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REVIEW

Comparison of intraoperative procedures for isolation of clinical grade stromal vascular fraction for regenerative purposes: a systematic review

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Abstract

Intraoperative application of the 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) that still contain the extracellular matrix. Pubmed, EMBASE and the Cochrane central register of controlled trials databases were searched for studies that compare procedures for intraoperative isolation of SVF (searched 28 September 2016). Outcomes of interest were cell yield, viability of cells, composition of SVF, duration, cost and procedure characteristics. Procedures were subdivided into procedures resulting in a cSVF or tSVF. Thirteen out of 3038 studies, evaluating 18 intraoperative isolation procedures, were considered eligible. In general, cSVF and tSVF intraoperative isolation procedures had similar cell yield, cell viability and SVF composition compared to a nonintraoperative (i.e. culture laboratory-based collagenase protocol) control group within the same studies. The majority of intraoperative isolation procedures are less time consuming than nonintraoperative control groups, however. Intraoperative isolation procedures are less time-consuming than nonintraoperative control groups 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 the preferred procedure to isolate SVF. Copyright © 2017 John Wiley & Sons, Ltd.

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1. 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 anaesthesia. Several clinical trials have been published using ASCs for soft tissue reconstruction (Tanikawa *et al*., 2013), cardiac repair (Perin *et al*., 2014), pulmonary repair (Tzouvelekis *et al*., 2013) and cartilage repair (Jo *et al*., 2014). 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 (Bourin *et al*., 2013), which is a relatively 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 (Bourin *et al*., 2013; Eto *et al*., 2009). ASCs *in vivo* are characterized as CD31min/CD45min/CD34pos/CD90pos/CD105low cells (Yoshimura *et al*., 2006). 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 (Gir *et al*. 2012; Suga *et al*., 2007). In case of cell culturing, only ASCs and their precursor cells (supra-adventitial cells and pericytes) are able to adhere and survive (Zimmerlin *et al*., 2010; Zuk *et al*., 2001). Upon passaging *in vitro*, the phenotype of ASCs starts to deviate from their *in vivo* phenotype (Spiekman *et al*., 2017): in this process CD34 surface expression is lost, while CD105 expression is upregulated to mention a few (Corselli *et al*., 2012; Yoshimura *et al*., 2006). Alternatively, administration of the enzymatically prepared vascular stromal fraction of adipose tissue might have a therapeutic capacity that is

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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 laboratory (good manufacturing practice facility), which is not available in most peripheral hospitals (Gimble *et al*., 2010). 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 (Aronowitz *et al*., 2015b; Oberbauer *et al*., 2015). 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 (ECM). In most of the nonenzymatic intraoperative isolation procedures, the cell–cell communications remain intact, resulting in an end product with more volume (tSVF). Various studies have 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 (nonintraoperative culture laboratory-based collagenase protocols that require culture good manufacturing practice facilities for clinical use, referred to as nonintraoperative isolation protocol). Recently, new intraoperative isolation procedures have been introduced and tested. It is not clear yet if intraoperative isolation procedures generate a similar quality and quantity of SVF as nonintraoperative 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.

2. Materials and methods

2.1. Protocol and registration

This study was performed using the PRISMA protocol (Moher *et al*., 2009). The search strategy for this systematic review was based on a population, intervention, comparison and outcome framework (Schardt *et al*., 2007). The study was not registered.

2.2. Eligibility criteria

Studies were included when at least two different types of intraoperative isolation procedures or one intraoperative isolation procedure with a nonintraoperative 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).

2.3. Information sources and search

Pubmed, EMBASE (OvidSP) and the Cochrane central register of controlled trials databases were searched (searched 28 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, nonenzymatic or mechanical.

2.4. Study selection and data collection process

Two authors (J.A.D., A.J.T.) selected studies independently based on the eligibility criteria. Inconsistencies were discussed during a consensus meeting. In case of disagreement, the senior author (M. C.H.) gave a binding verdict.

2.5. Data items

Search term was partly based on a population, intervention, comparison, outcome 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 vs. regular nonintraoperative isolation protocols. Differences in harvesting procedure were not taken into account.

2.6. Risk of bias in individual studies

It is known that the quality of ASCs depends on age and harvest location of the donor (Di Taranto *et al*., 2015; Dos-Anjos Vilaboa *et al*., 2014; Engels *et al*., 2013; Maredziak *et al*., 2016). The inclusion of young healthy

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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	Noncomparative 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	Mesenchymal cells derived from other source than adipose tissue
Intraoperative procedures	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)

patients may positively affect the results. Therefore, detailed information about demographics are described in this review.

