FACIAL FAT GRAFTING

Technique and outcomes

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COLOPHON

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01

General Introduction

FAT GRAFTING

Facial appearance is an important function of the face.[1] Within the spectrum of surgical procedures with an aesthetic objective, facial fat grafting is an established technique to restore facial volume, to correct volume deficiencies and to improve soft tissue contours in combination with, e.g., orthognathic surgery or reconstructive surgery.[2-4]

Fat grafting literally means transplantation of autologous adipose tissue to another part of the body. The term fat grafting is often used in the context of lipofilling: the injection of autologous adipose tissue, harvested by liposuction, into subcutaneous tissues.[5,6] Fat grafting can be used in different locations of the body, but is mostly applied in the face and breast.[7]

Fat grafting is a widely applicable technique because adipose tissue is abundantly available in most subjects and the fat needed for the grafting can be easily obtained through manual liposuction.[8,9] The injected adipose tissue, the so called fat graft, survives in the recipient site but, unfortunately, decreases in volume during the first year after transplantation.[10] Although fat grafting is commonly applied, uncertainty still exists about the percentage of the retained volume of the fat graft. Researchers are pursuing predictable results to attain a proper insight into retainable fat volumes one year after grafting is important for the surgical planning of the procedure in order to meet the expectations of the patient.[11,12]

Unfortunately, the mechanism of fat graft retention is not clearly understood at this moment but a few theories exist. The "host cell replacement theory" states that the fat graft will necrotize and will be replaced by fibrotic tissue and/or new metaplastic adipocytes.[13] The "cell survival theory" poses that the transplanted adipocytes will survive at the recipient site, particularly when viable adipocytes are transplanted to "favorable" recipient sites.[14] In 2012, Eto et al.[15] have introduced the "compensatory proliferation theory" which encompasses three different zones after fat grafting: the peripheral zone (adipocytes survive by plasmatic diffusion of oxygen and nutrients; adipose stromal cells (ASCs) survive), the regeneration zone (adipocytes die due to limited diffusion; ASCs survive) and the necrotic zone (both adipocytes and ASCs die). If ASCs survive in the regenerating zone, they become activated leading to the generation of adipocytes. In the latter theory, the thickness of the regenerating zone is an important factor for predicting the volume of the fat graft. However, the retention of adipocytes in the regenerating zone depends on the micro environmental conditions such as vascularity and attachment to the surrounding tissues.[15] This dynamic remodeling of the "compensatory proliferation theory" is currently the most adhered to in literature[16] whereupon it is hypothesized that adding ASCs to a fat graft may improve its volume retention.[7,11,17]

FACIAL FAT GRAFTING TECHNIQUES

Several fat grafting techniques are available for harvesting, processing and injecting the lipoaspirate. One has to work gently at the donor site to get a substance that can pass through a thin injection cannula as well as to maintain a sufficient fraction of living adipocytes and other cells during the whole procedure from harvesting to injecting (Figure 1).[8,9]

For a long time, the so called Coleman technique was considered the gold standard. This technique includes infiltrating the subcutaneous adipose tissue of the donor site with a tumescent solution (saline with a local anesthetic) followed by liposuction with a small cannula under manual negative pressure. To get an optimal injectable graft, the lipoaspirate must be processed to remove infiltrated fluid and blood. The Coleman technique involves centrifuging the harvested adipose tissue at 3000 rounds per minute for 3 minutes.[18] Many modifications to the Coleman technique have been proposed to improve the viability of the adipocytes and to optimize the graft: different tumescent solutions, different sizes of harvesting cannulas, different negative harvesting pressures and different processing techniques.[8,9,19] It is not clear yet which processing technique is the best to give the highest yield of viable adipocytes and the highest volume retention.

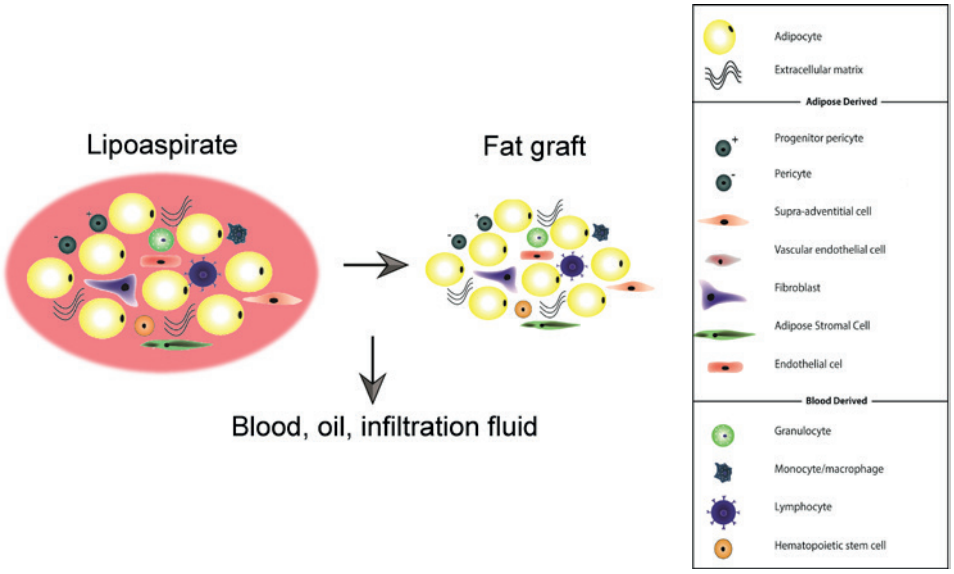

Figure 1: Schematic illustration of processing the lipoaspirate. The lipoaspirate contains adipocytes and other cell types, including ASCs, extracellular matrix, infiltrated fluid, blood and oil originating from ruptured adipocytes. The goal of processing the lipoaspirate is to optimize the fat graft by removing any blood, oil and infiltrated fluid by centrifugation, washing, decantation and filtering.

OUTCOMES OF FACIAL FAT GRAFTING

The outcome of facial fat grafting can be divided into objective (e.g., visible volumetric effect) and subjective (patients' satisfaction). Although many studies have tried to assess the effect of facial fat grafting, most did not use a validated measurement tool. This lack of a validated measurement tool hampers a valid comparison of the studies' results.

Volumetric effect

3D stereophotogrammetry is currently the most used imaging modality to measure the visible volumetric effects of facial fat grafting. 3D stereophotogrammetry is a quick, non-invasive, non-irradiating and patient friendly method to capture the facial surface in order to calculate volume differences.[20] Other modalities are computed tomography (CT) and magnetic resonance imaging (MRI), which focus more on volume retention of the graft than on the visible volumetric effect at the surface. Furthermore, on being injected into subcutaneous areas, the graft is not easy to distinguish from the other tissues present at the recipient site.

A 3D stereophotogrammetric image has a system accuracy of 0.2mm (euclidean distance).[21,22] However, additional factors may further decrease the accuracy in the clinical setting, such as facial expression during the imaging process and inaccuracies related to the analysis of sequential images (matching of pre- and post- operative surfaces or repetitive selection of target areas). An accumulation of system accuracies and clinical factors is defined as clinical accuracy but clinical accuracy is lower than the accuracy of the camera system itself.[23]

Most clinical studies that assessed the volumetric effects of facial fat grafting did not attempt to improve the clinical accuracy of 3D stereophotogrammetry. Many studies lack a protocol for standardized imaging.[4,24-27] Furthermore, most volumetric outcome assessments of facial fat grafting were from the full face[24,25] or from large, rather inaccurately, manually selected areas around the fat graft.[4,26,27] Since the current techniques selected large areas, it is still not clear whether the volumetric effect of facial fat grafting is region dependent. It has been hypothesized that there might be a difference in the volume gained between the target areas, e.g., between the zygoma and lips.[28]

Patients' satisfaction

Apart from the objective volumetric result of fat grafting, the subjective outcome (patients' satisfaction) after facial fat grafting is of utmost importance. Patient reported outcome measurements (PROMs) are increasingly used for subjective assessments of facial surgery. Although some studies assessed patients' satisfaction after facial fat grafting[25,28], none of them applied validated PROMs.

The most frequently used validated PROM for aesthetic facial procedures is the FACE-Q questionnaire[29-31]. The FACE-Q contains appraisal scales for different parts of the face as well as quality of life scales (including psychological function and social wellbeing).[29-31] The FACE-Q questionnaire has not yet been used to assess the outcome of facial fat grafting. Even more remarkable is that, of the studies that used the FACE-Q to assess the subjective outcome of aesthetic surgery, such as facelifts, blepharoplasties and orthognathic surgery[32-40], none of them included control groups. Thus, the question remains whether the observed effects on patient satisfaction are of clinical relevance. Normative FACE-Q data are not available, which is an omission since they could provide insights into the clinical relevance. Some of the issues that need to be answered are: Can an aesthetic procedure improve patient satisfaction? Are postoperative satisfaction scores comparable to pre-operative / non-operative levels?.

POTENTIAL REFINEMENT OF THE FAT GRAFTING TECHNIQUE

Over the last decade, adipose tissue has not only been considered to be a volume enhancer, but also that components of this tissue have potential regenerative effects. [7,11,41-44] It was hypothesized that adding ASCs to a fat graft might result in better volume retention.[7,11,17] A fat graft can be enriched with ASCs by adding the stromal vascular fraction (SVF) of the adipose tissue to the fat graft.

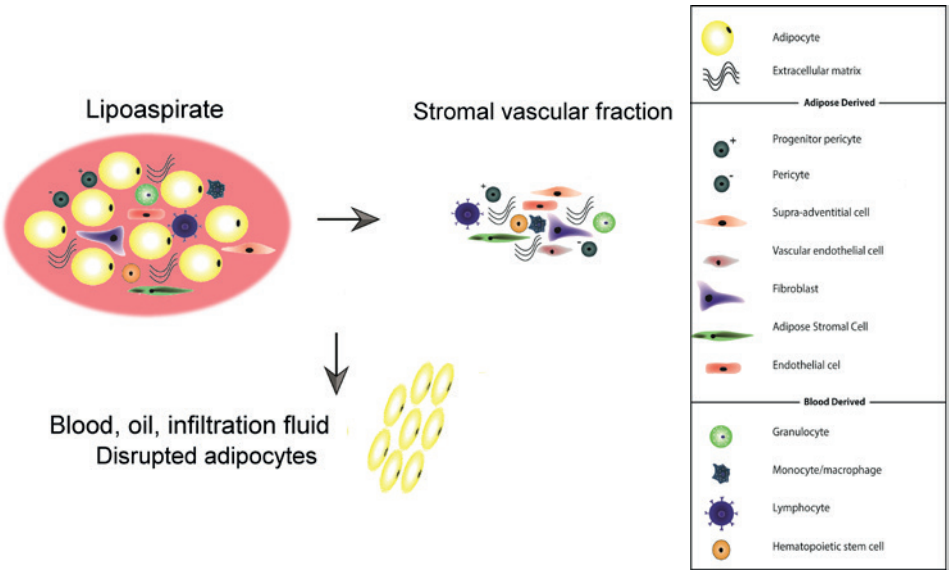

Figure 2. Schematic illustration of isolating a SVF from the lipoaspirate. Blood, infiltrated fluid, oil from dead adipocytes and disrupted adipocytes are separated from the SVF. The remaining SVF contains many different cell types, including ASCs.

A SVF contains all the non-adipocyte cell types found in adipose tissue including ASCs, endothelial cells, smooth muscle cells, immune cells and fibroblasts (Figure 2).[45] The precise mechanism that explains the volume enhancement of a SVF enriched graft is unclear, but animal studies have shown that some SVF cells in the connective tissue express the Von Willebrand factor, suggesting that increased angiogenesis could be a part of their mechanism of action.[46] SVF is isolated from the lipoaspirate by removing the adipocytes but as to which technique is most suitable clinically regarding cell yield, cell composition, duration, costs and the applicability in the clinic is not set yet.

AIMS

The general aim of the research described in this thesis was to reliably assess the clinical outcomes of facial fat grafting with respect to the visible volumetric effect of and the patients' satisfaction with facial fat grafting. Therefore, a number of studies were performed:

1. to select the best processing technique for facial fat grafting on the basis of a systematic review of the literature (Chapter 2). That technique was used for clinical evaluation in the studies described in Chapters 5 and 6;
2. to develop a valid method to measure volumetric changes in well-defined aesthetic areas as well as to assess the reproducibility of this technique when applied to the volunteers' sequential images after one year (Chapter 3);
3. to assess whether measuring facial appearance with different modules of the FACE-Q questionnaire is age related by asking different aged women who had never undergone any aesthetic facial procedures to fill in the questionnaire (Chapter 4). This study also provided normative values for the various modules of the FACE-Q with respect to the Dutch population;
4. to assess the overall and the local volumetric effects of facial fat grafting as well as to compare these effects with patients' satisfaction up to one year after fat grafting using the measurement tools developed in Chapter 3 (Chapter 5);
5. to assess whether pregnancy affects the visible volume of a facial fat graft (Chapter 6);

In addition, we recognized the potential of adding ASCs to the fat graft to optimize its retention, hence:

6. the literature was systematically reviewed to select the best technique to isolate SVF for clinical use (Chapter 7);
7. the sterility and purity of SVF, processed according to the best isolation technique resulting from the systematic literature review, were tested (Chapter 8).

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02

What is the current optimal fat grafting processing technique?

A systematic review

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ABSTRACT

Background: With the advents of new processing techniques and new graft survival theories in fat grafting, the question is: Which processing technique is of preference? This study systematically reviewed literature regarding current techniques for processing fat grafts.

Methods: PubMed, Embase, Cinahl, and Cochrane databases were searched until August 2015. Studies comparing different fat grafting processing techniques were included. Outcomes were viability of adipocytes, number of adipose-derived stromal/stem cells (ASC) and growth factors *in vitro*, volume and quality of the graft in animal studies, and satisfaction and volume retention in human studies.

Results: Thirty-five studies were included. Adipocyte viability and ASC numbers were the best using the gauze/towel technique (permeability principle) compared to centrifugation. With regard to centrifugation, the pellet contained more ASCs compared to the middle layer. The animal studies' and patients' satisfaction results were not distinctive. The only study assessing volume retention in humans showed that a wash-filter device performed significantly better than centrifugation.

Conclusion: Processing techniques using permeability principals prove superior to centrifugation (reinforced gravity principle) regarding viability and ASC number. Due to the variety in study characteristics and reported outcome variables, none of the processing techniques demonstrate any clinical evidence.

INTRODUCTION

Autologous fat transplantation (AFT) is a commonly applied procedure in reconstructive and aesthetic surgery.[1] Autologous subcutaneous fat is abundantly available in most patients, fully biocompatible and conceivably permanent.[2] AFT is used for facial rejuvenation and correction of volume deficiencies caused by trauma[3], congenital malformations[4], or after surgical procedures[2]. Moreover, AFT has been used increasingly for skin regeneration, e.g., in the case of burns and scars.[5]

Even though AFT has been performed for decades, no consensus exists about the best fat grafting technique.[6,7] Amongst others, location of donor sites, use of local anesthetics, harvesting methods, processing techniques, and injection techniques continue to be points of discussion.[6,8,9] Most studies analyzed the effects of fat processing techniques on adipocyte viability.[6] Currently used processing techniques are based on centrifugation, sedimentation, filter, or washing principles.[7,9] Recent theories focus more on the crucial role of adipose-derived stromal/stem cells (ASC)[10] and/or growth factors like vascular endothelial grow factor (VEGF)[11,12] in fat graft survival rather than adipocyte viability. These theories give the current literature another perspective.

This systematic review analyzed the effects of current processing techniques of fat grafting on adipocyte viability, levels of ASCs and growth factors *in vitro*, volume and quality of grafts in animals, as well as volume retention and patients' satisfaction in humans.

MATERIAL AND METHODS

Information sources and search

PubMed, Embase, Cochrane Central Register of controlled trials, and Cinahl electronic databases were searched (last search August, 10th 2015). Keywords used for the search were "fat graft", "fat transfer", "lipofilling", "autologous fat transplantation", or "subcutaneous fat transplant" in combination with either "processing", "harvesting", "centrifugation", "gauze", "mesh", "towel", "wash", "sieve", "sedimentation", or "decantation" (Appendix 1). The reference lists of the selected articles were screened for relevant studies missed in the search.

Eligibility criteria

Papers were eligible if at least 2 different types of fat graft processes were compared or 1 process was compared to a control group without a processing procedure. *In vitro*, animal, and human studies were included when studies assessed adipocyte viability, ASC levels, stromal vascular fraction (SVF) yield, or growth factors *in vitro*, the volume and quality of grafts

in animals, or the volume retention and patients' satisfaction in humans. Studies focusing on methods other than processing of the harvested lipoaspirate were excluded. Moreover, studies were rejected when different harvesting techniques were used between study groups within a study or when additional growth factors, SVF, or ASCs were added to the lipoaspirate. Case series (n<5), case-reports, and expert reviews were also excluded. No language restrictions were applied.

Assessment of quality of included studies

The methodological quality of the included studies was assessed using the criteria of the modified Methodological Index of Nonrandomized Studies (MINORS).[13] Table 1 describes the specific assessment criteria of the studies, specified for the current study. The authors (AJT, PD) predefined a MINORS score of ≤6 as being of insufficient quality; those studies were excluded for analysis.

Table 1. Individual MINORS criteria explained

1. Aim	Clearly stated aim. Comparison and endpoints need to be mentioned.
2. Inclusion	Clear inclusion and exclusion criteria of subjects.
3. Collection	Prospective collection of data. Protocol established before the beginning of the study
4. Endpoints	Endpoints need to be in accordance with the question/ aim of the study. Endpoints need to be clearly stated.
5. Unbiased assessment	Any form of blinding (double blind or single blind).
6. Follow up	Follow up period is sufficiently long to allow the assessment of the endpoints. *In vitro* studies = directly; *In vivo* > 28 days; *In vivo* "long term" endpoint >10 months.
7. Loss to follow up	All patients should be included in a follow up. Follow up loss may not exceed 5%.
8. Prospective calculation of the study size	A sample size calculation is performed before the start of the study.
9. Adequate control group	The control group should have a gold standard. In this assessment any form of centrifugation is 1 point.
10. Contemporary groups	Control and studied groups are managed for the same time period (no historical comparison).
11. Baseline equivalence	Study groups are similar . No confounding factors. Fat from same person, or age/gender matched fat donors/receivers.
12. Statistical analysis	Adequate reported statistical analysis.

** The items are scored 0 (not reported or reported inadequately) or 1 (reported and adequate). The ideal score for comparative studies is 12.*

Study selection

Study selection and quality assessment was done by two observers independently (AJT, PND). Disagreement was discussed during a consensus meeting. In the case of a persistent disagreement, an independent observer (AV) gave a binding verdict.

Data items

Processing techniques used in the included studies were categorized according to the following conditions: "centrifugation", "decantation", "gauze/towel", "devices", "metal sieve", "wash", "wash and centrifugation", and "negative control" (Table 2).

Table 2. Description of the processing categories

Processing categories	Code*	Principle	Further explanation
Centrifugation	c	Reinforced Gravity	Any time or g-force centrifugation. Distinct different layers in the aspirate.
Decantation	d	Gravity	Minimum of 2 minutes of decantation (sedimentation). Distinct different layers in the aspirate.
Device	dv	Wash, Permeability, (Gravity)	Using a manufactured device intended for fat grafting. Including devices for harvesting and processing in one.
Gauze/towel	g	Gravity, Permeability	Any technique using the principle of gravity through a gauze, mesh gauze or towel (fabric).
Metal sieve	s	Gravity, Permeability	Technique using the principle of gravity through a metal sieve.
Wash	w	Wash	Washing only, without any form of gravity or permeability.
Wash + centrifugation	wc	Wash, Reinforced Gravity	Combination of washing and centrifugation (any time, any g-force).
Negative control	n	-	No treatment. No distinct different layers.

Outcomes

Studies were classified based on their outcome *in vitro*, in animals, and/or in humans. *In vitro* studies analyzed adipocyte viability, number ASC or SVF yield, and growth factors. Animal studies focused on volume retention (or graft weight) and/or histologic findings in transplanted grafts such as cysts, inflammation, fibrosis, vascularization, and/or integrity. Human studies focused on volume retention using 3D imaging and/or patient or observer satisfaction using questionnaires or photographs.

Statistical analysis

Intra observer agreement for MINORS assessment was calculated by an absolute agreement score and a Cohen's kappa.

Publication bias of included studies

Publication bias could affect the results of this review. It might be more beneficial for research groups with an interest in processing devices to only publish studies with positive results of their devices. Devices were split into another subcategory in the data analysis.

Synthesis of centrifugal forces

Centrifugal forces can be displayed in revolutions per minute or g-force. Thus, to compare centrifugal forces of different studies, the relative centrifugal force (RCF) was used. If centrifugal forces were given in revolutions per minute (rpm), the RCF was calculated by the first author with the following formula[14]: RCF (in *x*g) $=1.12*10^{-5} * r * rpm^2$. This calculation means the articles had to include the radius (r) of the centrifuge or information about the specific centrifuge to then look-up the radius.

RESULTS

Included studies

In total, 401 papers were identified (Figure 1). After abstract-screening, 45 full-text studies remained and were assessed for eligibility. Three studies were excluded on the basis of the lack of comparison of at least two separate processing methods.[15-17] One study was excluded because other factors were added to the aspirate.[18] Two studies did not report an outcome of interest.[19,20] Thus, 38 studies remained for further analysis.

MINORS assessment of study quality

MINORS scores ranged from 12 to 5 (Appendix 2). All studies had a prospective collected study population, but only one study used a historical control group. Six studies reported blinded assessment of their results. Just 42% of the studies described their inclusion criteria properly. Three studies did not pass the minimum MINORS assessment score and were not analyzed further.[21-23] Thirty five studies were of sufficient methodological quality and thus compared. The absolute agreement of the MINORS score of the individual components between observers was 95%. The Cohen's kappa was 0.872 ($p<0.001$).

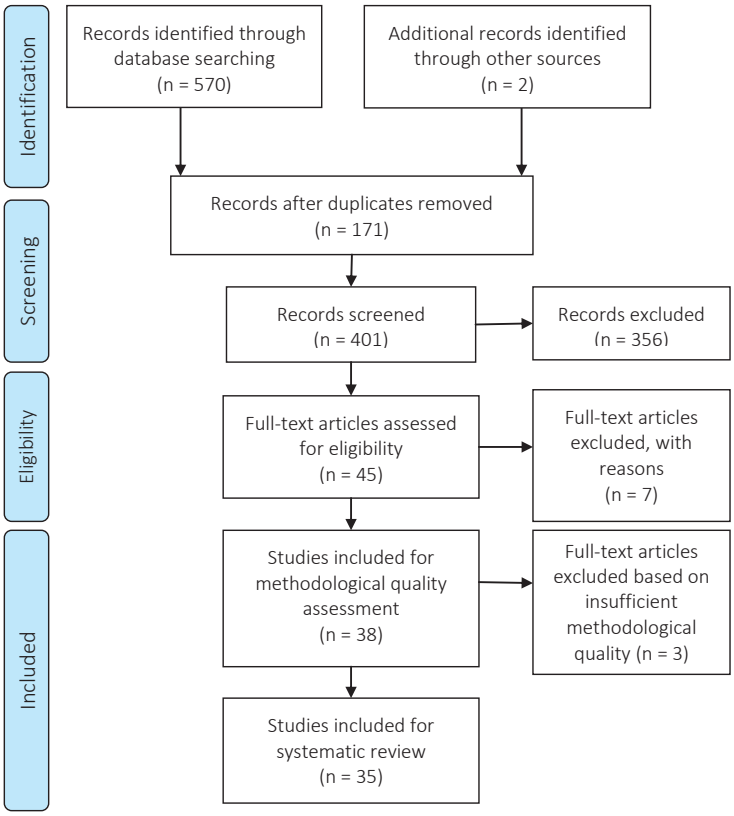

Figure 1. Flow diagram of study selection

Studies' characteristics

Of the 35 studies, two studies only analyzed processed animal fat lipoaspirate *in vitro* and 17 studies only analyzed processed human fat lipoaspirate *in vitro* (Table 3). Eight studies described processed human fat graft transplantation to animals and eight studies described a processed human fat graft transplantation to humans. Some of these *in vivo* studies (n=8) performed additionally an *in vitro* analysis of the processed lipoaspirate. Of the 26 studies in which gender was reported, 86% of the population was female (n=363 females). The characteristics of the study population, and infiltration and harvesting techniques are summarized in Table 4. Only descriptive analyses were performed since outcome variables and methods proved to be too diverse for other analysis. No meta-analyses could be conducted.

Table 3. Characteristics of the included studies according to study design

Study information					Donor characteristics					Infiltration		
First author	Year	MINORS	Design	outcomes	Total N (n females)	Average age (SD)	Age range	Primary liposuction aim	Donor site	Infiltration	Fluid	Lidocaine
Animal processed fat in vitro												
Gonzalez	2007	8	ws	V	5 rats	.	.	AFT	f	+	R	.
Piasecki	2007	7	ws	V	x mice	.	.	LS	t	.	.	.
Human processed fat in vitro												
Boschert	2002	8	ws	V	20 (16)	.	27-49	LS	a,f,h,k,t	.	.	.
Huss	2002	8	ws	V	8 (.)	.	.	LS	a,b	.	.	.
Rohrich	2004	8	ws	V	5 (.)	.	.	.	a,f,k,t	+	.	.
Rose	2006	9	bs	V	22 (.)	.	.	AFT	a	+	NaCl	50ml 1%
Kim	2009	8	ws	V	8 (.)	32 (6)	.	LS	a	+	HS	20ml 2%
Conde-Green, b	2010	10	ws	V	20 (20)	.	28-64	LS	a	+	NaCl	
Conde-Green, a	2010	9	ws	V	10 (10)	.	35-58	LS	a	+	NaCl	
Herold	2011	8	ws	V	9(5)	40 (.)	14-74	LS	a,b,h	+	NaCl	
Pulsfort	2011	8	ws	V	13 (11)	47 (11)	.	AFT/LS	.	+	NaCl	12.5ml 1% [b]
Duman	2013	7	ws	V	.	.	.	LS	a	+	NaCl	
Zhu	2013	10	ws	V	22 (22)	45 (12)	24-64	.	a,f,h	.	.	.
Kamel	2014	8	ws	V	20 (20)	31 (1)	20-41	LS	a,t	+	R	30ml 1%
Pfaff	2014	8	ws	V	5 (3)	38 (24)	12-68	.	a	+		10ml 1%
Iyyanki	2015	8	ws	V	19(19)	51(10)	41-61	AFT Breast	a, b, f	.	.	.
Osinga	2015	8	ws	V	6(3)	.	.	LS	a	+	NaCl	0.91mg/ml
Palumbo	2015	9	ws	V	5(5)	47	35-58	LS	t	+	NaCl	0.05%
Rubino	2015	8	ws	V	10(10)	.	.	AFT Breast	f	+	R	20ml 2%[c]
Human processed fat- to-animal transplantation												
Ramon	2005	11	bs	A	1 (1)	32	32	LS	b	+	R	20ml 2%
Smith	2006	10	bs	A,V	3 (3)	.	.	LS	a	+	R	30ml 1%
Kurita	2008	10	ws,bs	A,V	8 (8)	.	21-38	.	a, t	+	.	.
Minn	2010	7	bs	A,V	.	.	.	AFT Breast	a	+	R	50ml 1%
Fisher	2013	9	ws	A,V	1 (1)	57	57	LS	t	.	.	.
Hoareau	2013	10	ws	A,V	9 (9)	43 (9)	.	LS	.	+	R	40ml 2%
Ansorge	2014	12	ws	A,V	10 (9)	41(9)	30-35	LS	a	+	R	50mg 1%
Salinas	2014	7	.	A,V	9 (9)	48 (12)	29-63	LS	a,f,t	.	.	.
Human processed fat-to-human transplantation												
Butterwick	2002	8	ws	H	14 (14)	54 (.)	41-64	AFT Hands	h,k,t	+	NaCl	50ml 1%
Khater	2008	7	bs	H,V	30 (26)	.	15-47	AFT Face	t	.	.	.
Khater	2009	10	bs	H,V	51 (51)	33 (2)	16-55	AFT Face	t	.	.	.
Ferraro	2011	7	bs	H,V	30 (.)	.	30-50	AFT Buttock	h,k,t	.	.	.
Botti	2011	10	ws	H	25 (21)	46(.)	21-72	AFT Face	a,k,t	+	NaCl	0.25% [d]
Asilian	2014	11	bs	H	32 (.)	.	35-50	AFT Face	.	+	R	0.05%
Mestak	2014	9	bs	H	30 (30)	38 (.)	28-62	AFT Breast	a,f,t	+	NaCl	
Gerth	2014	9	bs	H	26(26)[a]	55 (11)	34-70	AFT Face	a,t	+	.	0.5%
												or 0.25%

		Aspiration				Processing
Epinephrine	NaHCO3	Cannula (in mm)	Cannula brand	syringe (cc)	pressure	Processing category
.	.	2;3	.	10/20/60	x neg	d, g
.	.	1.2	.	5	5cc neg	c (8x), d, g
.	.	2.0/3.0/5.0	Mercedes	sp	sp	c (4x)
.	.	5.0/6.0	Toomey	50	.	w, wc
.	.	.	Coleman	10	.	c, n
1 ml 1:000	+	.	Coleman	10	manual	c, d
0.5ml 0.1%	-	1.2	Coleman	.	.	c (8x), n
1: 500 000	-	3.0	Richter	10	manual	c, d, w
1: 500 000	-	3.0		10	.	c, d
1ml 1:1000	+	3.0	Coleman	→ TT	→-0.38 atm	c, dv, n
				→ 10	→ <2cc neg	c, dv, n
1:200 000	-	2.0	Coleman	10	.	c (7x), n
1:500 000	-	.	Lipokit	50	sp	d, dv
.	.	.	.	.	.	c, d, dv, n
1mg	-	3.0		60	→ manual	c, g
					→ 2-3 atm	c, g
1:100 000	-	.	.	10	manual	c, g
.	.	3.0	Coleman	10	manual	c, n
1.8µg/ml	+	4.0	Lenoir	10	manual	dv, n
1:100 000	+	2.0	.	sp	x neg	c (3x), d (2x)
0.5ml 1:200 000		→2.0	Coleman	10	manual	c, d
		→3.0	Mercedes	60	manual	c, d
1ml	-	2.0		10	.	c , g
1mg in 1 ml	-	.	Coleman	→ 10	→ manual	c, wc, w (2x), n
				→ sp	→ sp	c, wc, w (2x), n
.	.	.	Lipokit	50	sp	dv (5x), n
1 ml 1:1000	+	2.0	.	10	.	c, g, s
.	.	.	→ Shippert	→ TT	→-0.57 atm	c, dv, g
			→Coleman	→ 10	→.	c, dv, g
1mg/L	-	2.0	Inex	10	<2cc neg	c (6x), d
1ml 1:1000	-	3.3	VentX	sp	0,5 atm neg	c, d, dv
.	.	4.0	Mentor	.	1atm neg	c, g
1 ml 1:000	+	2.6	Klein	10	2cc neg	c, n
.	.	2.6	.	10	.	c, w
.	.	2.6	.	10	<2cc neg	c, w
.	.	3.0	.	20	x neg	c (3x), d
1:500 000	+	2.0	.	10	<2cc neg	c, s
1:1000 000	-	2.0		10	<2cc neg	c, s
1 ml	-	3.0	Mercedes	60	.	c, dv
1: 200 000	-	3.0	.	.	15 cc neg	c, dv
or 1:400.000						

*. not reported; / , more than one item used interchangeably; →, split design using different aspiration methods; ws, within subject design, more processing techniques used within one subject; bs, between subject design, only one processing technique used in individual subjects; V, in vitro outcome variable; A, animal outcome variable; H, human outcome variable; n, number of donors; x, unknown number; SD, standard deviation; Average age and SD rounded to the nearest whole number; LS, liposuction; AFT, autologous fat transplantation; a, abdomen; f, flank; h, hip; k, knee; t, thigh; +, with infiltration; -, without infiltration; NaCl, sodiumchloride; R, ringer lactate; HS, Hartman Solution; NaHCO3, sodium bicarbonate; sp, suction pump; TT, TissueTrans®; atm, atmosphere; processing category used in the study; c, centrifugation; d, decantation; dv, device; g, gauze/towel; n, no treatment; s, metal sieve; wc, washing+centrifugation; w, washing only; * Number processing categories used in the study[e] Only experimental group. 33 subjects in historical comparison average age of 54 (39-70), [b] prilocaïne , [c] lignocaïne, [d] mepivacaïne*

Table 4. Details processing techniques per study

First author	Year	Centrifugation force and time reported in study	(Calculated) Relative centrifugal force (xg)	Other techniques
Animal processed fat in vitro				
Gonzalez	2007	-	-	decantation; cotton towel (both 50g 5 min centrifugation)
Piasecki	2007	500,1000,1500,2000rpm 3 min;1000rpm 1,2,3,5,10 min	57xg, 228xg, 514xg, 913xg	decantation 15min; mesh gauze rinsed with 5cc ringer
Human processed fat in vitro				
Boschert	2002	50g 2,4,6,8 min	50ig	-
Huss	2002	wash + 200g 5min	200xg	2-4 times saline wash
Rohrich	2004	500g 2 min	500xg	no treatment
Rose	2006	3000rpm 3 min	6000xg	decantation; saline wash
Kim	2009	1500,3000,5000rpm 1,3,5 min	553xg, 2214xg, 6149xg[a]	no treatment
Conde-Green, b	2010	3000rpm 3 min	1150xg[a]	decantation; saline wash
Conde-Green, a	2010	3000rpm 3 min	1150xg[a]	decantation 30min
Herold	2011	920g 3min, 1840g 3min	920xg, 1840xg	no treatment; TissueTrans filtration
Pulsfort	2011	1000,1500,3000, 5000,7500,10.000, 15.000rpm (no duration reported)	92xg, 206xg, 825xg, 2292xg, 5157xg, 9168xg, 20.627xg	no treatment
Duman	2013	Lipokit® centrifugation 4000rpm 8min	.	no treatment
Zhu	2013	3000rpm 3min	1200xg	no treatment; decantation 20min; Puregraft® 250; Puregraft® 850
Kamel	2014	1000rpm 3min	.	mesh gauze without wash
Pfaff	2014	1500rpm 3min	.	Telfa rolling
Iyyanki	2015	3200rpm 2-3min	.	no treatment
Osinga	2015	-	-	no treatment; Shuffling though 3-way stoplock
Palumbo	2015	90g, 400g, 1500g 3min	90xg, 400xg, 1500xg	decantation 10,20,30 min
Rubino	2015	3000rpm 3 min	.	no treatment; decantation 30min
Human processed fat -to-animal transplantation				
Ramon	2005	1500rpm 2x5 min	.	cotton gauze 10min
Smith	2006	500g 2min; ringer wash + 500g 2min; saline wash + 500g 2min	500xg	no treatment; ringer wash; saline wash
Kurita	2008	Lipokit® centrifugation 400,700,1200,3000,4200g 3 min	400xg, 700xg, 1200xg, 3000xg, 4200xg	no treatment
Minn	2010	1800g 3 min	1800xg	cotton gauze, metal sieve
Fisher	2013	3000rpm 3 min	1200xg	cotton gauze; Tissuetrans filtration®
Hoareau	2013	100g 1s,1min; 400,900g 1min; 900g 3min; 1800g 10min	100xg, 400xg, 900xg, 1800xg	decantation 2 min
Ansorge	2014	1200g 3 min	1200xg	decantation 10min; Revolve system™
Salinas	2014	1200g 3 min	1200xg	mesh gauze
Human processed fat -to-human transplantation				
Butterwick	2002	3600rpm 3 min	.	no treatment
Khater	2008	3000rpm 3 min	.	saline wash
Khater	2009	3400rpm 3 min	.	saline wash
Ferraro	2011	3000rpm 3 min (1300rpm 5 min and 500rpm only in vitro analysis)	1500xg (250xg and 50xg only in vitro analysis)	decantation
Botti	2011	3000rpm 3 min	.	metal sieve + saline
Asilian	2014	3400rpm 3 min	.	metal sieve + saline
Mestak	2014	3000rpm 3 min	1150xg[a]	Puregraft® 250
Gerth	2014	unknown	.	Puregraft® 250, Purgraft®850

- no technique in this category; . no RCF calculation possible based on unknown centrifuge radius and/or RPM, insufficient data reported to calculate relative centrifugal force; [a], calculated relative centrifugal force based on the formula $RCF=1.12*10^{-5} * r * rpm^2$*. RCF= relative centrifugal force; r = radius of the centrifuge in centimeters reported in the article; rpm = revolutions per minute reported in the article.*

Processing techniques

Thirty-three studies applied some form of centrifugation (Table 3 and 4). The relative centrifugal force could not be generated from 11 studies due to insufficient information about the centrifuge. Eight studies used different types of centrifugation times and/or forces. Decantation as a processing method was applied in 15 studies, the gauze/towel in 10, devices in 11, and metal sieve in 3. Just washing was reported in 5 studies and a combination of washing and centrifugation in 4 studies.

