Journal of Cranio-Maxillo-Facial Surgery 44 (2016) 45–55

Contents lists available at ScienceDirect

Journal of Cranio-Maxillo-Facial Surgery

journal homepage: www.jcmfs.com

What is the current optimal fat grafting processing technique? A systematic review

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ARTICLE INFO

Article history:
Paper received 2 September 2015
Accepted 15 October 2015
Available online 23 October 2015

Keywords:
Systematic review
Fat graft
Processing
Viability
Adipose derived stromal/stem cells
Volume

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: In this study, processing techniques using permeability principles proved superior to centrifugation (reinforced gravity principle) regarding viability and ASC number. Due to the variety in study characteristics and reported outcome variables, however, none of the processing techniques in this study demonstrated clinical evidence of superiority.

1. Introduction

Autologous fat transplantation (AFT) is a commonly applied procedure in reconstructive and aesthetic surgery (Coleman, 1997). Autologous subcutaneous fat is abundantly available in most patients, fully biocompatible, and conceivably permanent (Coleman, 2006). AFT is used for facial rejuvenation and correction of volume deficiencies caused by trauma (Arcuri et al., 2013), congenital malformations (Guibert et al., 2013), or after surgical procedures (Coleman, 2006). Moreover, AFT has been used increasingly for skin regeneration, e.g., in the case of burns and scars (Gentile et al., 2014).

Even though AFT has been performed for decades, no consensus exists about the best fat-grafting technique (Gir et al., 2012; Gupta et al., 2015). Among others, location of donor sites, use of local anesthetics, harvesting methods, processing techniques, and injection techniques continue to be points of discussion (Gir et al., 2012; Lin et al., 2015; Strong et al., 2015). Most studies have analyzed the effects of fat processing techniques on adipocyte viability (Gir et al., 2012). Currently used processing techniques are based on centrifugation, sedimentation, filtering, or washing principles (Gupta et al., 2015; Strong et al., 2015). Recent theories focus more on the crucial role of adipose-derived stromal/stem cells (ASC) (Matsumoto et al., 2006) and/or growth factors such as vascular endothelial growth factor (VEGF) (Nishimura et al., 2000; Garza et al., 2015) in fat graft survival rather than adipocyte viability. These theories give the current literature another perspective.

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http://dx.doi.org/10.1016/j.jcms.2015.10.021
1010-5182/

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 animal studies, as well as volume retention and patient satisfaction in human studies.

2. Material and methods

2.1. Information sources and search

PubMed, Embase, Cochrane Central Register of controlled trials, and Cinahl electronic databases were searched (last search August 10, 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.

2.2. 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, volume and quality of grafts in animals, or volume retention and patient 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.

2.3. 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) (Slim et al., 2003). Table 1 describes the specific assessment criteria of the studies, specified for the current study. The authors.–> (A.J.T., P.N.D.) predefined a MINORS score of $\leq$6 as being of insufficient quality; those studies were excluded from analysis.

2.4. Study selection

Study selection and quality assessment was done by 2 observers independently (A.J.T., P.N.D.). Disagreement was discussed during a consensus meeting. In the case of a persistent disagreement, an independent observer (A.V.) gave a binding verdict.

2.5. 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).

2.6. 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 three-dimensional (3D) imaging and/or patient or observer satisfaction using questionnaires or photographs.

2.7. Statistical analysis

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

2.8. 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 publish only those studies with positive results of their devices. Devices were split into another subcategory in the data analysis.

2.9. 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: RCF (in *x*g) $= 1.12 \times 10^{-5} \times r \times rpm^2$ (Ohlendieck, 2010). This calculation means that the articles had to include the radius (r) of the

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.

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

Table 2
Description of the processing categories.

Processing category	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 min 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.

centrifuge or information about the specific centrifuge to then look up the radius.

