Does Secondary Mechanical Manipulation of Lipoaspirate Enhance the Vasculogenic Potential of Fat Grafts? A Systematic Review

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Background: Fat grafting is a highly versatile option in the reconstructive armamentarium but with unpredictable retention rates and outcomes. The primary outcome of this systematic review was to assess whether secondary mechanically processed lipoaspirate favorably enhances the vasculogenic potential of fat grafts when compared to unprocessed lipoaspirate or fat grafts prepared using centrifugation alone. The secondary outcome was to assess the evidence around graft retention and improved outcomes when comparing the aforementioned groups.

Methods: A search on MEDLINE, EMBASE, and the Cochrane Central Register of Controlled Trials was conducted up to February 2022. All human and animal research, which provided a cross-comparison between unprocessed, centrifuged, secondary mechanically fragmented (SMF) or secondary mechanically disrupted (SMD) fat grafts, was included.

Results: Thirty-one full texts were included. Vasculogenic potential was assessed by quantification of angiogenic growth factors and cellular composition. Cellular composition of mesenchymal stem cells, perivascular stem cells, and endothelial progenitor cells was quantified by fluorescence activated cell sorting (FACS) analysis. Fat graft volume retention rates and fat grafting to aid wound healing were assessed. Although the presence of industry-funded studies and inadequate reporting of methodological data in some studies were sources of bias, data showed SMF grafts contain an enriched pericyte population with increased vascular endothelial growth factor (VEGF) secretion. Animal studies indicate that SMD grafts may increase rates of fat graft retention and wound closure compared to centrifuged grafts; however, clinical studies are yet to show similar results.

Conclusions: In this systematic review, we were able to conclude that the existing literature suggests mechanically processing fat, whether it be through fragmentation or disruption, improves vasculogenic potential by enhancing angiogenic growth factor and relevant vascular progenitor cell levels. Whilst in vivo animal studies are scarce, the review findings suggest that secondary mechanically processed fat enhances fat graft retention and can aid with wound healing. Further clinical studies are required to assess potential differences in human studies.

Key Words: fat grafting, mechanical processing, centrifuged grafts, vascularity, systematic review

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Subcutaneous adipose tissue is a highly versatile soft tissue filler widely used by plastic surgeons due to its autologous nature, accessibility, and low donor site morbidity. The Coleman technique, or modifications of it, is widely used for adipose tissue harvesting and grafting.[1] In spite of the development of numerous techniques, fat grafting outcomes remain highly variable with no single method clearly superior.[2–4] Improving and standardizing fat graft retention would vastly improve outcomes and patient satisfaction. In avascular fat grafting, revascularization is essential for graft retention. As such, a review of the literature assessing the vasculogenic potential of harvest techniques will inform clinicians in their selection of grafting system from the multitude of commercially available systems and may suggest which processing methods would result in the best retention. Vasculogenic potential can be assessed by quantifying vasculogenic growth factors and cellular composition whilst reviewing the in vivo outcomes that are clinically relevant, such as volume retention, symptom relief, and wound healing rate.

The identification of adipose-derived mesenchymal stem cells (AD-MSCs) in adipose tissue by Zuk et al represented a paradigm shift in the field of adipose tissue research.[5] Later confirmation of cell-mediated anti-inflammatory[6] and angiogenic effects[7] of mesenchymal stem cells (MSCs) have since opened the possibility of utilizing AD-MSCs in a wide variety of regenerative fields.[8,9] The identification of MSC markers and comparable gene profiles on pericyte and adventitial stromal cells further suggest an in vivo reservoir of progenitors.[10,11] AD-MSCs have been shown to secrete pro-angiogenic factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), leading to improved formation of blood vessels in vivo, whilst having a higher tolerance to hypoxia compared to grafted adipocytes, whose viability reduces markedly from the periphery of grafts inwards.[12–15]

In 2013, Bourin et al characterized AD-MSCs within the stromal vascular fraction (SVF) of adipose tissue.[16] Cellular-SVF (c-SVF) is produced using collagenase digestion and differential centrifugation to yield a purely cellular derivative without the extracellular and perivascular adipose matrix. However, the enzymatic isolation of c-SVF does not meet the Food and Drug Administration (FDA) definition of "minimal manipulation" and is therefore subject to more stringent regulation,[17] limiting its use in clinical practice. Several nonenzymatic methods have been developed to isolate SVF cells in a way that complies with the FDA definition of "minimal manipulation." These methods use a variety of mechanical forces to produce tissue-SVF (tSVF) or "mechanically processed" lipoaspirate: a heterogeneous mixture of cellular debris, blood cells, and adipose extracellular matrix (ECM).[18]