Cell viability in vitro

Centrifugation time

Differences centrifugation time (2, 4, 6 or 8 minutes) at 50xg did not affect viability in one study.[24] An other study reported a reduction in the number of viable cells after centrifuging for 5 minutes (at three different speeds, approximately 553xg, 2214xg, and 6149xg).[25]

Centrifugation forces

The number of viable cells were reduced with an increase in relative centrifugal force, above 6149xg [25] and viable cells dropped between 228xg and 514xg [26]. In contrast, other studies did not find a reduction in the number of viable cells with an increase in centrifugation forces (above 20.627xg [27] and 4200xg [28]). In another study, viability was not affected by higher centrifugation forces, but more apoptotic and fewer necrotic cells were observed at 1500xg for 3 minutes compared to 50xg for 10 minutes and 250xg for 5 minutes.[29]

Centrifugation versus no centrifugation/decantation

Centrifugation resulted in significantly fewer intact cells[30-32] or more altered cells[33] compared to decantation. In contrast, one study found significantly better viability after centrifugation (57xg and 228xg 3min) and decantation[26] compared to the negative control whereas one study did not find a difference in viability[34] between centrifugation and the negative control.

Gauze/towel

Two studies reported a significantly higher number of viable cells using the mesh gauze technique compared to centrifugation (at 1000 and 1500 rpm 3 min, no RCF available).[35,36] Two other studies reported better viability with the gauze/towel technique compared to no treatment[26] and decantation[37]. In another study, no significant difference was found regarding viability between centrifugation (1800xg 3 min) and mesh gauze.[38] Additionally, both centrifugation and mesh gauze had significantly higher absorbance readings than the metal sieve technique in that study.

Devices

Adipocyte viability after processing with the TissueTrans® system (Shippert Medical Technology Corp Centennial, CO, USA) was 60%, which was significantly worse than after centrifugation (74% at 920xg 3min, 81% at 1840xg 3min).[39] Lipokit® centrifugation (Medikan Corp., Seoul, Korea) showed histologically small groups of adipocytes, while large intact adipocytes were present in the control intervention samples after centrifugation.[40] On the other hand, Puregraft® (Cytori Therapeutics Inc, San Diego, CA, USA), a closed wash/filter system, gave significantly better adipocyte viability than non-processed fat and centrifuged fat.[41]

Wash with/or without centrifugation

Washing showed, histologically, more pre-adipocytes than with centrifugation.[42] Although washing combined with centrifugation resulted in lower viability compared to sedimentation[30], washing without centrifugation[43,44] or centrifugation only[30,44] this lower viability trend was not significant in all studies.

Adipose derived stromal/stem cells (ASC) or stromal vascular fraction (SVF)

Different studies evaluated the ASC and SVF count between centrifugation and no treatment/decantation. The results varied and were generally inconsistent which technique performed best (Table 5).[28,29,31,32,45,46] Two studies found significantly higher ASC counts in the pellet of the centrifuged lipoaspirate than relating to the middle layer of the centrifuged lipoaspirate.[31,32]Two studies reported significantly better results for the gauze/towel technique compared with centrifugation based on ASC number[47] or SVF[36]. On the other hand, one study used a more strictly ASC marker profile and did not find significant differences in ASC count between the mesh gauze technique and centrifugation.[48]

Growth factors

One study did not find significant difference in the relative density unit of a broad variety of growth factors in lipoaspirates when comparing centrifugation to a closed wash/filter device (Zhu et al., 2013).[41] In another study, at 24 hours after injection in mice significantly higher concentrations of IL-6 and MCP-1 were found after centrifugation at 900xg for 3 minutes compared to centrifugation at 400xg for 1 minute and decantation.[49] No significant differences were found one week after injecting into mice.

Animal models: Graft volume and histology

All animal studies used xenografts (human fat transplanted into athymic animals, Table 6). Three out of seven studies reported a significant difference in volume or graft weight related to the different processing methods; these three studies also had shorter follow up times. Lipokit® centrifugation demonstrated significantly higher graft weight than no centrifugation.[28] A wash

filter device (Revolve system™, LifeCell Corp, Bridgewater, NJ, USA) and centrifugation had significantly better graft take than decantation, 73% and 68% respectively, compared to 38% of the fat weight before injection.[50] On the other hand in another study, the gauze/towel method gave significantly better results in graft volume with 70% retention compared to 47% retention after centrifugation.[47]

Table 5. Summary of records with ASC/SVF outcome variables

First author	Year	Method category	Outcome variable	Complement factor used for ASC measurement	Differentiation assay used	Outcome
Kurita	2008	c, n	SVF	.	no	n > c
Conde-Green, b	2010	c, d, wc	ASC, SVF	45-34+105+	no	w, c(p) > d , c(m)
Conde-Green, a	2010	c, d	ASC, SVF	45-34+105+	no	c(p) >d, c(m)
Ferraro	2010	c, n	ASC	34+90+105+	yes	c > n
Duman	2013	dv, n	SVF	.	no	dv > n
Fisher	2013	c, g	SVF	.	no	g > c
Pfaff	2014	c, g	ASC	73+105+, 73+44-, 73+90-, 90+44+	no	g > c
Salinas	2014	c, g	ASC	90+73+105-45-	no	g = c
Iyyanki	2015	c,n	ASC, SVF	11b- 45- 34+ D7FIB+ 90+	yes	c > n (only SVF)
Osinga	2015	dv, n	SVF	.	yes	dv = n
Palumbo	2015	c,d	ASC, SVF	45-105+90+	yes	c = d

. not reported; m, middle layer of the centrifuged lipoaspirate; p, pellet of the centrifuged lipoaspirate; processing category used in the study; c, centrifugation; d, decantation; dv, device; g, gauze/towel; n, negative control; s, metal sieve; wc, washing+centrifugation; w, washing only; = no difference reported between used processing categories; > significant difference reported in advantage of the category in front of the > symbol.

Histologically, only a few differences were found in animal recipient sites of fat grafts. One study found less fibrosis using gauze/towel versus centrifugation.[51] Another study found no differences using the gauze/towel technique related to centrifugation, but found less inflammation in the gauze/towel compared with the metal sieve.[38]

Table 6. Summary of records with animal outcome variables

First author	Year	Donors: number (females)	Diameter Injection cannula (in mm)	End of cannula	Volume (per side)	Number of animals	Location	Technique category	Time (weeks)	N per group	Volume	Weight	Cysts / vacuoles	Inflammation	Fibrosis	Vascularity	Integrity
Ramon	2005	1 (1)	2.1	sharp	1 ml	22	nuchal	c, g	16	11	=	=	=	=	g > c	=	=
Smith	2006	3 (3)	.	.	300mg	57	flank	c, n, wc, w	12	10-30	.	=	=	=	.	.	.
Kurita	2008	3(3)	1.2	.	1 ml	72	back	c, n	4	12	.	c > n	.	.	.	.	=
Minn	2010	. (.)	1.2	.	1 ml	18	nuchal	c, g, s	12	6	.	=	.	g > s	.	=	.
Fisher	2013	1 (1)	2.1	blunt	1 ml	.	back	c, dv, g	6	.	g > c, dv	.	.	.	.	=	.
Hoareau	2013	9 (9)	1.6	.	1ml	36	flank	c, n	4	6	.	.	c > n	.	.	.	.
Ansorge	2014	10 (9)	2.1	.	1 ml	240	flank	c, d, dv	4	80	.	dv > d	.	=	=	.	.
Salinas	2014	9 (9)	2.1	.	10-1000mg	.	flank	c, g	4-6	16-24	=	.	=	=	=	.	.

. not reported; [category] processing category used in the study; processing category used in the study; c, centrifugation; d, decantation; dv, device; g, gauze/towel; n, negative control; s, metal sieve; wc, washing+centrifugation; w, washing only; =, no difference reported between used processing categories; >, significant difference reported in advantage of the category in front of the > symbol.

Table 7. Summary of records with human outcome variables.

First author	Year	Donors (female)	Age range	Donor site	Injection diameter (mm)	End canulla	Location	Method	Evaluation	Time (months)	N per group	Patient satisfaction	Objective observer	Volume
Butterwick	2002	14 (14)	41-64	h, k, t	1.2	blunt	Hands	c, n	Side preference	1/3/5	14	c > n	c > n	.
Khater	2008	30 (26)	15-47	t	.		Face	c, w	Photographs	3/6/12	15	w > c	w > c	
Khater	2009	51 (51)	16-55	t	.	.	Face	c, w	Photographs	12	24,27	w > c	w > c	.
Ferraro	2010	30 (.)	30-50	h,k	.	.	Buttocks	c, n	Questionnaire	12	10	.	c > n	.
Botti	2011	25 (21)	21-72	a, k, t	1-2	blunt	Face	c, s	Photographs, Questionnaire	2/6/12/24	32	c = s	c = s	.
Asilian	2014	31 (.)	35-50	.	1-1.5	blunt	Nasolabial	c, s	Photgraphs	1/6/12	16	c = s	c = s	.
Mestak	2014	30 (30)	28-62	a,f,t	.	blunt	Breast	c, dv	Questionnaire	pre/12	15	c = dv	c = dv	.
Gerth	2014	26(26)*	34-70	.	.		Face	c, dv	3D scan	pre/12	26*	.	.	dv > c

*. not reported; a, abdomen; h, hip; f, flank; k, knee; t, thigh; pre, preoperative; [category] processing category used in the study; n, no treatment; d, decantation; c, centrifugation; g, gauze/towel; dv, device; s, metal sieve; wc, washing+centrifugation; w, washing only; = no difference reported between processing categories; > significant difference reported in advantage of the category in front of the > symbol. * only experimental group, 33 subjects in comparison group*

Human models: graft volume and patients' satisfaction

Eight studies covered autologous fat transfer in humans (Table 7). Five studies reported on facial augmentations, whereas three studies on hands, buttocks or breast augmentation. None of the studies used the gauze/towel technique. Only one study objectified different processing methods with regard to volume retention in humans. In this study, a significant better volumetric outcome (41.2% retention; SD 24.4) was found using a closed wash/filter device (Puregraft®) compared to centrifugation (31.8% retention; SD 20.3) in a historical control group.[52]

Patients' satisfaction was comparable with the outcome of objective observers. Two studies reported that centrifugation resulted in higher satisfaction than no centrifugation in hands and buttocks.[29,53] Washing was shown to be superior to centrifugation concerning patient satisfaction after facial augmentation.[42,54] In two studies no significant difference was found in patients' satisfaction between centrifugation, the use of the metal sieve technique and the closed wash/filter device.[55,56]

DISCUSSION

The vast majority of the 35 studies included in this systematic review analyzed centrifugation as a processing technique. Centrifugation is a commonly applied method in fat graft processing and usually serves as the gold standard. However, this systematic review demonstrates that the different processing techniques prove to be superior on several and diverse aspects. Especially with regard to cell viability, centrifugation resulted in more damaged adipocytes than other processing techniques. Both laboratory and animal studies showed that the gauze/towel technique and some devices based on permeability principles performed better than centrifugation for adipocyte viability, ASC count, volume retention and histology. Unfortunately, the gauze/towel technique was not used in all the eight clinical studies. As the survival mechanism of fat grafts in humans is not fully understood (yet), it is not exactly clear which of the evaluated *in vitro* outcome variables is crucial for the optimal survival of fat grafts.

Until recently, the fat graft survival theory by Peer was commonly accepted.[57] This theory stipulates that grafts tend to survive better when transplanted as complete cell identities in *favorable transplantation niches*. Disregarding favorable transplantation niches supposedly, higher numbers of damaged results in lower retention of fat grafts. Accordingly low graft survival can be linked to centrifugaton, because centrifugation is known to result in the highest percentages of damaged adipocytes. In contrast, the atraumatic gauze/towel technique appears to perform better regarding adipocyte viability. Unfortunately, data concerning volume retention in animal and human studies is lacking to confirm this survival theory.

Recently, new theories posed stating the interaction between the different components of fat grafts, and not the viability of adipocytes, is the principal factor in fat graft survival. One theory states that existing adipocytes die shortly after transplantation and new adipocytes will grow from stem or progenitor cell proliferation, the so-called compensatory proliferation.[58,59] Some recent articles presume that poor microvascular circulation conditions trigger ASCs to induce angiogenic growth factors like VEGF.[11,60] In this respect, the facilitation of the revascularization of the graft by angiogenic growth factors, and not the stem cells, will result in better long term survival. The highest numbers of ASCs in this review were in the fat processed with the gauze/towel technique and in the pellets post-centrifugation.

Although the opinion about the survival theory has changed, the most recent studies in this review focus on other endpoints than viability, such as ASC and growth factors *in vitro*. However, it is still not proven that these laboratory outcome variables result in better fat survival in humans. Of the 35 included studies, only one measured volume retention in humans in relation to processing techniques.[52] In that study, volume retention of the lipoaspirate was higher after processing with a closed filter device than after centrifugation as measured by 3D stereophotogrammetry. Unfortunately, the proportions of adipocytes, ASCs and growth factors in the fat graft after both processing methods were not measured.

Aside from the quest for the best processing technique, recent studies predominantly focus on lipoaspirate enrichement as well as ASCs or SVF before injection, the so-called cell-assisted lipotransfer. Studies on this technique showed better fat survival in enriched fat grafts compared to animals controls[10,61,62] and human[63]. These results further indicate that ASCs appear to play an important role in fat grafting. Although enrichment of the fat graft seems to result in a powerful improvement of the number of ASCs, efficient methods for cell assisted lipotransfer (isolation and supplementation) in clinical practice are still lacking.

It is still unclear whether the use of an optimal processing technique resulting in a slightly higher level of ASCs gives a significantly higher residual volume. Studies performing cell-assisted lipotransfer used extremely high ASC counts. For example, the study performed in humans, used a 2000 times higher ASC level than found in under physiological conditions.[63] In contrast to cell-assisted lipotransfer with high ASCs numbers, another study reported that human grafts with a physiologically higher proportion of ASCs resulted in greater survival in athymic mice.[64] In that study, small differences in ASCs led to significant differences in volumetric outcome.

Both the studies[63,64] used the Coleman method (centrifugation) as a processing technique. The middle layer of the centrifuged lipoaspirate was suboptimal for adipocyte viability and ASC numbers regarding the included articles in this review. Further research is necessary to

determine whether other processing techniques, other than centrifugation, can increase the number of viable adipocytes and ASCs in processed lipoaspirates, thereby improving long term survival of fat grafts in humans.

This review was not without limitations. The great variation in outcome variables, and the development of a variety of processing method, do not allow for a straightforward answer as to which processing technique is the best. Eight categories and seven outcome variables still remain, even after simplifying the outcome variables and processing techniques. Regarding centrifugal forces, a relative centrifugal force could not be extracted in eleven studies because of insufficient information, thereby making comparison impossible. Moreover, before fat processing takes place, other steps and decisions such as infiltration solution, size of cannulas and negative harvesting pressure may impact outcome.[6] Poor methods and materials description in the included studies made grouping impossible.

CONCLUSION

Centrifugation was the most commonly analyzed processing technique in this systematic review. Processing techniques using permeability principles were superior above the centrifugation technique in *in vitro* and animal studies in terms of viability, number of ASCs and fat graft retention. Such evidence of the superiority of these processing techniques is still missing in human studies. Clinically, there is no evidence of any best fat processing technique based on the results reported in the included studies, mainly due to the lack of evidence in humans and the great diversity in methods and outcome variables applied in these studies.

Appendix 1. Search terms

Search term Pubmed:
((lipofilling[Title/Abstract]) OR ("fat graft*"[Title/Abstract]) OR ("fat transfer"[Title/Abstract]) OR ("fat transplant*"[Title/Abstract]) OR ("Transplantation, Autologous"[Mesh] AND fat [Title/Abstract]) OR ("Subcutaneous Fat/transplantation"[Mesh])) AND ((process* [Title/Abstract]) OR ("Tissue and Organ Harvesting/methods"[Mesh]) OR ("centrifugation"[Mesh]) OR (centrifugation [Title/Abstract]) OR (gauze [Title/Abstract]) OR (wash* [Title/Abstract]) OR (sedimentation [Title/Abstract]) OR (decant* [Title/Abstract]) OR (mesh [Title/Abstract]) OR (sieve [Title/Abstract]) OR (towel [Title/Abstract]) OR (device [Title/Abstract]))
Search term Embase:
(lipofilling:ab,ti OR 'fat graft': ab,ti OR 'fat transplantation':ab,ti OR 'autologous fat transplant':ab,ti OR 'fat transfer':ab,ti) AND ('harvesting':ab,ti OR proces:ab,ti OR 'centrifugation'/exp OR 'centrifugation':ab,ti OR gauze:ab,ti OR mesh:ab,ti OR towel:ab,ti OR 'wash':ab,ti OR 'sedimentation':ab,ti OR sieve:ab,ti OR device:ab,ti)
Search term Cinahl:
1. lipofilling OR fatgraft OR fat transplantation OR subcutaneous fat transplantation OR autologous fat transplantation OR fat transfer 2. process OR harvesting OR centrifugation OR gauze OR mesh OR towel OR wash OR sedimentation OR decantation OR sieve OR device 3. #1 AND #2
Search term Cochrane Library:
(lipofilling or fat transfer or fat transplantation or fat graft) AND (process* or centrifugation or sedimention or gauze or mesh or towel or wash* or sedimentation or decant* or sieve or device)

Appendix 2. Ranking of studies according to MINORS score

	1. aim	2. inclusion	3 collection	4. endpoints	5. unbiased assessment	6. follow up	7. loss to follow up	8. prospective calculation	9. centrifugation control	10 contemporary groups	11 baseline equivalence	12 statistical analysis	Total score
Ansorge et al, 2014	1	1	1	1	1	1	1	1	1	1	1	1	**12**
Ramon et al, 2005	1	1	1	1	1	1	1		1	1	1	1	**11**
Asilian et al, 2014	1	1	1	1	1	1	1		1	1	1	1	**11**
Condé-Green et al, 2010	1	1	1	1	1	1			1	1	1	1	**10**
Botti et al, 2011	1	1	1	1		1	1		1	1	1	1	**10**
Kurita et al, 2008	1	1	1	1		1	1		1	1	1	1	**10**
Hoareau et al, 2013	1	1	1	1	1	1			1	1	1	1	**10**
Smith et al, 2006	1	1	1	1		1	1		1	1	1	1	**10**
Khater et al, 2009	1	1	1	1	1	1	1		1		1	1	**10**
Rose et al, 2006	1		1	1	1	1	1		1	1		1	**9**
Condé-Green et al, 2010	1	1	1	1		1			1	1	1	1	**9**
Fisher et al, 2013	1		1	1	1	1			1	1	1	1	**9**
Mestak et al, 2014	1		1	1		1	1		1	1	1	1	**9**
Gerth et al, 2014	1	1	1	1		1	1		1		1	1	**9**
Palumbo et al 2015	1	1	1	1		1			1	1	1	1	**9**
Zhu et al, 2013	1		1	1		1			1	1	1	1	**8**
Butterwick et al, 2002			1	1	1		1		1	1	1	1	**8**
Herold et al, 2011	1		1	1		1			1	1	1	1	**8**
Kamel et al, 2014	1		1	1		1			1	1	1	1	**8**
Pulsfort et al, 2011	1		1	1		1			1	1	1	1	**8**
Kim et al, 2009	1		1	1		1			1	1	1	1	**8**
Gonzalez et al, 2007	1	1	1	1		1				1	1	1	**8**
Pfaff et al, 2014	1		1	1		1			1	1	1	1	**8**
Huss et al, 2002	1		1	1		1			1	1	1	1	**8**
Rubino et al, 2015	1		1	1		1			1	1	1	1	**8**
Boschert et al, 2001	1		1	1		1			1	1	1	1	**8**
Rohrich et al, 2004	1		1	1		1			1	1	1	1	**8**
Iyyanki et al 2015	1	1	1	1		1			1	1		1	**8**
Osinga et al 2015	1	1	1	1		1				1	1	1	**8**
Salinas et al, 2014			1	1	1	1			1	1		1	**7**
Ferraro et al, 2011	1	1	1			1			1	1	1		**7**
Khater et al, 2008	1		1			1	1		1	1	1		**7**
Duman et al, 2013	1		1	1		1			1	1	1		**7**
Piasecki et al, 2007			1	1		1			1	1	1	1	**7**
Minn et al, 2010	1		1	1		1			1	1		1	**7**
Shiffman et al 2001 *	1		1			1			1	1	1		**6**
Guijarro-Martínez et al, 2011 *		1	1			1	1		1	1			**6**
Mikus et al, 1995 *	1		1			1			1	1			**5**
	34	**17**	**38**	**33**	**10**	**37**	**13**	**1**	**36**	**36**	**32**	**32**	

The items are scored 0 (not reported or inadequate reported) or 1 (reported and adequate). The ideal score for comparative studies is 12. Three studies with a total MINORS score of 6 or lower are not included in the ranking list.

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03

Three-dimensional facial volume analysis using algorithm based personalized aesthetic templates

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ABSTRACT

Background: Three-dimensional stereophotogrammetry is commonly used to assess volumetric changes after facial procedures. A lack of clear landmarks in aesthetic regions complicates reproduction of selected areas in sequential images. We developed a three-dimensional volumetric analysis based on a personalized aesthetic template. Accuracy and reproducibility of this method were assessed.

Methods: Six female volunteers were photographed using the 3dMDtrio system, according to a clinical protocol twice at baseline (T1) and after one year (T2). A styrofoam head was used as a control. A standardized aesthetic template was morphed over the baseline images of the volunteers using a coherent point drift algorithm. The resulting personalized template was projected over all sequential images to assess surface area differences, volume differences and RMS errors.

Results: In 12 well-defined aesthetic areas, mean average surface area and volume differences between the two T1 images ranged from 7.6 to 10.1mm^2 and -0.11 to 0.13cm^3 respectively. T1 RMS errors ranged between 0.24-0.68mm (sd 0.18-0.73). Comparable differences were found between the T2 images. An increase in volume between T1 and T2 was only observed in volunteers who gained in body weight.

Conclusion: Personalized aesthetic templates are an accurate and reproducible method to assess changes in aesthetic areas.

INTRODUCTION

Three-dimensional (3D) stereophotogrammetry is commonly used to assess volumetric changes after facial aesthetic procedures, e.g., fat grafting or fillers. Multiple 3D camera systems are available which are accurate up to 0.2mm.[1,2] However, clinical accuracy of 3D stereophotogrammetry is limited due to additional errors in the process such as the matching and analysis of the 3D images and patient-related errors such as variations in facial expression or body weight.[3-5]

To objectify volume changes in a specific area of the face, 3D images need to be analyzed by software systems. With the existing software systems based on manual selection using brush or lasso tools, it is difficult to reproduce the exact same target area on sequential images, especially in areas without reproducible landmarks (cheeks or jowls).[5-7] It becomes even more complicated when this target area has undergone changes, such as after fat grafting or fillers. This uncertainty has to be reduced to a minimum to allow for reliable comparison of sequential postoperative images with preoperative images and comparison of volume differences between different patients.[7-9]

To obtain better reproducible areas on 3D images after aesthetic facial procedures, we developed a method to measure volumetric changes of well-defined aesthetic areas using a personalized aesthetic template. The aim of this study was to assess the measurement error of a three-dimensional volumetric analysis based on the personalized aesthetic template as well as to assess its reproducibility when applied to sequential images of the volunteers after one year.

METHODS

A prospective study was designed at the departments of Oral and Maxillofacial Surgery of the University Medical Center Groningen, Groningen, The Netherlands and the Radboud University Medical Center, Nijmegen, The Netherlands. The study was approved by the medical ethical review board of the University Medical Center Groningen (protocol no. 201400179).

Subjects and control

A rigid, non-deformable styrofoam 3D head (mannequin) was used as a control of the measurement error of the 3dMDtrio system (3dMD, Atlanta, USA) and the software analysis. The mannequin was put in a fixed position in front of the 3D cameras for 26 photo series. Every photo series includes one 3D image at baseline (1A) and one 3D image directly after the first image session without changing position (1B).

Six female volunteers without facial deformities were then asked to participate. 3dMD images were captured following a newly developed clinical 3D photo protocol for this purpose with two photo sessions at baseline (T1, images 1A, 1B) and two sessions after one year (T2, images 2A, 2B). The second photo session (B) occurred directly after the first photo session (A) at baseline and after one year. Five photographs were taken per session: one test photo without instructions in order to get used to the environment and the flash of the camera. After this, four photos were taken with the instruction "relax your face, open your eyes and close your lips gently". The best fit image of every session, based on intended facial expression criteria, was chosen by two observers and used for the analysis (AJT, TL). In case of disagreement the third author (JM) gave binding verdict. The volunteers' body weight was measured at T1 and T2 to ensure that measured volume changes were not as a result of weight gain or loss.

Creation of the personalized aesthetic template and analysis

Preparation of 3D images

First, a standardized template (Figure 1,video 1) with 12 aesthetic regions per facial half was designed using MeshMixer 3D software (Autodesk MeshMixer, San Francisco, CA, USA). Second, the standardized aesthetic template was globally aligned with all the selected images of the subject using seven globally pointed landmarks. Five landmarks (pupil left/right, nasion, labial commissure left/right) were located on every 3D image using the Matlab (MATLAB v2017a, The Mathworks Inc., Natick, MA, USA) automatic landmark detection program[10]. Two additional landmarks were located manually on the baseline image by two observers at the most dorsal point of the skin surface at the frontozygomatic suture left and right (AJT, TGJL) using Vultus software (3dMD LCC, Atlanta, USA). The outer boundary of the personalized aesthetic template was applied to cut off and discard irrelevant regions of all 3D images.

Personalized aesthetic template application

A non-rigid transformation based on Coherent Point Drift (CPD) morphed the standardized aesthetic template towards the baseline 3D image (Video 1).[11] The CPD is an algorithm that is based on the spatial transformation of one set of points (template) to another existing set of points (3D image). CPD was set to 300 iterations and 200 degrees of freedom. The previously located landmarks were used to enhance the CPD algorithm with landmark guidance.[12] Using a ray casting algorithm, the corresponding points of all the template's vertices were located on the corresponding 1A, 1B, 2A and 2B image. As a result, 24 aesthetic areas were selected on every 3D image (Figure 2). The forehead and nose regions were used to perform a second more accurate surface registration to match the baseline with the sequential images, since they are subject to less variation and are not so likely to be involved in most aesthetic facial procedures (fat grafting, fillers, face lift).[4]

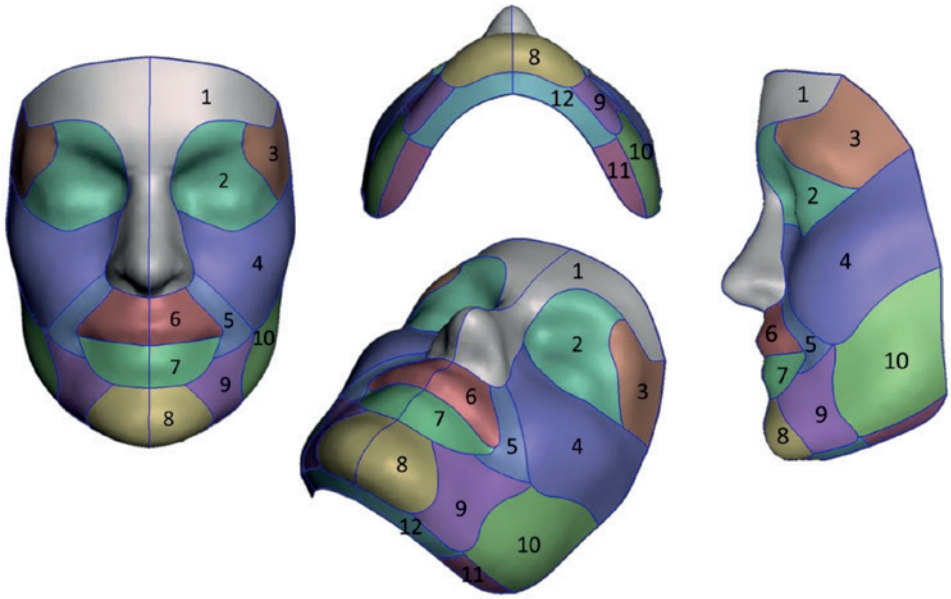

Figure 1: Standard aesthetic template with 12 areas per facial half. 1 forehead/nose; 2 eye; 3 temporal area; 4 zygomatic area/cheeks; 5 nasolabial; 6 upper lip; 7 lower lip; 8 chin; 9 prejowl area; 10 mandibular angle area; 11 submandibular area; 12 submental area.