3. Results

3.1. Included studies

In total, 401 papers were identified (Fig. 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 2 separate processing methods (Ferguson et al., 2008; Lee et al., 2013; Findik et al., 2007). One study was excluded because other factors were added to the aspirate (Moscona et al., 1994). Two studies did not report an outcome of interest (Dos-Anjos Vilaboa et al., 2013; Brzezienski and Jarrell, 2015). Thus, 38 studies remained for further analysis.

3.2. MINORS assessment of study quality

MINORS scores ranged from 12 to 5 (Appendix 2). All studies had a prospective collected study population, but only 1 study used a historical control group. Six studies reported blinded assessment of their results. Only 42% of the studies described their inclusion criteria properly. Three studies did not pass the minimum MINORS assessment score and were not analyzed further (Shiffman and Mirrafati 2001; Guijarro-Martinez et al., 2011; Mikus et al., 1995). A total of 35 studies were of sufficient methodological quality and thus compared. The absolute agreement of the MINORS score of the individual components between observers was 95%. Cohen's kappa was 0.872 ($p < 0.001$).

3.3. Study characteristics

Of the 35 studies, 2 analyzed only processed animal fat lipoaspirate in vitro and 17 studies analyzed only processed human fat lipoaspirate in vitro (Table 3). Eight studies described processed human fat graft transplantation to animals, and 8 studies described a processed human fat graft transplantation in humans. Some of these in vivo studies ($n = 8$) also performed 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 the 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 analyses. No meta-analyses could be conducted.

3.4. Processing techniques

Of the studies, 33 applied some form of centrifugation (Tables 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, gauze/towel in 10, devices in 11, and metal sieve in 3. Only washing was reported in 5 studies, and a combination of washing and centrifugation was reported in 4 studies.

3.5. Cell viability in vitro

3.5.1. Centrifugation time

Differences centrifugation time (2, 4, 6, or 8 min) at 50 *g* did not affect viability in 1 study (Boschert et al., 2002). Another study reported a reduction in the number of viable cells after centrifuging for 5 min at 3 different speeds (approximately 553 g, 2214 g, and 6149 g) (Kim et al., 2009).

3.5.2. Centrifugation forces

The number of viable cells was reduced with an increase in relative centrifugal force, above 6149 g (Kim et al., 2009), and viable cells dropped between 228 g and 514 g (Piasecki et al., 2007)). In contrast, other studies did not find a reduction in the number of viable cells with an increase in centrifugation forces (>20.627 g (Pulsfort et al., 2007) and 4200 g (Kurita et al., 2008)). In another study (Ferraro et al., 2011), viability was not affected by higher centrifugation forces, but more apoptotic and fewer necrotic cells were observed at 1500 g for 3 min compared to 50 g for 10 min and 250 g for 5 min.

3.5.3. Centrifugation versus no centrifugation/decantation

Centrifugation resulted in significantly fewer intact cells (Rose et al., 2006; Conde-Green et al., 2010b, 2010a) or more altered cells (Rubino et al., 2015) compared to decantation. In contrast, one study found significantly better viability after centrifugation (57 g and 228 g for 3 min) and decantation (Piasecki et al., 2007) compared to the negative control, whereas 1 study did not find a difference in viability (Rohrich et al., 2004) between centrifugation and the negative control.

3.5.4. 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) (Kamel et al., 2014; Pfaff et al., 2014). Two other studies reported better viability with the gauze/towel technique compared to no treatment (Piasecki et al., 2007) and decantation (Gonzalez et al., 2007). In another study (Minn et al., 2010), no significant difference was found regarding viability between centrifugation (1800 g for 3 min) and mesh gauze. Additionally, both centrifugation and mesh gauze had significantly higher absorbance readings than the metal sieve technique in that study.