Types of nonmechanically processed fat used clinically include crude lipoaspirate and centrifuged lipoaspirate. We suggest that mechanical processing strategies can be classified into "secondary mechanical disruption" (SMD) and "secondary mechanical fragmentation" (SMF), based on the impact they have on the adipose tissue matrix. SMD strategies, such as intersyringe shifting,[19] microfat harvesting,[20–23] or filtration using the Puregraft (Bimini Technologies, LLC, USA),[24] Fastem (CORIOS Soc.Coop., Italy),[25] or LipiVage (Genesis Biosystems Inc, Lewisville, TX)[26] devices, subject unprocessed or condensed lipoaspirate to mechanical forces, which do not fragment the adipose tissue matrix into its constituent parts. Instead, they utilize mechanical or gravitational forces to transfer the adipose matrix through multiple fixed apertures. The aperture size and the force applied to the lipoaspirate determine the extent of adipose matrix emulsification. In contrast, SMF techniques,

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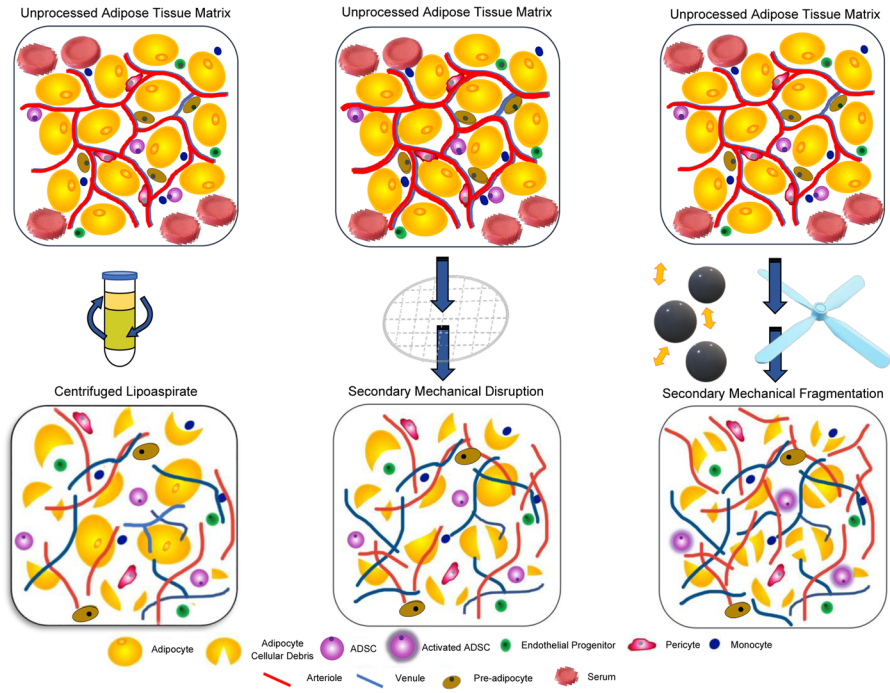

FIGURE 1. A visual representation of the increase in vascularity and adipose-derived stem cell (ADSC) activation that may occur due to the forces mediated by SMF processing compared to unprocessed lipoaspirate exposed to centrifugation or SMD.

such as Lipogems (Lipogems International SpA, Italy),[27] Filler Geller (Medikan, Seoul, Republic of Korea),[23] and Rigeneracons (Rigenera HBW, Torino, Italy),[28] fragment the adipose tissue matrix into its smaller constituent parts using a variety of mechanisms such as ball bearings[27] or blades.[23,28] The proposed effect of SMF and SMD processing on the adipose tissue matrix is visually summarized in Fig. 1.

There still exists no consensus as to the optimum method for mechanically processing lipoaspirate. We hypothesize that the key to the regenerative potential of fat grafts lies in its vasculogenic potential, which forms the basis for this systematic review. The aim of this review is to examine whether SMD or SMF techniques have a significant effect on the vasculogenic potential of fat grafts compared to unprocessed lipoaspirate or fat grafts prepared using centrifugation alone.