2.7. 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 nonintraoperative 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 that presented results in mean and standard deviation were analysed. 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.

2.8. Synthesis of results

In some studies, derivate numbers from 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. nonintraoperative protocols or other intraoperative procedures) in the same study, only CD marker expression was used. Studies evaluating a single CD marker expression to analyse 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 analyse the expression of stromal cells, pericytes, vascular endothelial cells/endothelial progenitor cells, endothelial cells, lymphocytes, leucocytes and haematopoietic 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 (Bianchi *et al.*, 2013).

2.9. 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 International Federation of Adipose Therapeutics and Science (IFATS)/International Society of Cellular Therapy (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.

2.10. 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

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on the position statement of the IFATS and ISCT (Bourin *et al.*, 2013). 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 (J.A.D., A.J.T.) 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.

3. Results

3.1. Included studies

A total of 3038 studies were identified after database searching. There were 2955 articles excluded after abstract screening. Fifty-nine full text studies were assessed on eligibility criteria. Fourteen studies were excluded based on the use of a nonintraoperative protocol for isolation as experimental method (Al Battah *et al.*, 2011; Carvalho *et al.*, 2013; Chen *et al.*, 2014; Condé-Green and Lamblet 2012; Doi *et al.*, 2014; Escobedo-Lucea *et al.*, 2013; Fink *et al.*, 2011; Jiang *et al.*, 2007; Okura *et al.*, 2012; Pilgaard *et al.*, 2008; Seaman *et al.*, 2015; Siciliano *et al.*, 2013; Vykoukal *et al.*, 2008; Yoshimura *et al.*, 2006). Seven studies described isolation protocols in general but gave no results (Bernacki *et al.*, 2008; Buehrer and Cheatham 2013; Dubois *et al.*, 2008; Hicok and Hedrick, 2011; Yu *et al.*, 2011; Zachar *et al.*, 2011; Zhu *et al.*, 2013). Seven studies were excluded based on the lack of a control group (i.e. nonintraoperative isolation protocols or other intraoperative isolation procedures) (Dos-Anjos Vilaboa *et al.*, 2014; Inoue *et al.*, 2014; Raposio *et al.*, 2016; Sadighi *et al.*, 2014; Van Pham *et al.*, 2014; Zeng *et al.*, 2013; Zuk *et al.*, 2001). Four studies were excluded based on their study design (Aronowitz and Hakakian 2015; Bertheuil and Chaput 2015; Kim 2014; Marincola 2014). Three studies were excluded based on the use of culture methods to isolate ASCs, because culture methods are incompatible with intraoperative applications (Busser *et al.*, 2014; Priya *et al.*, 2014; Wu *et al.*, 2012). Four studies used only centrifugation or RBC lysis buffer as isolation protocol and were thereby excluded (Amirkhani *et al.*, 2016; Baptista *et al.*, 2009; Markarian *et al.*, 2014; Raposio *et al.*, 2014). Three studies used the blood saline fraction of lipoaspirate and were thereby excluded (Cicione *et al.*, 2016; Francis *et al.*, 2010; Shah *et al.*, 2013). Four studies did not describe an outcome of interest (Aronowitz *et al.*, 2015a,b; Fraser *et al.*, 2014; Reshak *et al.*, 2013; Yi *et al.*, 2014). Four additional studies were identified through other sources (Figure 1). Thus, 13 studies with 18 intraoperative isolation procedures remained for analysis.