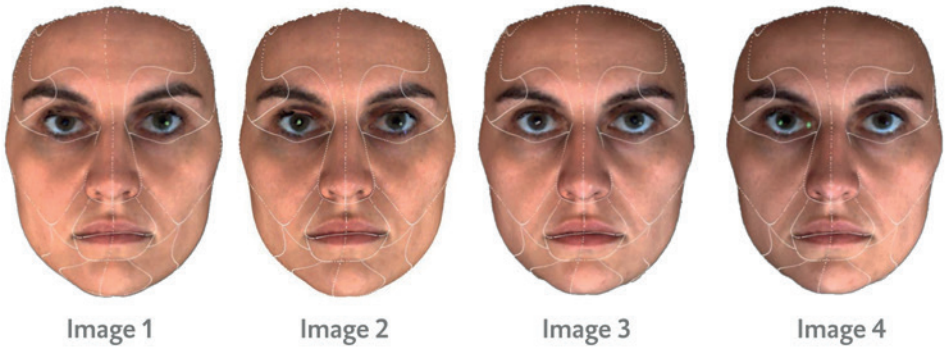

Figure 2: Example of the application of the personalized template on 4 different 3D images of a test person.

Volume measurements

3D stereophotogrammetry results in a 3D image (a shell) without a volume, therefore an additional step was performed in Matlab to assess volume differences between two 3D images. To calculate the volumes of different aesthetic areas, a virtual backplane (reference backplane)

was created by moving a copy of the baseline image (1A) 2 mm posterior in the direction of the point of gravity (Figure 3) to prevent overlap between the tested images. This results in a space between the reference backplane and the 1A, 1B, 2A and 2B images. Closing the borders between the 3D image and the corresponding reference backplane resulted in a bounded volume. Volume calculations were performed for every aesthetic zone of all images. All images used the same backplane (copy of 1A).

To secure the quality of the system and the software after one year (T2), a quality sub-analysis with the 3D images after one year (2A and 2B) was performed using 2A as a personalized aesthetic template and reference backplane.

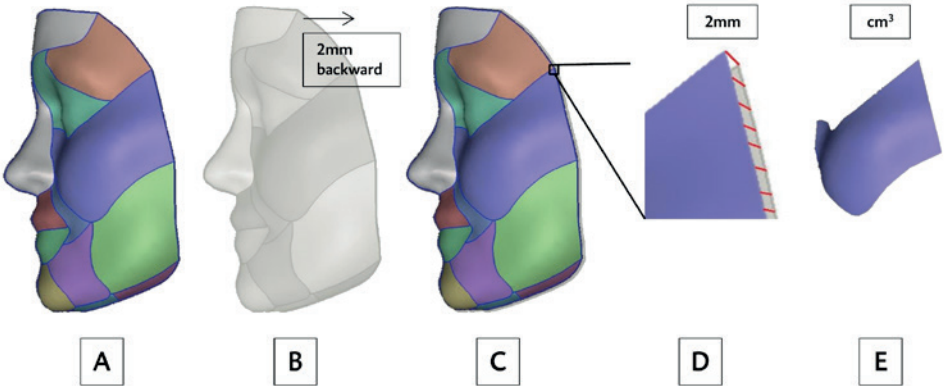

Figure 3: Schematic illustration of volume calculations using the personalized aesthetic template. A. 3D image with personalized aesthetic template. B. The reference plane which is a copy of the baseline images moved 2mm backwards. C. All sequential 3D images are projected onto the reference plane. D. The borders of the 3D images and the reference plane are closed resulting in a bounded volume per aesthetic area. E. Volume calculations are performed per aesthetic area.

Data analysis

The alignment of all the personalized templates (mannequin and volunteers) was checked by two observers (AJT, TGJL). The mannequin's aesthetic personalized template was projected onto image 1A and 1B, the volunteers' aesthetic personalized template onto images 1A, 1B, 2A and 2B. Differences in surface area of the aesthetic areas were calculated. The volumes of the 1B, 2A and 2B aesthetic areas were subtracted from the 1A volume to calculate volume differences compared to baseline (1A). Root mean square (RMS) error was calculated by dividing the volume difference by the surface area resulting in a measurement error per aesthetic area in mm.

Statistical analysis

Descriptive analysis was performed on the surface area differences, volume differences, and RMS errors per aesthetic area of the mannequin and the volunteers at baseline using IBM SPSS Statistics, Version 23.0 (Armonk, NY: IBM Corp). A Wilcoxon signed ranked test was applied for quality sub-analysis of the system at different time points. For the assessment of measured differences between both images of each individual aesthetic area at T2 (2A and 2B) compared to baseline also a Wilcoxon signed rank test was performed.

RESULTS

Measurement error of the system and analysis

No visible problems, such as wrongly projected or faulty discarded irrelevant regions of the template were objectified with the automatic application of the aesthetic template to the baseline image of the styrofoam head. The average surface areas, volume differences and RMS errors are given in Table 1A.

Table 1A: Results of mannequin at T1 (1B).

	Count	Area (mm^2)	Δ Area (mm^2)	sd	% area Δ	sd	Δ Volume (cm^3)	sd	RMS error (mm)	sd
1. Forehead/Nose	52	3302	7.08	82.33	0.21	2.51	0.28	2.38	0.55	0.45
2. Eye	52	1301	0.69	9.51	0.05	0.74	0.05	0.54	0.33	0.26
3. Temporal	52	1405	0.81	34.43	0.10	2.36	0.02	1.00	0.55	0.42
4. Zygomatic/Cheeks	52	3676	1.85	40.82	0.05	1.12	0.13	2.12	0.47	0.33
5. Nasolabial	52	509	-0.11	10.81	-0.03	2.06	0.04	0.49	0.69	0.66
6. Upper lip	52	648	5.77	23.94	0.89	5.41	0.08	0.71	0.74	0.70
7. Lower lip	52	567	-3.28	5.84	-0.57	4.97	0.06	0.47	0.59	0.57
8. Chin	52	929	-0.62	20.49	-0.10	2.24	0.05	0.89	0.73	0.59
9. Prejowl	52	1004	0.10	20.60	0.02	2.06	0.01	0.84	0.69	0.45
10. Mandibular angle	52	2151	12.30	43.42	0.59	1.02	0.06	1.28	0.48	0.33
11. Submandibular	52	404	9.57	20.22	2.26	3.66	0.10	0.76	1.46	1.63
12. Submental	52	230	4.23	11.32	1.98	2.15	0.06	0.76	2.55	1.87

Δdifference; % percentage; RMS Root Mean Square; sd Standard deviation

Table 1B: Results of 6 volunteers at T1 (1B).

	Count	Area (mm^2)	Δ Area (mm^2)	sd	% area Δ	sd	Δ Volume (cm^3)	sd	RMS error (mm)	sd
1. Forehead/Nose	12	4060	-1.11	89.30	-0.03	2.21	-0.04	2.32	0.45	0.35
2. Eye	12	1693	10.05	20.71	0.64	1.34	-0.01	0.49	0.24	0.18
3. Temporal	12	1800	-1.65	47.08	-0.08	2.66	0.11	1.44	0.64	0.46
4. Zygomatic/Cheeks	12	4037	-3.74	68.23	-0.10	1.67	0.12	2.91	0.56	0.39
5. Nasolabial	12	521	2.37	3.85	0.44	0.73	-0.01	0.28	0.42	0.28
6. Upper lip	12	621	-1.86	14.39	-0.42	2.31	-0.04	0.24	0.31	0.26
7. Lower lip	12	490	-0.75	6.24	-0.22	1.18	-0.06	0.19	0.25	0.29
8. Chin	12	793	-7.63	27.56	-0.96	3.32	-0.11	0.36	0.33	0.33
9. Prejowl	12	908	-0.19	21.48	-0.11	2.30	-0.01	0.56	0.48	0.34
10. Mandibular angle	12	2195	1.43	57.42	0.04	2.52	0.13	1.87	0.64	0.45
11. Submandibular	12	325	1.76	6.91	0.70	2.30	0.02	0.25	0.62	0.73
12. Submental	12	341	-0.64	5.41	-0.23	1.59	0.01	0.20	0.43	0.37

Δdifference; % percentage; RMS Root Mean Square; sd Standard deviation

Table 1C: Results of 3 volunteers **(without weight change)** at T2 (2A and 2B).

	Count	Area (mm^2)	Δ Area (mm^2)	sd	% area Δ	sd	Δ Volume (cm^3)	sd	RMS error (mm)	sd
1. Forehead/Nose	12	3975	42.92	111.29	1.22	2.88	1.28	2.99	0.65	0.48
2. Eye	12	1666	4.17	9.25	0.26	0.55	0.05	0.58	0.29	0.18
3. Temporal	12	1712	0.36	32.80	0.04	1.96	0.05	0.93	0.41	0.35
4. Zygomatic/Cheeks	12	3905	-18.58	45.35	-0.48	1.21	-0.50	1.91	0.40	0.30
5. Nasolabial	12	496	0.66	10.16	0.06	1.94	0.10	0.24	0.43	0.30
6. Upper lip	12	615	12.61	17.62	2.08	2.93	0.09	0.25	0.35	0.21
7. Lower lip	12	477	7.17	12.56	1.61	2.98	0.05	0.18	0.32	0.18
8. Chin	12	758	0.86	32.52	-0.01	4.59	0.10	0.28	0.29	0.26
9. Prejowl	12	873	2.36	17.27	0.13	2.05	0.13	0.37	0.39	0.24
10. Mandibular angle	12	2122	-10.69	38.07	-0.58	1.81	-0.40	1.07	0.46	0.28
11. Submandibular	12	283	0.35	11.00	0.20	4.45	-0.03	0.23	0.66	0.45
12. Submental	12	329	-9.70	16.78	-2.92	5.02	-0.07	0.27	0.68	0.51

Δdifference; % percentage; RMS Root Mean Square; sd Standard deviation

Table 1D: Results of 3 volunteers **(with weight change)** at T2 (2A and 2B).

	Count	Area (mm^2)	Δ Area (mm^2)	sd	% area Δ	sd	Δ Volume (cm^3)	sd	RMS error (mm)	sd
1. Forehead/Nose	12	4357	124.44	189.16	2.86	4.45	5.48	6.20	1.57	0.91
2. Eye	12	1728	20.00	15.38	1.17	0.88	0.37	1.13	0.58	0.33
3. Temporal	12	1967	81.16	138.36	3.97	6.98	1.98	2.96	1.24	0.96
4. Zygomatic/Cheeks	12	4179	19.12	116.97	0.44	2.68	1.67	4.21	0.78	0.63
5. Nasolabial	12	539	3.18	19.85	0.71	3.84	0.22	0.48	0.78	0.54
6. Upper lip	12	667	10.50	37.67	1.70	5.59	0.20	0.52	0.70	0.43
7. Lower lip	12	501	-6.49	9.39	-1.23	1.79	0.02	0.34	0.56	0.38
8. Chin	12	850	11.30	45.19	1.35	5.46	0.20	0.58	0.57	0.39
9. Prejowl	12	951	9.23	29.30	1.00	3.04	0.33	0.82	0.66	0.60
10. Mandibular angle	12	2261	6.12	70.47	0.29	2.98	0.31	2.25	0.60	0.68
11. Submandibular	12	396	19.07	46.00	5.69	14.16	0.18	0.43	0.75	0.80
12. Submental	12	348	8.33	14.04	2.60	4.25	0.12	0.29	0.66	0.58

Δdifference; % percentage; RMS Root Mean Square; sd Standard deviation

Validation of the clinical protocol with female volunteers

Results at T 1

The demographics of the six female volunteers are given in Table 2. The average surface area differences, volume differences and RMS errors ranged between, -7.6 to 10.1 mm^2 (sd 3.9-89.3 mm^2), -0.11 to 0.13 cm^3 (sd 0.19-2.91 cm^3) and 0.24-0.64 mm (sd 0.18-0.73 mm) respectively, meaning that any differences caused by physical movements were limited and were comparable to the Styrofoam head (Table 1B). Relatively low surface area deviations (sd <2%) were seen in the nasolabial area, the zygoma/cheek area, and the lower lip. In general, the standard deviation of the surface area and volume differences were larger in the aesthetic areas with a greater surface area, such as the zygoma/cheek and forehead/nose. When the volume differences were corrected for the surface area (RMS error), the measurement errors between the different aesthetic areas were comparable.

Table 2. Demographics of test persons.

	Gender (M/F)	Age	Height (cm)	Weight T1 (kg)	BMI T1	Weight T2 (kg)
1	F	63	178	79	24.9	79
2	F	27	172	58	19.6	58
3	F	27	177	70	22.3	72
4	F	44	180	70	21.6	72
5	F	43	173	75	25.1	77
6	F	26	175	68	22.2	68

Results at T2

The same analysis method as at T1 was used for quality sub-analysis of the system at T2. Average volume differences between baseline (1B versus 1A) and one year (2B versus 2A) were comparable (p=0.660). There were no significant differences between the measured volume differences of images 2A and 2B compared to the baseline image (1A), when using the baseline image (1A) for backplane and template (p=0.122).

Differences between T1 and T2

After one-year, the overall volume difference of all aesthetic areas increased from 0.01 cm^3 at baseline to 0.50 cm^3 after one year. To find an explanation for this difference, an extra analysis was performed. An increase in volume was observed in three volunteers who had gained 2 kg in body weight between T1 and T2 (Tables 1C, 1D, 2), while the body weight and volume difference of the other three volunteers was stable. The average volume difference after one year between volunteers who had weight gain and those who had not was 0.92 cm^3 and to 0.07 cm^3, respectively.

DISCUSSION

This study introduced a new, accurate three-dimensional analysis method to evaluate sequential 3D images, based on personalized aesthetic templates. The use of the designed 3D clinical photo protocol to reduce the influence of physiological differences, such as facial expression, resulted in volume differences that are comparable to those obtained with a styrofoam head.

In this 3D technique, measurement errors are an accumulation of errors of 3D photo acquisition, template projection and matching of the 3D surfaces. Moreover, physiological differences in the face can influence the variation of measurements. RMS errors are often used to evaluate measurement errors, because absolute volume differences are dependent on the size of the selected area. A study by Maal et al. on the accuracy of 3D stereophotogrammetry found a

variation of 0.25mm (0.21-0.27 mm) based on 100 images of one person.[4] An additional variation of approximately 0.15 mm was found over 6 weeks. Our study did not show additional variation after one year. In our opinion, a selection of different photos and following the strict instructions minimize the influence of facial expression over time. However, the Maal et al. variation was still lower after 6 weeks than our RMS error variation after one year, which was 0.29-0.68 mm. In the study of Maal et al., only one person was used for 100 3D photos. The use of a single test person might explain the lower RMS error because, in another study, Maal et al. found higher variations in a clinical test group of 15 volunteers of around 0.5mm RMS error after 3 weeks. [3] The results of this clinical test group were comparable to our results.

This is the first study using an individualized template to automatically determine specific aesthetic regions on sequential images from the same person. The personalized aesthetic template method was designed to replace the rather inaccurate lasso or brush tool method to encircle target aesthetic areas manually on sequential images. Many previous clinical studies which evaluated aesthetic facial procedures using 3D imaging, had inaccuracies in the encircled areas at different time points.[6-8] Manual selection of the target area could result in selection bias and unreliable volumetric outcomes. In this study, there was no human interference (and potential selection bias) in the selection of the aesthetic areas. Moreover, especially in regions without obvious landmarks, such as the zygoma/cheek and nasolabial area, this technique showed the smallest variation in surface area differences after one year.

The projection of the aesthetic template onto the 3D image was performed using an algorithm based on the coherent point drift.[11] This algorithm uses coherent movement of surface points (standard aesthetic template) to other surface points (baseline image) in order to preserve the topological structure of the template. Since the algorithm is based on this coherent point drift and uses a total set of points of a standard model instead of only a few landmarks, the assumption is that the template will at least also suit faces with minor deformities (mild craniofacial microsomia, after trauma, minor scarring). The advantage of algorithm based personalized templates is that volumetric changes, especially in regions without clear landmarks, can be compared objectively between patients.

The clinical 3D photo protocol of this study included instructions to relax facial expression, which is known to be the most reproducible one. [13] In order to reduce the effect of facial expression even more, the best image of the session was used. The protocol measurement errors are comparable to those attained with a fixed Styrofoam head. Although we proclaimed earlier that we prefer to keep inaccuracies by human intervention as low as possible, this selection step has not been automatized yet. No software programs or algorithms are available that are as good as the human eye to determine subtle differences in facial expression. Hence, human intervention remains unavoidable for the selection of the images.

In conclusion, a new three-dimensional protocol to evaluate 3D images reliably, based on personalized aesthetic templates, was introduced and tested. It is an accurate automated method to evaluate specific aesthetic areas of the face. Measurement errors comparable to a Styrofoam head, were achieved using the developed clinical 3D photo protocol by focusing on the standardization of facial expression.

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04

Effect of age on satisfaction with facial appearance in women based on the FACE-Q in a Dutch normative population

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SUMMARY

Background: Patient reported outcome measurements such as FACE-Q are often used to assess facial aesthetic procedures. It is poorly researched if age effects the normal satisfaction of facial appearance. Therefore, the effect of age to average facial appearance satisfaction was assessed in women who never received any kind of aesthetic facial procedures.

Methods: Dutch women aged between 18 and 85 years from all over the country were randomly asked to participate. Exclusion criteria were a history of facial surgery or facial cosmetic procedures (e.g., facelift, orthognatic surgery, injectables, blepharoplasty). Fourteen modules of the validated FACE-Q questionnaire were examined. The data were analyzed as a function of age (18-30 years, 30-39 years, 40-49 years, 50-59 years, 60+) by a Kruskal-Wallis test.

Results: 155 of the 180 volunteers who signed the informed consent completed the FACE-Q questionnaires. The median satisfaction of the "Facial appearance overall" module was 59 (IQR 51-70). Although older women gave significantly higher scores for the aging face modules such as wrinkles, lip-lines, upper eye lids, and nasolabial folds, there was no significant association between age and the scores for the module "facial satisfaction overall" ($p=0.776$). Low psychological wellbeing scores were strongly associated with low satisfaction scores with overall facial appearance (0.621, $p<0.001$).

Conclusion: Satisfaction with overall facial appearance is not associated with age in women who have had not been subjected to any kind of aesthetic facial procedures.

INTRODUCTION

The popularity of facial aesthetic procedures has increased significantly over the last few decades.[1] Both objective and subjective evaluation of the final outcome of facial aesthetic procedures is essential to enable comparison of different procedures. Objective assessments of changes after aesthetic facial procedures are commonly achieved by photographs and three-dimensional imaging. Patient reported outcome measurements (PROMs) are used for subjective assessment. Nowadays, the most frequently used and validated PROM for aesthetic facial procedures is the FACE-Q questionnaire.[2-4] Clinical studies using this FACE-Q to evaluate patient satisfaction generally focus on the difference between pre- and post-operative satisfaction rate without taking age into account.[5-8] It is known that a strong inverse relationship exists between perceived attractiveness and age.[9] Although attractiveness is not similar to being satisfied with one's facial appearance, it might quite well be possible that satisfaction with facial appearance is also affected by age. If patients' satisfaction is indeed affected by age, then study groups in clinical trials with a significant different mean age might have biased postoperative satisfaction results.

The validation studies of different FACE-Q modules did assess the effect of age in general, but did not assess the average scores per age group.[2-4] Moreover, patients used for FACE-Q validation studies all underwent different aesthetic facial procedures, such as blepharoplasty, face lifts, fillers etcetera.[2-4] Patients that have had an aesthetic facial procedure might have a different self-perception in comparison with the normative population: individuals that never received any aesthetic facial procedure. To date, no publications are available studying the influence of age on satisfaction based on the FACE-Q questionnaire in a normative population. To correct for the influence of age during post-hoc analysis when aesthetic facial procedures are evaluated in clinical studies, the effect of age on patients' satisfaction rates needs to be known.

Therefore, this study assessed if facial appearance measured by different modules of the FACE-Q questionnaire was age related in women who never received any aesthetic facial procedures.

MATERIALS AND METHODS

Subject enrollment

A prospective cross-sectional study was performed by the Departments of Oral and Maxillofacial Surgery and Plastic Surgery of the University Medical Center Groningen, the

Netherlands, between December 2017 and October 2018. The study was approved by the medical ethical review board of the University Medical Center Groningen (protocol no. 201700392).

Female volunteers, 18 years of age and over, with Dutch nationality, and a good understanding of the Dutch language were asked to participate and to complete the FACE-Q questionnaire. Exclusion criteria were a medical history of any facial surgical procedures (e.g., orthognathic surgery, oncologic surgery, trauma reconstructive procedures, facelift, lipofilling) or minimal invasive aesthetic procedures (e.g., injectables). Patients were not associated with the Departments of Oral and Maxillofacial Surgery and Plastic Surgery of the University Medical Center Groningen. An equal distribution in age was pursued resulting in 180 participants.

FACE-Q questionnaire

The original English version of the validated FACE-Q questionnaire was translated into Dutch after receiving approval from the FACE-Q editorial board, resulting in fourteen different modules (Table 1). [2-4] The translation was performed following the Mapi Research Institute (Lyon, France) linguistic validation protocol. All the participants received the FACE-Q questionnaire by email after signing and providing informed consent. Non-responders received a reminder email after one week.

Data analysis

The FACE-Q questionnaire is developed based on Rasch Measurements Theory Models.[2] Following the FACE-Q protocol, raw sum scores per individual were translated to an equivalent Rasch transformed score using the corresponding FACE-Q conversion tables. Accordingly, only the Rasch scores per module were analyzed in this study. We subdivided the fourteen modules into four categories based on the aim of the module: Overall satisfaction, appraisal of specific areas, age-related modules, and quality of life modules (Table 1).

The population was subdivided into five age categories: 18-29 years; 30-39 years; 40-49 years; 50-59 years; ≥60 years. Descriptive statistics were performed. Association between overall satisfaction and age were assessed by Spearman's Rho test. Differences in FACE-Q scores between the age groups were assessed by a Kruskal-Wallis test. Associations between the different FACE-Q modules were assessed with a Spearman Rho test.

Table 1: Examined modules of the FACE-Q questionnaire

A. Overall satisfaction:
A1. Satisfaction with Facial Appearance overall
B. Appraisal of specific facial areas:
B1. Satisfaction with Skin
B2. Satisfaction with Cheeks
B3. Satisfaction with Lips
B4. Satisfaction with Eyes
B5. Satisfaction with Lower Face and Jawline
C. Age-related modules
C1. Aging Appearance Appraisal
C2. Appraisal of Area Under Chin
C3. Appraisal of Nasolabial Folds
C4. Lines-Lips
C5. Appraisal of lines Overall
C6. Appraisal of Crow's Feet
C7. Age Appraisal-Visual Analog Scale
D. Quality of life modules:
D1. Psychological Well-Being
D2. Social Function

RESULTS

Subject demographics

A total of 155 out of the 180 participants who signed the informed consent completed the FACE-Q questionnaires. The response percentages per age group ranged from 69.4% to 83.8% (Table 2). The N per age group ranged from 25 to 36 participants. Twenty-five of the volunteers did not complete the questionnaires (without giving an explanation), and three volunteers only completed the questionnaires partially.

Table 2: Distribution of the surveyed population's age (years)

Age Group	% response	N	Mean age	Median age	Youngest of age group	Oldest of age group
18-29	76.6	36	25.3	26.2	18.1	29.7
30-39	82.1	32	34.0	33.4	30.4	39.8
40-49	79.5	31	46.1	46.9	40.6	49.9
50-59	83.8	31	55.2	55.2	50.1	59.9
60+	69.4	25	66.9	64.6	60.3	80.4
Total	78.3	155	43.9	44.4	18.1	80.4

Overall satisfaction with facial appearance

The median score of overall satisfaction with facial appearance was 59 (interquartile range (IQR) 51-70). Satisfaction with facial appearance was not significantly associated with age (p = 0.776). No significant differences were seen between the age groups (p = 0.994 Kruskal-Wallis test) (Table 3, Figure 1, supplementary Table 1).

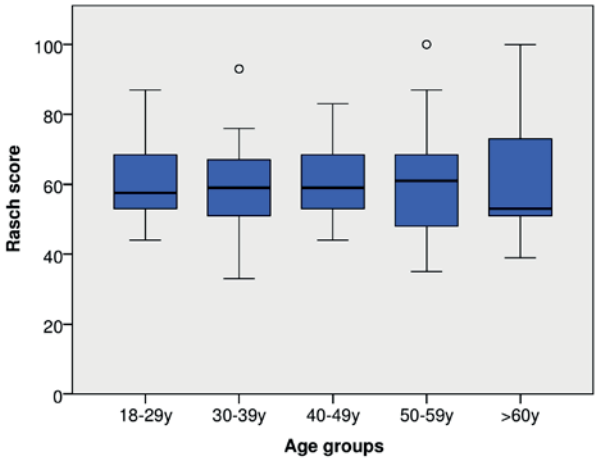

Figure 1: FACE-Q scores of module 'Satisfaction with facial appearance overall'

Satisfaction with specific areas

The percentiles of the FACE-Q scores for the different parts of the face, such as cheeks, lips, lower face and jawline, eyes and skin are shown in Table 3. The FACE-Q 'satisfaction with lips'

module was significantly different between age groups (p = 0.036) (Table 3, supplementary Table 1). Interestingly, the lowest scores for satisfaction with lips were seen in the youngest (18-29 years) and oldest (≥60 years) age groups (supplementary Table 1).

Table 3: Percentile scores of the FACE-Q A,B and D modules of the total population.

	N	P5	P25	Median	P75	P95	Age Groups p-value
A1. Satisfaction with Facial Appearance overall	155	39	51	59	70	87	0.994
B1. Satisfaction with Skin	155	41	51	59	63	93	0.304
B2. Satisfaction with Cheeks	155	35	63	70	100	100	0.399
B3. Satisfaction with Lips	155	.	.	.	.	.	0.036*
B4. Satisfaction with Eyes	155	39	59	72	86	100	0.080
B5. Satisfaction with Lower Face and Jawline	155	28	52	66	92	100	0.377
C1. Aging Appearance Appraisal	155	.	.	.	.	.	0.001*
C2. Appraisal of Area Under Chin	155	0	0	18	36	69	0.277
C3. Appraisal of Nasolabial Folds	155	.	.	.	.	.	0.001*
C4. Lines-Lips	155	.	.	.	.	.	0.000*
C5. Appraisal of lines Overall	155	.	.	.	.	.	0.006*
C6. Appraisal of Crow's Feet	155	0	0	0	23	43	0.277
C7. Age Appraisal-Visual Analog Scale	155	.	.	.	.	.	0.002*
D1. Psychological Well-Being	155	42	58	71	88	100	0.880
D2. Social Function	155	34	52	66	81	100	0.579

(P) Percentile; Independent-samples Kruskal-Wallis test was performed to test differences between age groups. (*) p-value < 0.05 was seen as a significant difference between age groups; (.) no value displayed due to significant difference between age groups. See supplementary Table 1 for percentiles per age group.

Age-related modules

The scores were significantly different between groups regarding the age-related modules, such as appraisal of upper eyelids, nasolabial folds, lines overall, and lip lines (Table 3, supplementary Table 1). All the age-related modules, except crow's feet, were positively correlated with age, whereby older women reported that they were more bothered by their aging face. The interquartile ranges of the complaints scores were broad in the over 50 age groups. The age appraisal-visual analogue scale showed that older women (age groups 50-59 and ≥60) rated themselves as younger than their actual age.

Quality of life modules

The average scores of the quality of life modules are displayed in Table 3. No significant differences with regard to psychological well-being (p=0.695) and social function (p=0.506) were observed between the age groups.

Relations between different FACE-Q modules

Correlations were seen between almost all the modules of the FACE-Q questionnaire. The highest two correlations were seen between psychological well-being and satisfaction with overall facial appearance (0.569; p<0.000) and psychological wellbeing and social function (0.669; p<0.001) (Supplementary Table 2, Figure 2).

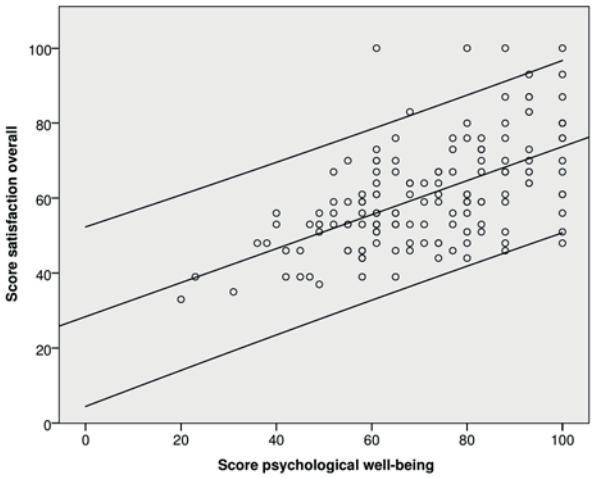

Figure 2: Correlation of FACE-Q scores of overall facial appearance and psychological well-being.

DISCUSSION

This cohort study of 155 Dutch women, who have never had any aesthetic facial procedures, clearly demonstrated that the overall satisfaction with their facial appearance, based on the FACE-Q questionnaire was not age related. The average for overall satisfaction with facial appearance is therefore suitable for all age groups from 18 to 85 years old. There was a strong positive correlation in the various modules of the FACE-Q questionnaire between overall satisfaction of facial satisfaction and psychological well-being. Moreover, these data might

be of great value when assessing how much a potential cosmetic patient deviates from the average and how much a specific facial aesthetic procedure may restore satisfaction towards "normal i.e. average", or even supersedes it.

The definition of satisfaction in general comprises a fulfilment of wishes, expectations, needs and the pleasure derived from it.[10] Differences in wishes, expectations and needs between women of different age are probably the main reason why the overall satisfaction was not age related in a normative patient population. Although older women reported that they were more bothered by features of their aging face, such as wrinkles, upper eye lids, eyes, and nasolabial folds, it did not affect the satisfactions with overall facial appearance. In this general Dutch population, the wishes, expectations and needs adapt apparently with age.

It is not surprising that that psychological well-being does have a major influence on satisfaction with facial appearance in this study. In a state being less psychologically comfortable, healthy or happy, the components 'fulfilment' and 'pleasure' of the definition satisfaction might be more difficult to achieve and might result in less satisfaction with facial appearance.

The strong positive correlation between the overall facial appearance and psychological wellbeing in this normative population was also reported in specific patient populations such as orthognathic and rhinoplasty patients.[11,12] This correlation indicates that psychological wellbeing is probably a valuable predictor for overall facial appearance in both the general population and specific patient populations undergoing aesthetic facial procedures. This finding is of major concern for aesthetic facial procedures. In our opinion, the standard use of validated questionnaires, such as the FACE-Q, is essential to evaluate the baseline level of satisfaction with facial appearance and psychological well-being. Such an ideal work-up will indicate those patients that will be most satisfied with an aesthetic facial procedure.

The given average scores for overall satisfaction with facial appearance in the general population, enable comparison of the differences between pre- and post-operative scores of a treated patient population with the range of controls, thereby balancing the true value of specific aesthetic procedures. It is also possible to evaluate the true value of the given scores after facial cosmetic procedures of previously published clinical studies. Eleven clinical studies assessed the subjective outcome of aesthetic facial procedures by means of the FACE-Q 'satisfaction with facial appearance overall' module (Table 4). A variety of surgical procedures were evaluated, such as facelift [5,6,8,13], blepharoplasty [6,14], orthognathic surgery [6,12], fat grafting[15] and fillers [7]. Unfortunately, half of these studies did not report the pre-operative FACE-Q scores.[13,14,16,17] The preoperative scores that were reported were lower than our study's median of 59, while their

Table 4: Review of literature: Average score for the 'Satisfaction with Facial Appearance Overall' Module.