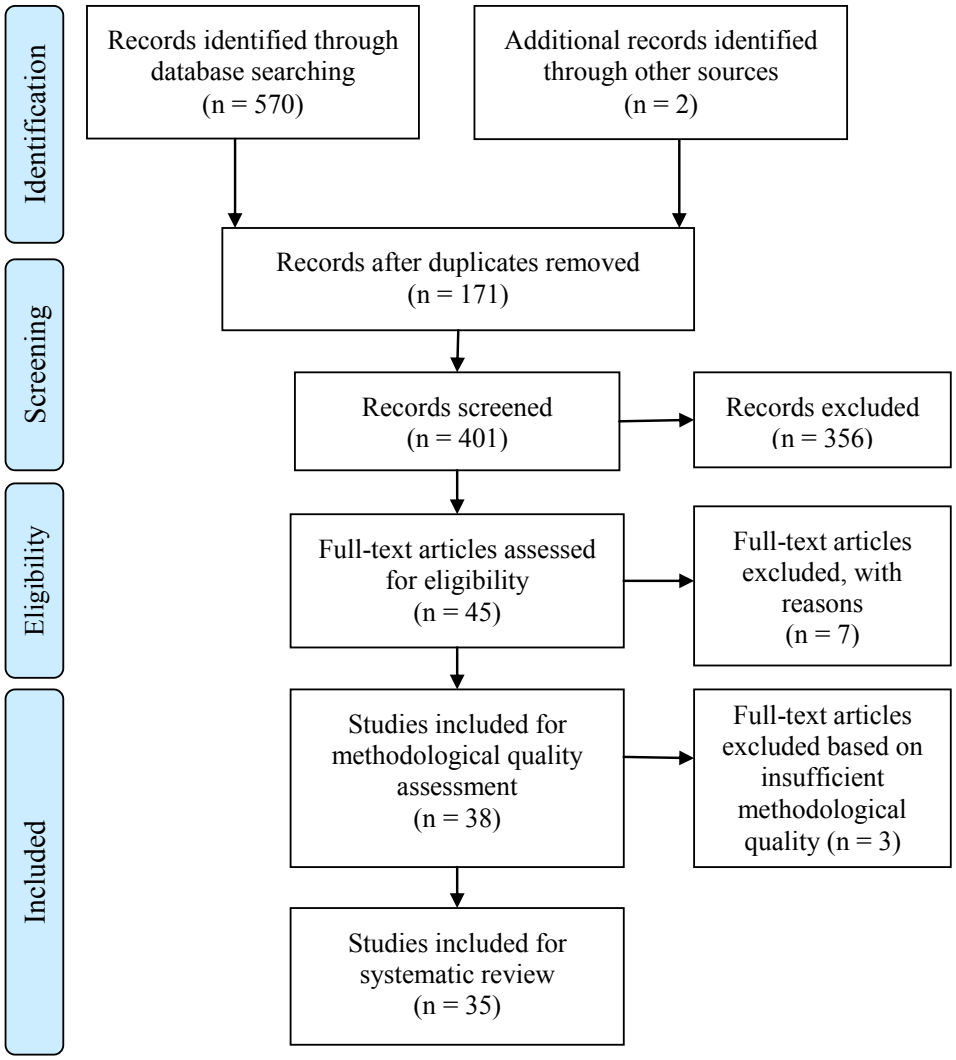

Fig. 1. Flow diagram of study selection.

3.5.5. 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 920 *g* for 3 min and 81% at 1840 *g* for 3 min) (Herold et al., 2011). Lipokit® centrifugation (Medikan Corp., Seoul, Korea) showed histologically small groups of adipocytes, whereas large intact adipocytes were present in the control intervention samples after centrifugation (Duman et al., 2013). On the other hand, Puregraft® (Cytori Therapeutics Inc, San Diego, CA, USA), a closed wash/filter system, gave significantly better adipocyte viability than nonprocessed fat and centrifuged fat (Zhu et al., 2013).

3.5.6. Wash with/or without centrifugation

Washing showed, histologically, more preadipocytes than with centrifugation (Khater et al., 2009). Although washing combined with centrifugation resulted in lower viability compared to sedimentation (Rose et al., 2006), washing without centrifugation (Huss and Kratz, 2002; Smith et al., 2006), or centrifugation only (Rose et al., 2006; Smith et al., 2006), this lower viability trend was not significant in all studies.

