for precise information to be extracted about volume gain, or the quality MRI volumetric measurements of these techniques that are said to be the most accurate and objective method available when it comes to AFG of the breast.[51] According to

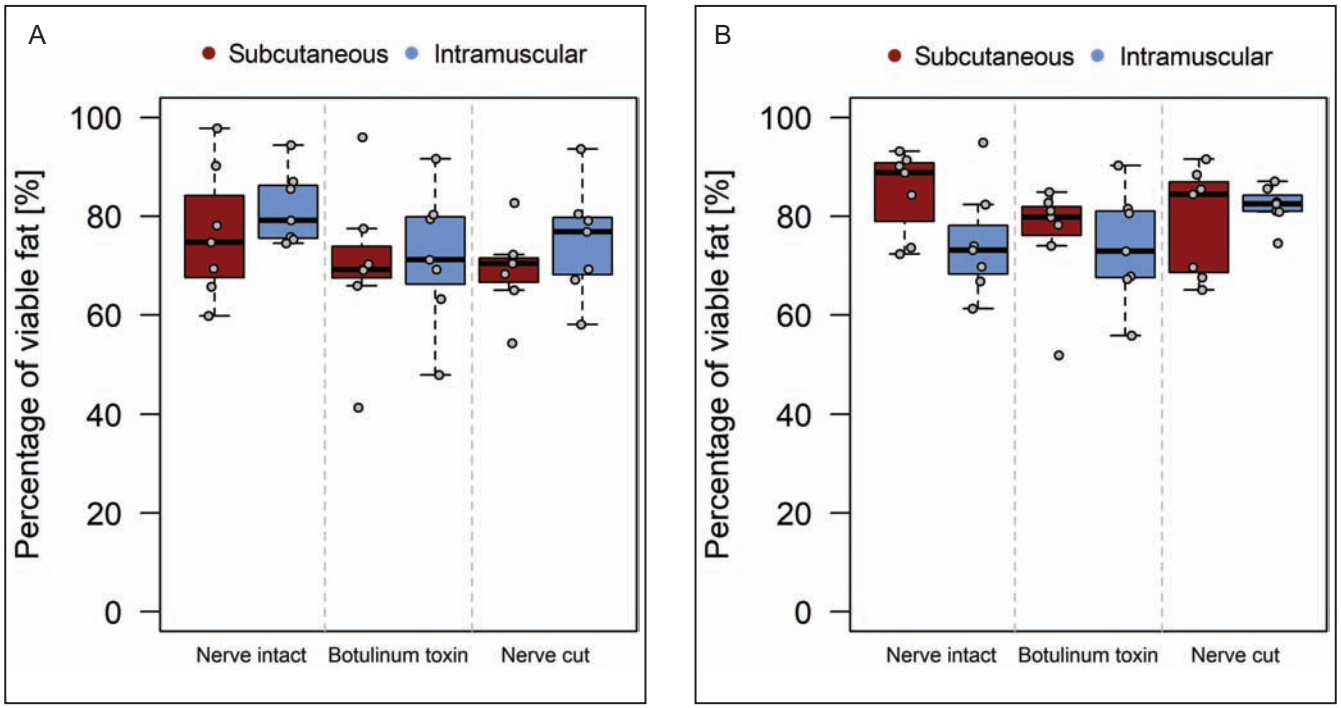

Figure 6. Percentage of viable fat cells within one representative histological section stratified for injection layer and nerve treatment (A) 2 weeks after the injection and (B) 6 weeks after the injection. Boxes represent first and third quartiles with the median central. The whiskers represent fifth and 95th percentiles, and the observed data are represented by the white dots. The injection layer is color-coded.

Herold et al,[52] MRI volumetry is indeed a useful tool for the fast, exact, and reproducible analysis of breast tissue volume. As demonstrated by the results of our study, MRI proved to be an excellent method for quantification and quality measurement.

To calculate the retention ratio in an animal study, the wet weight of the extracted grafts of mice was measured by Shi et al.[10] In a rat model, Condé-Green et al[53] evaluated graft survival through serial measurements of volume retention. A disadvantage of these strategies is that accuracy might be limited when measuring subcutaneous volume from the body surface. Besides our work, the 2007 study of Kruschewsky et al[50] is a rare example of objective fat volume measurement by MRI in an animal experiment.