	Name	Origin	Procedure	Goal	N	% F	Pre-op	sd	Post op	sd	P-value	Follow up
2019	Ozak	EU	Fat grafting	C	14	100	28.4	23.3	90.3	17.5	<0.001	>9m
2019	Berger	EU	Face-lift	C	36	86	33.8	11.4	67.2	17.0	<0.001	12m
2018	Wang	A	Face-lift	C	x	100	80.6	8.7	93.4	5.3	<0.001	12m
2018	Kant	EU	Scar treatment	R	x	64	.	.	80.4	13.2	.	36-48m
2017	Klassen	US/EU	Rhinoplasty	C,R	54	72	42.6	15.7	73.1	19.4	<0.01	4
2017	Tan	A	Orthognatic surgery	R	100	57	43*	x*	65*	x*	.	x
2017	Weinkle	US	Fillers	C	93	96	41.2	12	72.9	18.3	<0.001	4m
2017	Chen	A	Blepharoplasty	C	200	x	.	.	81.7	18.3	.	x
2016	Chang	US	Botulinum Toxin	C	57	100	x	x	x	x	<0.001	14d
2015	Sinno	US	Face-lift	C	53	100	.	.	80.7	22.3	.	3-72m
			Only presurgical data									
2016	East	US/EU	Orthognatic surgery	x	34	x	40	x				
			Minimal invasive	x	72	x	52	x				
			Rhinoplasty	x	97	x	45	x				
			Face-lift/ blepharoplasty	x	76	x	44	x				
			Body contouring	x	90	x	40	x				

*(A) Asian; (EU) European; (US) United States and/or Canada; * No RASCH Scores were reported. Raw scores 22.8 (sd 4.6) preoperative and 31.39 (sd 5.7) postoperative transformed to RASCH; (% F) percentage female; (.) Analysis not performed; (x) results not described in the paper or unclear; C cosmetic purposes; R Reconstructive purposes; sd Standard deviation; m follow up time in Months; d follow up time in days.*

post-operative scores were comparable with or higher than the normal range of the general population in our study. This finding suggests that aesthetic facial procedures result in a shift of facial satisfaction towards the normal range.[5-8,15,16]

This study calculated the average FACE-Q scores of a general Dutch population (Northern Europe). Intercultural differences in average may reflect variations regarding aesthetic ideals and aesthetic demands among cultures.[18] It is debatable if this Dutch population is comparable to other population such as the Northern American population. In previous validation studies, the Dutch population tended to report slightly lower raw scores in cross-cultural validation studies with other types of questionnaires regarding complaints or distress, compared to the Northern American population.[19-21] Therefore, there is certainly a need for cross-cultural validation studies to assess the normal range of the FACE-Q questionnaire for different cultural groups, especially between populations from Asia, Europe, North America, and Latin America.

This study focused on females' satisfaction with facial appearance, because according to the American Society of Plastic Surgeons 91.6% of the aesthetic facial procedures were performed on female patients in 2017.[1] Most previous clinical studies evaluating the facial satisfaction by means of the FACE-Q included predominantly female subjects, but occasionally combined FACE-Q scores of both sexes.[7,11,12,17] The study of Tan et al. was the only study that evaluated the differences between sexes and showed no significant differences in satisfaction scores given by both male and female orthognathic surgery patients.[12] However, the number of male patients seeking aesthetic facial procedures is increasing hence, normative data for the average satisfaction with facial appearance in males is warranted in the future.

CONCLUSION

In conclusion, the overall average satisfaction with facial appearance, as determined by the FACE-Q, is not influenced by age in women that have not been subjected to any kind of aesthetic facial procedures. However, older women reported that they were more bothered by features of their aging face such as wrinkles, upper eye lids, eyes, and nasolabial folds. Although, it did not result in a decrease of the average overall satisfaction. The obtained normative dataset from the FACE-Q questionnaire can be compared with pre- and post-operative FACE-Q values after aesthetic facial procedures.

Supplementary Table 1. Rasch scores of all FACE-Q modules per age group

		A1. Satisfaction with facial appearance overall				
	N	Median	p 05	p 25	p 75	p 95
18-29	36	58	46	53	69	87
30-39	32	59	39	51	67	93
40-49	31	59	46	53	70	83
50-59	31	61	37	48	70	87
60+	25	53	39	51	73	100

		B1. Satisfaction with Skin					B2. Satisfaction with Cheeks					B3. Satisfaction with Lips				
	N	Median	p 05	p 25	p 75	p 95	Median	p 05	p 25	p 75	p 95	Median	p 05	p 25	p 75	p 95
18-29	36	56	36	48	63	76	74	35	63	100	100	79	37	64	94	100
30-39	32	58	43	49	63	84	70	44	59	100	100	89	50	74	100	100
40-49	31	63	47	53	65	93	77	55	63	100	100	86	45	59	100	100
50-59	31	55	26	51	63	72	63	25	55	100	100	83	37	56	100	100
60+	25	57	41	47	63	100	63	35	44	100	100	64	40	56	72	100

		B4. Satisfaction with Eyes					B5. Satisfaction with Lower Face				
	N	Median	p 05	p 25	p 75	p 95	Median	p 05	p 25	p 75	p 95
18-29	36	77	47	63	92	100	66	28	49	92	100
30-39	32	75	43	63	92	100	66	0	63	100	100
40-49	31	68	51	59	81	92	66	34	66	92	100
50-59	31	63	35	51	86	100	66	34	46	92	100
60+	25	63	35	47	81	100	66	28	46	66	100

		C1. Aging Appearance Appraisal					C2. Appraisal of Area Under Chin					C3. Appraisal of Nasolabial folds				
	N	Median	p 05	p 25	p 75	p 95	Median	p 05	p 25	p 75	p 95	Median	p 05	p 25	p 75	p 95
18-29	36	100	77	90	100	100	91	30	63	100	100	100	76	91	100	100
30-39	32	100	56	80	100	100	91	10	69	100	100	100	69	100	100	100
40-49	31	77	56	70	100	100	91	36	63	100	100	100	47	100	100	100
50-59	31	83	46	60	100	100	83	24	63	100	100	100	24	83	100	100
60+	25	83	53	56	100	100	76	36	52	100	100	83	36	63	100	100

		C4. Appraisal of Lip-lines					C5. Appraisal of Lines overall					C6. Appraisal of Crow's Feet				
	N	Median	p 05	p 25	p 75	p 95	Median	p 05	p 25	p 75	p 95	Median	p 05	p 25	p 75	p 95
18-29	36	100	81	100	100	100	93	55	82	100	100	100	64	87	100	100
30-39	32	100	65	100	100	100	87	45	75	100	100	100	55	80	100	100
40-49	31	100	6	76	100	100	87	57	68	100	100	100	55	74	100	100
50-59	31	100	59	76	100	100	74	13	60	100	100	93	55	69	100	100
60+	25	71	25	54	100	100	68	38	50	80	100	93	47	64	100	100

		C7. Age Appraisal-Visual Analog Scale				
	N	Median	p 05	p 25	p 75	p 95
18-29	36	-1	-4	-2	0	3
30-39	32	0	-6	-4	0	4
40-49	31	-2	-6	-4	0	2
50-59	31	-4	-10	-5	0	2
60+	25	-3	-10	-7	-1	2

		D1. Psychological wellbeing					D2. Social Function				
	N	Median	p 05	p 25	p 75	p 95	Median	p 05	p 25	p 75	p 95
18-29	36	73	49	58	88	100	70	34	52	92	100
30-39	32	67	23	52	88	100	66	27	49	77	100
40-49	31	68	47	58	83	100	55	31	46	77	100
50-59	31	74	49	61	88	100	62	44	52	81	100
60+	25	74	47	61	83	93	62	38	55	77	100

Supplementary Table 2. Correlations between FACE-Q modules

		A1	B1	B2	B3	B4	B5	C1	C2	C3	C4	C5	D1
A1 Overall	CC	1.000											
	Sig.												
B1. Skin	CC	.419**											
	Sig.	0.000											
B2. Cheeks	CC	.492**	.408**										
	Sig.	0.000	0.000										
B3. Lips	CC	.406**	.258**	.405**									
	Sig.	0.000	0.001	0.000									
B4. Eyes	CC	.369**	.372**	.339**	.302**								
	Sig.	0.000	0.000	0.000	0.000								
B5. Lower face	CC	.497**	.408**	.512**	.452**	.374**							
	Sig.	0.000	0.000	0.000	0.000	0.000							
C1 Aging	CC	.416**	.280**	.363**	.188*	.431**	.372**						
	Sig.	0.000	0.000	0.000	0.019	0.000	0.000						
C2 Area under chin	CC	.442**	.282**	.409**	.297**	.264**	.526**	.239**					
	Sig.	0.000	0.000	0.000	0.000	0.001	0.000	0.003					
C3. Nasolabial	CC	.256**	.303**	.357**	.231**	.297**	.196*	.321**	.193*				
	Sig.	0.001	0.000	0.000	0.004	0.000	0.015	0.000	0.016				
C4. Lip-lines	CC	.300**	.176*	.269**	.373**	.237**	.236**	.385**	.276**	.427**			
	Sig.	0.000	0.028	0.001	0.000	0.003	0.003	0.000	0.001	0.000			
C5. Crow's feet	CC	0.142	0.136	0.083	0.078	.175*	0.105	.283**	.336**	.198*	.347**		
	Sig.	0.078	0.092	0.303	0.333	0.029	0.193	0.000	0.000	0.013	0.000		
D1. Psychological Wellbeing	CC	.569**	.359**	.428**	.371**	.461**	.483**	-.285**	-.384**	-0.125	-0.151	-0.067	
	Sig.	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.121	0.061	0.408	
D2. Social Function	CC	.433**	.170*	.295**	.420**	.372**	.287**	-0.115	-.217**	-0.133	-0.102	-0.063	.669**
	Sig.	0.000	0.035	0.000	0.000	0.000	0.000	0.155	0.007	0.100	0.208	0.436	0.000

(CC) Correlation coefficient; (Sig.) 2-tailed significance. (*) Correlation is significant at the 0.05 level (2-tailed).(**) Correlation is significant at the 0.01 level (2-tailed).

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05

Volumetric effect and patients' satisfaction of facial fat grafting

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ABSTRACT

Background: Facial fat grafts decrease in volume after transplantation. This observation is based on overall facial 3D analyses, since there is sparse information on volume changes in well-defined aesthetic areas. We aimed to assess the overall and more specifically the local volumetric effects of facial fat grafting and relate these effects to patient satisfaction up to one year after treatment.

Methods: All consecutive adult female patients who were scheduled for facial fat grafting without additional surgical procedures were asked to participate. In all patients the same fat grafting method was used. An algorithm based personalized aesthetic template was applied to define specific aesthetic areas on the preoperative 3D image (3dMD). Objective outcome parameters (3D volume differences, patient satisfaction (FACE-Q questionnaire)) were measured at baseline, and 6 weeks, 6 months and 12 months after fat grafting.

Results: Of 33 female patients that underwent a facial fat graft procedure, 23 patients had complete 3D data and were eligible for analysis. Highest volume gain was observed 6 weeks after grafting and was followed by a gradual loss thereafter. Overall and in the zygomatic area, a substantial gain in volume was still present 1 year after grafting, while this effect was lost in the lip area. FACE-Q scales "Satisfaction with facial appearance overall" and "satisfaction with cheeks" improved too, while "lines lips" returned to baseline levels. The improvement in FACE-Q scales was in agreement with the objective change in volume.

Conclusion: Gain in overall and local volumetric effects is accompanied by comparable changes in patient satisfaction.

INTRODUCTION

Autologous facial fat grafting is a widely used procedure in aesthetic and reconstructive facial surgery. It is a simple and safe method using subcutaneous autologous fat harvested from donor sites such as thighs, hips and lower abdomen to correct tissue volume deficiencies.[1] Unfortunately, the gain in volume after facial fat grafting is not stable as it has been shown to decrease with time.[1-4] It is unclear whether this is an overall loss of volume or if it is site specific. For example, it has been assumed that the recipient location[5] and the recipient tissue type[6], e.g., injection in fat pads, subcutaneous or intramuscular, might affect the amount of fat volume loss in the grafted area.

Objective assessment of the volume retention of the fat graft and the resulting visible volumetric effect have always been a challenge. Since the introduction of three-dimensional (3D) stereophotogrammetry in the clinical setting, this technique has evolved to the leading tool for assessing visible volumetric effects as a function of time.[4,7-9] Most clinical studies used 3D stereophotogrammetry to assess the overall visible volumetric effect, i.e., the volumetric effect of the total face.[10,11] Occasionally, attempts have been made to assess local effects by manually selecting large, not strictly predefined areas on 3D images.[12-14] It is rather difficult to reproduce and analyse such, manually selected, specific aesthetic facial areas on sequential images. Recently, we developed an automatized 3D method to study overall and local volumetric changes in predefined local aesthetic areas of the face.[15] This 3D method uses an algorithm[16] to morph a standardized aesthetic template to a patients' face resulting in a personalized template that can be used to measure changes in volume on sequential images. Using this personalized template, overall and local volume differences of well-defined aesthetic areas can be calculated as a function of time.

Besides the objectively measured visible volumetric effect, it is very important to know how patients rate the change, if any, in their facial appearance after the facial fat graft procedure. The validated FACE-Q questionnaire is a common patient reported outcome measurement (PROM) in aesthetic facial surgery that can be used for this purpose.[17-19] An advantage of the FACE-Q questionnaire is that different modules exist assessing overall satisfaction as well as satisfaction related to local areas of the face such as the cheeks, the lips and the nasolabial area.[17-19]

Yet, to the best of our knowledge, no studies are available in the literature linking local volume changes to patients' perception of these changes. Therefore, the aim of the current study was to assess the overall and more specifically the local volumetric effects of facial fat grafting and to relate them to patient satisfaction up to one year after fat grafting.

METHODS

A prospective observational study was performed at the department of Oral and Maxillofacial Surgery of the University Medical Center Groningen, Groningen, The Netherlands. The study was approved by the medical ethical review board of the University Medical Center Groningen (protocol no. NL51511.042.14) and registered in the Dutch Clinical Trial Center (NTR5325).

Patient selection

All consecutive female patients, above 18 years, that were scheduled for a facial fat grafting procedure without any additional facial surgical or cosmetic procedure at the department of Oral and Maxillofacial surgery in the University Medical Center Groningen were asked to participate in this study. Patients underwent the procedure either for aesthetic reasons or to restore a volume deficiency resulting from previous cancer surgery or facial trauma. Exclusion criteria were pregnancy at the moment of the procedure, ASA classification 3 or higher, use of anti-coagulants that could not be stopped and a medical history of body dysmorphic disorder.

Baseline data

At baseline, during the preoperative consultation, information about demographics, medical history, medication and smoking/alcohol habits were collected. Bodyweight and height were measured. A baseline 3D photo series was captured using the 3dMDtrio system (3dMD LLC, Atlanta, USA) following a standardized clinical 3D photo protocol.[15] In this 3D photo protocol, 1 test photo and 4 photos with instructions "relax your face, open your eyes, close your lips gently" were taken. In addition, the following scales of the FACE-Q were examined: "Satisfaction with facial appearance overall", "Psychological wellbeing", "Social function", "Satisfaction with cheeks", "Satisfaction with lips", "Lines lips" before the start of the procedure.[17-19]

Preoperatively, a blood sample was collected to measure cotinine to objectify smoking, and 17ß-estradiol and anti-Müllerian hormone (AMH) levels to objectify menopausal status.

Fat grafting procedure

The fat graft procedure was performed under local or general anaesthesia depending on the patient's preference. The donor site (abdomen, flank or inner knee) was infiltrated with tumescent solution (5ml xylocaine 2% in 45ml Ringers lactate). The adipose tissue was manually harvested under negative pressure of 2cc using a Sorensen cannula (Tulip Medical, San Diego, USA). The harvested tissue was processed with the PureGraft 50 closed wash system (Cytori, San Diego, USA) according to the manufacturer's protocol. Processed adipose tissue was injected with a 0.9 mm blunt cannula (Tulip Medical, San Diego, USA) in different subcutaneous layers. Data of the procedure, such as total harvested volume and injected volumes per aesthetic area, was collected.

Follow-up

Follow-up visits were scheduled 6 weeks, 6 months and 12 months after the fat grafting procedure. During these visits, adverse effects, complications were queried, bodyweight was measured, and standardized 3D photos series were made according to the baseline 3D photo protocol (1 test photo and 4 photos with specific instructions). All preoperative FACE-Q questionnaires were examined as well as two additional FACE-Q outcome scales: "Satisfaction with outcome", and "Satisfaction with decision".

Control group for satisfaction with facial appearance

155 predominantly Caucasian women, around The Netherlands, between 18 and 80 years old, who had not undergone any type of aesthetic facial surgery were randomly asked to complete the same baseline FACE-Q questionnaire modules to create normative values of satisfaction with facial appearance.[20]

3D volume measurements

After all the 3D photo sessions of the patient were completed, the most similar images per session based on facial expression were selected for further analysis according to our previously published protocol[15]. A standard aesthetic template (Figure 1) was morphed to the baseline 3D image of the patient using a coherent point drift algorithm[16] in Matlab (MATLAB v2017a, The Mathworks Inc., Natick, MA, USA) resulting in a personalized aesthetic template. This personalized template was used to determine specific aesthetic areas on all consecutive 3D images of the same patient. The personalized template was aligned to all 3D images using a ray-casting algorithm[21] in Matlab. All post-operative 3D images (with the aesthetic template) were matched to the baseline image. This matching was based on the forehead and nose region. Volume differences were calculated between the baseline image and the 3 post-operative 3D images.

Data analysis

Only patients with a complete 3D follow up dataset were included for analysis. Raw FACE-Q scores were translated to the equivalent RASCH score following the protocol of the FACE-Q editorial board.[17] FACE-Q scores were compared to scores of the control group. The outcome differences between treatment for aesthetic reasons or for reconstructive reasons were analysed.

For sub-analysis, volumetric and satisfaction results of patients that received fat injection in the zygomatic area or lip area were used. These areas were chosen because the lip and zygomatic areas were most often injected. The volumetric effect of the zygomatic area was calculated by the sum of volume differences of both zygomatic areas (left/right). This volumetric effect

was compared to the FACE-Q "satisfaction with cheeks". The volumetric effect in the lips was calculated by the sum of upper and lower lip areaThis volume effect was compared to FACE-Q "Satisfaction with lips" and "Lines lips".

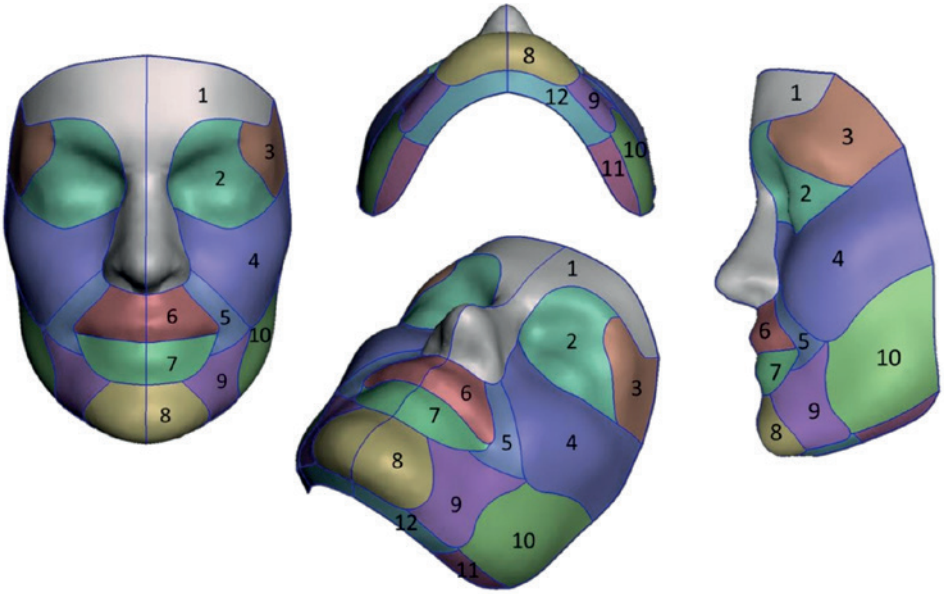

Figure 1: Standard aesthetic template with 12 aesthetic areas per facial half used for 3D analysis. Standard aesthetic template 1 forehead/nose; 2 eye; 3 temporal area; 4 zygomatic area/cheeks; 5 nasolabial; 6 upper lip; 7 lower lip; 8 chin; 9 prejowl area; 10 mandibular angle area; 11 submandibular area; 12 submental area.

Statistical analysis

Descriptive analysis was performed of the patients with complete 3D data. Due to differences in injected volume standard error of the mean was calculated for 3D volume analysis. Demographics of missing patients was compared with the study group. Between subject factors such as the patient population (aesthetic/reconstructive), menopausal status and smoking to volumetric effect and satisfaction were analysed by a repeated measurements linear model. Paired T-tests were performed to calculate differences between preoperative and postoperative FACE-Q scores. Predictors of FACE-Q modules 'satisfaction with facial appearance overall' and 'satisfaction with result' were evaluated by linear multiple regression to assess the effect of volumetric outcome, population (aesthetic or reconstructive), and the preoperative psychological well-being.

RESULTS

All eligible 33 consecutive patients agreed to participate, none of them was excluded based on the exclusion criteria. Of the 33 patients that underwent facial fat grafting, 23 patients had complete 3D data that could be used for analysis. Of the 10 patients with incomplete data, two patients were lost to follow up, one patient due to pregnancy and one patient due to recurrence of a tumour in the oral cavity, the other 8 patients missed one of the three follow up appointments. No significant differences were seen in baseline data (age, injection volume, FACE-Q scores, aesthetic/reconstructive purpose) between the 10 missing patients and the 23 patients included in the analysis which was therefore totally at random.

Table 1: Demographics of included patients.

	Total
Number	23
Age, years	49.4 (sd 14.0)
Length, cm	168 (sd 6)
Weight, kg	63.9 (sd 6.7)
Weight change during follow up	
No	20 (87%)
Yes, between +2 and +4kg	1 (4%)
Yes, between -2 and -4kg	2 (9%)
Medication use, yes	14 (61%)
Psychological treatment	
No	17 (74%)
Yes, current treatment	2 (9%)
Yes, in the past	4 (17%)
Smoking (serum level cotinin >0.5µg/ml)	6 (26%)
Menopause (serum level AMH <0.1µg/ml)	15 (65%)
Previous facial procedures or surgery	
Congenital disorders	2 (9%)
Tumors and neoplasm	8 (35%)
Trauma	1 (4%)
Population	
Aesthetic patients	12 (52%)
Reconstructive patients	11 (48%)

Demographics of the included patients with a complete follow up are described in Table 1. Twelve patients received fat grafts for aesthetic purposes and 11 patients for reconstructive purposes. On average, a total of 20.4 cm^3 processed adipose tissue was injected in different aesthetic areas (Table 2).

Table 2: Details of fat grafting.

Anaesthesia	
Local	20 (87%)
General	3 (13%)
Harvest location	
Abdomen	16 (80%)
Knee	4 (17%)
Flank	2 (9%)
Hip	2 (9%)
Thigh	1 (4%)
Total harvested volume (cm^3)	72.2 (sd 27.8)
Total injected volume (cm^3)	20.1 (sd 14.7)
Injected volume in injected zygoma areas (N=16)	12.8 (sd 7.6)
Injected volume in injected lips (N=12)	3.6 (sd 2.3)

Objective volume effect

The highest volumetric effect was seen 6 weeks postoperatively, with an average increase in visible volume of 23.9 cm^3 (Figure 2). Between 6 weeks and 12 months postoperative, the average gain in visible volume decreased to 10.4 cm^3 in comparison with the pre-operative situation. The visible volumetric effect was independent of the reconstructive or aesthetic nature of the procedure, menopause and smoking (Table 3).

Subjective patient satisfaction

Satisfaction with overall facial appearance had significantly increased after 6 weeks (p=0.001), 6 months (p=0.003) and one year (p=0.005) compared to baseline (Figure 2, Table 4). Psychological well-being and social functioning had increased significantly at all time points after grafting (Figures 3 and 4, Table 4). The increase of "Satisfaction with facial appearance overall" was comparable between aesthetic and reconstructive patients after facial fat grafting (Figure 5). Both groups had preoperative scores lower than the average scores of satisfaction in the control group, but had scores within the normal range one year after the fat graft procedure.

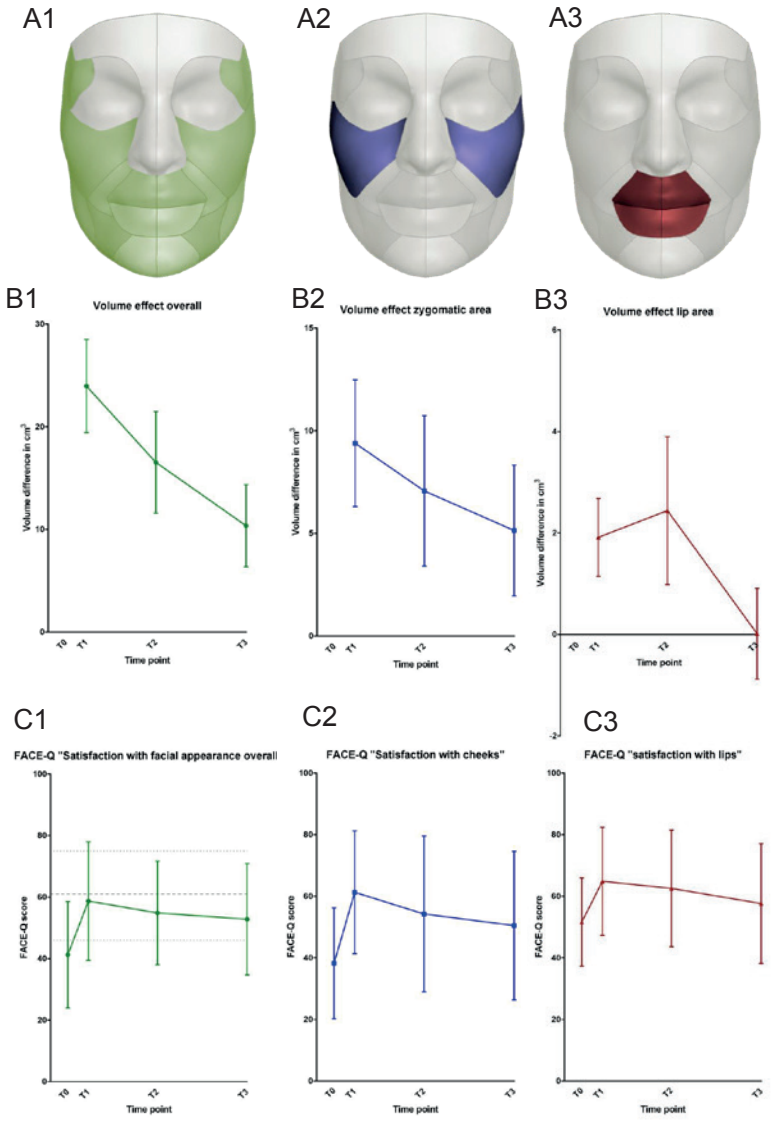

Figure 2: Volumetric effect (mean ± SEM, B) and patient satisfaction (FACE-Q score (mean ± SD), C) per injected aesthetic area (A). Group 1 (green): all patients with facial fat grafting (n=23); Group 2: patients with fat grafting in zygomatic area (n=16); Group 3 (red): patients with fat grafting in the lip area (n=12). **A1**. All fields of the aesthetic template except eye, forehead/nose, submental and submandibular area (green) (n=23 patients). **A2** Zygomatic areas (blue) (n=16 patients). **A3** Lip area (upper lip and lower lip) (red) (n=12 patients). **B1-B3:** Volume differences compared to the T0 3D image as a function of time in cm^3. Therefore, volume effect at T0 was not displayed.. **C1-C3** Patient satisfaction as a function of time. C1: "Satisfaction with facial appearance overall" (green). The dashed line represents the mean and the dotted lines the standard deviation of the controls. C2: "Satisfaction with cheeks" (blue). C3: "Satisfaction with lips" (red). T0 baseline; T1 6 weeks after fat grafting; T2 6 months after fat grafting; T3 one year after fat grafting.

Table 4. Patient satisfaction measured by FACE-Q questionnaires at baseline (T0) and after 6 weeks (T1), 6 months (T2) and one year (T3).

	N	Average	SD	p-value*
Total (N=23)				
"Satisfaction with facial appearance"				
Control	155	60.8	14.0	
T0	22	41.3	17.3	
T1	20	58.7	19.3	0.001*
T2	22	54.9	16.9	0.003*
T3	21	52.8	18.1	0.005*
"Psychological well-being"				
Control	155	71.5	17.8	
T0	22	54.3	16.7	
T1	20	63.1	19.0	0.006*
T2	22	62.7	19.9	0.018*
T3	21	60.8	14.7	0.028*
"Social Function"				
Control	155	66.1	19.8	
T0	22	48.0	23.0	
T1	20	57.1	19.8	0.001*
T2	22	60.4	23.3	0.001*
T3	21	62.3	20.6	0.022*
Sub-analysis				
Zygomatic area (N=17)				
"Satisfaction with cheeks"				
Control	155	74.2	21.7	
T0	17	38.2	18.0	
T1	14	61.3	20.0	0.002*
T2	16	54.3	25.7	0.009*
T3	16	50.5	24.1	0.047*
Lip area (N=12)				
"Satisfaction with Lips"				
Control	n/a**	n/a**		
T0	11	51.6	14.4	
T1	9	64.9	17.5	0.015*
T2	11	62.5	19.0	0.027*
T3	12	57.7	19.5	0.227
"Lines Lip"				
Control	n/a**	n/a**		
T0	9	47.1	26.2	
T1	6	76.3	27.8	0.013*
T2	7	55.0	24.4	0.039*
T3	8	49.5	21.8	0.688

**statistically significant to baseline score based on paired T-test*

*** Control values not given due to differences between age groups of normative controls*

Preoperative psychological well-being was strongly associated with "Satisfaction with facial appearance overall" and "Satisfaction with result" after one year ($p<0.005$). In contrast, the visible volumetric effect and the aesthetic or reconstructive purpose were not associated to those FACE-Q modules (Table 5).

Table 3. Analysis of possible associated factors to visible volumetric effect one year after fat grafting by multiple regression analysis. No associated factors were objectified.

Multiple regression analysis	Coefficient	p-value
Measured volumetric effect after one year		
Menopause (yes 1; no 0)	-0.267	0.230
Smoking (yes 1; no 0)	-0.307	0.943
Goal: reconstructive (1) / aesthetic (0)	-0.256	0.873

Sub-analysis of zygomatic and lip area

Of the 23 patients, 17 patients had fat grafting in the zygomatic area and 12 patients in the lip area. These patients were used for sub-analysis of these specific aesthetic areas (Figure 2). The average gain in visible volume in the zygomatic area was 5.1 cm^3 after one year, the trend line of the volumetric effect was comparable to the trend line of the total volumetric effect. This is in contrast with the lip area, where no gain in visible volume (0.0 cm^3) was measured after one year. Satisfaction with cheeks was significantly increase at all time points after grafting (Figure 2, Table 4), while the FACE-Q modules "satisfaction with lips" and "Lines lips" only showed a temporary increase (Table 4, Figures 2 and 6). No differences in volumetric effect were objectified between aesthetic patients and reconstructive patients.

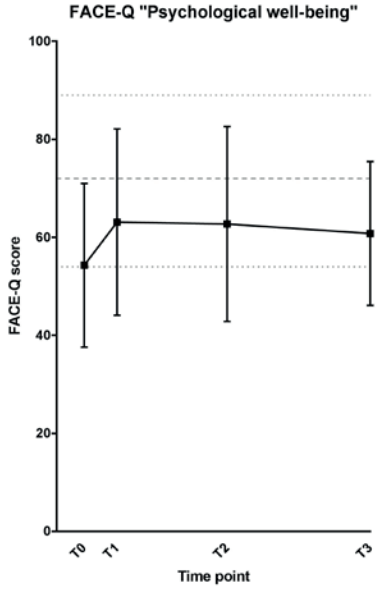

Figure 3: FACE-Q score on psychological well-being during follow up. The range of the normative controle group is displayed by the horizontal lines (mean with standard deviation).

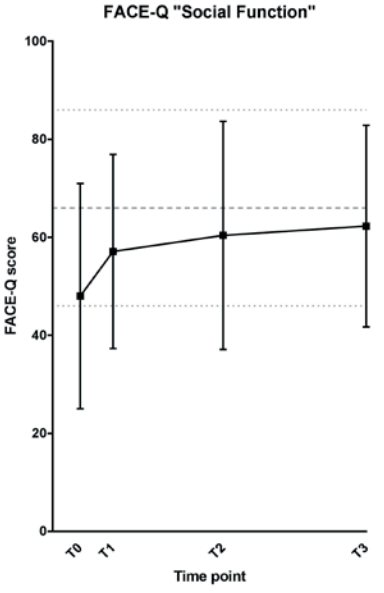

Figure 4: FACE-Q score on social function during follow up (mean ± SD). The dashed line represents the mean and the dotted lines the standard deviation of the controls

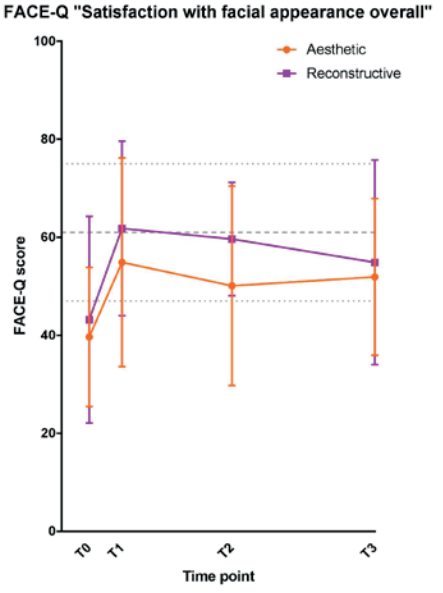

Figure 5: Satisfaction with facial appearance overall separated in reconstructive and aesthetic goal of the procedure (mean ± SD).