3.6. Adipose-derived stromal/stem cells or stromal vascular fraction

Different studies evaluated adipose-derived stromal/stem cells (ASC) and stromal vascular fraction (SVF) count between centrifugation and no treatment/decantation. The results varied and were generally inconsistent as to which technique performed best (Conde-Green et al., 2010b, 2010a; Iyyanki et al., 2015; Palumbo et al., 2015; Kurita et al., 2008; Ferraro et al., 2011) (Table 5). Two studies found significantly higher ASC counts in the pellet of the centrifuged lipoaspirate than relating to the middle layer of the centrifuged lipoaspirate (Conde-Green et al., 2010b, 2010a). Two studies (Pfaff et al., 2014; Fisher et al., 2013) reported significantly better results for the gauze/towel technique compared with centrifugation based on ASC number (Fisher et al., 2013) or SVF (Pfaff et al., 2014). 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 (Salinas et al., 2014).

3.7. Growth factors

One study did not find a significant difference in the relative density unit of a broad variety of growth factors in lipoaspirates

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

Study information					Donor characteristics					Infiltration					Aspiration				Processing
First author	Year	MINORS	Design	Outcomes	Total N (n females)	Average age (SD)	Age range	Primary liposuction aim	Donor site	Infiltration	Fluid	Lidocaine	Epinephrine	NaHCO3	Cannula (in mm)	Cannula brand	Syringe (cc)	Pressure	Processing category
Animal processed fat in vitro																			
Gonzalez	2007	8	ws	V	5 rats	.	.	AFT	f	+	R	.	.	.	2;3	.	10/20/60	x neg	d, g
Piasecki	2007	7	ws	V	x mice	.	.	LS	t	.	.	.	.	.	1.2	.	5	5 cc neg	c (8x), d, g
Human processed fat in vitro																			
Boschert	2002	8	ws	V	20 (16)	.	27–49	LS	a,f,h,k,t	.	.	.	.	.	2.0/3.0/5.0	Mercedes	sp	sp	c (4x)
Huss	2002	8	ws	V	8 (.)	.	.	LS	a,b	.	.	.	.	.	5.0/6.0	Toomey	50	.	w, wc
Rohrich	2004	8	ws	V	5 (.)	.	.	.	a,f,k,t	+	.	.	.	.	.	Coleman	10	.	c, n
Rose	2006	9	bs	V	22 (.)	.	.	AFT	a	+	NaCl	50 ml 1%	1 ml 1:000	+	.	Coleman	10	Manual	c, d