Our histological evaluation demonstrates that 6 weeks postoperatively, the remaining proportion of the absolute fat volume was less than 20%. Interestingly, at both time points (2 and 6 weeks), the proportion of viable fat cells was of good quality, at approximately 81%. Most oil cysts were detected after 6 weeks in the nerve intact group, which seems to further demonstrate the disadvantages of muscle contraction when focusing on graft survival.

Combining the MRI and histology data of this study showed a reduction of inflammatory signs over time. This is in line with the findings of Mikus et al,[49] who observed that 12 weeks following AFG, there was relatively little inflammation present in the tissue surrounding the injected fat, suggesting a stable fat graft volume.

Limitations

A limitation of our study is that subcutaneously injected fat can be difficult to distinguish from the surrounding native fat tissue. This appears to be a well-known problem, because Yu et al[47] reviewed a lack of strong enough research evidence to measure fat survival rate in the subcutaneous tissue due to the less quantifiable measurement. Moreover, the subcutaneous layer in rats is quite different from that in humans, limiting its comparative value for the clinical setting.

A further limitation is that the harvest of the inguinal fat pad followed by mincing of the fat, prior to injection, does not expose adipocytes to the same type of sheer stress as suction lipectomy, nor does it involve lidocaine or epinephrine, both of which can impact adipocyte physiology in clinical practice.

Ideally, we aim to include a cellular viability assay in a future study to allow for a more reliable analysis of the viability of adipocytes. To exclude only partial denervation by nerve excision or BTX injection, future studies should include loss of motor endplates with, eg, alpha-bungarotoxin (α-BTX) staining.

Table 2. Model Estimates*

Parameter	Estimate	95% CI	*P*-value
(Constant)	76.54	[68 to 85.07]	< 0.001
Time			
6 weeks vs 2 weeks	8.27	[-3.8 to 20.35]	0.17
Nerve treatment			
After 2 weeks			
Botulinum toxin vs. nerve intact	-6.63	[-18.71 to 5.44]	0.27
Nerve cut vs. nerve intact	-7.43	[-19.51 to 4.64]	0.22
After 6 weeks			
Botulinum toxin versus nerve intact	-8.72	[-20.79 to 3.35]	0.15
Nerve cut vs. nerve intact	-5.93	[-18 to 6.15]	0.33
Layer intramuscular vs subcutaneous			
After 2 weeks			
When nerve is intact	5.13	[-5.77 to 16.02]	0.35
When nerve is Botulinum toxin treated	1.95	[-8.94 to 12.84]	0.72
When nerve is cut	5.81	[-5.08 to 16.7]	0.29
After 6 weeks			
When nerve is intact	-10.21	[-21.1 to 0.68]	0.07
When nerve is Botulinum toxin treated	-2.35	[-13.24 to 8.54]	0.66
When nerve is cut	3.19	[-7.7 to 14.09]	0.56

* The estimated effects on the percentage of viable fat cells in the graft of the injection layer after 2 and 6 weeks in the different nerve treatment settings, as well as the effect of time and the effect of nerve treatments themselves for the different time points of measurement. The reference categories used are subcutaneous, nerve intact, and 2 weeks after treatment.

Another limitation of our study is that because only one anesthesia per animal was authorized by the animal ethics committee, different rats had to be compared at the two time points. For the same reason, BTX was injected at the time of AFG, despite its onset of action being 2 to 5 days after injection.

Our original study outline aimed to carry out the experiments over 3 months. However, because no similar experiment had been carried out in our national facilities, the animal ethics committee expressed some concerns regarding the timeframe, requesting us to start with the shorter time frame of 2 and 6 weeks instead. To best simulate clinical experience, fat survival should ideally be measured after a period of 3 months. It is our clinical experience, however, that most resorption takes place before postoperative week 6. We are optimistic that, following the successful finalization of the current study, we will be authorized to conduct observation over a 3-month period in future studies.

Unfortunately, this study did not detect statistically relevant differences because of the limited number of animals per group. Clinically relevant effects are, however, possible, because confidence intervals included clinically relevant differences.

CONCLUSION

This study indicates a trend that utilizing intramuscular injection on an intact nerve leads to unfavorable results compared with subcutaneous injection. On the other hand, if the nerve is cut or treated with BTX, intramuscular injection tends to be superior to subcutaneous injection. The observed ratio of viable fat cells within the grafts stayed at approximately 81 % 6 weeks after AFG. Signs of inflammation diminished between weeks 2 and 6. MRI proved to be an excellent method for quantification and quality measurement, because it allowed for depiction of the entire breast tissue.