Table 5. Analysis of possible associated factors to patients' satisfaction one year after fat grafting by multiple regression analysis. Preoperative psychological well-being was significantly associated to patients' satisfaction.

Multiple regression analysis	Coefficient	p-value
FACE-Q 'Satisfaction facial appearance overall' after one year		
Volume difference after one year	0.204	0.292
Goal (aesthetic (0) or reconstructive (1))	0.233	0.227
Preoperative psychological well-being	0.609	0.005*
FACE-Q 'Satisfaction with result' after one year		
Volume difference after one year	-0.041	0.832
Goal (aesthetic or reconstructive)	0.393	0.053
Preoperative psychological well-being	0.577	0.008*

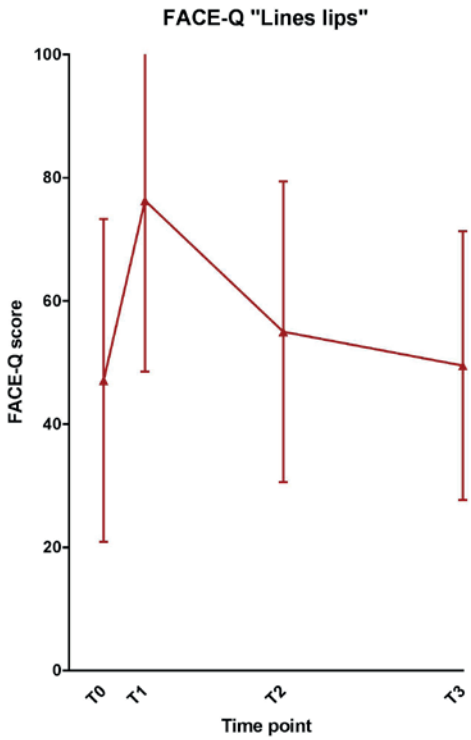

Figure 6: FACE-Q module "Lines lips" in patients with fat grafting in lip area (mean ± SD). Caution: Question of the module "Lines lips" are asked in the opposite way of the satisfaction modules: "How much have you been bothered by lip lines".

DISCUSSION

In this prospective observational study, overall and local visible volumetric effects of fat grafting were measured with a follow up of one year after grafting. This study showed that a substantial volumetric effect was still present overall and in the zygomatic area, while this effect was lost in the lip area. Patient satisfaction was consistent with these overall and local volumetric effects.

The overall visible volumetric effect up to one year after grafting observed in our study matches the observed overall effect in previous studies using 3D stereophotogrammetry.[7,10,12-14,22] A one to one comparison of the observed effects in the various studies is difficult to perform because of differences in injected volumes, fat grafting techniques, and different evaluated regions between the studies. Notwithstanding these differences in design of the studies, similar effects were reported in general. Almost all studies evaluated the full face[10,14] or roughly manually defined large local areas such as the zygoma/cheek[12,13] and temporal area[22]. In none of these studies an automated objective 3D analysis was performed and therefore selection bias due to manual selection of areas of interest could not be ruled out.

As we objectified local effects between predefined aesthetic areas, we were able to show that the volumetric gain of the lip was more likely to decrease than the volumetric gain in the zygomatic area. This finding might indicate that the recipient location influences the visible volumetric effect over time. In the zygomatic area, fat is usually injected mainly in adipose fat pads, in contrast to the lip area, where fat is injected in intramuscular, subcutaneous and submucosal layers. The role of the different tissue components into which the graft is injected on retention of fat is not clear yet. Factors such as vascularization, pro-adipogenic circumstances and physical/mechanical forces to adipocytes[23,24], are considered to have influence on the balance between degeneration and regeneration of the grafted adipose tissue at the recipient site, and thus on the final volume retention. Although vascularization is often mentioned as an important factor at the recipient site,[24] it has been shown that it is not the only factor influencing volume retention of the fat graft. Despite the fact that intramuscular layers are better vascularized, a lower volume retention of fat grafts was reported in mice after injection into intramuscular layers compared with injection into fat pads.[6] Shi et al.[6] posed that tissue-resident adipose stromal/progenitor cells presumably play a leading role in fat graft retention in fat pads by generating pro-adipogenic circumstances. This hypothesis supports the results shown in our study. Furthermore, physical or mechanical forces to adipocytes at the recipient site, such as pressure or movement, could be linked to differences in visible fat graft retention between the lips and the zygomatic area. Adipocytes are mechanosensitive cells. It is posed that external mechanical forces or movement might negatively affect tissue growth and cellular

function and thus influence fat graft survival.[23] Supportive to this hypothesis is the finding that fat graft retention in mice was better in muscular layers that were immobilized by denervation compared to muscular layers which were not immobilized.[25]

Based on our findings and the recently published literature[6,23-25], the role of fat grafting as a stable volume enhancer of the lips is arguable. It has been reported that patients were "less satisfied" with their lips than with their cheeks/malar area using non-validated measurement tools 23 months after facial fat grafting.[5] Another study showed that hyaluronic acid, a powerful (non-permanent) filler, resulted in higher and longer lasting improvement of FACE-Q scores of lip line satisfaction than the improvement found after fat grafting of the same area in our study.[26] Unfortunately, no studies comparing the volumetric effect of facial fat grafting and hyaluronic acid fillers are available yet.

Patients with low preoperative scores on psychological well-being had significantly lower scores on the FACE-Q module "satisfaction with the result" after fat grafting. The preoperative psychological score had more influence on this module than the objective volumetric result of the procedure itself or the indication for the fat grafting (aesthetic or reconstructive). This implies that patients with low preoperative psychological well-being scores are more likely to be less satisfied with the obtained. The phenomenon that preoperative psychological well-being is a strong predictive factor for satisfaction with the result is of great concern for facial aesthetic surgery with regard to patient selection. Further research is needed to select only those patients for a fat grafting procedure who are presumed to benefit from a facial aesthetic procedure.

This study was designed as an observational study in a broad patient population to assess the overall volumetric effect and the patient satisfaction during one year follow up. This study was not powered to compare differences in local volumetric effects, but results of our study indicate that great differences exist in obtained volumetric effects between different aesthetic areas. This comparison is in need for further study. The same need for further study applies to the effects of smoking and menopausal state on volumetric effects.

In conclusion, an increase in overall and local volumetric effects up to one year after facial fat grafting is accompanied by comparable changes in patient satisfaction.

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06

Volumetric effect of pregnancy on a unilateral fat graft

A case report

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ABSTRACT

Introduction: Weight gain can affect the volume of a facial fat graft, resulting in unfavorable asymmetries. Weight gain during pregnancy is more complex and does not just entail an increase in adipose tissue. This case report objectifies whether pregnancy results in volume changes of a facial fat graft.

Case: A 24-year-old female received a fat graft (7ml) in the mandibular area to mask a volume deficiency. This deficiency occurred after a fibula reconstruction of a mandibular defect resulting from the removal of an ameloblastoma. The patient became pregnant 3 weeks after the fat graft procedure. Standardized three-dimensional photographs (3dMD) were available preoperatively, and at 7 weeks (first trimester), 6 months (second trimester), 9 months (third trimester), and 14 months (4 months after delivery) postoperatively. Three-dimensional analysis revealed that no substantial volume changes of the fat graft occurred during pregnancy other than the overall proportional gain in facial volume.

Conclusion: Pregnancy apparently does not affect the volume of a small unilateral fat graft applied in the facial region.

INTRODUCTION

Weight gain is associated with an increase of the facial fat graft volume in young patients.[1] In case of unilateral fat grafting, volume changes of the fat graft can result in new undesirable asymmetry. In young female patients, pregnancy can be expected. Weight gain during pregnancy is more complex and does not just entail an increase in adipose tissue.[2] The aim of this case report was to objectify the volumetric effect of pregnancy on a facial fat graft.

CASE PRESENTATION

A 24-year-old female was diagnosed with an ameloblastoma on the right side of the mandible at the age of 20. After reconstruction with a free vascularized fibula graft with dental implants, a soft tissue deficiency remained in the region of the right mandibular body and angle (Figure 1; T0).

Fat graft procedure

Fat grafting was performed under local anesthesia. The donor site, the inner knee on both sides, was infiltrated with tumescent solution (5ml xylocaine 2% in 45ml Ringers lactate). Adipose tissue was harvested manually using a Sorensen cannula (Tulip medical, San Diego, USA) under negative pressure. The harvested tissue was processed with Puregraft 50 (Cytori, San Diego, USA) according to the manufacturer's protocol. A total of 7ml of processed adipose tissue was injected with a 0.9mm blunt cannula subcutaneously in the right mandibular region. Preoperative photographs and three-dimensional stereophotogrammetry (3dMD, London, United Kingdom) pictures were taken.

Follow-up

At the first routine control visit, 7 weeks after the procedure, the patient reported that she was approximately 3 weeks pregnant. Additional regular and three-dimensional photographs were taken at 7 weeks (first trimester, T1), 6 months (second trimester, T2), 9 months (third trimester, T3), and 14 months (4 months after delivery, T4) after grafting (Figure 1). The patient's weight changed from 64kg preoperatively to, 61kg (T2), 74kg (T3), 79kg (T4) and 70kg (T5) (Table 1). Weight gain and general facial volume gain were most evident in the second and third trimester (Figure 1 and 2). The fat graft in the mandibular region showed the highest positive intensity (red; + 3-4mm) on all postoperative images that were projected over the preoperative three-dimensional photograph (Figure 2A). The gain in volume of the fat graft was equal to the gain in other areas such as the zygomatic region during pregnancy (Figure 2B).

Table 1: Follow up details

Time	Visit	Time in relation to pregnancy	Days after procedure	Weight (in kg)	17β estradiol level serum (nmol/L)	Accuracy 3D analysis: RMS to T0 *(Figure 2A)*	Accuracy 3D analysis: RMS to T2 *(Figure 2B)*
T0	Preoperative	-3 weeks	0	64	0.0179*	-	-
T1	1st trimester	+3 weeks	42	61		0.32	-
T2	2nd trimester	+22 weeks	175	74		0.37	0.31
T3	3rd trimester	+37 weeks	280	79		0.37	0.43
T4	After delivery	8 weeks after delivery	357	70		0.33	0.38

**not pregnant: reference first trimester level 0.563-11.6 nmol/L; RMS: Root Mean Square. The matches of the 3dMD photographs were based on a T-shaped area of the forehead and nose. A RMS under 0.50 was assumed to represent an accurate match.*

Figure 1: Regular photographs of the lower face per visit. T0 Preoperative, T1 first trimester of pregnancy, T2 second trimester of pregnancy, T3 third trimester of pregnancy.

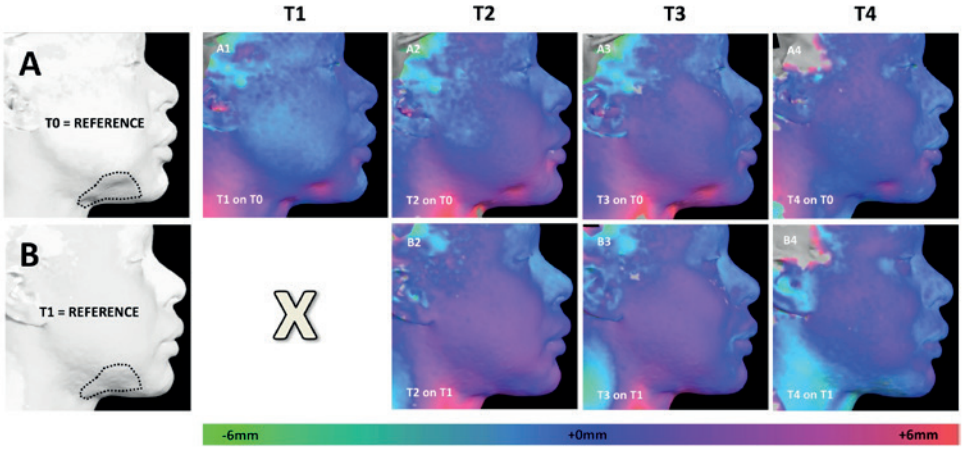

Figure 2: Three-dimensional volumetric analysis of the facial fat graft during pregnancy 2A: Color map of the postoperative 3D photographs projected over the preoperative 3D photograph (T0). The matches of the 3dMD. Color scale: Green is -6mm distance in relation to the T0 3D photograph, blue is no difference in relation to T0 the 3dMD photograph; red is +6mm distance in relation to the T0 3D photograph. Red/purple colored areas were detected at the injection place of the fat graft at the mandibular region on the right side in all postoperative images (T1, T2, T3, T4). Extra purple areas were detected around the cheeks, especially in T2 and T3. **2B:** Color map of the first 3D photograph after fat grafting projected over the first trimester 3D photograph (T1). No extra red/purple colored areas were detected in the area of the fat graft in relation to the cheek area. photographs were based on a T-shaped area of the forehead and nose. All RMS scores were lower than 0.5. RMS under 0.50 was assumed to represent an accurate match.

DISCUSSION

Despite hormonal and weight changes during pregnancy, substantial volume changes were not detected in the facial fat graft applied in the mandibular region. The changes in the fat graft area were comparable to the changes in other tissues in the facial region during pregnancy in terms of volume gain.

As mentioned earlier, Taupin et al. reported that young patients with unilateral fat grafts are at risk of undesirable volume changes of a fat graft after weight gain.[1] Growth in length and width cannot always be predicted for future life. Nevertheless, knowledge about weight gain and pregnancy in relation to fat grafting would be helpful in order to prevent undesirable asymmetries in young patients. Based on our case, pregnancy does not seem to be a major factor.

The average gain in body weight during pregnancy is 10.8-12kg, with an estimated increase of 6-7% of body fat.[3] The percentage of fat tissue increases slowly until the 24th week of gestation and remains stable after that until the time of delivery.[2] In contrast to fat percentage gain, extracellular fluid increases from the 24th week until the 40th week of gestation, resulting in a weight gain of approximately 1.5 kilograms.[2] In our case, the extra volume gain around the cheeks on both sides was observed in the second and third trimester. It is unclear whether the fat or the extracellular fluid caused this bilateral volume gain in the face.

In our case, subcutaneous adipose tissue from the inner knee was used for fat grafting. In women, femoral subcutaneous adipose tissue is comparable to abdominal subcutaneous adipose tissue with regard to fat local thickness and number of adipocytes.[4,5] Although no literature is available about changes in subcutaneous femoral adipose tissue during pregnancy, if any, it has been shown that the increase of abdominal fat during pregnancy is a result of an accumulation of visceral adipose tissue, and not caused by accumulation of subcutaneous abdominal adipose tissue during pregnancy.[6,7] This conclusion is in line with our finding that the subcutaneous fat graft did not increase in volume during pregnancy.

An animal study by Mok et al.[8] stated that high estrogen levels during fat graft transplantation did not lead to higher volume retention in mice. High estrogen is related to a lower acute inflammation response as it inhibits neutrophils and M1 macrophages. However, in their study, some mice had low and some high estrogen levels at the time of transplantation and were followed up at 4 and 12 weeks. In our case, high estrogen levels occurred three weeks after the transplantation due to pregnancy onset at that time. We presume that the acute inflammation response was not lower due to this three-week gap between injection of the fat graft and the conception.

The fat graft did not increase disproportionally during pregnancy, but this observation can be criticized. First, it is possible that the fat graft increased in volume due to pregnancy, but at the same time decreased due to physiological fat graft remodeling. It is known that during the first months after transplantation volume of a fat graft will decrease. [9-11] Second, a low amount of 7 ml of fat was injected and changes within the graft might not become visible. However, with the very accurate three-dimensional imaging techniques we applied minor changes were detected in this case. Lastly, the unnoticeable difference in volume could be a result of the presence of scar tissue of the reconstructed area.

Our case showed that a unilateral small facial fat graft did not undergo noticeable volumetric changes during pregnancy. This presumption is based on a single case, however. To improve scientific evidence, larger studies are needed that to objectify possible volume changes of facial fat grafts during pregnancy.

INFORMED CONSENT: The patient was included in the prospective study "predictors of volumetric outcome and patient satisfaction of lipofilling" registered under number NTR5325 in the Dutch Trial Register. The patient signed an extra informed consent to publish photographs in this article.

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07

A comparison of intraoperative procedures for isolation of clinical grade stromal vascular fraction for regenerative purposes

A systematic review

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ABSTRACT

Background: Intraoperative application of stromal vascular fraction (SVF) of adipose tissue, requires a fast and efficient isolation procedure of adipose tissue. This review was performed to systematically assess and compare procedures currently used for the intraoperative isolation of cellular SVF (cSVF) and tissue SVF (tSVF) which still contains the extracellular matrix.

Methods: Pubmed, EMBASE and The Cochrane Central Register of controlled trials databases were searched for studies that compare procedures for intraoperative isolation of SVF (searched 28th of September, 2016). Outcomes of interest were cell yield, viability of cells, composition of SVF, duration, cost and procedure characteristics. Procedures were subdivided in procedures resulting in a cSVF or tSVF.

Results: Thirteen out of 3038 studies were included, evaluating eighteen intraoperative isolation procedures, were considered eligible. In general, cSVF and tSVF intraoperative isolation procedures had comparable cell yield, cell viability and SVF composition compared to a non-intraoperative (*i.e. culture lab-based collagenase protocol)* control group within the same studies. The majority of intraoperative isolation procedures are less time consuming than non-intraoperative control groups, however.

Conclusion: Intraoperative isolation procedures are less time-consuming than non-intraoperative control group with similar cell yield, viability of cells and composition of SVF and therefore more suitable for use in the clinic. Nevertheless, none of the intraoperative isolation procedures could be designated as preferred procedure to isolate SVF.

INTRODUCTION

Adipose tissue seems to be an outstanding source for regenerative therapies, since it is an easy accessible source for adipose-derived stem or stromal cells (ASCs). Adipose tissue can easily be harvested with liposuction, a low risk procedure that can be performed under local anesthesia. Several clinical trials have been published using ASCs for soft tissue reconstruction[1], cardiac repair[2], pulmonary repair[3] and cartilage repair[4]. All these trials show promising results for future use of ASCs in tissue repair and regeneration.

To harvest ASCs, adipose tissue or lipoaspirate is subjected to enzymatic dissociation followed by several centrifugation steps[5], which is a relative long-lasting procedure that cannot be performed during surgery. The cell population obtained by this enzymatic digestion and centrifugation is the stromal vascular fraction (SVF), containing ASCs, endothelial cells, supra-adventitial cells, lymphocytes and pericytes.[5, 6] ASCs *in vivo* are characterized as CD31min/CD45min/CD34pos/CD90pos/CD105low cells.[7] After isolation, the SVF can either be used directly in clinical procedures or can be cultured to increase the number of cells before using them in the clinic.[8, 9] In case of cell culturing, only ASCs and their precursor cells (supra-adventitial cells and pericytes) are able to adhere and survive.[10, 11] Upon passaging *in vitro*, the phenotype of ASCs starts to deviate from their *in vivo* phenotype: in this process CD34 surface expression is lost, while CD105 expression is up-regulated to mention a few.[7, 12] Alternatively, administration of the enzymatically prepared vascular stromal fraction of adipose tissue might have a therapeutic capacity that is similar to cultured ASCs. Although, no formal scientific evidence exists, the consensus is, that the therapeutic benefit of SVF predominantly relies on the abundantly present ASCs.

The current protocol to isolate and culture ASCs from adipose tissue involves enzymatic digestion with collagenase. This is a laborious and time consuming protocol and requires a specialized culture lab (Good Manufacturing Practice facilities (cGMP)), which is not available in most peripheral hospitals.[13] Therefore, intraoperative procedures for SVF isolation are warranted, in particular systems that do not employ enzymatic treatment, such as mechanical dissociation.

At present, several (commercial) procedures are available for intraoperative isolation of SVF.[14, 15] These intraoperative isolation procedures differ in various aspects: isolation of a single cell SVF (cellular SVF (cSVF)) resulting in a pellet with hardly any volume or isolation of SVF cells containing intact cell-cell communications (tissue SVF (tSVF). Most of the enzymatic intraoperative isolation procedures result in a cSVF, because of the loss of cell-cell communications and extracellular matrix. In most of the non-enzymatic intraoperative isolation procedures the cell-cell communications remain intact, resulting in an end product with more volume (tSVF). Different studies assessed the cell yield and phenotype of the isolated cSVF

or tSVF of the various intraoperative isolation procedures compared to other intraoperative (commercial) procedures or to the gold standard for SVF isolation (non-intraoperative culture lab-based collagenase protocols which require cGMP facilities for clinical use, referred to as 'non-intraoperative isolation protocol'). Recently, new intraoperative isolation procedures are introduced and tested. It is not clear yet if intraoperative isolation procedures generate a similar quality and quantity of SVF as non-intraoperative isolation protocols. Next to this, the distinction between end products of intraoperative isolation procedures, *e.g.* cSVF and tSVF have never been studied. Therefore, a systematic review was performed to assess the efficacy of intraoperative isolation procedures of human SVF based on number of cells, cell viability and composition of SVF. In addition, duration and costs of the intraoperative isolation procedures were compared.

MATERIAL & METHODS

Protocol and registration

This study was performed using the PRISMA protocol.[16] The search strategy for this systematic review was based on a Population, Intervention, Comparison, and Outcome (PICO) framework.[17] The study was not registered.

Eligibility criteria

Studies were included when at least two different types of intraoperative isolation procedures or one intraoperative isolation procedure with a non-intraoperative isolation protocol were assessed using human adipose tissue to isolate SVF. Studies need to use the adipose fraction of lipoaspirate. Studies only evaluating centrifugation forces, sonication or red blood cell (RBC) lysis buffer were excluded. Studies focusing on processing methods of adipose tissue for the use in fat grafting were excluded as well as case reports, case series and reviews. Searches were not limited to date, language or publication status (Table 1).

Information sources and search

Pubmed, EMBASE (OvidSP) and The Cochrane Central Register of controlled trials databases were searched (searched 28th September, 2016). The search was restricted to human studies. The search terms (Table 2) were based on three components: (P) adipose stromal cell, adipose stem cell, stromal vascular fraction, autologous progenitor cell, or regenerative cell in combination with (I) cell separation, isolation, dissociation, digestion, emulsification, isolation system, cell concentrator and finally connected with (C) enzymatic, non-enzymatic, or mechanical.

Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Clinical trials	Case reports
Comparative studies	Case series
Full text available	Reviews
All languages	Letters to editor
Human studies	Non-comparative studies
	No full text available
≥2 different types of SVF isolation procedures	Processing methods for fat grafting Protocols using centrifugation or RBC lysis buffer only
1 SVF isolation procedure compared with control group Intraoperative procedures	Mesenchymal cells derived from other source than adipose tissue Blood saline fraction used instead of adipose fraction of the lipoaspirate Laboratory based enzyme protocols as experimental group No outcome of interest: SVF composition (CD markers), cell yield, viability of SVF

Table 2. Specific search terms of databases

Search term Pubmed
((((Adipose Tissue [Mesh] OR Adipocytes [Mesh] OR Fat [tiab] OR Lipoaspirate* [tiab])) AND (Cell separation [Mesh] OR Isolat* [tiab] OR Dissociat* [tiab] OR Emulsification [tiab] OR Concentrat* [tiab] OR Digest* [tiab] OR Obtained [tiab])) AND (Stem cells [Mesh] OR Stromal cells [Mesh] OR Autologous progenitor cell* [tiab] OR Stromal vascular* [tiab] OR Regenerative cell* [tiab] OR Vascular stroma [tiab])) *Restriction: Only human*
Search term Embase
('adipose tissue':ab,ti OR 'adipocytes':ab,ti OR 'fat':ab,ti OR lipoaspirate*:ab,ti AND ('cell separation' OR isolat*:ab,ti OR dissociat*:ab,ti OR 'emulsification':ab,ti OR concentrat*:ab,ti OR digest*:ab,ti OR 'obtained':ab,ti) AND ('stem cells':ab,ti OR 'stromal cells':ab,ti OR 'autologous progenitor cell':ab,ti OR 'autologous progenitor cells':ab,ti OR 'stromal vascular':ab,ti OR 'stromal vascular fraction':ab,ti OR 'regenerative cell':ab,ti OR 'regenerative cells':ab,ti OR 'vascular stroma':ab,ti)) AND [embase]/lim NOT [medline]/lim AND 'article'/it *Restriction: Only EMBASE*
Search term Cochrane Library
(adipose tissue OR adipocytes OR fat OR lipoaspirate*) AND (cell separation OR Isolat* OR Dissociat* OR Emulsification OR Concentrat* OR Digest* OR Obtained) AND (stem cells OR stromal cells OR autologous progenitor cell* OR stromal vascular* OR regenerative cell* OR vascular stroma)

Study selection and data collection process

Two authors (JAD, AJT) selected studies independently based on the eligibility criteria. Inconsistencies were discussed during a consensus meeting. In case of disagreement, the senior author (MCH) gave a binding verdict.

Data items

Search term was partly based on a Population, Intervention, Comparison, Outcome (PICO) framework. Outcomes of interest were not included in the search term. For this review the outcomes of interest were cell yield, viability of the nucleated cells, composition of the SVF and duration, cost and characteristics of the intraoperative isolation procedures. Effect sizes were calculated on cell yield and viability in studies with a comparison of intraoperative isolation procedures versus regular non-intraoperative isolation protocols. Differences in harvesting procedure were not taken into account.

Risk of bias in individual studies

It is known that the quality of ASCs depends on age and harvest location of the donor.[18-21] The inclusion of young healthy patients may positively affect the results. Therefore, detailed information about demographics are described in this review.

Summary measurements

Effect sizes were calculated of the outcome variables cell yield and percentage of viable nucleated cells from cSVF between enzymatic intraoperative isolation procedures and non-intraoperative isolation protocols (gold standard). The following effect size formula was used: effect size = (difference in mean outcomes between enzymatic intraoperative isolation procedures and gold standard) / (standard deviation of the gold standard). Studies which presented results in mean and standard deviation were analyzed. Intraoperative isolation procedures focusing on tSVF instead of cSVF were not taken into account in the effect size of cell yield, because of different start volumes of lipoaspirate and end volumes of tSVF.

Synthesis of results

In some studies, derivate numbers of graphs are used when the actual number of outcomes was not given. Cell types within the SVF can be distinguished based on CD marker expression or immuno-staining. To compare SVF compositions between different studies and to compare intraoperative procedures with their control (*i.e.* non-intraoperative protocols or other intraoperative procedures) in the same study, only CD marker expression was used. Studies evaluating a single CD marker expression to analyze different cell types were seen as insufficient distinctive and were excluded. Cells were divided into two major groups: CD45min (adipose tissue-derived) and CD45pos (blood derived) cells to analyze the expression of

stromal cells, pericytes, vascular endothelial cells/endothelial progenitor cells, endothelial cells, lymphocytes, leukocytes and hematopoietic stem cells. All other cells are placed in the category: other cell types. The CD34pos/CD146pos population is excluded from analysis because of the inability to discriminate between progenitor pericytes and progenitor endothelial cells.[22]

Risk of bias across studies

Included studies could present different outcome variables related to SVF analysis. There is a risk that studies did not present a full SVF characterization and thereby bias their results. In order to provide an overview of the used outcome variables per study, a Modified IFATS/ISCT Index Score was used (see 2.10). The risk of publication bias of positive results might be expected in those articles were the authors have benefits in the investigated products. Disclosure agreements were reviewed for each study.

Modified IFATS/ISCT Index Score for the measurement of adipose tissue-derived stromal vascular fraction

Studies were assessed based on the reported outcome variables. The assessment of quality was evaluated based on the position statement of the International Federation of Adipose Therapeutics and Science (IFATS) and the International Society of Cellular Therapy (ISCT).[5] The IFATS and ISCTS proposed guidelines to develop reproducible standardized endpoints and methods to characterize ASCs and SVF cells. For each of the following characterization methods a grade was given by the authors (JAD, AJT) to an article if the characterization was carried out: viability of nucleated cells, flow cytometry of SVF cells, flow cytometry of ASCs (CD13, CD29, CD31, CD34, CD44, CD45, CD73, CD90, CD105, CD235a), proliferation and frequency (CFU-F) and functional assays (adipogenic, osteogenic and chondrogenic differentiation assays) of ASCs. The maximum score in case of a full characterization was 5.

RESULTS

Included studies

A total of 3038 studies were identified after database searching. 2955 articles were excluded after abstract screening. 59 full text studies were assessed on eligibility criteria. Fourteen studies were excluded based on the use of a non-intraoperative protocol for isolation as experimental method.[7, 23-35] Seven studies described isolation protocols in general but gave no results.[36-42] Seven studies were excluded based on the lack of a control group (*i.e.* non-intraoperative isolation protocols or other intraoperative isolation procedures).[10, 18, 43-47] Four studies were excluded based on their study design.[48-51] Three studies were excluded based on the use of

culture methods to isolate ASCs, because culture methods are incompatible with intraoperative applications.[52-54] Four studies used only centrifugation, centrifugation or RBC lysis buffer as isolation protocol and were thereby excluded.[55-58] Three studies used the blood saline fraction of lipoaspirate and were thereby excluded.[59-61] Four studies did not describe an outcome of interest.[62-65] Four additional studies were identified through other sources (Figure 1). Thus, thirteen studies with eighteen intraoperative isolation procedures remained for analysis.

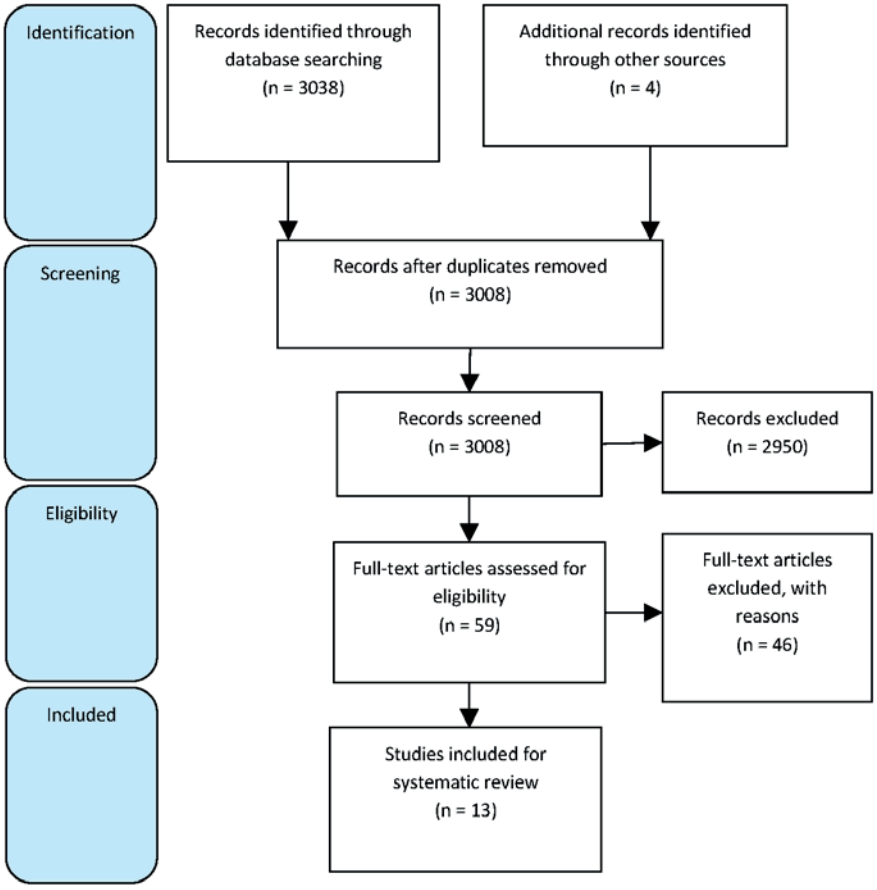

Figure 1. Flow diagram of study selection

Study characteristics

In total, 93 subjects were enrolled in the thirteen studies. Nine studies reported gender of which 95% was female (n=58). Nine studies reported the mean age or age variance of the subjects and ten other studies described the use of infiltration (Table 1, supplemental content). No meta-analysis could be performed because the metrics and outcomes were too diverse.