Materials and Methods

A search on MEDLINE, EMBASE, and the Cochrane Central Register of Controlled Trials was conducted up to February 2022 for articles pertaining to mechanical fragmentation or disruption of fat, the Coleman technique, and adipose tissue. The full search strategy can be found in Supplementary Table 1 http://links.lww.com/SAP/B17), which details all search terms used. We included all human and animal clinical research, which compared any of the 4 interventions—crude lipoaspirate, centrifuged lipoaspirate, SMD, or SMF. Texts that used enzyme-derived SVF or cell-assisted lipotransfer as a comparator were excluded. Two reviewers independently removed any duplicate results and screened titles and abstracts against the eligibility criteria. The reference lists of included studies and relevant reviews were also searched for further relevant studies. The resulting abstracts were transferred into a Microsoft Excel spreadsheet, their titles alphabetized, and duplicates removed. Full texts were sourced for all studies that met these criteria or where clarification was needed.

Quality assessments of controlled trials, observational studies, and preclinical animal studies were undertaken using the Consolidated Standards of Reporting Trials (CONSORT),[29] Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklists,[30] and the Animal Research: Reporting In Vivo Experiments 2.0 (ARRIVE 2.0) guidelines,[31] respectively. The ARRIVE 2.0 guidelines[31–34] were adapted to assess the quality of in vitro studies included in the review (Supplementary Table 2, http://links.lww.com/SAP/B17).

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Review and Meta-analyses (PRISMA) statement,[35] and a protocol was preregistered on PROSPERO (CRD42020190628).[36]

RESULTS

There was a total of 6708 records after duplicates were removed. Of those, 132 full text articles were accessed for eligibility and 31 were included in the study (Fig. 2). Included studies consisted of 17 in vitro studies, 10 preclinical animal trials, and 4 human studies. Human studies consisted of 1 randomized single-center study, 2 nonrandomized pilot trials, and 1 case series. Twelve papers compared centrifuged lipoaspirate against SMD strategies, 9 compared crude lipoaspirate against SMF strategies, and 3 compared both SMF and SMD techniques against centrifuged lipoaspirate. Two papers were found comparing each of the following groups: (i) crude lipoaspirate against SMD, (ii) centrifuged lipoaspirate against SMF methods, and (iii) SMD and SMF techniques. One further paper was identified, which compared centrifuged lipoaspirate and crude lipoaspirate. All included papers are detailed fully in Supplementary Table 3 (http://links.lww.com/SAP/B17). Meta-analysis of results was not possible due to multiple variations in fat derivative preparation and the diversity of biological effects noted in the literature, as summarised in Figure 3. Fig. 3. All statistically significant positive biological effects demonstrated with fat derivatives examined in this review are summarized in Fig. 4.

Comparison of Vasculogenic Potential In Vitro

Vasculogenic Growth Factors

Bianchi et al used reverse transcription polymerase chain reaction (RT-PCR) to quantify vasculogenic growth factors in adipocyte stem cells (ASCs) derived from cadaveric and live donors for SMF