Finally, this study contributed to proving the scientific value of performing AFG in rats, in response to our initial question, namely, what remains in a patient after AFG. Many questions remain, and issues such as processing optimization, choice of recipient area, and engraftment rates will have to be studied further to make the outcomes of AFG to the breast more reproducible and predictable.

Acknowledgements

The authors would like to thank Dr Arne Fischmann for his invaluable support with the MRI and evaluation of the generated data. The authors would also like to thank Katerina I. Kappos for proofreading the manuscript. Patricia E. Engels and Elisabeth A. Kappos contributed equally to this manuscript and should be listed as shared first authors.

Disclosures

The authors declared no conflicts of interest with respect to the research, authorship, and publication of this article.

Funding

The authors received no financial support for the research, authorship, and publication of this article.

REFERENCES

1. Coleman SR. Long-term survival of fat transplants: controlled demonstrations. *Aesthetic Plast Surg*. 1995;19(5):421-425.
2. Costantini M, Cipriani A, Belli P, et al. Radiological findings in mammary autologous fat injections: a multi-technique evaluation. *Clin Radiol*. 2013;68(1):27-33.
3. Cozzo AJ, Fuller AM, Makowski L. Contribution of adipose tissue to development of cancer. *Compr Physiol*. 2017;8(1):237-282.
4. Claro F Jr, Moreira LR, Morari J, et al. Assessment of the cancer risk of the fat-grafted breast in a murine model. *Aesthet Surg J*. 2017;37(5):603-613.