Characteristics of the intraoperative isolation procedures

All intraoperative isolation procedures are divided into two categories: enzymatic and non-enzymatic procedures resulting in cSVF and tSVF respectively (Table 3A and table 3B). Eight of the eighteen intraoperative isolation procedures were based on enzymatic digestion and ten isolation procedures were based on non-enzymatic procedures. Two non-enzymatic procedures, the Residual tissue of emulsified fat procedure and the Fractionation of adipose tissue procedure, are named differently, but are almost identical. One intraoperative isolation procedure, the Filtrated fluid of emulsified fat, is a combined procedure of two other intraoperative isolation procedures *i.e.* the Fractionation of adipose tissue procedure and the Nanofat procedure.[66-68]

Table 3A. Duration, costs and procedure characteristics of intraoperative isolation procedures focusing on cSVF

Name	Author	Enzymatic/ Non-enzymatic (E/N)	Automatic/ Manual/ Semi (A/M/S)	Open/ closed (O/C)	Isolation details		Time (min)	Disposable (D)/ reusable (R) cost (Dollar)	Volume processed (ml)	Capacity (ml)	End volume (ml)	Maximum volume processed / maximum end volume
AIS	SundarRaj et al. 2015	E	A	C	Tissue digestion, heating and agitation, three-stage filter system (100 micron, 35 micron, 5 micron porosity)	-	133	-	-	500	10.8 [4-20]	-
CHA	Aronowitz et al. 2013	E	S	C	Fat bag, adapter, centrifugation, shaking incubator and tissue digestion, cell strainer, cell counter	Collagenase	88+/23	D710	80-180	180	-	-
CYT	Aronowitz et al. 2013	E	A	C	Washing (lactated Ringer), tissue digestion and agitation, centrifugation	Celase/ Reagent A	90 +/16	D1950	100-180	360	-	-
	Aronowitz et al. 2016						89.4 [85-93]	D2400 per 120-360 ml	126 [90-150]	360	5 [5]	30
	Domenis et al. 2015						60	D	250	-	Pellet	-
	Lin et al. 2008						90	-	-	-	Pellet	-
GID-SVF2	Aronowitz et al. 2016	E	M	C	Disposable canister for harvesting, filtration, separation and concentration	GIDzyme-50	71.4 [68-75]	D1000 per 20-120 ml	53.2 [32-88]	120	7.2 [6-9]	13.3
LIPOK	Domenis et al. 2015	E	S	C	1200 xg centrifugation (with a weight-mesh filter piston), celltibator	Liberase (collagenase mixture)	-	-	-	-	-	-
	Aronowitz et al. 2013					Collagenase	111+/-18	D530	60-100	100	-	-
	Aronowitz et al. 2016					Time Machine accelerator	120.8 [99-149]	D450 per 100 ml	71.4 [40–97]	400	20 [15-25]	3.9
PNC	Aronowitz et al. 2013	E	M	O	Centrifugation, shaking incubator, clean bench, HEPA filter, UV-lamp	Collagenase	115+/-13	D460	100-150	400	-	-
	Aronowitz et al. 2016						65.4 [59-74]	D250 per 100 ml	105.6 [68-150]	800	12.2 [10.5-15]	10
SEPAX	Güven et al. 2012	E	A	C	Tissue digestion, priming and straining, centrifugation, washing	0.15% NB6 GMP Grade Collagenase	90-120	-	40-400	-	Pellet	-
TGCIS	Doi et al. 2012	E	A	C	Tissue digestion, centrifugation, washing, 700 xg centrifugation	0.075% collagenase	65	D	20-60	-	Pellet	-

AIS Automated Isolation System; CHA-station (CHA-Biotech); CYT Celution System Enzymatic (Cytori); GID SVF2 (GID Europe); LIPOK Lipokit System (Medi-khan); PNC Multi station (PNC); SEPAX Sepax (Biosafe); TGCIS Tissue Genesis Cell Isolation System (Tissue Genesis)

Table 3B. Duration, costs and procedure characteristics of intraoperative concentration procedures focusing on tSVF

Name	Author	Enzymatic/ Non-enzymatic (E/N)	Automatic/ Manual/ Semi (A/M/S)	Open/ closed (O/C)	Isolation details		Time (min)	Disposable (D)/ reusable (R) cost (Dollar)	Volume processed (ml)	Capacity (ml)	End volume (ml)	Maximum volume processed / maximum end volume
FAT	Van Dongen et al. 2016	N	M	O	3000 rpm (radius 9.5 cm) centrifugation, shuffling through a 1.4 mm hole connector, 3000 rpm (radius 9.5 cm) centrifugation	n/a	8-10	R	10	10	0.96 [0.75 - 1.75]	10.4
FAST	Domenis et al. 2015	N	M	-	Filterbag (120 micron filter), 400 xg centrifugation	n/a	-	-	-	-	10	-
FEF	Mashiko et al. 2016	N	M	O	1200 xg centrifugation, shuffling through a connector with three small holes 30 times, 1200 xg centrifugation, fluid of decanting filtration (500-µm pore size) used	n/a	-	-	-	-	-	9.9+/-2.0
LIPOG	Bianchi et al. 2013	N	M	C	Filtering, decantation, stainless steel marbles to mix layers (oil, adipose tissue, blood, saline), washing, decantation, reversing devices, filtering	n/a	20	D	40-130	130	60-100	1.3
NANO	Tonnard et al. 2013	N	M	O	Shufling adipose tissue through a female-to-female luerlok 30 times, filtering	n/a	-	-	-	-	-	-
REF	Mashiko et al. 2016	N	M	O	1200 xg centrifugation, shuffling through a connector with three small holes 30 times, 1200 xg centrifugation, residual tissue of decanting filtration (500-µm pore size) used	n/a	-	-	-	-	-	2.5+/-0.2
SF	Mashiko et al. 2016	N	M	O	1200 xg centrifugation, squeeze using automated slicer, 1200 xg centrifugation	n/a	-	-	-	-	-	2.1+/-0.2
SHUF5	Osinga et al. 2015	N	M	O	Shuffling lipoaspirate through female-to-female luerlok 30 times	n/a	5 sec.	-	10	-	-	-
SHUF30	Osinga et al. 2015	N	M	O	Shuffling lipoaspirate through female-to-female luerlok 30 times	n/a	30 sec.	-	10	-	-	-
STCELL	Milan et al.	N	M	C	1000 xg centrifugation	n/a	-	-	400	500	Pellet	-

FAT Fractionation of Adipose Tissue procedure; FAST Fastem Corios (Corios); FEF Filtrated fluid of emulsified fat; LIPOG Lipogems (Lipogems); NANO Nanofat procedure; REF Residual tissue of emulsified fat; SF Squeezed fat; SHUF5 Shuffling 5 times; SHUF30 Shuffling 30 times

Start volume versus end product

The Automated isolation system, GID SVF2, Lipokit system and Multi station are enzymatic intraoperative isolation procedure that resulted in large average amounts of SVF (7.2 ml – 20 ml), suggesting inefficient enzymatic digestions.[69, 70] The non-enzymatic intraoperative isolation procedures resulted in larger end volumes than only a pellet. Prior the Lipogems procedure, 130 ml of adipose tissue can be obtained to mechanical dissociate to 100 ml of lipoaspirate. Hence, this a reduction of the volume of 1.3 times, suggesting an inefficient mechanical dissociation to our opinion.[22] In contrast, the Fractionation of adipose tissue procedure resulted in a 10.4-fold volume reduction.[67] For all other intraoperative isolation procedures, no data is mentioned about the end volume of the lipoaspirate (Table 3A and table 3B).

Duration and costs

Duration of the intraoperative isolation procedures varied from 5 seconds to 133 minutes (n=12). Isolation with the Automated isolation system was the longest intraoperative isolation procedure.[69] Shuffling lipoaspirate 5 or 30 times through a luer-to-luer lock syringe will take 5 or 30 seconds respectively and were therefore the fastest procedures.[71] In general, the tested non-enzymatic procedures take less time than the enzymatic procedures (Table 3A and table 3B).

The costs of only enzymatic procedures Celution system (2013: $1950 and 2016: $2400), CHA-station ($710), Multi station (2013: $460 and 2016: $250), Lipokit system (2013: $530 and 2016: $450) and GID SVF2 ($1000) are mentioned, the enzymatic Celution system being the most expensive.[70, 72] No data of non-enzymatic intraoperative procedures were available (Table 3A and table 3B).

Cell yield

Thirteen studies evaluated the cell yield of eighteen different intraoperative isolation procedures [22, 66-77] (Table 2A and table 2B, supplemental content). The reported cell yield after those different procedures varied between 0.19 – 11.7 x 10^5 cells per ml in enzymatic intraoperative isolation procedures and between 1.8 – 22.6 x 10^5 cells per ml in non-enzymatic intraoperative isolation procedures. Non-enzymatic intraoperative procedures yielded higher number of cells since the cell yield was based on 1ml of end volume, whereas the enzymatic intraoperative isolation cell yield was based on the obtained pellet per 1 ml start volume of lipoaspirate. Of the enzymatic intraoperative isolation procedures, the Celution system, Multi station and Lipokit system were evaluated by more than one group of authors.[70, 72-74] Interestingly, obvious different yields were seen using the same procedure in different studies.[70, 72-74] Reproducibility is thereby questioned in our opinion. The cell yield using the enzymatic Celution system was significantly higher as compared to the Lipokit system (p=0.004), the Multi station (p=0.049)

and CHA-station ($p<0.001$).[72] In contrast, Domenis et al. did not find a statistical difference between the enzymatic Celution system and Lipokit system. Moreover, Aronowitz et al. again compared the enzymatic Celution system with the Lipokit system and Multi station. This time, Multi station and the Lipokit system resulted in significant more cells as compared to the Celution system ($p<0.05$).[70]

In the non-enzymatic intraoperative isolation procedures, the Squeezed fat, Residual fluid of emulsified fat and Fractionation of fat procedures resulted in the relative highest cell yields per ml harvested lipoaspirate.[66, 67] Non-enzymatic intraoperative isolation procedures such as shuffling (5 times and 30 times), the Nanofat procedure and Fastem did not mention the begin and end volumes, so the relative yield by isolation cannot be calculated.[68, 71, 74] Osinga et al, reported that most of the adipocytes remain intact after shuffling 5 or even 30 times.[71] Consequently, to our opinion, the effect of shuffling only cannot be stated as an isolation procedure. We deem it possible that the lipoaspirate after both two procedures did not differ from the initial lipoaspirate obtained at the start of the procedure. However, the benefit might be at a different level, because shuffling does improve the injectability of lipoaspirates as shown by Tonnard et al..[68]

More interesting than comparing intraoperative isolation procedures evaluated in different studies might be the comparison between an intraoperative isolation procedure and a non-intraoperative isolation protocol (gold standard) starting from the same lipoaspirate. Six studies reported the results of such comparisons (Table 4A).[69, 73-77] The Automated isolation system and Tissue genesis cell isolation system resulted in the same cell yield as the non-intraoperative isolation protocol control (effect size, respectively, 0.07 and 0.00).[69, 76] Sepax isolated a higher cell yield compared to a non-intraoperative isolation protocol (effect size 1.11) (Table 4A).[75] Lower cell yield was seen after using the Lipokit system compared to the non-intraoperative isolation protocol control (effect size -0.52).[74] Interestingly, the highest positive as well as the most negative effect sizes were seen with the enzymatic Celution system related to regular isolation with a non-intraoperative isolation protocol.[73, 74]

Table 4A: Effect sizes of studies evaluating enzymatic intraoperative isolation procedures regarding cell yield

	Enzymatic isolation procedure			Non-intraoperative isolation protocol			
Study	**N**	**Cell yield x10^5 cells**	**SD**	**N**	**Cell yield x10^5 cells**	**SD**	**Effect size**
AIS, SundarRaj, 2015	11	1.17	0.5	11	1.15	0.30	0,07
CYT, Domenis, 2015	9	11.7	5.0	16	6.7	3.30	1,52
CYT, Lin, 2008	6	3.7	0.9	3	4.96	0.72	-1,75
LIPOK, Domenis, 2015	9	5.0	3.0	16	6.7	3.30	-0,52
SEPAX, Güven, 2012	6	2.6	1.2	6	1.6	0.90	1,11
TGCIS, Doi, 2012	6	7.0	1.9	6	7.0	2.43	0,00

AIS Automated Isolation System; CYT Celution System Enzymatic (Cytori); LIPOK Lipokit System (Medi-khan); SEPAX Sepax (Biosafe); TGCIS Tissue Genesis Cell Isolation System (Tissue Genesis)

Table 4B. Effect sizes of studies evaluating viable nucleated cells

	Procedure			Non-intraoperative isolation protocol			
Study	**N**	**% viable cells**	**SD**	**N**	**% viable cells**	**SD**	**Effect size**
Enzymatic							
AIS, SundarRaj, 2015	11	97.5	2.8	11	97.3	1.5	0.13
CYT, Lin, 2008	3	89.2	1.1	3	90.8	1.3	-1.23
TGCIS, Doi, 2012	6	80.7	7.1	6	82.4	7.7	-0.22
Non-enzymatic							
FEF, Mashiko, 2016	10	39.3	9.1	10	93.8	1.2	-45.4
REF, Mashiko, 2016	10	90.6	2.8	10	93.8	1.2	-2.67
SF, Mashiko, 2016	10	89.9	4.6	10	93.8	1.2	-3.25
STCELL, Millan, 2014 [a]	3	87.7	8.9	3	74.5	20.1	0.66

[a] *No exact data described in text, data extracted from figures by authors JAD and AJT. AIS Automated Isolation System; CYT Celution System Enzymatic (Cytori); FEF Filtrated fluid of emulsified fat; REF Residual tissue of emulsified fat; SF Squeezed fat; STCELL StromaCell; TGCIS Tissue Genesis Cell Isolation System (Tissue Genesis)*

Viability of nucleated cells

Eight studies described viabilities from 39% to 98% of nucleated cells in the SVF. No big differences in viability were seen between enzymatic and non-enzymatic intraoperative isolation procedures. The Filtrated fluid of emulsified fat procedure showed the lowest viability [66], while the Automated isolation system showed the highest viability of nucleated cells of 98% after isolation (Table 2A and table 2B, supplemental content) [69]. Three enzymatic and three

non-enzymatic intraoperative isolation procedures were compared to a non-intraoperative isolation protocol regarding the viability of nucleated cells (Table 4B).[69, 73, 76] The viability of five intraoperative isolation procedures was comparable to their non-intraoperative isolation protocol controls; the effect sizes were close to zero in many studies (Table 4B). Only the Filtrated fluid of emulsified fat procedure showed an effect size of -45.4.[66] In general, viability did not differ between non-intraoperative isolation protocols and the individual intraoperative isolation procedures tested.

Composition of stromal vascular fractions

The SVF compositions is reported in nine studies evaluating six enzymatic procedures and three non-enzymatic procedures. The stromal cell population is larger in the SVF isolated by the enzymatic Celution system, Sepax and Tissue genesis cell isolation system and the non-enzymatic Residual of emulsified fat and Squeezed fat procedures compared to other intraoperative isolation procedures [66, 72, 75, 76] (Table 5, supplemental content). The percentage of stromal cell population of the SVF isolated by the enzymatic Celution system only differs with 25.2% between two studies [72, 74] and 32.8% between two other studies, both evaluated by Aronowitz et al. [70, 72]. In general, non-enzymatic procedures yielded same amounts of CD31min/CD34pos stromal cells.

The stromal cell population, including pericytes, ASCs and supra-adventitial cells, are the most important cell types in regenerative therapies because of their paracrine effect and multi-lineage differentiation capacity.[10, 78]

Pericytes defined using other CD markers than to define the stromal cell population are placed separately in the table. The enzymatic Celution system evaluated by Lin et al. resulted in the lowest percentage of pericytes in the SVF (0.8%), but used more than three CD markers to detect pericytes.[73] SundarRaj et al. resulted in a higher percentage (2.0%) of pericytes in SVF obtained by the Automated isolation system, but used only two CD markers to determine the pericyte population and other cell types.[69] The use of multiple CD markers results in a more specific population than the use of less CD markers and so a lower percentage of that specific cell type e.g. pericytes.[22] Bianchi et al. used CD34min/CD146pos/CD90pos to detect the pericyte-like population in the SVF and isolated the highest percentage of pericytes using the non-enzymatic Lipogems procedure as compared to other intraoperative isolation procedures.[22] However, Bianchi et al. mostly used other combinations of CD markers in comparison to other studies.[22] This renders their SVF composition incomparable with SVF compositions obtained by other intraoperative isolation procedures.

The enzymatic procedures: Automated isolation system, Tissue genesis cell isolation system and Sepax isolated more endothelial progenitor cells in comparison to other intraoperative isolation

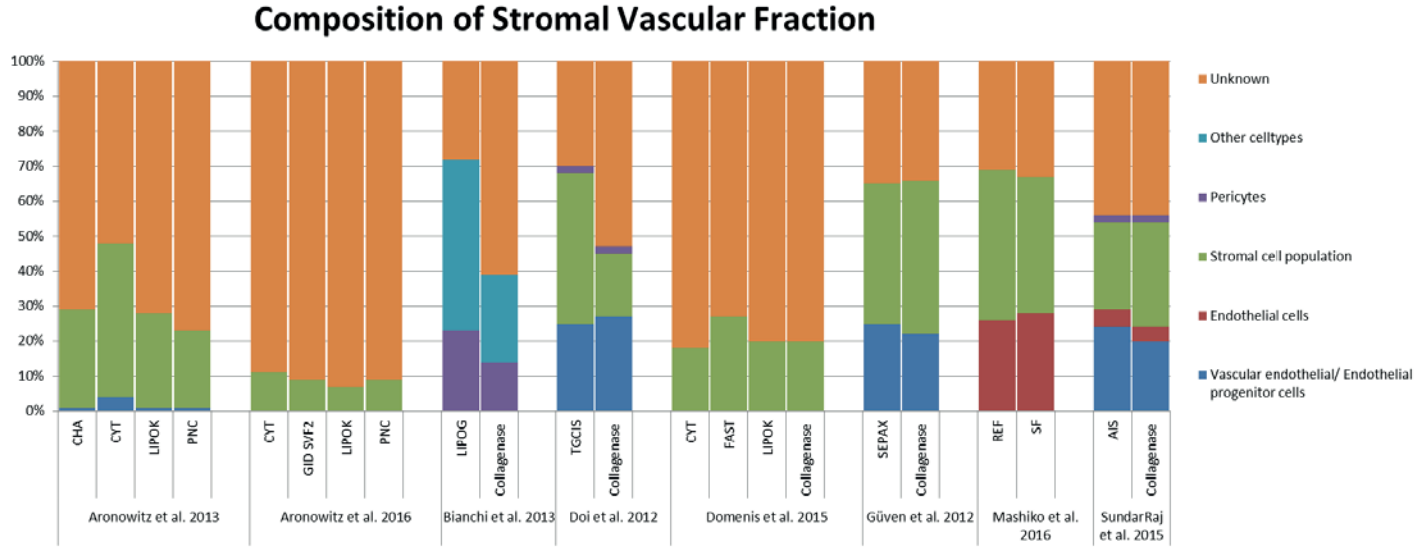

Figure 2. SVF composition (CD marker) of procedures comparing an intraoperative isolation procedure with a non-intraoperative isolation protocol or with other intraoperative isolation procedures within one study. Stromal cell population (CD31min/CD34pos) consists of supra-adventitial cells, ASCs and pericytes, only pericytes defined as CD31min/CD146pos, CD31min/CD34min/pos or CD34min/CD146pos/CD90pos are placed separately in the table. Endothelial cells and vascular/progenitor endothelial cells are described as respectively, CD31pos/CD34min and CD31pos/CD34pos. No exact data described in text by Aronowitz et al., Bianchi et al., Domenis et al., Güven et al. and Mashiko et al., data is extracted from figures by authors JAD and AJT. AIS Automated Isolation System; CHA-station (CHA-Biotech); CYT Celution System Enzymatic (Cytori); FAST Fastem Corios (Corios); GID SVF2 (GID Europe); LIPOK Lipokit System (Medi-khan); PNC Multi station (PNC); REF Residual tissue of emulsified fat; SEPAX Sepax (Biosafe); SF Squeezed fat;Tissue Genesis Cell Isolation System (Tissue Genesis)

Table 5. Modified IFATS index score for the measurement of adipose tissue-derived stromal vascular fraction

			Flow cytometry of cultured ASCs										Functional assasys			
Studies	**Viability**	**Flow cytometry of SVF**	**CD13**	**CD29**	**CD31**	**CD44**	**CD45**	**CD73**	**CD90**	**CD105**	**CD235a**	**CFU-F**	**Adipogenic**	**Osteogenic**	**Chondrogenic**	**Total Score**
Aronowitz et al. 2013	1	1										1				3.00
Aronowitz et al. 2016	1	1										1				3.00
Bianchi et al. 2013	1	1										0	1/3	1/3	1/3	3.00
Doi et al. 2012	1	1										0				2.00
Domenis et al. 2015	0	1	1/9	1/9		1/9	1/9	1/9	1/9	1/9		1				2.78
Van Dongen et al. 2016	1	0		1/9	1/9	1/9	1/9		1/9	1/9		1	1/3	1/3		3.33
Güven et al. 2012	1	1		1/9		1/9		1/9	1/9	1/9		1	1/3	1/3	1/3	4.56
Lin et al. 2008	1	1										1	1/3	1/3		3.67
Mashiko et al. 2016	1	1										0				2.00
Millan et al.	1	0				1/9		1/9	1/9	1/9		0				1.44
Osinga et al. 2015	1	0										1	1/3	1/3	1/3	3.00
SundarRaj et al. 2015	1	1										1				3.00
Tonnard et al. 2013	0	1										0	1/3			1.33

procedures.[69, 75, 76] Nonetheless, more endothelial progenitor cells were not corresponding to less stromal cells or pericytes. In all differently obtained SVF, the origin of large numbers of cells remains unidentified. This is partly because not every study identified both adipose tissue-derived and blood-derived cell types, but probably not every subpopulation of all cell types is already known as well.

When donor variability is neutralized by the use of the same lipoaspirate, intraoperative isolation procedures resulted in different SVF compositions. Lipogems isolated significantly more pericytes and stromal cells than the non-intraoperative isolation protocol control ($p<0.05$) [22] (Figure 2). The enzymatic Celution system resulted in significantly more endothelial progenitor cells in comparison with the CHA-system, Lipokit system and Multi station, which is not necessarily preferred ($p=0.003$).[72] All other intraoperative isolation procedures compared with non-intraoperative isolation protocols showed no significant differences.

Modified IFATS/ISCT Index Score for the measurement of adipose tissue-derived stromal vascular fraction

Modified IFATS/ISCT index scores ranged from 1 to 4.6 out of 5. Güven et al. scored 4.6 and presented the most complete characterization of the SVF and ASCs [75] (Table 5). Tonnard et al. scored 2 points, but had only used CD34 as a marker to identify a subpopulation in the SVF.[68] Two studies used other methods than flow cytometry to determine the composition of SVF.[67, 71] No studies were excluded based on a low number of outcomes of interest measured by the modified IFATS/ISCT Index Score, because five out of thirteen studies scored less than half of the possible points given. This high number of low scores given to studies underlines the need for standardization.

Disclosure agreements of included articles

A disclosure agreement of support by the manufacturer was provided in five of the thirteen studies [22, 72, 73, 75, 76] (Table 6, supplemental content). The company, which was mostly involved in the studies, was Cytori, the manufacturer of the enzymatic Celution system.

DISCUSSION

Grafting of lipoaspirates and of SVF in particular, is a rapidly evolving treatment modality for scars and other skin defects, arthritis, neuropathy, diabetic ulcers to mention a few. Many of these, initially small scale, single center studies, are on the verge of expansion to multicenter placebo-controlled double-blind randomized clinical trials. An important prerequisite is the use of an efficient and standardized intraoperative isolation procedure of SVF. This systematic review shows that none of these procedures supersedes other procedures in terms of cell

yield, viability and SVF composition while being time and cost efficient too when analyzed using the same lipoaspirate. However, three intraoperative isolation procedures (shuffling 5 times, shuffling 30 times and Lipogems) showed only a minimal reduction of the volume of lipoaspirate, implicating that most of the adipocytes still are intact. Consequently, these three procedures are methods of processing rather than isolation procedures.[22, 71] Moreover, there is a wide variation in cell yield, viability of cells and composition of SVF when all intraoperative isolation procedures are compared together. Study characteristics showed small and varied sample sizes regarding the number, sex and age of the donors. It is known that the cell yield and viability of SVF differ among donors, depending on age, harvest location and co-morbidities, such as obesity, of the donors.[18-21, 79] This interdonor variability is a possible explanation for the variations found between several studies. To avoid variation bias, isolation procedures should be investigated using identical lipoaspirates in the same study. There are, however, differences between non-enzymatic and enzymatic isolated SVFs on a different level. Non-enzymatic isolation procedures resulted in larger volumes (tSVF) than the resulting pellets (cSVF) after enzymatic intraoperative isolation procedures. Because the final products of both types of isolation procedures are different, the clinical purpose of the use of SVF is an important factor which isolation procedure suits best. In some cases, such as the intra-articular injection of SVF in temporomandibular joints requires very small volumes, whereas the end volume of SVF enriched lipofilling is less relevant. Isolation procedures of SVF of adipose tissue are based on reduction of large volume containing tissue or cells, such as ECM and/or adipocytes to concentrate the stromal vascular fraction. Non-enzymatic isolation of SVF results in a smaller volume of adipose tissue containing intact ECM and cell-cell communications between SVF cells (tSVF), because the shear forces are too low to disrupt cell to cell and cell to ECM adhesions.[12, 80] Therefore, the tissue structure of lipoaspirate is still intact in the tSVF. Enzymatic procedures, however, likely result in a single cell cSVF, because enzymes likely disrupt all cell-cell interactions and ECM (Figure. 3).[15] This is may not happen in the Automated isolation system, GID SVF2, Lipokit system and Multi station, possibly due to insufficient enzymatic digestion.[69, 70]

Clinical use of tSVF has several advantages over the use of cSVF in different clinical applications of regenerative medicine. It is well known that single cells migrate within 24 hours after application.[81] The ECM, containing a microvasculature structure, might function as a natural scaffold for cells like ASCs and most likely also augments rapid vascularization and reperfusion. This will probably increase cell retention rates after injection and enhance clinical effects. In case of early scar formation, wound healing, or organ fibrosis, tSVF might therefore be more an appropriate therapy, which implicates that non-enzymatic procedures are more suitable as compared to enzymatic isolation procedures. In case of excessive pre-existing scar formation, the ECM in the SVF might not be appropriate and therefore the application of a cSVF or ASCs might be more eligible. ASCs could remodel excessive scar formation by immunomodulation or instruction of resident cells.

Characterization of subpopulations in the SVF depends upon selection of appropriate markers. Selection of an insufficient number of markers will give a disfigured image of the actual SVF composition (Figure 3). SVF of adipose tissue can be divided into two major subpopulations based on the expression of CD45, which is a hematopoietic cell marker: adipose derived (CD45min) and blood derived (CD45pos).[7] Adipose derived cell populations can be divided into endothelial cells (CD31pos) and stromal cells (CD31min).[7] Three important subpopulations of the stromal cell population (CD45min/CD31min) are supra-adventitial cells: CD34pos/CD146min, pericytes: CD34pos/min/CD146pos and ASCs: CD34pos/CD90pos/CD105low.[7, 11, 12, 82] Supra-adventitial cells and pericytes are both identified as precursor cells of ASCs, although there remains some controversy about this item.[11, 12, 80, 83] Ideally, to discriminate between those three cell types within the CD45min/CD31min subpopulation, CD146 and/or CD90 markers should be used additionally. However, in most studies two CD markers or inappropriate combinations of CD markers have been used to determine cell types; only Lin et al. used all the aforementioned combinations.[73] Because Lin et al. focus mainly on blood derived cells and not on the stromal cell population or pericytes, this did not affect their results. Doi et al. ascribed CD31min/CD34min/CD45min to the pericyte population, so therefore the CD34pos pericytes will be missed.[76] SundarRaj et al. and Güven et al. used CD34pos/CD31min to determine the number of ASCs [69, 75], while pericytes and supra-adventitial cells also express CD34. Therefore, the number of ASCs contains pericytes and supra-adventitial cells as well.[7, 11] To cover pericytes, supra-adventitial cells and ASCs, Domenis et al., Aronowitz et al. and Mashiko et al. used CD34pos/CD31min/CD45min to determine the stromal cell population.[66, 70, 72, 74] CD34pos is frequently used as a marker to describe cells with stem cell characteristics in both hematopoietic and non-hematopoietic stem cells.[84] The differences in use of CD marker expression to determine pericytes and the stromal cell population might be a possible explanation for the large variations found in SVF between different studies. No solid conclusions could be made about which isolation procedure generates the most stromal cells or pericytes.

Unfortunately, a limited number of commercially available intraoperative SVF isolation procedures not yet have reached scientific validation at an acceptable level. The American Society for Aesthetic Plastic Surgery (ASAPS) and the American Society of Plastic Surgeons (ASPS) published a position statement in 2012 on fat grafting and stem cells.[85] All specialized equipment for the use of stem cell extraction should be fully verified regarding efficacy and safety before use in clinical settings. In 2013, the IFATS and ICTS proposed guidelines with standardized endpoints and methods to verify and compare SVF isolation procedures.[5] None of the included studies fully verified their isolation procedure according to these IFATS and ICTS guidelines. Moreover, viability was measured in different ways among studies (*e.g.* directly on obtained SVF or after an extra non-intraoperative isolation protocol) and lipoaspirate was processed differently prior to isolation (*e.g.* centrifugation or decantation). For those reasons,

we propose new adjusted IFATS and ICTS guidelines to validate intraoperative isolation procedures (Figure. 3). All intraoperative isolation procedures should be validated using centrifuged adipose tissue to determine the actual volume of lipoaspirate prior to isolation. It is known that increased centrifugal forces have a harmful effect on the viability of fat grafts.[86, 87] However, the use of centrifuged adipose tissue is necessary to determine the actual cell yield after an isolation procedure. Furthermore, cell viability of tSVF should be determined directly on tSVF, instead of using an extra non-intraoperative isolation protocol which possibly results in more cell damage. However, the proposed adjusted standardized endpoints and methods by IFATS and ICTS are time-consuming and expensive since it requires cultured ASCs. In order to quickly verify isolation procedures intraoperatively during clinical trials, the end product of non-enzymatic intraoperative isolation procedures should be centrifuged to separate the oily fraction from the tSVF and pellet fraction based on density. For enzymatic intraoperative isolation procedures, microscopy can be used to visualize single cells. In this way, isolation procedures can be quickly evaluated during clinical trials.

A large number of SVF isolation procedures without applying a full verification according to the IFATS and ICTS guidelines is available.[14] Oberbauer et al. presented a narrative overview of enzymatic and non-enzymatic intraoperative SVF isolation procedures.[14] In twenty-one out of thirty (both enzymatic as well as non-enzymatic) intraoperative isolation procedures reported in their study, there was a lack of verification data. In two studies intraoperative isolation procedures without scientific evidence *e.g.* viability of SVF, flow cytometry of SVF cells and ASCs, were used to treat patients. One study used SVF obtained by ultrasonic cavitation to treat patients with migraine and tension headache.[88] Another study used SVF in combination with platelet rich plasma for meniscus repair.[89] Hence, it cannot be guaranteed that the isolation procedures indeed isolate SVF, which is clinical safe for use. It seems that the use of most SVF isolation procedures with its concomitant clinical application is far ahead of a sound scientific base upon which these procedures should be used.

Moreover, the clinical safety of isolated SVF or ASCs is not clear yet, especially regarding clinical use in patients with any kind of malignancy. It is demonstrated, *in vitro*, that ASCs influence growth, progression and metastasis of cancer cell lines through *e.g.* promoting angiogenesis and differentiation of ASCs into carcinoma-associated fibroblasts.[90] Zimmerlin et al. showed *in vitro* that ASCs influence growth of active malign cell lines, but this is not seen in latent cancer cell lines.[91] Clinical data suggest that the use of isolated SVF or ASCs is safe in patients without an oncological history.[92] *In vitro* studies often use higher concentrations of ASCs as compared to clinical studies and this might be the cause of differences found between *in vitro* and *in vivo* studies.[92] However, to test clinical safety it is important to reach scientific validation of the commercially available procedures at an acceptable level. In this review it

become clear that the reproducibility of the procedures as well as characterization of the SVF had shortcomings. If this is reached, further scientific research with proper controls with regard to the clinical effect and safety of SVF or ASCs are definitely wanted.

CONCLUSION

There is no evidence thus far that any intraoperative isolation procedure could be designated as preferred procedure for isolating SVF. However, three isolation procedures are rather processing techniques than isolation procedures. Enzymatic and non-enzymatic procedures had comparable results as it comes to cell yield, viability, and SVF composition. Non-enzymatic isolation procedures end products resulted had greater volumes (tSVF) than the pellets (cSVF) of the enzymatic isolation procedures. The results of intraoperative isolation procedures are comparable with those of the gold standard, the collagenase based non-intraoperative isolation protocol. Since intraoperative isolation procedures are less time-consuming, but as efficient as the non-intraoperative isolation protocol, the use of intraoperative isolation procedures seems to be more suitable for clinical purposes. However, only small sample sizes have been used to validate the isolation procedures. To test clinical safety, it is important to reach scientific validation of the commercially available procedures at an acceptable level. Regarding to this review, this level is not yet reached by many procedures.