Kim	2009	8	ws	V	8 (.)	32 (6)	.	LS	a	+	HS	20 ml 2%	0.5 ml 0.1%	–	1.2	Coleman	.	.	c (8x), n
Conde-Green, b	2010	10	ws	V	20 (20)	.	28–64	LS	a	+	NaCl		1:500 000	–	3.0	Richter	10	Manual	c, d, w
Conde-Green, a	2010	9	ws	V	10 (10)	.	35–58	LS	a	+	NaCl		1: 500 000	–	3.0		10	.	c, d
Herold	2011	8	ws	V	9(5)	40 (.)	14–74	LS	a,b,h	+	NaCl		1 ml 1:1000	+	3.0	Coleman	→ TT → 10	→−0.38 atm →<2 cc neg	c, dv, n c, dv, n
Pulsfort	2011	8	ws	V	13 (11)	47 (11)	.	AFT/LS	.	+	NaCl	12.5 ml 1%[b]	1:200 000	–	2.0	Coleman	10	.	c (7x), n
Duman	2013	7	ws	V	.	.	.	LS	a	+	NaCl		1:500 000	–	.	Lipokit	50	sp	d, dv
Zhu	2013	10	ws	V	22 (22)	45 (12)	24–64	.	a,f,h	.	.	.	.	.	.	.	.	.	c, d, dv, n
Kamel	2014	8	ws	V	20 (20)	31 (1)	20–41	LS	a,t	+	R	30 ml 1%	1 mg	–	3.0		60	→ manual →2–3 atm	c, g c, g
Pfaff	2014	8	ws	V	5 (3)	38 (24)	12–68	.	a	+		10 ml 1%	1:100 000	–	.	.	10	Manual	c, g
Iyyanki	2015	8	ws	V	19(19)	51(10)	41–61	AFT breast	A, b, f	.	.	.	.	.	3.0	Coleman	10	Manual	c, n
Osinga	2015	8	ws	V	6(3)	.	.	LS	a	+	NaCl	0.91 mg/ml	1.8 μg/ml	+	4.0	Lenoir	10	Manual	dv, n
Palumbo	2015	9	ws	V	5(5)	47	35–58	LS	t	+	NaCl	0.05%	1:100 000	+	2.0	.	sp	x neg	c (3x), d (2x)
Rubino	2015	8	ws	V	10(10)	.	.	AFT breast	f	+	R	20 ml 2%[c]	0.5 ml 1:200 000		→2.0 →3.0	Coleman Mercedes	10 60	Manual Manual	c, d c, d
Human processed fat- to-animal transplantation																			
Ramon	2005	11	bs	A	1 (1)	32	32	LS	b	+	R	20 ml 2%	1 ml	–	2.0		10	.	c, g
Smith	2006	10	bs	A,V	3 (3)	.	.	LS	a	+	R	30 ml 1%	1 mg in 1 ml	–	.	Coleman	→ 10 → sp	→manual →sp	c, wc, w (2x), n c, wc, w (2x), n
Kurita	2008	10	ws,bs	A,V	8 (8)	.	21–38	.	a, t	+	.	.	.	.	.	Lipokit	50	sp	dv (5x), n
minn	2010	7	bs	A,V	.	.	.	AFT Breast	a	+	R	50 ml 1%	1 ml 1:1000	+	2.0	.	10	.	c, g, s
Fisher	2013	9	ws	A,V	1 (1)	57	57	LS	t	.	.	.	.	.	.	→ Shippert →Coleman	→ TT → 10	→−0.57 atm →.	c, dv, g c, dv, g
Hoareau	2013	10	ws	A,V	9 (9)	43 (9)	.	LS	.	+	R	40 ml 2%	1 mg/L	–	2.0	Inex	10	<2 cc neg	c (6x), d
Ansorge	2014	12	ws	A,V	10 (9)	41(9)	30–35	LS	a	+	R	50 mg 1%	1 ml 1:1000	–	3.3	VentX	sp	0.5 atm neg	c, d, dv
Salinas	2014	7	.	A,V	9 (9)	48 (12)	29–63	LS	a,f,t	.	.	.	.	.	4.0	Mentor	.	1 atm neg	c, g

(*continued on next page*)

Table 3 (*continued*)