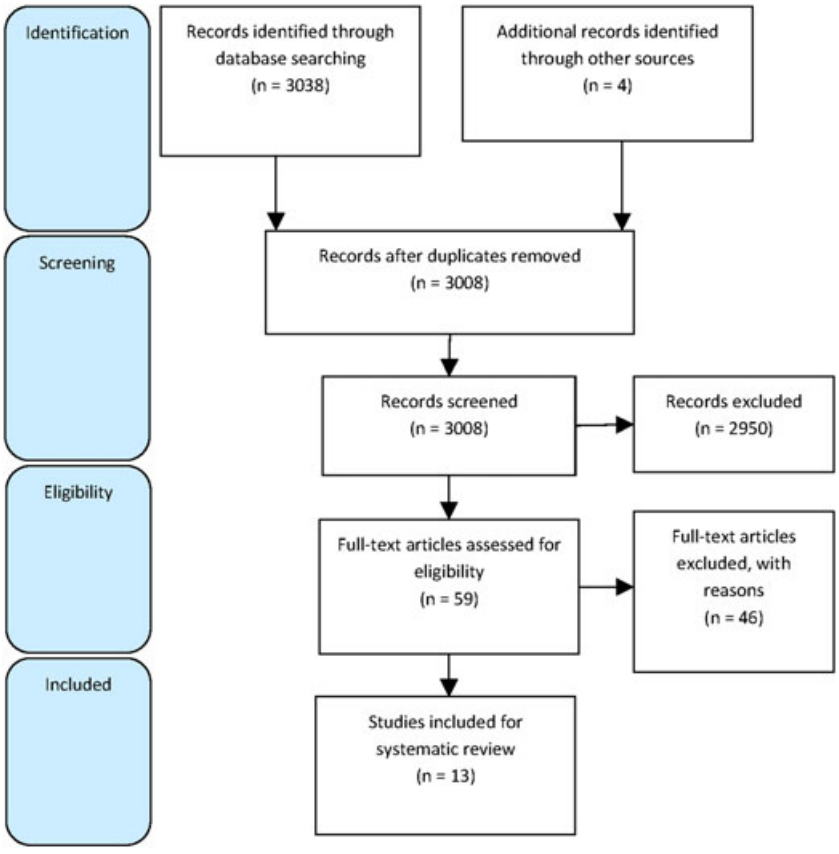

Figure 1. Flow diagram of study selection [Colour figure can be viewed at wileyonlinelibrary.com]

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3.2. Study characteristics

In total, 93 subjects were enrolled in the 13 studies. Nine studies reported sex, of which 95% was female ($n = 58$). Nine studies reported the mean age or age variance of the subjects and 10 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.

3.3. Characteristics of the intraoperative isolation procedures

All intraoperative isolation procedures are divided into two categories: enzymatic and nonenzymatic procedures resulting in cSVF and tSVF respectively (Table 3A, B). Eight of the 18 intraoperative isolation procedures were based on enzymatic digestion and 10 isolation procedures were based on nonenzymatic procedures. Two nonenzymatic 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 filtered 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 (Mashiko *et al*., 2017; Tonnard *et al*., 2013; van Dongen *et al*., 2016).

3.3.1. Start volume vs. end product

The Automated Isolation System (AIS), GID SVF2, Lipokit system and Multi station are enzymatic intraoperative isolation procedures that resulted in large average amounts of SVF (7.2–20 ml), suggesting inefficient enzymatic digestion (Aronowitz *et al*., 2016; SundarRaj *et al*., 2015). The nonenzymatic intraoperative isolation procedures resulted in larger end volumes than only a pellet. Prior to the Lipogems procedure, 130 ml of adipose tissue can be obtained to mechanical dissociate to 100 ml of lipoaspirate. This is a 1.3-fold reduction of the volume, suggesting an inefficient mechanical dissociation to our opinion (Bianchi *et al*., 2013). In contrast, the fractionation of adipose tissue procedure resulted in a 10.4-fold volume reduction (van Dongen *et al*., 2016). For all other intraoperative isolation procedures, no data are mentioned about the end volume of the lipoaspirate (Table 3A, B).

3.3.2. Duration and costs

Duration of the intraoperative isolation procedures varied from 5 s to 133 min ($n = 12$). Isolation with the AIS was the longest intraoperative isolation procedure (SundarRaj *et al*., 2015). Shuffling lipoaspirate 5 or 30 times through a luer-to-luer lock syringe will take 5 or 30 s, respectively, and were therefore the fastest procedures (Osinga *et al*., 2015). In general, the tested nonenzymatic procedures take less time than the enzymatic procedures (Table 3A, B).

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 (Aronowitz and Ellenhorn 2013, Aronowitz *et al*., 2016). No data of nonenzymatic intraoperative procedures were available (Table 3A, B).

3.4. Cell yield

Thirteen studies evaluated the cell yield of eighteen different intraoperative isolation procedures (Aronowitz and Ellenhorn 2013; Aronowitz *et al*., 2016; Bianchi *et al*., 2013; Doi *et al*., 2013; Domenis *et al*., 2015; Güven *et al*., 2012; Lin *et al*., 2008a; Mashiko *et al*., 2017; Millan, 2008; Osinga *et al*., 2015; SundarRaj *et al*., 2015; Tonnard *et al*., 2013; van Dongen *et al*., 2016) (Table 2, supplemental content). The reported cell yield after those different procedures varied from 0.19 to 11.7×10^8 cells/l in enzymatic intraoperative isolation procedures and from 1.8 to 22.6×10^8 cells/l in nonenzymatic intraoperative isolation procedures. Nonenzymatic intraoperative procedures yielded higher number of cells since the cell yield was based on 1 ml 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 (Aronowitz and Ellenhorn 2013; Aronowitz *et al*., 2016; Domenis *et al*., 2015; Lin *et al*., 2008a). Interestingly, obvious different yields were seen using the same procedure in different studies (Aronowitz and Ellenhorn 2013; Aronowitz *et al*., 2016; Domenis *et al*., 2015; Lin *et al*., 2008a). 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 ($p < 0.001$) (Aronowitz and Ellenhorn 2013). In contrast, Domenis *et al*. (2015) did not find a statistical difference between the enzymatic Celution system and Lipokit system. Aronowitz *et al*. (2016) 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 ($p < 0.05$) (Aronowitz *et al*., 2016).