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Supplemental table 1. Study characteristics

Name	Author	Female (F)/ Male (M)	Age mean +sd (y)	Age variance (y)	Liposuction	Donor site	Infiltration (1/0)	Cannula (mm)	Pressure
CHA CYT LIPOK PNC	Aronowitz et al. 2013	5F	-	-	Tumescent liposuction	Abdominal	1	2.5 blunt	25-28 mmHg Vacuum
CYT GID SVF2 LIPOK PNC	Aronowitz et al. 2016	5F	32.4 +/-5.9	25-37	Tumescent liposuction	Abdominal, flank, back, arms, buttocks, inner thighs	1	-	-
LIPOG	Bianchi et al. 2013	4	-	-	Liposuction	Abdominal	1	19 cm blunt, 3 mm OD, 5 oval holes (1x2 mm)	manually or clamping
TGCIS	Doi et al. 2012	6F	-	-	Liposuction	-	0	-	-
CYT	Domenis et al. 2015	9	46	41-70	Liposuction	Hips, Trochanteric, abdominal	1	3 blunt	0.4 bar
FAST		6	52	19-74		Hips, Trochanteric, abdominal			
LIPOK		5	52	41-74		Hips, abdominal			
FAT	van Dongen et al. 2016	11F	-	-	Liposuction	-	1	Sorenson cannula	-
SEPAX	Güven et al. 2012	11F	-	20-65	Tumescent liposuction	Abdominal	1	-	-
CYT	Lin et al. 2008	6F	38.7+/-16.3	18-60	Plastic Surgery	-	0	-	-
FEF REF SF	Mashiko et al. 2016	10F	41.0+/-8.2	-	Liposuction	Thigh	1	3-mm multiport cannula, holes of 2 mm	-
STCELL	Millan et al.	3	-	-	Liposuction	-	1	-	-
SHUF5 SHUF30	Osinga et al. 2015	3F/3M	M57, F61	-	Liposuction	Abdominal	1	4 mm blunt cannula, oval opening 2 x 4mm	manual
AIS	SundarRaj et al. 2015	11	30.86	17-47	-	Abdominal, thigh, hip	0	-	-
NANO	Tonnard et al. 2013	1F	40	n/a	Abdominoplasty liposuction	Abdominal	1	3-mm sharp nultiport cannula, holes of 1 mm	high-negative

AIS Automated Isolation System; CHA-station (CHA-Biotech); CYT Celution System Enzymatic (Cytori); FAST Fastem Corios (Corios); FAT Fractionation of Adipose Tissue procedure; FEF Filtrated fluid of emulsified fat; GID SVF2 (GID Europe); LIPOG Lipogems (Lipogems); LIPOK Lipokit System (Medi-khan); NANO Nanofat procedure; REF Residual tissue of emulsified fat; PNC Multi station (PNC); SEPAX Sepax (Biosafe); SF Squeezed fat; SHUF5 Shuffling 5 times; SHUF30 Shuffling 30 times; STCELL StromaCell; TGCIS Tissue Genesis Cell Isolation System (Tissue Genesis); 1 = used infiltration prior to liposuction, 0 = not mention the use of infiltration or did not use infiltration prior to liposuction

Supplemental table 2A: Cell yield and viability per milliliter start volume of lipoaspirate of all intraoperative isolation procedures per study

Enzymatic isolation procedure	Cell yield x10^5 cells/ml	SD	Viability nucleated cells (%)	SD
AIS (SundarRaj, 2015)	1,2	0,5	98%	21
CHA (Aronowitz, 2013)	0,6	0,15	87%	12
CYT (Aronowitz, 2013)	2,4*	0,32	93%	2
CYT (Aronowitz, 2016) ¤	1	0,16	84%*	1
CYT (Domenis, 2015)	11,7	0,5		
CYT (Lin, 2008)	3,7	0,86	89%	1
GID SVF2 (Aronowitz, 2016) ¤	2,9	0,65	69%	6
LIPOK (Domenis, 2015)	5	3		
LIPOK (Aronowitz, 2013)	0,3	0,15	72%	15
LIPOK (Aronowitz, 2016) ¤	6,2*	0,25	50%	10
PNC (Aronowitz, 2013)	1,1	0,49	57%	21
PNC (Aronowitz, 2016) ¤	5,4*	1,64	82%*	5
SEPAX (Güven, 2012)	2,6*	1,2		
TGCIS (Doi, 2012)	7	1,89	81%	

**Significantly best procedure tested in their study (p>0.05); ¤ No exact data mentioned in text, data extracted from figures by authors JAD and AJT. AIS Automated Isolation System; CHA-station (CHA-Biotech); CYT Celution System Enzymatic (Cytori); GID SVF2 (GID Europe); LIPOK Lipokit System (Medi-khan); PNC Multi station (PNC); SEPAX Sepax (Biosafe); TGCIS Tissue Genesis Cell Isolation System (Tissue Genesis)*

Supplemental table 2B: Cell yield per milliliter of end volume, viability and concentration of concentration procedures.

Non-enzymatic isolation procedure	Cell yield x10^5 cells/ml	SD	Viability nucleated cells (%)	SD	Start volume	End volume	End as % of start volume	Y:C ratio*
FAST (Domenis, 2015)	4,6	2,9	-	-	-	-	-	-
FAT (van Dongen, 2016)	22,6	4,5	-	-	10	1	10%	2.3
FEF (Mashiko, 2016)	1,8[a]	0,5[a]	39%	9	-	-	10%	0.2
NANO (Tonnard, 2013)	-[b]	-	-	-	100	-	-	-
REF (Mashiko, 2016)	6,5[a]	0,8[a]	91%	3	-	-	39%	2.5
SF (Mashiko, 2016)	8,0[a]	0,4[a]	90%	5	-	-	48%	3.8
SHUF5 (Osinga, 2015)	13		65%	-	10	-	-	-
SHUF30 (Osinga, 2015)	11		63%	-	10	-	-	-
STCELL (Millan, 2015)[a]	18,8	4,7	87%	-	100	15	15%	2.8

** Y:C ratio: Yield: Concentration ratio; relative cell yield per processed lipoaspirate volume. [a] No exact data mentioned in text, data extracted from figures by authors JAD and AJT. [b] Cell yield 2*10^6 cells per 100 ml of processed fat by the Nanofat procedure. No cell yield per ml end product can be calculated. FAST Fastem Corios (Corios); FAT Fat procedure; FEF Filtrated fluid of emulsified fat; NANO Nanofat procedure; REF Residual tissue of emulsified fat; SF Squeezed fat; SHUF5 Shuffling 5 times; SHUF30 Shuffling 30 times; STCELL StromaCell.*

Supplemental table 3. Stromal Vascular Fraction composition (CD marker) of intraoperative isolation procedures in all studies

	Enzymatic isolation procedures														Non-enzymatic isolation procedures			
	AIS (SundarRaj, 2015)	°CHA (Aronowitz, 2013)	°CYT(Aronowitz, 2013)	CYT (Aronowitz, 2016)	°CYT (Domenis, 2015)	CYT (Lin, 2008)	GID SVF2 (Aronowitz, 2016)	°LIPOK (Aronowitz, 2013)	LIPOK (Aronowitz, 2016)	°LIPOK (Domenis, 2015)	°PNC (Aronowitz, 2013)	PNC (Aronowitz, 2016)	°SEPAX (Güven, 2012)	TGCIS (Doi, 2012)	FAST (Domenis, 2015)	°LIPOG (Bianchi, 2013)	°REF (Mashiko 2016)	°SF (Mashiko 2016)
Adipose derived cells (CD45-)																		
Vascular endothelial/Endothelial progenitor cells (CD31+/CD34+)	24%	1.1%	4.2%	-	-	8%	-	0.7%	-	-	0.9%	-	25%	24.5%	-	-	-	-
Endothelial cells (CD31+/CD34-)	5%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	26%	28%
Pericytes (CD31-/CD146+) (CD31-/CD34-/+)(CD34-/CD146+/CD90+)	2%	-	-	-	-	0.8%	-	-	-	-	-	-	-	2.2%	-	23.2%	-	-
Stromal cell population (CD31-/CD34+)	25%	28%	43.5%	10.7%	18.3%	-	8.9%	26.5%	7.2%	20%	22%	9%	40%	43.2%	26.7%	-	43%	39%
Blood derived cells (CD45+)																		
Lymphocytes (CD31+/CD34-/CD90-/CD105-/CD146-)	-	-	-	-	-	19%	-	-	-	-	-	-	-	-	-	-	-	-
Leukocytes (CD31-/CD34-/CD90-/CD105-/CD146-)	-	-	-	-	-	7.6%	-	-	-	-	-	-	-	-	-	-	-	-
Hematopoetic stem cell (CD31dim/CD34+/CD90-/CD105-/CD146-)	-	-	-	-	-	4.6%	-	-	-	-	-	-	-	-	-	-	-	-
Other cell types (CD markers)		-	-	-	-	-	-	-	-	-	-	-	-	-	-	49.4%	-	-
Unknown	44%	70.9%	52.3%	89.3%	81.7%	60%	91.1%	72.8%	92.6%	80%	77.1%	91%	35%	30.1%	73.3%	27.4%	31%	33%

Stromal cell population (CD31min/CD34pos) consists of supra-adventitial cells, ASCs and pericytes, only pericytes defined as CD31min/CD146pos, CD31min/CD34min/pos or CD34min/CD146pos/CD90pos are placed separately in the table. ° *No exact data described in text, data extracted from figures by authors JAD and AJT. AIS Automated Isolation System; (CHA-Biotech); CYT Celution System Enzymatic (Cytori); FAST Fastem Corios (Corios); GID SVF2 (GID Europe); LIPOG Lipogems (Lipogems); LIPOK Lipokit System (Medi-khan); PNC Multi station (PNC); REF Residual tissue of emulsified fat; SEPAX Sepax (Biosafe); SF Squeezed fat;TGCIS Tissue Genesis Cell Isolation System (Tissue Genesis)*

Supplemental table 4. Disclosures of included studies

Articles	Disclosures
Aronowitz et al. 2013	Loan devices CHA and Cytori
Aronowitz et al. 2016	No disclosures
Bianchi et al. 2013	Lipogems
Doi et al. 2012	Kaneca, Inc
Domenis et al. 2015	No disclosures
van Dongen et al. 2016	No disclosures
Güven et al. 2012	Biosafe SA and loan Sepax
Lin et al. 2008	Cytori Therapeutic Inc
Mashiko et al. 2016	No disclosures
Millan et al. 2014	No disclosures
Osinga et al. 2015	No disclosures
SundarRaj et al. 2015	No disclosures
Tonnard et al. 2013	No disclosures

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08

Sterility and endotoxin levels after mechanical isolation of stromal vascular fraction by the FAT-procedure

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ABSTRACT

Background: The therapeutic use of stromal vascular fraction (SVF) from adipose tissue has increased significantly, such as the intra-articular injection of SVF in the temporomandibular joint in osteoarthritis. This increased applicability requires additional quality standards regarding the SVF isolation procedure as well as the final product. Therefore we assessed the sterility and purity of a SVF isolation procedure: the fractionation of adipose tissue (FAT procedure).

Methods: The FAT-procedure was performed following three elective clinical liposuction procedures. Two aliquots of tissue (A and B) were obtained from of each of the four different FAT procedure phases per patient (n=3) (in total 24 samples) and tested for bacterial growth using Agar plates and a non-selective highly sensitive Fastidious Bacteria (FB) broth. The supernatant from the tissue samples of two different FAT procedure phases were subjected to an endotoxin test (in total 12 samples).

Results: None of the samples yielded bacterial outgrowth on standard Agar plates. In the additional FB broth, contamination was detected in 4 out of 24 samples. In one sample, an endotoxin level of 1.75EU/ml was detected.

Conclusion: The FAT procedure can be safely applied for therapeutic use from a sterility and purity point of view.

INTRODUCTION

Over the last decade, the therapeutic use of stromal vascular fraction (SVF) from adipose tissue has increased significantly. SVF was initially used as a stromal cell-enrichment procedure for fat grafting. The mechanism behind the clinical effect of SVF is partly ascribed to adipose derived stromal cells (ASCs). ASCs can act anti-inflammatory, anti-fibrotic and remodel extracellular matrix by secretion of cytokines, growth factors and exosomes.[1]

Therefore, clinical application of SVF has been increased, such as for intra-articular applications in osteoarthritic joints.[2] It is hypothesized that an injection with SVF might reduce inflammation of osteoarthritic joints, based on this anti-inflammatory effect.[3-6] Recently, stromal cell injections have been suggested as treatment for TMJ osteoarthritis in a systematic review[2] and this suggestion was supported by in vitro studies[3,6,7]. A recent published clinical study showed promising results of less pain and improved movement of the jaw after the injection of SVF in the temporomandibular joint in a case serie.[8]

This increased applicability of SVF, such as intra-articular application in the TMJ, requires additional quality standards regarding the SVF isolation procedure as well as the final product. Although many studies have been performed to validate SVF isolation procedures for clinical use, data about sterility and the possible presence of bacterial endotoxin is still lacking of almost all SVF isolation procedures. Hitherto, only Aronowitz et al. evaluated bacterial contamination of adipose tissue after four different isolation procedures[9] whereby the isolated tissue from three out of five patients were contaminated with bacteria. In this current study, we assessed sterility and endotoxin levels after another SVF isolation procedure namely, the fractionation of adipose tissue (FAT).[10]

METHODS

The FAT-procedure[10] was performed following three elective clinical liposuction procedures under general anesthesia. After manual liposuction, the decanted adipose tissue was centrifuged at 960g for 2.5 minutes (Thermo Scientific Medilite™) and then stroked 30 times backwards and forwards through the Fractionator (ETLLLL 1.4, Tulip, Medical Products®, San Diego, CA, USA). Subsequently, the processed lipoaspirate was centrifuged again at 960g for 2.5 minutes.

Two aliquots of tissue (A and B) were obtained from of each of the four different FAT procedure phases per patient (n=3) (in total 24 samples) and tested for bacterial growth using agar plates (Brucella agar + 5% sheep blood/vitamin K/hemin medium (BBA); blood agar + 5% sheep blood medium (BA); and chocolate medium (CHOC)). Additionally, in order to detect a low

Table 1. Sterility during the FAT-procedure in different subjects.

	Subject 1		Subject 2		Subject 3	
Agar plates (BBA, BA, CHOC)	**A**	**B**	**A**	**B**	**A**	**B**
1. Lipoaspirate after harvesting	neg.	neg.	neg.	neg.	neg.	neg.
2. Lipoaspirate after first centrifugation	neg.	neg.	neg.	neg.	neg.	neg.
3. Lipoaspirate after fractionation	neg.	neg.	neg.	neg.	neg.	neg.
4. SVF	neg.	neg.	neg.	neg.	neg.	neg.
FB Broth (high sensitivity test)						
1. Lipoaspirate after harvesting	pos: Staph	pos: Staph.	neg.	neg.	neg.	pos: Staph
2. Lipoaspirate after first centrifugation	neg.	neg.	neg.	neg.	neg.	neg.
3. Lipoaspirate after fractionation	neg.	neg.	neg.	neg.	neg.	neg.
4. SVF	neg.	neg.	neg.	neg.	neg.	pos: Staph

BBA: Brucella agar + 5% sheep blood/ vitamin K /hemin medium); BA: blood agar + 5% sheep blood medium; CHOC: chocolate medium. Neg = negative. Pos = positive. Staph = staphylococcus bacteria; SVF = stromal vascular fraction. Staph: Staphylococcus. FB broth is a highly sensitive non-selective medium, no species can be given.

Table 2. Endotoxin levels during the FAT-procedure for 3 different subjects.

	Subject 1				Subject 2				Subject 3			
	A		**B**		**A**		**B**		**A**		**B**	
	EU/ml	**PPC**	**EU/ml**	**PPC**	**EU/ml**	**PPC**	**EU/ml**	**PPC**	**EU/ml**	**PPC**	**EU/ml**	**PPC**
2. Supranatant after first centrifugation	<0.05	87%	<0.05	117%	<0.05	77%	<0.05	81%	<0.05	113%	<0.05	119%
4. Supranatant SVF product	1.75	106%	<0.05	93%	<0.05	68%	<0.05	62%	<0.05	108%	<0.05	116%

EU: Endotoxin Units; PPC positive product control; SVF: stromal vascular fraction; Endotoxin test is adequate if PPC value is between 50-200%. Endotoxin FDA standard for parenteral use is 2.5 EU/ kg body weight.

bacteria load, a non-selective Fastidious Bacteria (FB) broth (10ml tube) was used (Table 1). The samples were incubated at 35°C for 7 days. The supernatant from the tissue samples of two different FAT procedure phases were also subjected to an endotoxin test performed with BioTekElx808 (Cambrex, NJ, USA) (see Table 2).

RESULTS

None of the samples yielded bacterial outgrowth on standard agar plates (Table 1). In the additional FB broth, contamination was detected in 4 out of 24 samples. Samples A and B of subject 1 only had Staphylococci species growth after harvesting. Bacterial growth occurred in one of the two samples from subject 3. A low endotoxin level of 1.75 EU/ml was detectable in only one of the two samples from subject 1. Endotoxin was undetectable in all the other samples (Table 2).

DISCUSSION

Based on the aforementioned results, we conclude that the small amount of staphylococcal contamination objectified in a few samples of the FB broth cannot be explained by a specific phase of the FAT-procedure.

Contamination with staphylococci is often linked with skin contamination. Skin contamination is a more general non-technique related concern that occurs in all types of surgery. Higher contamination rates have been found on trocars in orthopedic surgery than in our present study.[11] Relating our findings to Aronowitz's study, higher bacterial contamination rates were seen after their four isolation procedures compared to our FAT procedure, but their contamination rate was more patient related than technique related.[9] Endotoxin levels were undetectable, except in one sample, but still below the FDA standards.[12]

Therefore, to our opinion, the FAT procedure can be safely applied for therapeutic use from a sterility and purity point of view. In general, sterility and purity of all types of SVF isolation procedures should be assessed before intra-articular clinical use to prevent technique related contamination.

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09

General Discussion

GENERAL DISCUSSION

Facial fat grafting is used to restore volume deficiencies in the face as well as to improve soft tissue contours. In the maxillofacial region fat grafting can be used as a solitary procedure, but can also be used as an adjunct in orthognathic surgery and reconstructive surgery. Unfortunately, the volumetric effect after a facial fat graft procedure decreases, particularly during the first postoperative year.[1-3]

Thus far, studies aiming to evaluate the effect of facial fat grafting have used a variety of grafting techniques and non-validated measurement tools to assess the outcome. This variety impedes comparisons of the results of fat grafting between studies. Hence, there is still uncertainty as to which current fat grafting technique is the best and what volumetric result can be expected after facial fat grafting.[4-6]

The overall aim of the research described in this thesis was to assess the volumetric outcome of and patient satisfaction with facial fat grafting when applying the best current processing technique and validated measuring tools. First, the most optimal fat grafting technique was deducted from a systematic review of the literature. Next, a valid measurement tool was developed to reliably assess both the overall volumetric outcome and the local outcomes of facial fat grafting. After validating this measurement tool, the volumetric outcome and patient satisfaction after facial fat grafting were assessed in a clinical study. In the evolving field of facial fat grafting, the potential of adding a stromal vascular fraction (SVF) to a fat graft to improve graft retention was explored through a systematic review of the literature. Finally, the sterility and endotoxins of the fractionation of adipose tissue was tested. This is needed for the non-homologous use of SVF in clinical studies.

Processing techniques

As deducted from the systematic review of the literature (Chapter 2), processing techniques with the least mechanical stress, such as filtering and washing, are superior to centrifugation regarding adipocyte cell viability. This is supported by a study of routine fat grafting procedures which describes that adipocyte membrane leakage and disruption occurs due to centrifugation forces.[7] This is an important observation as adipocytes are the main component of adipose tissue, making up more than 90% of its volume.[8,9] Most processing techniques aim to keep the adipocytes viable to maintain the volume of the graft, but the question is whether processing techniques that yield a higher percentage of viable adipocytes *in vitro* also result in a higher retention of facial fat graft volume *in vivo*. It is still unknown whether the surviving adipocytes of the fat graft or the growth of new pre-adipocytes are responsible for the volume retention.

The review revealed that closed washing systems have a slight advantage, supported by the only human 3D volumetric comparison study.[10] Therefore, in 2015, we decided to use the closed washing system (PureGraft) for our clinical study. Ever since, a number of clinical studies was published that assessed the visible volumetric effect after different processing techniques.[11-13] Our choice for the closed-filter technique was supported by Wang et al.[6] who concluded in their recent systematic review that, with regard to retention of the visible volumetric volume, the sedimentation technique is also inferior to filter-wash and centrifugation techniques. Although uncertainty remains as to which processing technique is the best for clinical studies, due to differences in study design, we consider the filter-wash (PureGraft) system that we have used for our clinical trial is one of the most optimal processing techniques to obtain a good volumetric effect.[6]

Measurement tools

Visible volume analysis

In the study described in Chapter 3, we used stereophotogrammetry to assess visible volume changes of the surface of the face. This technique has evolved into the leading tool for assessing visible volumetric effects as a function of time in facial fat grafting because it is patient friendly and easy to use.[10-12,14,15] Many of the recently published articles on volumetric outcomes after facial fat grafting use the term volume retention after applying 3D stereophotogrammetry.[6,10-12,16] However, in our opinion, 'volume retention' is an incorrect term to use in fat graft studies because it encompasses more than visible chances of the surface, which cannot be measured with 3D stereophotogrammetry. True volume retention can be assessed by other modalities such as directly assessing the fat graft after excision (often used in animal studies) or indirectly by using MRI (often used in breast fat grafting)[17-21]. The final outcome of facial fat grafting is the visible effect at the skin surface which is, of course, very important for patients. Therefore, we used 3D stereophotogrammetry as a tool to measure what we termed the visible volumetric effect.

Chapter 3 describes the 3D stereophotogrammetry method that we developed and validated to measure volumetric changes of well-defined aesthetic areas in the face. This method uses personalized aesthetic templates to define the aesthetic areas. Previous clinical studies did not use standardized protocols that took facial expression into account and/or applied an inadequate reproducible determination of target areas.[10,12,16,22,23] Doing a volumetric analysis from the developed clinical 3D protocol and applying an algorithm based projection of a standardized template was adequate for both overall and local assessments.

The developed 3D method was shown to be very sensitive. In fact, it was so sensitive that even a slight gain in body weight resulted in an increase in visible volume of the face. This finding should be taken into account when interpreting the results of facial fat grafting as a function of time in future studies.

Patient satisfaction

The FACE-Q questionnaire used in the study described in Chapter 4 was designed for the assessment and validation of the outcome of aesthetic facial surgery. [24-26] First, it was translated and validated for application in The Netherlands. We tested the FACE-Q questionnaire on women who had not been subjected to any kind of aesthetic facial procedures and showed that satisfaction with overall facial appearance is not associated with age. Thus, it is in line with the original validation studies of the FACE-Q that assessed age-relatedness in patient populations that underwent aesthetic facial surgery.[24-26]

The obtained normal values for the Dutch population (Chapter 4) are important when measuring and comparing satisfaction with facial appearance after aesthetic surgical procedures. Namely, it is important to be able to judge whether the obtained effect is comparable with what is considered to be 'normal' in the Dutch population. A limitation of our study is that the Dutch average scores we obtained were not corrected for social economic status and cultural differences. The latter is an omission since intercultural differences may result in different normal scores with regard to aesthetic ideals and aesthetic demands.[27] The great variety of subcultures and nationalities in The Netherlands makes it nearly impossible to define them all and to correct for any possible deviation from the Dutch average.

Clinical outcomes

The study described in Chapter 5 shows that patient satisfaction after fat grafting coincides with the overall and local visible volumetric effects of fat grafting. An increase in patient satisfaction was seen after 6 weeks followed by a slight decrease up to one year post surgery. This decrease in patient satisfaction was in line with the decrease in visible volumetric effects. Notwithstanding this decrease, overall patient satisfaction and visible volumetric effects were still significantly higher after one year compared to the preoperative scores.

Our study is one of the first studies to compare volumetric outcome and patient satisfaction as a function of time up to one year after facial fat grafting. Most studies either assessed the visible volumetric outcome[11,12,28] or patient satisfaction[29,30] as a function of time. The only other study that assessed both outcomes also reported a good match between volumetric outcome and patient satisfaction, but only measured them at two time points, preoperatively and one year postoperatively whereas we measured them at three time points, preoperatively and then 6 weeks followed by one year postoperatively.[16]

An interesting observation was that the recipient site appears to be a factor influencing the visible volumetric effect of the graft. The zygomatic area showed better volume retention after one year than the lip area. Although the important role of the recipient site with regard to retention of the fat graft was suggested by an earlier clinical study, it was based on differences in patient satisfaction for different areas.[29] To the best of our knowledge, differences in the effect of fat graft volumes between different aesthetic facial areas in humans have never been objectified before in clinical studies using 3D volumetric measurement tools.

The role of the recipient site has gained more interest over the last few years. Currently, recipient site aspects such as vascularization, pro-adipogenic factors and physical/mechanical forces on adipocytes[31] are considered as factors that influence the balance between degeneration and regeneration of the grafted adipose tissue as well as the final volume retention of a fat graft.

Although vascularization is often mentioned as an important recipient site factor influencing fat graft volume retention,[32] Shi et al.[33] showed that other factors are also involved. Despite intramuscular layers having better vascularization, the retained volume of the fat grafts injected into mice' intramuscular layers was lower than in the fat pads. They suggested that tissue-resident adipose stromal/progenitor cells play a leading role in fat graft retention in the fat pads by generating pro-adipogenic circumstances. Their hypothesis supports our finding (Chapter 5) that the visible fat graft retention in the zygomatic area (a fat pad site) was better than in the lip area (a subcutaneous and muscle layer site).

Physical or mechanical forces on adipocytes at the recipient site, such as pressure or movement of the recipient site, can also be linked to differences in visible fat graft retention between the lips and the zygomatic area. Adipocytes are mechanosensitive cells and it is posed that external mechanical forces on or the movement of adipocytes might negatively affect tissue growth and cellular function and thus influence fat graft survival.[31] Supporting this hypothesis is the finding that muscular layers in mice retain a fat graft better after being immobilized by denervation compared to muscular layers which have not been immobilized.[34]

Another important observation (Chapter 5) was that the patients' FACE-Q scores for 'preoperative psychological well-being' were associated more strongly with the FACE-Q score 'satisfaction with the result' than the objective volumetric visible effect of the fat grafting procedure. Even though the objective result of the fat graft may be good, a patient may remain dissatisfied. This does not mean that surgeons should not operate on patients with low psychological well-being, but they should definitely be aware of the fact that the expected satisfaction with the results after facial fat grafting can be lower compared to patients with a

normal or high level of psychological well-being. Surgeons should discuss this issue with their patients and use the outcome of the discussion in their decision making as to whether they should or should not operate on patients with low psychological well-being.

On comparing the fat grafted patient population's results with the control population's normative dataset, a similar strong association was found between psychological well-being and satisfaction with the overall facial appearance among women (Chapter 3). The women with low psychological well-being ratings gave significantly lower scores for overall satisfaction with their facial appearance. Apparently those two factors go hand-in-hand in all women. However, a major difference was that the average preoperative scores of the fat grafting patient population were lower than the controls' scores.

Stromal vascular fraction

The last part of this thesis focused on the clinical application of stromal vascular fraction (SVF). According to the the systematic review (Chapter 7) it can be concluded that non-enzymatic intraoperative isolation procedures (tissue derived SVF (tSVF), also containing extracellular matrix) are less time-consuming than intraoperative enzymatic isolation procedures (single cell SVF (cSVF)) and that they result in similar cell yields, cell viability and composition of tissue-derived SVF. Therefore, non-enzymatic isolation procedures (tSVF) are currently considered to be the most suitable for clinical use.

It is very important to point out the difference between the isolation techniques to obtain a SVF and the processing techniques to obtain a fat graft. Whereas the aim of the processing techniques is to maintain the adipocytes, the aim of the isolation techniques is to disrupt and remove the adipocytes in order to only obtain SVF. In contrast to the processing techniques where many different procedures are used, such as decantation, filtration or centrifugation, almost all studies recommend using a form of centrifugation for the SVF isolation procedures.

Besides the use of SVF as an additive to facial fat grafting, the potential regenerative capacities of SVF in fibrosis and osteoarthritis have gained attention in the last few years.[35-38] Sterility and purity tests are mandatory for non-homologous clinical SVF applications i.e., therapeutic use in other structures than subcutaneous layers. However, those test are not mandatory for homologous use of SVF, such as subcutaneous injection. In the study described in Chapter 8, the sterility and purity (level of endotoxins) of the Fractionation of Adipose Tissue[39], also known as the FAT-procedure, were tested. The FAT-procedure was chosen because this is one of the most optimal SVF isolation techniques (Chapter 7) and is already being used in a number of Dutch clinical trials for homologous use (subcutaneous). No contamination was seen in the standard contamination tests.

Future perspectives

Outcomes

We introduced a reproducible measurement tool in Chapter 3 to measure volume differences between different aesthetic areas with the use of a personalized aesthetic template. This measurement tool enables a more standardized and automated selection of aesthetic areas and can be used in future clinical trials to compare different predefined aesthetic areas and to assess the role of the different recipient sites in facial fat grafting.

Although our 3D analysis method is accurate and can be used in the clinic, the template projection technique should be optimized further in order to reduce measurement variation. Moreover, standardized templates could also be designed for other types of facial surgery such as in oncologic resection patients, Graves ophthalmopathy patients, and cleft and syndromatic patients.

The clinical study (Chapter 5) focused on volumetric outcome as the primary aim and patient satisfaction as a secondary aim. It can be assumed that with the increased attention to personalized medicine, patient satisfaction will replace the volumetric outcome as the primary outcome of studies assessing the results of fat grafting.

The incorporation of personalized care as a leading decision factor in aesthetic surgery in order to adapt the procedure and expectations of the procedure to the perception of the patient was suggested by Selvaggi et al.[40]. The suggestion is only operate on those patients who are expected to appreciate the outcome of aesthetic facial surgery. The importance of this suggestion is underlined by our observation (Chapter 5) that patients with a low psychological well-being are more likely to be dissatisfied with the result of the facial fat graft. Nowadays, it is not standard for clinics to assess the preoperative psychological well-being with validated questionnaires. We feel that such an examination should be incorporated in the standard of care in the future. Preoperative questionnaires, such as the psychological modules of the FACE-Q, and their correlation to the expected results, may help patients and surgeons to gain better insight into the expected post-operative satisfaction. Expectation management can influence the decision on whether or not an aesthetic facial surgery will meet the patient's goals. Further research is needed to refine the predictors for optimal patient satisfaction.

In addition to patient satisfaction and volumetric outcome, other factors have been suggested as outcome measurements after facial fat grafting, e.g., skin quality and pore size.[41-45] These outcome variables are linked to the hypothesis that certain components of a fat graft, e.g., adipose stromal cells, have a regenerative potential. Chapter 3 reported that women within the normative dataset are very conscious of their skin quality. Apparently, no woman is perfectly satisfied with her facial skin, in contrast to the other parts of the face such as cheeks and lips.

Therefore, women in the general population are more likely to undergo facial fat grafting or an SVF injection to improve the skin than to correct volume deficiencies. Once fat grafting and / or SVF injections are indeed capable of improving skin quality significantly, skin improvement will potentially become another important indication to perform facial fat grafting for facial rejuvenation.

Techniques

As described in Chapter 5, and as discussed before, fat grafting had different volumetric effects during the follow-up period among the different recipient sites of the face. Chapter 5's study design was observational and not specifically to assess the volumetric effects of fat grafting on different aesthetic areas. Further research is needed to get more evidence on whether there is a difference between aesthetic areas. We hypothesize that areas with fewer mechanical forces and containing adipose tissue (such as fat pads) provide a better environment for transplanted adipocytes to survive and may therefore result in better fat retention.

Optimization of the circumstances for fat grafts to retain their volume in the recipient area will gain increasing attention in studies on facial fat grafting, e.g., to create a better environment for adipocytes to survive or for pre-adipocytes to grow within the transplantation niche. Theoretically, this can be accomplished by optimizing the recipient site or by optimizing the graft itself.