5. Cohen O, Lam G, Karp N, Choi M. Determining the oncologic safety of autologous fat grafting as a reconstructive modality: an institutional review of breast cancer recurrence rates and surgical outcomes. *Plast Reconstr Surg*. 2017;140(3):382e-392e.
6. Rieck B, Schlaak S. Measurement in vivo of the survival rate in autologous adipocyte transplantation. *Plast Reconstr Surg*. 2003;111(7):2315-2323.
7. Kononas TC, Bucky LP, Hurley C, May JW Jr. The fate of suctioned and surgically removed fat after reimplantation for soft-tissue augmentation: a volumetric and histologic study in the rabbit. *Plast Reconstr Surg*. 1993;91(5):763-768.
8. Guerrerosantos J, Gonzalez-Mendoza A, Masmela Y, Gonzalez MA, Deos M, Diaz P. Long-term survival of free fat grafts in muscle: an experimental study in rats. *Aesthetic Plast Surg*. 1996;20(5):403-408.
9. Constantine RS, Harrison B, Davis KE, Rohrich RJ. Fat Graft Viability in the Subcutaneous Plane versus the Local Fat Pad. *Plast Reconstr Surg Glob Open*. 2014;2(12):e260.
10. Shi Y, Yuan Y, Dong Z, Gao J, Lu F. The fate of fat grafts in different recipient areas: subcutaneous plane, fat pad, and muscle. *Dermatol Surg*. 2016;42(4):535-542.
11. Delay E, Streit L, Toussoun G, La Marca S, Ho Quoc C. Lipomodelling: an important advance in breast surgery. *Acta Chir Plast*. 2013;55(2):34-43.
12. Losken A, Pinell XA, Sikoro K, Yezhelyev MV, Anderson E, Carlson GW. Autologous fat grafting in secondary breast reconstruction. *Ann Plast Surg*. 2011;66(5):518-522.
13. Locke MB, Zhong T, Mureau MA, Hofer SO. Tug 'O' war: challenges of transverse upper gracilis (TUG) myocutaneous free flap breast reconstruction. *J Plast Reconstr Aesthet Surg*. 2012;65(8):1041-1050.
14. Tremp M, Meyer Zu Schwabedissen M, Kappos EA, et al. The regeneration potential after human and autologous stem cell transplantation in a rat sciatic nerve injury model can be monitored by MRI. *Cell Transplant*. 2015;24(2):203-211.
15. Pinheiro J, Bates D, DebRoy S, et al. NLME: linear and nonlinear mixed effects models. 2016. Available: http://CRAN.R-project.org/package=nlme.
16. RCore Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. 2017. Available: https://www.R-project.org.
17. Kling RE, Mehrara BJ, Pusic AL, et al. Trends in autologous fat grafting to the breast: a national survey of the american society of plastic surgeons. *Plast Reconstr Surg*. 2013;132(1):35-46.
18. Longo B, Laporta R, Sorotos M, Pagnoni M, Gentilucci M, Santanelli di Pompeo F. Total breast reconstruction using autologous fat grafting following nipple-sparing mastectomy in irradiated and non-irradiated patients. *Aesthetic Plast Surg*. 2014;38(6):1101-1108.
19. Hoppe DL, Ueberreiter K, Surlemont Y, Peltoniemi H, Stabile M, Kauhanen S. Breast reconstruction de novo by water-jet assisted autologous fat grafting–a retrospective study. *Ger Med Sci*. 2013;11:Doc17.
20. Howes BH, Fosh B, Watson DI, et al. Autologous fat grafting for whole breast reconstruction. *Plast Reconstr Surg Glob Open*. 2014;2(3):e124.
21. Bayti T, Panouilleres M, Tropet Y, Bonnetain F, Pauchot J. Fat grafting in breast reconstruction. Retrospective study of satisfaction and quality of life about 68 patients. *Ann Chir Plast Esthet*. 2016;61(3):190-199.
22. de Blacam C, Momoh AO, Colakoglu S, Tobias AM, Lee BT. Evaluation of clinical outcomes and aesthetic results after autologous fat grafting for contour deformities of the reconstructed breast. *Plast Reconstr Surg*. 2011;128(5):411e-418e.
23. Schmauss D, Machens HG, Harder Y. Breast Reconstruction after Mastectomy. *Front Surg*. 2015;2:71.
24. Pinsolle V, Chichery A, Grolleau JL, Chavoin JP. Autologous fat injection in Poland's syndrome. *J Plast Reconstr Aesthet Surg*. 2008;61(7):784-791.
25. Khouri RK, Khouri RK Jr, Rigotti G, et al. Aesthetic applications of Brava-assisted megavolume fat grafting to the breasts: a 9-year, 476-patient, multicenter experience. *Plast Reconstr Surg*. 2014;133(4):796-807; discussion 808–809.
26. Ueberreiter K, von Finckenstein JG, Cromme F, Herold C, Tanzella U, Vogt PM. BEAULI™–a new and easy method for large-volume fat grafts. *Handchir Mikrochir Plast Chir*. 2010;42(6):379-385.
27. Li FC, Chen B, Cheng L. Breast augmentation with autologous fat injection: a report of 105 cases. *Ann Plast Surg*. 2014;73(Suppl 1):S37-S42.
28. Rietjens M, De Lorenzi F, Rossetto F, et al. Safety of fat grafting in secondary breast reconstruction after cancer. *J Plast Reconstr Aesthet Surg*. 2011;64(4):477-483.
29. Li X, Guo X. Progressive fat necrosis after breast augmentation with autologous lipotransfer: a cause of long-lasting high Fever and axillary lymph node enlargement. *Aesthetic Plast Surg*. 2015;39(3):386-390.
30. Rosing JH, Wong G, Wong MS, Sahar D, Stevenson TR, Pu LL. Autologous fat grafting for primary breast augmentation: a systematic review. *Aesthetic Plast Surg*. 2011;35(5):882-890.
31. Eser C, Temiz G, Dulgar AG, Gencel E, Yavuz M. Reconstruction of acquired breast hypoplasia by subcutaneous scar releasing and repeated fat grafting combination. *Plast Reconstr Surg Glob Open*. 2015;3(6):e408.
32. Doren EL, Parikh RP, Laronga C, et al. Sequelae of fat grafting postmastectomy: an algorithm for management of fat necrosis. *Eplasty*. 2012;12:e53.
33. Mu DL, Luan J, Mu L, Xin MQ. Breast augmentation by autologous fat injection grafting: management and clinical analysis of complications. *Ann Plast Surg*. 2009;63(2):124-127.
34. Cleveland EC, Albano NJ, Hazen A. Roll, spin, wash, or filter? processing of lipoaspirate for autologous fat grafting: an updated, evidence-based review of the literature. *Plast Reconstr Surg*. 2015;136(4):706-713.
35. Condé-Green A, de Amorim NF, Pitanguy I. Influence of decantation, washing and centrifugation on adipocyte and mesenchymal stem cell content of aspirated adipose tissue: a comparative study. *J Plast Reconstr Aesthet Surg*. 2010;63(8):1375-1381.
36. Seaman SA, Tannan SC, Cao Y, Peirce SM, Lin KY. Differential effects of processing time and duration of