In the nonenzymatic 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 (Mashiko *et al*., 2017; van Dongen *et al*., 2016). Nonenzymatic intraoperative isolation procedures such as shuffling (five times and 30 times), the Nanofat procedure and Fastem did not mention the initial and

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

Name	Author	Enzymatic/ nonenzymatic (E/N)	Automatic/ Manual/ Semi (A/M/S)	Open/closed (O/C)	Isolation details		Time (min)	Disposable (D)/ reusable (R) cost (US$)	Volume processed (ml)	Capacity (ml)	End volume (ml)	Maximum volume processed/ maximum end volume (ml)
AIS	SundarRaj *et al.*, 2015	E	A	C	Tissue digestion, heating and agitation, three-stage filter system (100 μm, 35 μm, 5 μm 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's), 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.*, 2008a						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 × *g* 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.*, 2013	E	A	C	Tissue digestion, centrifugation, washing, 700 × *g* 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/ Nonenzymatic (E/N)	Automatic/ Manual/ Semi (A/M/S)	Open/ closed (O/C)	Isolation details	Time (min)	Disposable (D)/reusable (R) cost (US$)	Volume processed (ml)	Capacity (ml)	End volume (ml)	Maximum volume processed /maximum end volume (ml)
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	8–10	R	10	10	0.96 [0.75 – 1.75]	10.4
FAST	Domenis *et al.*, 2015	N	M	-	Filterbag (120 μm filter), 400 × *g* centrifugation	n/a	–	–	–	10	–
FEF	Mashiko *et al.*, 2017	N	M	O	1200 × *g* centrifugation, shuffling through a connector with three small holes 30 times, 1200 × *g* 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.*, 2017	N	M	O	1200 × *g* centrifugation, shuffling through a connector with three small holes 30 times, 1200 × *g* centrifugation, residual tissue of decanting filtration (500-μm pore size) used	n/a	–	–	–	–	–	2.5 ± 0.2
SF	Mashiko *et al.*, 2017	N	M	O	1200 × *g* centrifugation, squeeze using automated slicer, 1200 × *g* 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 s	–	10	–	–	–
SHUF30	Osinga *et al.*, 2015	N	M	O	Shuffling lipoaspirate through female-to-female luerlok 30 times	n/a	30 s	–	10	–	–	–
STCELL	Millan, 2008	N	M	C	1000 × *g* centrifugation	n/a	–	–	400	500	Pellet	–

FAT, fractionation of adipose tissue procedure; FAST, Fastem Corios (Corios); FEF, filtered fluid of emulsified fat; LIPOG, Lipogems; NANO, nanofat procedure; REF, residual tissue of emulsified fat; SF, squeezed fat; SHUF5, shuffling 5 times; SHUF30, shuffling 30 times.

final volumes, so the relative yield by isolation cannot be calculated (Domenis *et al*., 2015; Osinga *et al*., 2015; Tonnard *et al*., 2013). Osinga *et al*. (2015), reported that most of the adipocytes remain intact after shuffling five or even 30 times. Consequently, the effect of shuffling only cannot be stated as an isolation procedure. It is 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*. (2013).

More interesting than comparing intraoperative isolation procedures evaluated in different studies might be the comparison between an intraoperative isolation procedure and a nonintraoperative isolation protocol (gold standard) starting from the same lipoaspirate. Six studies reported the results of such comparisons (Table 4A) (Millan, 2008; Doi *et al*., 2013; Domenis *et al*., 2015; Güven *et al*., 2012; Lin *et al*., 2008a; SundarRaj *et al*., 2015). The AIS and Tissue Genesis cell isolation system resulted in the same cell yield as the nonintraoperative isolation protocol control (effect size, respectively, 0.07 and 0.00) (Doi *et al*., 2013; SundarRaj *et al*., 2015). Sepax isolated a higher cell yield compared to a nonintraoperative isolation protocol (effect size 1.11; Table 4A) (Güven *et al*., 2012). Lower cell yield was seen after using the Lipokit system compared to the nonintraoperative isolation protocol control (effect size –0.52) (Domenis *et al*., 2015). 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 nonintraoperative isolation protocol (Domenis *et al*., 2015; Lin *et al*., 2008a).