Study information					Donor characteristics			Infiltration					Aspiration			Processing
First author	Year	MINORS	Design	Outcomes	Total N (n females)	Average age (SD)	Age range	Primary liposuction aim	Donor site	Infiltration	Fluid	Lidocaine	Epinephrine	NaHCO3	Cannula (in mm)	Cannula brand	Syringe (cc)	Pressure	Processing category
Human processed fat-to-human transplantation																			
Butterwick	2002	8	ws	H	14 (14)	54 (.)	41–64	AFT Hands	h,k,t	+	NaCl	50 ml 1%	1 ml 1:000	+	2.6	Klein	10	2 cc neg	c, n
Khater	2008	7	bs	H,V	30 (26)	.	15–47	AFT Face	t	.	.	.	.	.	2.6	.	10	.	c, w
Khater	2009	10	bs	H,V	51 (51)	33 (2)	16–55	AFT Face	t	.	.	.	.	.	2.6	.	10	<2 cc neg	c, w
Ferraro	2011	7	bs	H,V	30 (.)	.	30–50	AFT Buttock	h,k,t	.	.	.	.	.	3.0	.	20	x neg	c (3x), d
Botti	2011	10	ws	H	25 (21)	46(.)	21–72	AFT Face	a,k,t	+	NaCl	0.25%[d]	1:500 000	+	2.0	.	10	<2 cc neg	c, s
Asilian	2014	11	bs	H	32 (.)	.	35–50	AFT Face	.	+	R	0.05%	1:1000 000	–	2.0		10	<2 cc neg	c, s
Mestak	2014	9	bs	H	30 (30)	38 (.)	28–62	AFT Breast	a,f,t	+	NaCl		1 ml	–	3.0	Mercedes	60	.	c, dv
Gerth	2014	9	bs	H	26(26)[a]	55 (11)	34–70	AFT Face	a,t	+	.	0.5% or 0.25%	1: 200 000 or 1:400.000	–	3.0	.	.	15 cc neg	c, dv

. 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, sodium chloride; 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.

[a] Only experimental group. 33 subjects in historical comparison average age of 54 (39–70).
[b] Prilocaïne.
[c] Lignocaïne.
[d] Mepivacaïne.

when comparing centrifugation to a closed wash/filter device (Zhu et al., 2013). In another study (Hoareau et al., 2013), at 24 h after injection in mice, significantly higher concentrations of IL-6 and MCP-1 were found after centrifugation at 900 *g* for 3 min compared to centrifugation at 400 *g* for 1 min and decantation. No significant differences were found 1 week after injection into mice.

3.8. Animal models: Graft volume and histology

All animal studies used xenografts (human fat transplanted into athymic animals) (Table 6). Three of 7 studies reported a significant difference in volume or graft weight related to the different processing methods; these 3 studies also had shorter follow-up times. Lipokit® centrifugation (Kurita et al., 2008) demonstrated significantly higher graft weight than no centrifugation. 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 (Ansorge et al., 2014). 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 (Fisher et al., 2013).

Histologically, only a few differences were found in animal recipient sites of fat grafts. One study (Ramon et al., 2005) found less fibrosis using gauze/towel versus centrifugation. Another study (Minn et al., 2010) found no differences using the gauze/towel technique related to centrifugation, but found less inflammation in the gauze/towel compared with the metal sieve.

3.9. Human models: Graft volume and patient satisfaction

Eight studies covered autologous fat transfer in humans (Table 7). Five studies reported on facial augmentations, whereas 3 studies concentrated on hands, buttocks, or breast augmentation. None of the studies used the gauze/towel technique. Only 1 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 (Gerth et al., 2014).

Patient satisfaction was comparable with the outcome of objective observers. Two studies (Butterwick, 2002; Ferraro et al., 2011) reported that centrifugation resulted in higher satisfaction than no centrifugation in the hands and buttocks. Washing was shown to be superior to centrifugation in regard to patient satisfaction after facial augmentation (Khater et al., 2008, 2009). In two studies (Asilian et al., 2014; Botti et al., 2011) no significant difference was found in patient satisfaction among centrifugation, the use of the metal sieve technique, and the closed wash/filter device.

4. 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 in several and diverse respects. 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,

Table 4
Details processing techniques per study.