collagenase digestion on human and murine fat grafts. *Plast Reconstr Surg*. 2015;136(2):189e-199e.
37. Osinga R, Menzi NR, Tchang LA, et al. Effects of intersyringe processing on adipose tissue and its cellular components: implications in autologous fat grafting. *Plast Reconstr Surg*. 2015;135(6):1618-1628.
38. Niddam J, Vidal L, Hersant B, Meningaud JP. Primary Fat Grafting to the Pectoralis Muscle during Latissimus Dorsi Breast Reconstruction. *Plast Reconstr Surg Glob Open*. 2016;4(11):e1059.
39. Pilanci O, Basaran K, Aydin HU, Cortuk O, Kuvat SV. Autologous fat injection into the pectoralis major as an adjunct to surgical correction of gynecomastia. *Aesthet Surg J*. 2015;35(3):NP54-NP61.
40. Biazus JV, Falcão CC, Parizotto AC, et al. Immediate reconstruction with autologous fat transfer following breast-conserving surgery. *Breast J*. 2015;21(3):268-275.
41. Claro F Jr, Pereira FL, Pinto-Neto AM. Fat Grafting to the breast, a simple procedure for a very complex reconstruction. *Plast Reconstr Surg Glob Open*. 2016;4(1):e594.
42. Coudurier J, Ho Quoc C, Ismail M, Dlimi C, Tourasse C, Delay E. Long-term outcome of lipomodeling in Poland's syndrome: about our first case with an eleven-years' follow-up. *Ann Chir Plast Esthet*. 2015;60(1):65-69.
43. Zhu L, Mohan AT, Vijayasekaran A, et al. Maximizing the volume of latissimus dorsi flap in autologous breast reconstruction with simultaneous multisite fat grafting. *Aesthet Surg J*. 2016;36(2):169-178.
44. Chang DW, Youssef A, Cha S, Reece GP. Autologous breast reconstruction with the extended latissimus dorsi flap. *Plast Reconstr Surg*. 2002;110(3):751-9; discussion 760.
45. Coleman SR. Structural fat grafting: more than a permanent filler. *Plast Reconstr Surg*. 2006;118(3 Suppl):108S-120S.
46. Kølle SF, Fischer-Nielsen A, Mathiasen AB, et al. Enrichment of autologous fat grafts with ex-vivo expanded adipose tissue-derived stem cells for graft survival: a randomised placebo-controlled trial. *Lancet*. 2013;382(9898):1113-1120.
47. Yu NZ, Huang JZ, Zhang H, et al. A systemic review of autologous fat grafting survival rate and related severe complications. *Chin Med J (Engl)*. 2015;128(9):1245-1251.
48. Ho Quoc C, Taupin T, Guérin N, Delay E. Volumetric evaluation of fat resorption after breast lipofilling. *Ann Chir Plast Esthet*. 2015;60(6):495-499.
49. Mikus JL, Koufman JA, Kilpatrick SE. Fate of liposuctioned and purified autologous fat injections in the canine vocal fold. *Laryngoscope*. 1995;105(1):17-22.
50. Kruschewsky Lde S, de Mello-Filho FV, dos Santos AC, Rosen CA. Autologous fat graft absorption in unilateral paralyzed canine vocal folds. *Laryngoscope*. 2007;117(1):96-100.
51. Del Vecchio DA, Bucky LP. Breast augmentation using preexpansion and autologous fat transplantation: a clinical radiographic study. *Plast Reconstr Surg*. 2011;127(6):2441-2450.
52. Herold C, Ueberreiter K, Cromme F, Busche MN, Vogt PM. The use of mamma MRI volumetry to evaluate the rate of fat survival after autologous lipotransfer. *Handchir Mikrochir Plast Chir*. 2010;42(2):129-134.
53. Condé-Green A, Wu I, Graham I, et al. Comparison of 3 techniques of fat grafting and cell-supplemented lipotransfer in athymic rats: a pilot study. *Aesthet Surg J*. 2013;33(5):713-721.