3.5. Viability of nucleated cells

Eight studies described viabilities from 39% to 98% of nucleated cells in the SVF. No major differences in viability were seen between enzymatic and nonenzymatic intraoperative isolation procedures. The filtered fluid of emulsified fat procedure showed the lowest viability (Mashiko *et al*., 2017), while the AIS

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

Study	Enzymatic isolation procedure			Nonintraoperative isolation protocol			Effect size
	n	Cell yield ($\times 10^5$ cells)	SD	*n*	Cell yield ($\times 10^5$ cells)	SD	
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); SD, standard deviation.

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showed the highest viability of nucleated cells of 98% after isolation (Table 2A, B, supplemental content) (SundarRaj *et al*., 2015). Three enzymatic and three nonenzymatic intraoperative isolation procedures were compared to a nonintraoperative isolation protocol regarding the viability of nucleated cells (Table 4B) (Doi *et al*., 2013; Lin *et al*., 2008a; SundarRaj *et al*., 2015). The viability of five intraoperative isolation procedures was comparable to their nonintraoperative isolation protocol controls; the effect sizes were close to zero in many studies (Table 4B). Only the filtered fluid of emulsified fat procedure showed an effect size of –45.4 (Mashiko *et al*., 2017). In general, viability did not differ between nonintraoperative isolation protocols and the individual intraoperative isolation procedures tested.

3.6. Composition of stromal vascular fractions

The SVF compositions is reported in nine studies evaluating six enzymatic procedures and three nonenzymatic 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 nonenzymatic residual of emulsified fat and squeezed fat procedures compared to other intraoperative isolation procedures (Aronowitz and Ellenhorn, 2013; Doi *et al*.; 2013; Güven *et al*., 2012; Mashiko *et al*., 2017) (Table 3, 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 (Aronowitz and Ellenhorn, 2013, Domenis *et al*., 2015) and 32.8% between two other studies, both evaluated by Aronowitz and Ellenhorn (2013) and Aronowitz *et al*. (2016). In general, nonenzymatic 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 multilineage differentiation capacity (Pawitan 2014; Zuk *et al*., 2001).

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

Study	Procedure			Nonintraoperative isolation protocol			Effect size
	n	% viable cells	SD	*n*	% viable cells	SD	
Enzymatic							
AIS, SundarRaj *et al*., 2015	11	97.5	2.8	11	97.3	1.5	0.13
CYT, Lin *et al*., 2008a	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
Nonenzymatic							
FEF, Mashiko *et al*., 2017	10	39.3	9.1	10	93.8	1.2	–45.4
REF, Mashiko *et al*., 2017	10	90.6	2.8	10	93.8	1.2	–2.67
SF, Mashiko *et al*., 2017	10	89.9	4.6	10	93.8	1.2	–3.25
STCELL, Millan, 2008[1]	3	87.7	8.9	3	74.5	20.1	0.66

[1]No exact data described in text, data extracted from figures by authors J.A.D. and A.J.T.
AIS, Automated Isolation System; CYT, Celution System Enzymatic (Cytori); FEF, filtered fluid of emulsified fat; REF, residual tissue of emulsified fat; SF, squeezed fat; STCELL, StromaCell; TGCIS Tissue Genesis Cell Isolation System (Tissue Genesis)

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*. (2008a) resulted in the lowest percentage of pericytes in the SVF (0.8%), but used more than three CD markers to detect pericytes. SundarRaj *et al*. (2015) found 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. The use of multiple CD markers results in a more specific population than the use of fewer CD markers and so a lower percentage of that specific cell type, e.g. pericytes (Bianchi *et al*., 2013). Bianchi *et al*. (2013), used CD34min/CD146pos/CD90pos to detect the pericyte-like population in the SVF and isolated the highest percentage of pericytes using the nonenzymatic Lipogems procedure as compared to other intraoperative isolation procedures. However, Bianchi *et al*. (2013) mostly used other combinations of CD markers in comparison to other studies. This renders their SVF composition incomparable with SVF compositions obtained by other intraoperative isolation procedures.

The enzymatic procedures AIS, Tissue Genesis cell isolation system and Sepax isolated more endothelial progenitor cells in comparison to other intraoperative isolation procedures (Doi *et al*., 2013; Güven *et al*., 2012; SundarRaj *et al*., 2015). 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 nonintraoperative isolation protocol control ($p < 0.05$; Figure 2) (Bianchi *et al*., 2013). 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 ($p = 0.003$) (Aronowitz and Ellenhorn, 2013). All other intraoperative isolation procedures compared with nonintraoperative isolation protocols showed no significant differences.