Evidence for recipient site optimization, particularly through the enhancement of vascularization by soft-tissue expansion or carbon dioxide laser, is mostly based on preclinical evidence.[46-48] The only published human study described external tissue expansion before breast fat grafting.[19]

Optimization of a fat graft could be obtained by adding SVF to the fat graft (Figure 1). Some animal and human studies show that combining a fat graft with SVF results in significant improvement of the volumetric effects.[12,18,21,49-52] The leading hypothesis for this effect is that the ASCs that are present in the added SVF secrete stimulating factors at the recipient site. These factors can improve adipocyte survival and/or stimulate pre-adipocytes to grow from the transplanted fat graft. Furthermore, SVF contains factors with pro-angiogenic and anti-inflammatory effects[31,53,54]. Yet, an unanswered question is: What is the most optimal concentration of SVF that has to be added?. Recently, no differences were reported when fat grafts were enriched with intra-operatively derived SVF or with different doses of lab-cultured ASCs (up to 20 times more cells).[18]

Another important research question is: Will the addition of SVF to a fat graft result in better visible volumetric effects than fat grafting alone when studied in a randomized placebo controlled clinical trial using validated 3D volumetric measurement tools?. Based on the

research in this thesis, our suggested optimal technique for enriching the fat graft would be a filter/wash processing method (Chapter 2) in combination with mechanical isolation to obtain tissue derived SVF (tSVF, Chapter 7). Both the FAT (fractionation of adipose tissue)[55] and the REF (residual tissue of emulsified fat) [56] mechanical isolation procedures have given good results. These procedures can be combined with facial fat grafting using a filter/wash method. None of the clinical studies published so far have used this combination for fat graft enrichment for humans.

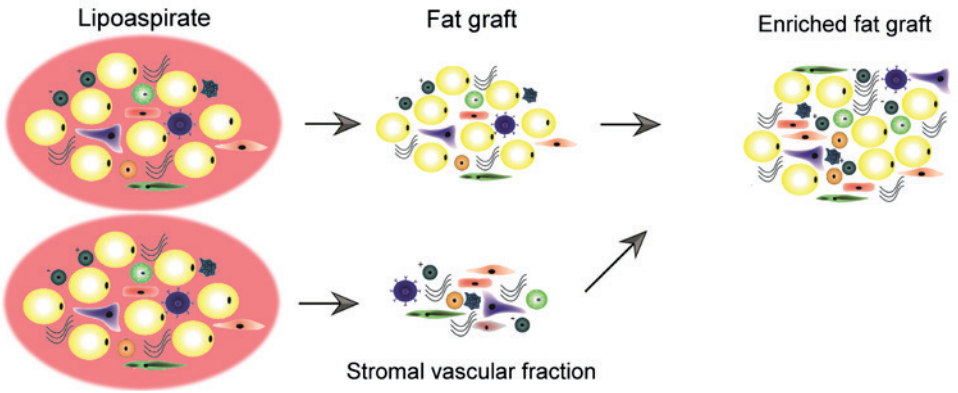

Figure 1: Schematic illustration of fat graft enrichment. The lipoaspirate contains adipocytes, other cell types, including ASCs, and extracellular matrix in a mix of infiltration fluid, blood and oil originating from ruptured adipocytes. A fat graft results from a removal of blood, oil and infiltration fluid from the lipoaspirate. Stromal vascular fraction (SVF) results from an enzymatic or mechanical removal of adipocytes next to removal of infiltration fluid, blood and oil from the lipoaspirate. To enrich the fat graft, SVF is added to the fat graft.

As stated before, the potential of SVF is developing beyond its use as an additive to a fat graft. SVF derived from adipose tissue was shown to have anti-inflammatory properties.[57,58] Therefore, it is hypothesized that injecting SVF into osteoarthritic joints (knee, temporomandibular joint (TMJ)) might reduce inflammation.[59-62] This hypothesis is supported by an animal study where the exosomes of injected stromal cells induced an early reduction in inflammation (less pain and tissue degeneration) followed by restoration of the TMJ osteochondral tissues with increased matrix synthesis.[63] Many bridges have to be crossed before the potential of SVF injections in TMJ osteoarthritis in human is met. Currently, the preparation and injection of SVF into the TMJ is labeled by the Dutch Central Committee on Research Involving Human Subjects as an Advanced Therapeutic Medicinal Product (ATMP). An intra-articular injection is seen as a non-homologous application of SVF (thus ATMP) whereas a SVF injection in a subdermal layer, as used in enriched fat grafting, is considered to be a homologous application. A non-

homologous application requires additional sterility and purity tests during the SVF isolation procedure, such as performed in Chapter 8, which is mandatory before starting any clinical trial.

Conclusion

We designed and validated a 3D measurement tool to evaluate the visible volumetric outcome of and patient satisfaction with facial fat grafting. We proved an increase in both visible volumetric effect and patient satisfaction one year after regular facial fat grafting. Furthermore, satisfaction with the result is strongly associated with the patient's preoperative psychological wellbeing. We provide clinicians with new insights for their decision making on whether or not to perform facial fat grafting in the individual subject.

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10

Summary

SUMMARY

Facial fat grafting is used to restore volume deficiencies of the face as well as for improvement of the appearance of soft tissue after other surgical procedures. Fat grafts can add volume at the recipient site, but unfortunately some of the added volume decreases with time, particularly during the first year after transplantation. Commonly, studies that have researched facial fat grafting use different grafting techniques and non-validated measurement tools to assess the outcome of facial fat grafting. Therefore, a comparison of the results of fat grafting between studies is difficult. The overall aim of the research described in this thesis was to assess the volumetric outcome and patients' satisfaction of facial fat grafting when applying the currently best processing technique and validated measuring tools.

In **Chapter 2**, a systematic review of literature was described to find the most optimal processing technique for facial fat grafting. PubMed, Embase, Cinahl, and Cochrane databases were searched until August 2015. Studies comparing different fat grafting processing techniques were included that assessed the outcomes viability of adipocytes, number of adipose-derived stromal/stem cells (ASC) and growth factors in vitro, volume and quality of the graft in animal studies, and satisfaction and volume retention in human studies. Thirty-five studies were included in this systematic review. Adipocyte viability and ASC numbers were optimal using the gauze/towel technique (permeability principle) compared to centrifugation. The animal studies' and patients' satisfaction results were not distinctive. The only study assessing volume retention in humans showed that a wash-filter device performed significantly better than centrifugation. It was concluded that processing techniques using permeability principals prove superior to centrifugation (reinforced gravity principle) regarding viability and ASC number. Due to the variety in study characteristics and reported outcome variables, none of the processing techniques demonstrate any clinical evidence. Based on the outcome of the systematic review, it was decided to use the wash/filter technique in our clinical study.

The goal of **Chapters 3 and 4** was to develop reliable measurement tools to assess the clinical objective and subjective outcome of facial fat grafting. In **Chapter 3** a new three-dimensional (3D) volumetric analysis based on a personalized aesthetic template is presented. Accuracy and reproducibility of this new 3D method were assessed. Six female volunteers were photographed using the 3dMDtrio system, according to a clinical protocol twice at baseline (T1) and after one year (T2). A styrofoam head was used as a control. A standardized aesthetic template was morphed over the baseline images of the volunteers using a coherent point drift algorithm. The resulting personalized template was projected over all sequential images to assess surface area differences, volume differences and RMS errors. It was shown that in the 12 well-defined aesthetic areas, the mean average surface area and volume differences between the two T1 images ranged from 7.6 to 10.1mm^2 and -0.11 to 0.13cm^3 respectively. T1 RMS

errors ranged between 0.24-0.68mm (sd 0.18-0.73). Comparable differences were found between the T2 images. An increase in volume between T1 and T2 was only observed in volunteers who gained in body weight. It was concluded that personalized aesthetic templates are an accurate and reproducible method to assess changes in aesthetic areas.

Subjective outcome after aesthetic facial surgery can be assessed by patient reported outcome measurements such as the often used FACE-Q questionnaire. It is poorly researched what the average scores of normal satisfaction are and if age effects the normal satisfaction of facial appearance. Therefore, in the study described in **Chapter 4,** the effect of age to average facial appearance satisfaction was assessed in women who never received any kind of aesthetic facial procedures. Dutch women aged between 18 and 85 years from all over the country were randomly asked to participate and fourteen modules of the validated FACE-Q questionnaire were examined. The data were analyzed as a function of age (18-30 years, 30-39 years, 40-49 years, 50-59 years, 60+) by a Kruskal-Wallis test. 155 of the 180 volunteers who signed the informed consent completed the FACE-Q questionnaires. The median satisfaction of the "Facial appearance overall" module was 59 (IQR 51-70). Although older women gave significantly higher scores for the aging face modules such as wrinkles, lip-lines, upper eye lids, and nasolabial folds, there were no significant association between age and the scores for the module "facial satisfaction overall" ($p=0.776$). Low psychological wellbeing scores were strongly associated with low satisfaction scores with overall facial appearance (0.621, $p<0.001$). It was concluded that satisfaction with overall facial appearance was not associated with age in women who have had not been subjected to any kind of aesthetic facial procedures.

The overall aim of this thesis was to assess the volumetric outcome and patients' satisfaction of facial fat grafting when applying the currently best processing technique and validated measuring tools. This research question is researched in **Chapter 5**. Therefore, an observational study is described assessing the overall and more specifically the local volumetric effects of facial fat grafting. These effects were related to patients' satisfaction up to one year after grafting. Equal fat grafting methods were used in all patients. Outcome parameters (3D volume differences (3dMD), patient satisfaction (FACE-Q questionnaire)) were measured at baseline, and 6 weeks, 6 months and 12 months after fat grafting. Of the 33 female patients that underwent a facial fat graft procedure, 23 patients had complete 3D data and were eligible for analysis. Highest volume gain was observed 6 weeks after grafting and was followed by a gradual loss thereafter. Overall and in the zygomatic area, a substantial gain in volume was still present 1 year after grafting, while this effect was lost in the lip area. FACE-Q scales "Satisfaction with facial appearance overall" and "satisfaction with cheeks" improved

too, while "lip lines" returned to baseline levels. The improvement in FACE-Q scales was in agreement with the objective change in volume. It was concluded that the gain in overall and local volumetric effects is accompanied by comparable changes in patients' satisfaction.

One of the patients initially included in the study described in **Chapter 5** became pregnant 3 weeks after the fat grafting procedure. It is known that weight gain can affect the volume of a facial fat graft, resulting in unfavorable asymmetries. Weight gain during pregnancy is even more complex and does not just entail an increase in adipose tissue. Therefore, in **Chapter 6** we objectified in this patient whether pregnancy results in volume changes of a facial fat graft. The 24-year-old female received a fat graft (7ml) in the mandibular area to mask a volume deficiency. This deficiency occurred after a fibula reconstruction of a mandibular defect resulting from the removal of an ameloblastoma. Standardized three-dimensional photographs (3dMD) were available preoperatively, and at 7 weeks (first trimester), 6 months (second trimester), 9 months (third trimester), and 14 months (4 months after delivery) postoperatively. Three-dimensional analysis revealed that no substantial volume changes of the fat graft occurred during pregnancy other than the overall proportional gain in facial volume. It was concluded from this case that pregnancy apparently does not affect the volume of a small unilateral fat graft applied in the facial region.

It has been hypothesized that addition of adipose derived stromal cells (ASCs), e.g. as present in stromal vascular fraction (SVF), to a regular fat graft may improve the volume retention of the fat graft. Therefore, we assessed in the studies described in **Chapters 7 and 8** whether it might be feasible to use SVF for future trials. Intraoperative application of stromal vascular fraction (SVF) of adipose tissue requires a fast and efficient isolation procedure of adipose tissue. In a similar fashion to selecting the best method for facial fat grafting, we systematically reviewed the literature to assess and compare procedures currently used for the intraoperative isolation of the enzymatic processed single cell SVF (cSVF) and mechanically processed tissue-derived SVF (tSVF) that also contains extracellular matrix (**Chapter 7**). Pubmed, EMBASE and The Cochrane Central Register of controlled trials databases were searched for studies that compare procedures for intraoperative isolation of SVF (searched 28th of September, 2016). Outcomes of interest were cell yield, viability of cells, composition of SVF, duration, cost and procedure characteristics. Procedures were subdivided in procedures resulting in a cSVF or tSVF. Thirteen out of 3038 studies were included, evaluating eighteen intraoperative procedures, were considered eligible. In general, cSVF and tSVF intraoperative procedures had comparable cell yield, cell viability and SVF composition compared to a non-intraoperative (*i.e. culture lab-based collagenase protocol)* control group within the same studies. The majority of intraoperative isolation procedures are less time consuming than non-intraoperative laboratory procedures. We can conclude that intraoperative isolation procedures are less time-consuming

than non-intraoperative procedures with similar cell yield, viability of cells and composition of SVF and therefore more suitable for use in the clinic. Nevertheless, none of the intraoperative isolation procedures could be designated as preferred procedure to isolate SVF.

Before intra-operative isolation procedure (tSVF) can be applied in the clinic for non-homologous use it has to be assessed whether the sterility and purity of a fractionation of adipose tissue (FAT procedure) procedure for obtaining SVF is acceptable (**Chapter 8**). The FAT-procedure was performed following elective clinical liposuction procedures in three patients. Two aliquots of tissue (A and B) were obtained from of each the four phases of the FAT procedure (in total 24 samples). Each aliquot was tested for bacterial growth using Agar plates and a non-selective highly sensitive Fastidious Bacteria (FB) broth. The supernatant from the tissue samples was subjected to an endotoxin test (in total 12 samples). None of the samples yielded bacterial outgrowth on standard Agar plates. In our opinion, the FAT procedure can be safely applied for therapeutic use from a sterility and purity point of view.

In the general discussion (**Chapter 9**) the results of the studies described in **Chapters 2-8** were discussed in a broader perspective and some perspectives for future studies are given.

11

Summary in Dutch

NEDERLANDSE SAMENVATTING

Lipofilling is een chirurgische procedure waarbij lichaamseigen vetweefsel (autologe vettransplantatie) vanuit een donorgebied wordt getransplanteerd naar een ander gebied van het lichaam (receptorgebied). In het gelaat wordt lipofilling meestal gebruikt voor het aanvullen van een bestaand volumetekort. Dit kan zowel als opzichzelfstaande procedure als in combinatie met andere chirurgische ingrepen van het aangezicht. Het doel van lipofilling is het optimaliseren van de contour van de weke delen. Het aangebrachte vet geeft een zichtbare volume toename van het gelaat. Dit volume-effect is geen één op één weergave van de hoeveelheid vet die is aangebracht, maar de aan het oppervlak van de huid te meten toename in volume. Dit volume-effect is helaas niet stabiel. Gedurende het eerste jaar na de lipofilling neemt het zichtbare volume-effect (voor een deel) weer af. Het is nog onvoldoende duidelijk hoe groot deze afname precies is en of deze afname afhankelijk is van het gebied waar het vet in het gelaat is aangebracht. Het is van groot belang om hier meer inzicht in te krijgen waardoor het te bereiken eindresultaat beter is te voorspellen.

In de studies die tot dusverre het te bereiken zichtbare volume-effect en de afname hiervan in de tijd hebben onderzocht geven onvoldoende uitsluitsel. De verschillende studies maken gebruik van verschillende technieken voor lipofilling gebruikt en hebben het zichtbare volume-effect veelal gemeten met niet-gevalideerde meetinstrumenten. Deze diversiteit aan toegepaste lipofilling technieken en meetinstrumenten laat een goede vergelijking van de uitkomsten tussen studies niet toe. Derhalve was het doel van het in dit proefschrift beschreven onderzoek om het zichtbare volume-effect en de patiënttevredenheid na lipofilling van het gelaat te onderzoeken. Alvorens dit te kunnen onderzoeken, moesten gevalideerde meetinstrumenten voor het bepalen van het volume-effect en de patiënttevredenheid worden ontwikkeld en gevalideerd, en de beste lipofilling techniek voor klinische toepassing worden geselecteerd.

In **hoofdstuk 2** worden de uitkomsten van een systematisch literatuuronderzoek beschreven. Het doel van dit onderzoek was het bepalen van de beste techniek om geoogst vet te bewerken voor lipofilling. Met behulp van vier verschillende literatuurzoekmachines (Pubmed, Embase, Cinahl, Cochrane) werd gezocht naar artikelen die minimaal twee procestechnieken met elkaar vergeleken en tenminste één van de volgende uitkomstmaten beschreven: de hoeveelheid levende vetcellen (adipocyten), de hoeveelheid in het vetweefsel aanwezige stromale cellen (adipocyte stromal cell: ASC), het volume van het transplantaat en/of de patiënttevredenheid. Vijvendertig van de 401 gevonden studies werden geschikt bevonden voor nadere analyse. Uit de *in-vitro* onderzoeken kwam naar voren dat meer overlevende adipocyten en ASC's in het te transplanteren vet aanwezig zijn wanneer het vettransplantaat wordt opgewerkt met een filtratietechniek (filtratie van het vettransplantaat door gaasjes of filters) dan wanneer het vettransplantaat wordt gecentrifugeerd. Uit de enige, geschikt bevonden, klinische studie

kwam naar voren dat een transplantaat dat wordt opgewerkt middels een gesloten was/filter techniek een beter volume-effect geeft na lipofilling dan wanneer het transplantaat wordt opgewerkt door middel van centrifugeren. Op grond van deze bevindingen werd besloten om voor de in dit proefschrift beschreven klinische studie naar de uitkomsten van lipofilling in het gelaat, het vettransplantaat op te werken voor transplantatie middels een was/filtertechniek (**hoofdstukken 5 en 6**).

Het doel van het in de **hoofdstukken 3 en 4** beschreven onderzoek was het vinden van betrouwbare meetinstrumenten voor het meten van het zichtbare volume-effect en de patiënttevredenheid na een lipofilling behandeling in het gelaat. In **hoofdstuk 3** wordt een nieuwe driedimensionale (3D) volume analyse geïntroduceerd. Deze 3D volume analyse techniek maakt gebruik van een zogenaamd gepersonaliseerd esthetisch raster. Dit is een raster dat wordt gevormd naar het gezicht van een individu. Het gelaat wordt hierbij onderverdeelt in 12 goed omschreven esthetische zones. De nauwkeurigheid en reproduceerbaarheid van de ontwikkelde 3D methode werden geanalyseerd. Voor de validatie van de methodiek werden zes vrouwelijke proefpersonen volgens een gestandaardiseerd protocol gefotografeerd met behulp van het 3dMDtrio camerasysteem. Met dit camerasysteem kan een 3D weergave van het oppervlak van het gelaat worden gemaakt zonder hierbij gebruik te hoeven maken van ioniserende straling. Van elke proefpersoon werden op twee verschillende tijdstippen twee gestandaardiseerde fotoseries gemaakt (uitgangssituatie (T1) en na 1 jaar (T2)). Als controle werd een uit piepschuim vervaardigd dummy hoofd gebruikt. Voor iedere proefpersoon en de dummy werd een persoonlijk esthetisch raster gevormd. Met behulp van dit persoonlijke raster werden vervolgens de verschillen in het oppervlak, de verschillen in het volume en de kwadratische gemiddelde afwijking van elke esthetische regio berekend tussen de beide fotoseries die op T1 en T2 waren vervaardigd. De kwadratisch gemiddelde afwijking in volume tussen beide fotoseries en tussen beide tijdstippen werd door middel van de root mean square (RMS) berekend. RMS is een standaardmaat voor nauwkeurigheid van de afwijking. De verschillen in oppervlakte en volume tussen de beide op T1 gemaakte fotoseries varieerden van 7.6 tot 10.1mm^2 en -0.11 tot 0.13cm^3. De RMS fouten varieerden tussen 0.24 en 0,68mm (standaard deviatie 0.18-0.73mm). Deze verschillen kwamen overeen met de gemeten verschillen tussen de beide gemaakte fotoseries op T2. Een opmerkelijke bevinding was dat bij proefpersonen die na 1 jaar niet in gewicht waren veranderd geen verschil in volume werd gevonden tussen de metingen op T1 en T2. Bij proefpersonen die in lichaamsgewicht waren toegenomen, werd daarentegen een toename in volume gevonden. Op basis van de uitkomsten van dit onderzoek werd de ontwikkelde 3D volume analyse techniek als geschikt bevonden om met voldoende nauwkeurigheid en reproduceerbaarheid toe te kunnen passen voor klinisch onderzoek.

De patiënttevredenheid na esthetische ingrepen, zoals bijvoorbeeld lipofilling, wordt gewoonlijk geëvalueerd met behulp van vragenlijsten. De van oorsprong Amerikaanse FACE-Q vragenlijst is een vragenlijst die veel wordt gebruikt voor onderzoek naar de uitkomsten van esthetische aangezichtschirurgie. Hoewel deze vragenlijst alom wordt toegepast, ontbreken gegevens over gemiddelde scores voor de verschillende modules van de FACE-Q voor personen die geen ingreep aan het gelaat hebben ondergaan. Ook is niet bekend of de factor leeftijd invloed heeft op de tevredenheid met het uiterlijk. In het in **hoofdstuk 4** beschreven onderzoek wordt in deze omissie voorzien. De FACE-Q vragenlijst werd voorgelegd aan 180 Nederlandse vrouwen tussen 18 en 85 jaar. Dit waren allen vrouwen die nooit een ingreep aan het gelaat hadden ondergaan. De antwoorden op de vragen werden voor een aantal leeftijdscategorieën (18-29 jaar, 30-39 jaar, 40-49 jaar, 50-59 jaar, 60+) geanalyseerd. Van de 180 vrijwilligers die hadden ingestemd om deel te nemen aan dit onderzoek, stuurden 155 vrijwilligers een volledig ingevulde vragenlijst terug. De mediane score van de module 'tevredenheid met het gelaat in het algemeen' was 59 (afstand tussen 1^{e} en 3^{e} kwartiel (IQR): 51-70). Hoewel oudere vrouwen zich significant meer stoorden aan kenmerken van het ouder wordende gelaat, zoals rimpels, liplijntjes, hangende bovenoogleden en diepe nasolabiale plooien, kon geen verband tussen de factor leeftijd en de module 'tevredenheid met het gelaat in het algemeen' ($p=0,776$) worden aangetoond. Daarnaast bleek een lage score op de module 'psychologisch welbevinden' samen te hangen met een lage score op de module 'tevredenheid met het gelaat in het algemeen' (0,621, $p<0,001$). Op grond van deze bevindingen werd geconcludeerd dat leeftijd niet van invloed is op de algemene tevredenheid over het gelaat bij vrouwen die geen ingreep hebben ondergaan.

Zoals eerder vermeld was het doel van het in dit proefschrift beschreven onderzoek om de zichtbare volume-effecten en de patiënttevredenheid na lipofilling te onderzoeken. In **hoofdstuk 5** worden de uitkomsten beschreven van een observationele studie onder patiënten die alleen een lipofilling procedure in het gelaat hadden ondergaan. Het opgetreden zichtbare volume-effect na de lipofilling procedure werd zowel voor het gehele gelaat als voor een aantal specifieke regio's berekend. Voor deze metingen werd de in **hoofdstuk 3** beschreven 3D volume analysetechniek gebruikt. Het gemeten zichtbare volume-effect werd vergeleken met de tevredenheid van de patiënt zoals gemeten door de FACE-Q vragenlijst. Zowel de objectieve metingen als het bepalen van de tevredenheid van de patiënt werden preoperatief en 6 weken, 6 maanden en 1 jaar na de lipofilling bepaald. Van de 33 patiënten die een lipofilling procedure hadden ondergaan, was van 23 patiënten een volledige 3D data set beschikbaar. Het grootste zichtbare volume-effect werd 6 weken na de lipofilling gezien gevolgd door een geleidelijk verlies aan volume. Een zichtbaar volume-effect van zowel het gehele gelaat als specifiek voor de zygoma regio was 1 jaar na de ingreep nog steeds aanwezig, terwijl dit effect ter plaatse van de lippen was verdwenen. Ook de scores op de FACE-Q modules 'algemene tevredenheid' en 'tevredenheid met de wangen' waren

na 1 jaar nog steeds hoger dan de uitgangswaarde, dat wil zeggen de tevredenheid voor de lipofilling. De score op de module 'tevredenheid met lippen' verschilde na 1 jaar niet van de preoperatieve uitgangswaarde. Deze subjectieve bevindingen kwamen overeen met de zichtbare volume-effecten in de onderzochte regio's.

Eén van de patiënten, die deelnam aan het in **hoofdstuk 5** beschreven onderzoek, bleek een aantal weken na de lipofilling zwanger te zijn. Zoals in **hoofdstuk 3** is beschreven, kan een gewichtsverandering van invloed zijn op het zichtbare volume-effect van een vettransplantaat. In geval van een unilaterale lipofilling zou dit kunnen leiden tot een onwenselijke asymmetrie. De gewichtstoename tijdens een zwangerschap verloopt immers anders dan een normale toename in lichaamsgewicht. In geval van een zwangerschap is sprake van meer dan alleen een toename van het volume van vetweefsel. In **hoofdstuk 6** zijn de waargenomen volume-effecten gedurende de zwangerschap beschreven bij deze patiënte. De patiënte was een 24-jarige vrouw die een lipofilling aan de linkerzijde van de onderkaak had ondergaan ter correctie van een door een oncologische ingreep ontstaan volume defect. Van deze patiënte waren gestandaardiseerde 3D foto's beschikbaar van voor de lipofilling, en na 7 weken (eerste trimester), 6 maanden (tweede trimester), 9 maanden (derde trimester) en 14 maanden (na de partus) na de lipofilling. Uit de 3D volume analyse kwam naar voren dat er geen extra volume verandering was opgetreden in de regio waar het vettransplantaat was aangebracht anders dan de algehele volume toename van het gelaat.

In de recente literatuur wordt steeds vaker melding gemaakt dat het toevoegen van stromale cellen uit vetweefsel (ASC) aan een vettransplantaat een positieve rol kan spelen bij het behoud van volume retentie van dat vettransplantaat. Deze cellen bevinden zich in de stromale vasculaire fractie (SVF) van vetweefsel. SVF bevat mix van, onder andere, endotheelcellen, supra-adventitiale cellen, pericyten, stromale cellen en lymfocyten. SVF kan worden verkregen door alleen de vetcellen te verwijderen uit het geoogste vetweefsel. Er blijft dan de mix van de SVF cellen over. In de literatuur wordt beschreven dat de paracriene functie van in SVF aanwezige cellen kan bijdragen aan optimaal milieu voor het overleven en groeien van vetcellen. Bovendien zouden de in SVF aanwezige cellen anti-inflammatoire en anti-fibrotische groeifactoren uitscheiden.

In hoofdstukken 7 en 8 wordt een tweetal onderzoeken beschreven waarvan de uitkomsten kunnen worden gebruikt voor studies naar de klinische toepassing van SVF. Op dit moment is de gouden standaard voor de isolatie van SVF een uitgebreide enzymatische isolatie. Voor deze uitgebreide isolatie is een laboratoriumsetting nodig. Bovendien is deze enzymatische isolatie zeer tijdrovend en vormt ze een logistieke uitdaging. Deze wijze van isolatie is hierdoor minder geschikt voor klinische toepassing.

In het in **hoofdstuk 7** beschreven systematische literatuuronderzoek werd nagegaan welke van de beschikbare intra-operatieve SVF procedures het meest geschikt is voor klinische toepassing en het beste resultaat geeft in vergelijking met de gouden standaard. Met behulp van vier literatuurzoekmachines (Pubmed, Embase, Cinahl, Cochrane) werd gezocht naar artikelen waarin intra-operatieve SVF isolatieprocedures werden beschreven en waarbij de volgende uitkomsten werden onderzocht: het aantal cellen dat wordt verkregen bij de isolatieprocedure, de overleving van deze cellen, de samenstelling van de SVF, en/of de duur en de kosten van deze procedures. In totaal werden 3038 artikelen gevonden waarvan 13 artikelen voldeden aan de inclusie criteria. Deze 13 artikelen werden nader geanalyseerd. De intra-operatieve isolatie procedures werden onderverdeeld in procedures die gebruik maakten van een (intra-operatieve) enzymatische isolatie en procedures waarbij mechanische isolatie werd toegepast. De uitkomsten van de intra-operatieve enzymatische en mechanische isolatieprocedures waren vergelijkbaar met betrekking tot zowel het aantal cellen dat kon worden geïsoleerd als de overleving en samenstelling van deze cellen. Deze intra-operatieve enzymatische isolatie procedure is een modificatie van de uitgebreide enzymatische isolatie waarvoor een laboratoriumsetting noodzakelijk is. De resultaten van beide, voor klinische toepassing geschikte, isolaties procedures bleken vergelijkbaar met die van de gouden standaard, dat wil zeggen de in het laboratorium uitgevoerde isolatie procedure. Met andere woorden, er is geen verschil tussen de opbrengsten van een intra-operatieve enzymatische of mechanische isolatie van SVF. Voor klinische toepassing genieten de intra-operatieve isolatie procedures vanwege het gebruiksgemak de voorkeur boven isolatieprocedures in het laboratorium.

Voordat het SVF kan worden toegepast voor een niet-homologe toepassing in klinische studies moeten aanvullende kwalitatieve testen worden gedaan. Niet-homologe toepassing wil zeggen dat SVF wordt aangebracht in een weefselniche waar van nature geen vetweefsel aanwezig is. Hierbij kan bijvoorbeeld worden gedacht aan een intra-articulaire injectie van SVF. Deze verrichte kwalitatieve testen betroffen zowel steriliteitstesten als de bepaling van de aanwezigheid en hoeveelheid endotoxines (celwandbestanddelen van bacteriën) in SVF. In **hoofdstuk 8** zijn de uitkomsten van deze testen beschreven voor de zogenaamde *FAT-procedure*. De *FAT-procedure* is een mechanische isolatie procedure waarbij gecentrifugeerd vetweefsel wordt fijn gedrukt door dit vetweefsel heen en weer te bewegen tussen twee 10 milliliter spuiten. De spuiten zijn verbonden door een koppelstuk (fractionator) met 3 gaten. Door de uitgevoerde mechanische druk gaan membranen van vetcellen wel en de membranen van de SVF cellen niet kapot. De olie die daarbij uit de vetcellen vrijkomt kan worden verwijderd door het weefsel te centrifugeren. In het in **hoofdstuk 8** beschreven onderzoek is voor de *FAT-procedure* gekozen, omdat uit het systematische literatuuronderzoek naar voren was gekomen dat deze procedure goede resultaten kent.

Het onderzoek naar de steriliteit van en aanwezigheid van endotoxinen in middels de *FAT-procedure* werd uitgevoerd tijdens drie electieve liposuctie procedures bij drie vrouwelijke patiënten. De FAT-procedure werd twee keer per patiënt uitgevoerd tijdens de operatie. Tijdens iedere stap van de FAT-procedure (4 stappen in totaal) werd 1 milliliter materiaal verzameld ten behoeve van de kwalitatieve testen. Voor het steriliteitsonderzoek werden de 24 monsters (3x2x4=24 monsters) zowel gekweekt op AGAR platen als in een bouillon medium. Op de AGAR platen werden geen contaminaties gezien, dit in tegenstelling tot in 4 van de 24 in bouillon medium gekweekte monsters. Deze contaminatie was niet te herleiden naar een specifieke stap van de *FAT-procedure* of een bepaald individu. De aanwezigheid van en hoeveelheid endotoxines werd in het cel-arme supernatant bepaald. In 1 monster konden endotoxines worden gedetecteerd (1.75 endotoxine units/ml). Deze endotoxine waarde was lager dan de maximaal toegestane endotoxine waardes volgens Europese richtlijnen.

In de overkoepelende discussie (**hoofdstuk 9**) worden de in de verschillende hoofdstukken beschreven studies in een breder kader geplaatst en bediscussieerd. Tenslotte worden ideeën voor toekomstig onderzoek geopperd, voor zowel het optimaliseren van het oogsten, opwerken en toepassen ten behoeve van lipofilling in het gelaat als voor indicaties voor toekomstig gebruik van SVF.

AP

Appendices

DANKWOORD

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CURICULUM

VITAE

Jorien Tuin was born in Zwolle, The Netherlands, on the 8th of March 1989. In 2007 she finished the Gymnasium of the Thomas a Kempis College, Zwolle, the Netherlands, cum laude. The same year, she started her bachelor Medicine at the University of Groningen, Groningen, the Netherlands. During her Medicine study, she was an active member of student communities and she finished her minor Public Administration at the faculty of Law of the University of Groningen and a course in Epidemiology at the University of Twente, Enschede, the Netherlands. From September 2013 to April 2014 she performed a research fellowship at the Department of Plastic and Reconstructive Surgery of the Children's Hospital of Philadelphia, University of Pennsylvania, Philadelphia, USA. Her research in Philadelphia focused on the diagnosis, clinical features and treatment of hemifacial microsomia. After finishing her master Medicine in 2014, she started a PhD project at the Department of Oral and Maxillofacial Surgery of the University Medical Center Groningen, Groningen, the Netherlands, concerning the techniques and clinical outcomes of facial fat grafting. She combined her PhD project with a Dentistry study at the University of Groningen. In 2019 she started her residency in Oral and Maxillofacial Surgery at the University Medical Center Groningen. She was awarded twice with a BOOA research grant from the Dutch Society of Oral and Maxillofacial Surgery.

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