First author	Year	Centrifugation force and time reported in study	(Calculated) relative centrifugal force (g)	Other techniques
Animal processed fat in vitro				
Gonzalez	2007	-	-	Decantation; cotton towel (both 50 g 5 min centrifugation)
Piasecki	2007	500,1000,1500,2000 rpm 3 min; 1000 rpm 1,2,3,5,10 min	57 g, 228 g, 514 g, 913 g	Decantation 15 min; mesh gauze rinsed with 5 cc ringer
Human processed fat in vitro				
Boschert	2002	50 g 2,4,6,8 min	50ig	-
Huss	2002	wash + 200 g 5 min	200 g	2–4 times saline wash
Rohrich	2004	500 g 2 min	500 g	No treatment
Rose	2006	3000 rpm 3 min	6000 g	Decantation; saline wash
Kim	2009	1500,3000,5000 rpm 1,3,5 min	553 g, 2214 g, 6149 g[a]	No treatment
Conde-Green, b	2010	3000 rpm 3 min	1150 g[a]	Decantation; saline wash
Conde-Green, a	2010	3000 rpm 3 min	1150 g[a]	Decantation 30 min
Herold	2011	920 g 3 min, 1840 g 3 min	920 g, 1840 g	No treatment; tissue trans filtration
Pulsfort	2011	1000,1500,3000, 5000,7500,10.000, 15.000 rpm (no duration reported)	92 g, 206 g, 825 g, 2292 g, 5157 g, 9168 g, 20.627 g	No treatment
Duman	2013	Lipokit® centrifugation 4000 rpm 8 min	.	No treatment
Zhu	2013	3000 rpm 3 min	1200 g	No treatment; decantation 20 min; Puregraft® 250; Puregraft® 850
Kamel	2014	1000 rpm 3 min	.	Mesh gauze without wash
Pfaff	2014	1500 rpm 3 min	.	Telfa rolling
Iyyanki	2015	3200 rpm 2–3 min	.	No treatment
Osinga	2015	-	-	No treatment; Shuffling though 3-way stoplock
Palumbo	2015	90 g, 400 g, 1500 g 3 min	90 g, 400 g, 1500 g	Decantation 10,20,30 min
Rubino	2015	3000 rpm 3 min	.	No treatment; decantation 30 min
Human processed fat -to-animal transplantation				
Ramon	2005	1500 rpm 2 × 5 min	.	Cotton gauze 10 min
Smith	2006	500 g 2 min; ringer wash +500 g 2 min; saline wash +500 g 2 min	500 g	No treatment; ringer wash; saline wash
Kurita	2008	Lipokit® centrifugation 400,700,1200, 3000,4200 g 3 min	400 g, 700 g, 1200 g, 3000 g, 4200 g	No treatment
Minn	2010	1800 g 3 min	1800 g	Cotton gauze, metal sieve
Fisher	2013	3000 rpm 3 min	1200 g	Cotton gauze; tissue trans filtration®
Hoareau	2013	100 g 1 s,1 min; 400,900 g 1 min; 900 g 3 min; 1800 g 10 min	100 g, 400 g, 900 g, 1800 g	Decantation 2 min
Ansorge	2014	1200 g 3 min	1200 g	Decantation 10 min; Revolve system™
Salinas	2014	1200 g 3 min	1200 g	Mesh gauze
Human processed fat-to-human transplantation				
Butterwick	2002	3600 rpm 3 min	.	No treatment
Khater	2008	3000 rpm 3 min	.	Saline wash
Khater	2009	3400 rpm 3 min	.	Saline wash
Ferraro	2011	3000 rpm 3 min (1300 rpm 5 min and 500 rpm only in vitro analysis)	1500 g (250 g and 50 g only in vitro analysis)	Decantation
Botti	2011	3000 rpm 3 min	.	Metal sieve + saline
Asilian	2014	3400 rpm 3 min	.	Metal sieve + saline
Mestak	2014	3000 rpm 3 min	1150 g[a]	Puregraft® 250
Gerth	2014	Unknown	.	Puregraft® 250, Puregraft®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 = $1.12 \times 10^{-5} \times r \times rpm^2$. RCF = relative centrifugal force; r = radius of the centrifuge in centimeters reported in the article; rpm = revolutions per minute reported in the article.

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.

Table 6
Summary of records with animal outcome variables.