3.7. 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*. (2012) scored 4.6 and presented the most complete characterization of the SVF and ASCs (Table 5). Tonnard *et al*. (2013) scored 2 points, but had only used CD34 as a marker to identify a subpopulation in the SVF. Two studies used other methods than flow cytometry to determine the composition of SVF (Osinga

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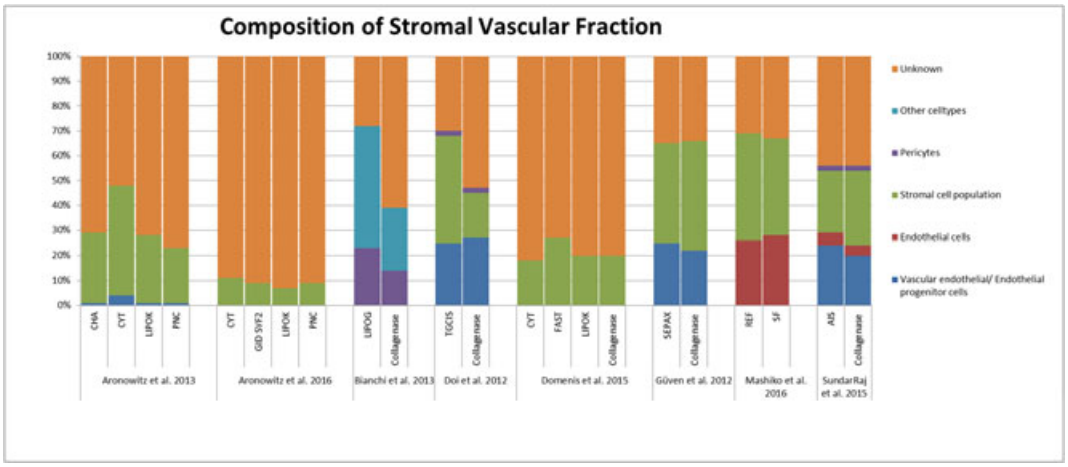

Figure 2. SVF composition (CD marker) of procedures comparing an intraoperative isolation procedure with a nonintraoperative isolation protocol or with other intraoperative isolation procedures within one study. Stromal cell population (CD31min/CD34pos) consists of supra-adventitial cells, ASCs and pericytes, 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 were described in the text of cited papers; data are extracted from figures by authors J.A.D. and A.J.T. 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) [Colour figure can be viewed at wileyonlinelibrary.com]

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

Studies	Viability	Flow cytometry of SVF	Flow cytometry of cultured ASCs									CFU-F	Functional assays			Total Score
			CD13	CD29	CD31	CD44	CD45	CD73	CD90	CD105	CD235a		Adipogenic	Osteogenic	Chondrogenic	
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.*, 2013	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.*, 2008a	1	1										1	1/3	1/3		3.67
Mashiko *et al.*, 2017	1	1										0				2.00
Millan, 2008	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

et al., 2015; van Dongen *et al.*, 2016). 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 13 studies scored less than half of the possible points given. This high number of low scores given to studies underlines the need for standardization.

3.8. Disclosure agreements of included articles

A disclosure agreement of support by the manufacturer was provided in five of the 13 studies (Lin *et al.*, 2008a, b; Güven *et al.*, 2012; Aronowitz and Ellenhorn, 2013; Bianchi *et al.*, 2013; Doi *et al.*, 2013) (Table 4, supplemental content). The company that was mostly involved in the studies, was Cytori the manufacturer of the enzymatic Celution system.

4. Discussion

Grafting of lipoaspirates and of SVF in particular, is a rapidly evolving treatment modality for scars and other skin defects, arthritis, neuropathy, and diabetic ulcers to mention a few. Many of these, initially small scale, single-centre studies, are on the verge of expansion to multicentre 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 analysed using the same lipoaspirate. However, three intraoperative isolation procedures (shuffling five 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 (Bianchi *et al.*, 2013; Osinga *et al.*, 2015). 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

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SVF differ among donors, depending on age, harvest location and comorbidities, such as obesity, of the donors (Di Taranto *et al*., 2015; Dos-Anjos Vilaboa *et al*., 2014; Engels *et al*., 2013; Maredziak *et al*., 2016; Pachon-Pena *et al*., 2016). 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 nonenzymatic and enzymatic isolated SVFs on a different level. Nonenzymatic 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 for 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. Nonenzymatic 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–cell and cell–ECM adhesions (Corselli *et al*., 2012; Lin *et al*., 2008b). Therefore, the tissue structure of lipoaspirate is still intact in the tSVF. Enzymatic procedures, however, probably result in a single cell cSVF, because enzymes are likely to disrupt all cell–cell interactions and ECM (Figure 3) (Aronowitz *et al*., 2015a,b). This is may not happen in the AIS, GID SVF2, Lipokit system and Multi station, possibly due to insufficient enzymatic digestion (Aronowitz *et al*., 2016; SundarRaj *et al*., 2015).

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 h after application (Parvizi and Harmsen 2015). The ECM, containing a microvasculature structure, might function as a natural scaffold for cells such as ASCs and probably 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 implies that nonenzymatic 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 haematopoietic cell marker: adipose derived (CD45min) and blood derived (CD45pos) (Yoshimura *et al*., 2006). Adipose-derived cell populations can be divided into endothelial cells (CD31pos) and stromal cells (CD31min) (Yoshimura *et al*., 2006). 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 (Corselli *et al*., 2012, 2013; Yoshimura *et al*., 2006; Zimmerlin *et al*., 2010). Supra-adventitial cells and pericytes are both identified as precursor cells of ASCs, although there remains some controversy about this item (Corselli *et al*., 2012; Lin *et al*., 2008b; Traktuev *et al*., 2008; Zimmerlin *et al*., 2010). 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*. (2008a) used all the aforementioned combinations. Because Lin *et al*. (2008a) focus mainly on blood derived cells and not on the stromal cell population or pericytes, this did not affect their results. Doi *et al*. (2013) ascribed CD31min/CD34min/CD45min to the pericyte population, so therefore the CD34pos pericytes will be missed. SundarRaj *et al*. (2015) and Güven *et al*. (2012) used CD34pos/CD31min to determine the number of ASCs, while pericytes and supra-adventitial cells also express CD34. Therefore, the number of ASCs contains pericytes and supra-adventitial cells as well (Yoshimura *et al*., 2006; Zimmerlin *et al*., 2010). To cover pericytes, supra-adventitial cells and ASCs, some authors used CD34pos/CD31min/CD45min to determine the stromal cell population (Aronowitz and Ellenhorn, 2013; Aronowitz *et al*., 2016; Domenis *et al*., 2015; Mashiko *et al*., 2017). CD34pos is frequently used as a marker to describe cells with stem cell characteristics in both hematopoietic and nonhematopoietic stem cells (Suga *et al*., 2009). 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 and the American Society of Plastic Surgeons published a position statement in 2012 on fat grafting and stem cells (Eaves *et al*., 2012). 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 (Bourin *et al*., 2013).