First author	Year	Donors: Number (females)	Diameter injection cannula (imm)	End of cannula	Volume (per side)	No. of animals	Location	Technique category	Time (wk)	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)	.	.	300 mg	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	.	1 ml	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	.	10–1000 mg	.	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.

and histology. Unfortunately, the gauze/towel technique was not used in all 8 clinical studies. As the survival mechanism of fat grafts in humans is not yet fully understood, 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 (Peer, 1955). 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 result in lower retention of fat grafts. Accordingly, low graft survival can be linked to centrifugation, 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 are lacking to confirm this survival theory.

Recently, new theories have been proposed stating that the interaction among 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 that new adipocytes grow from stem or progenitor cell proliferation, the so-called compensatory proliferation (Eto et al., 2012; Sunaga et al., 2013). Some recent articles presume that poor microvascular circulation conditions trigger ASCs to induce angiogenic growth factors such as VEGF (Nishimura et al., 2000; Garza et al., 2015). In this respect, the facilitation of the revascularization of the graft by angiogenic growth factors, and not 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 postcentrifugation.

Although the opinion about the survival theory has changed, the most recent studies in this review focus on endpoints other than viability, such as ASC and growth factors in vitro. However, it has still not been proved that these laboratory outcome variables result in better fat survival in humans. Of the 35 included studies, only 1 study measured volume retention in humans in relation to processing techniques (Gerth et al., 2014). 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 animal controls (Matsumoto et al., 2006; Zhu et al., 2010; Lu et al., 2009) and human (Kolle et al., 2013). 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 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 (Kolle et al., 2013). In contrast to cell-assisted lipotransfer with high ASCs numbers, another study (Philips et al., 2013) reported that human grafts with a physiologically higher proportion of ASCs resulted in greater survival in athymic mice. In that study,

Table 7
Summary of records with human outcome variables.

First author	Year	Donors (female)	Age range	Donor site	Injection diameter (mm)	End cannula	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	Photographs	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)[a]	34–70	.	.		Face	c, dv	3D scan	pre/12	26[a]	.	.	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.

[a] Only experimental group, 33 subjects in comparison group.

small differences in ASCs led to significant differences in volumetric outcome.

Both of the studies (Kolle et al., 2013; Philips et al., 2013) used the Coleman method (centrifugation) as a processing technique. The middle layer of the centrifuged lipoaspirate was suboptimal for adipocyte viability and ASC numbers with regard to the articles included in this review. Further research is necessary to determine whether other processing techniques besides 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 methods do not allow a straightforward answer as to which processing technique is the best. Eight categories and 7 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 11 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 affect outcomes (Gir et al., 2012). Poor descriptions of methods and materials in the included studies made grouping impossible.

5. Conclusion

Centrifugation was the most commonly analyzed processing technique in this systematic review. Processing techniques using permeability principles were superior to the centrifugation technique in both 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.

Authorship participation and contributions

A. Jorien Tuin, MD: First reviewer, design of study, data analysis, data interpretation, manuscript preparation.

Patrick N. Domerchie, MD: Second reviewer, design of study, data analysis, data interpretation, manuscript preparation.

Rutger H. Schepers, MD, DMD: Data analysis, design of study, data interpretation, manuscript preparation.

Joep C.N. Willemsen, MD: Data analysis, design of study, data interpretation, manuscript preparation.

Pieter U. Dijkstra, PT, PhD: Data analysis, design of study, data interpretation, manuscript preparation.

Fred K.L. Spijkervet, DMD, PhD: Design of study, data interpretation, manuscript preparation.

Arjan Vissink, MD, DMD, PhD: Design of study, data interpretation, manuscript preparation.

Johan Jansma, MD, DMD, PhD, FEBOMFS: Design of study, data interpretation, manuscript preparation.

Appendix 1. Search terms used in this study.

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 fat graft 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 sedimentation 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. End points	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., 2010b	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., 2010a	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, 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., 2007	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 and Kratz, 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., 2002	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 and Mirrafati, 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	

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 less are not included in the ranking list.

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