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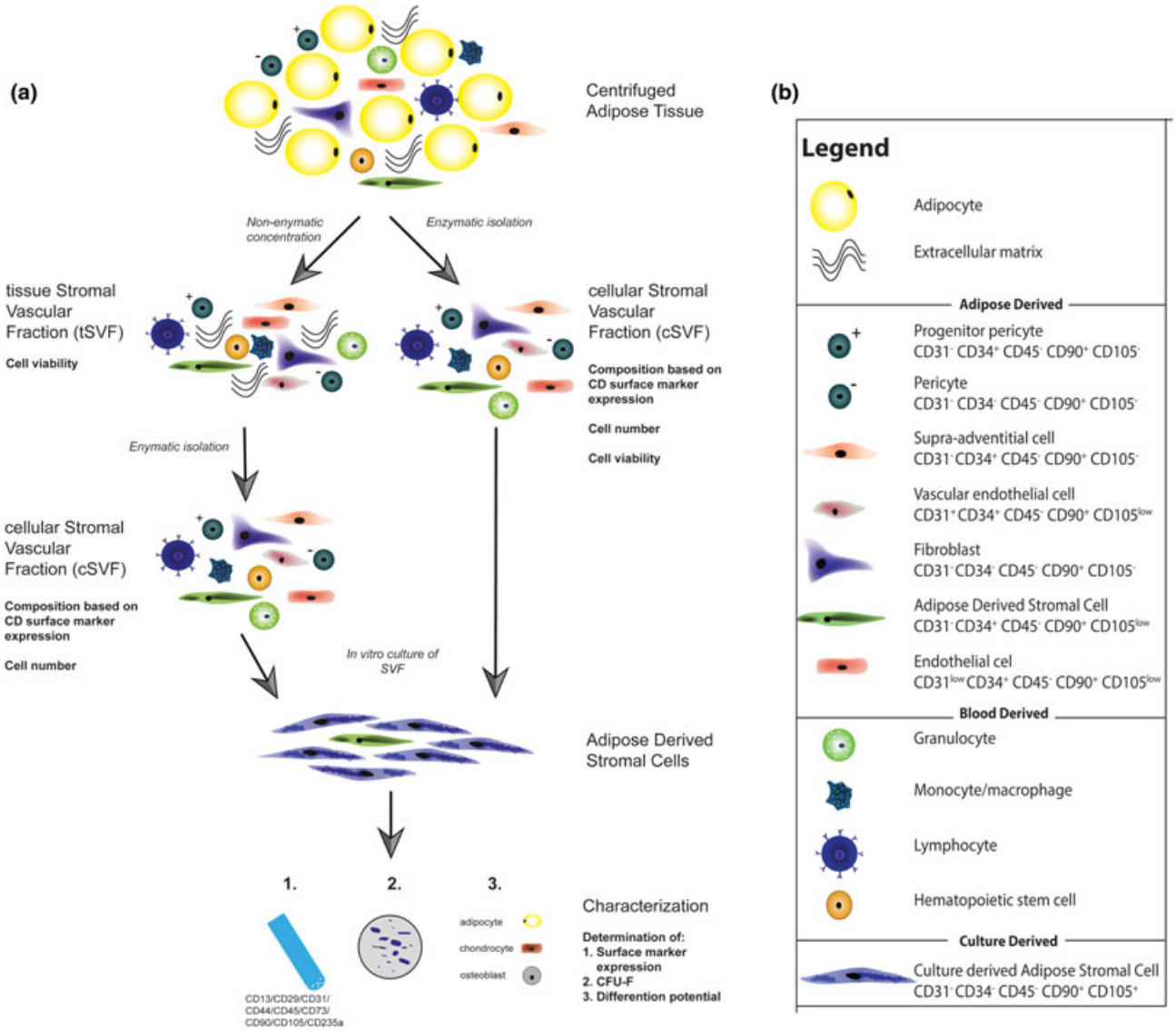

Figure 3. (A) Schematic overview of enzymatic vs. nonenzymatic intraoperative isolation and characterization of the obtained cSVF or tSVF. (B) Legend for Figure 3A [Colour figure can be viewed at wileyonlinelibrary.com]

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 nonintraoperative isolation protocol) and lipoaspirate was processed differently prior to isolation (e.g. centrifugation or decantation). For those reasons, new adjusted IFATS and ICTS guidelines are proposed 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 (Tuin *et al*., 2016; Xie *et al*., 2010). 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 nonintraoperative 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 they require cultured ASCs. In order to verify isolation procedures quickly intraoperatively during clinical trials, the end product of nonenzymatic 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.

Many SVF isolation procedures without applying a full verification according to the IFATS and ICTS guidelines are available (Oberbauer *et al*., 2015). Oberbauer *et al*. (2015) presented a narrative overview of enzymatic and nonenzymatic intraoperative SVF isolation procedures. In 21 out of 30 (both enzymatic as well as nonenzymatic) intraoperative isolation procedures reported in their study, there was a lack of verification data. In two studies, intraoperative isolation procedures without scientific

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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 (Bright *et al*., 2014). Another study used SVF in combination with platelet rich plasma for meniscus repair (Pak *et al*., 2014). Hence, it cannot be guaranteed that the isolation procedures indeed isolate SVF, which is clinically 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 (Freese *et al*., 2015). Zimmerlin *et al*. (2011) showed *in vitro* that ASCs influence growth of active malign cell lines, but this is not seen in latent cancer cell lines. Clinical data suggest that the use of isolated SVF or ASCs is safe in patients without an oncological history (Charvet *et al*., 2015). *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 (Charvet *et al*., 2015). 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 has become clear that the reproducibility of the procedures as well as characterization of the SVF has 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 warranted.

5. 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 nonenzymatic procedures had comparable results as it comes to cell yield, viability, and SVF composition. Nonenzymatic 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 nonintraoperative isolation protocol. Since intraoperative isolation procedures are less time-consuming, but as efficient as the nonintraoperative 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 this review, this level is not yet reached by many procedures.

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Conflict of interest

The authors have no conflict of interest to disclose in relation to the content of this work.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article.

Table S1. Study characteristics

Table S2A: Cell yield and viability/ml start volume of lipoaspirate of all intraoperative isolation procedures per study

Table S2B: Cell yield/ml of end volume, viability and concentration of concentration procedures.

Table S3. Stromal vascular fraction composition (CD marker) of intraoperative isolation procedures in all studies

Table S4. Disclosures of included studies

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