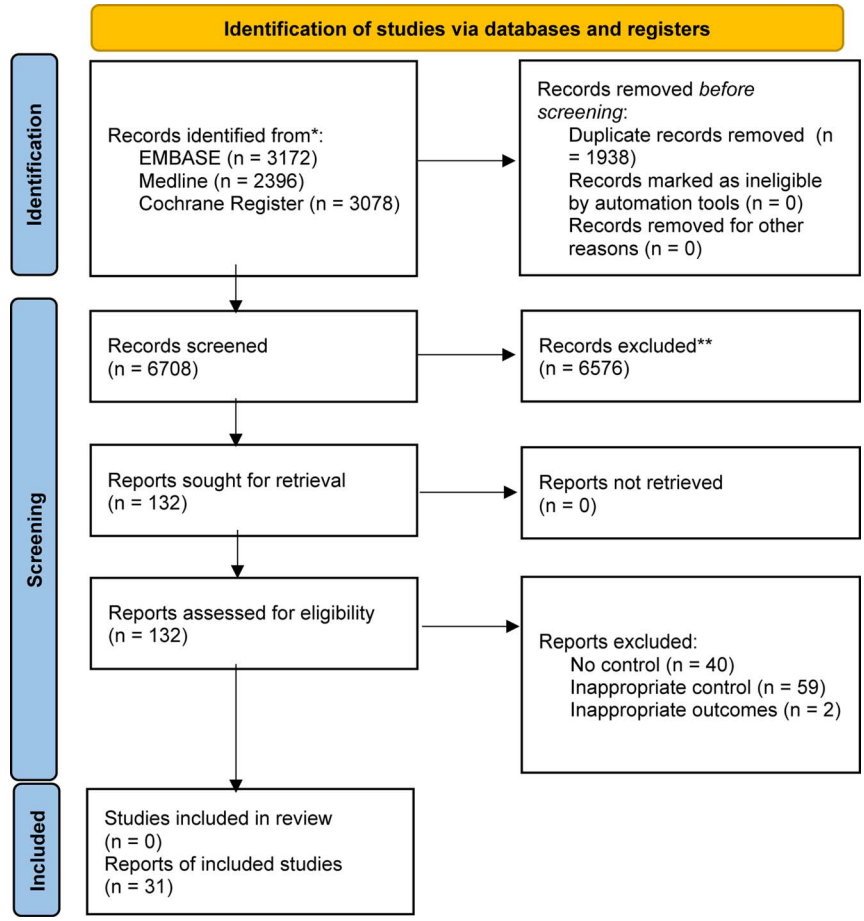

FIGURE 2. Flowchart of systematic search strategy carried out in accordance with PRISMA guidelines.[35] *Consider, if feasible to do so, reporting the number of records identified from each database or register searched (rather than the total number across all databases/registers). **If automation tools were used, indicate how many records were excluded by a human and how many were excluded by automation tools. Adapted from Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 2021;372:n71. doi: 10.1136/bmj.n71. For more information, visit: http://www.prisma-statement.org/

(Lipogems) and unprocessed lipoaspirate that had been exposed to a mixture of hyaluronan, butyric, and retinoic acids, previously shown to increase expression of vasculogenic genes[38] for 24–72 hours.[27] In the SMF group, there was a significant increase in hepatocyte growth factor (HGF) and kinase insert domain receptor (KDR) mRNA levels on day 1 ($P < 0.05$), but this difference was not sustained by day 3. VEGF mRNA levels showed a significant increase on day 3 compared to unprocessed lipoaspirate ($P < 0.05$). RT-PCR was also used by Casari et al to demonstrate that freshly processed Lipogems expresses significantly higher levels of bFGF than freshly processed centrifuged lipoaspirate ($P = 0.02$).[39]

Nava et al used multiplex bead analysis to quantify the secretome of conditioned media (CM) derived from Lipogems (MFAT-CM) and unprocessed lipoaspirate (LP-CM) after 28 days of culture.[40] VEGF was secreted at a significantly higher level in LP-CM after 7 days of culture (1446 ± 799) compared to conditioned media derived from MFAT-CM (308 ± 30) ($P < 0.05$). There was then a rapid decline in LP-CM secretion (123 ± 97 at 14 days and 63 ± 18 at 28 days). VEGF secretion in MFAT-CM was significantly higher at days 14 (749 ± 68; $P < 0.05$) and 28 (479 ± 39; $P < 0.01$).

Enzyme-linked immunosorbent assay (ELISA) and bicinchoninic acid protein assays were used in 4 SMD procedures against a variety of other SMF and centrifuged derivatives. Despite overlapping confidence intervals, Alharbi et al reported a significant increase ($P < 0.05$) in VEGF concentration in centrifuged fat (52 ± 29 pg/mg) compared to microfat (35 ± 18 pg/mg).[41] In the remaining papers, there were no significant differences in VEGF content in SMD [lipoaspirate processed with the Puregraft (Bimini Technologies, LLC) device vs centrifuged grafts][42] or SMF [micronized cellular adipose matrix (MCAM) lipoaspirate vs scissored lipoaspirate].[43]

Comparison of Cellular Composition In Vitro

Adipose-Derived Stem Cells/Mesenchymal Stem Cells

Using flow cytometric (FACS) analysis, several groups demonstrated a significant increase ($P < 0.05$) in the percentage of MSCs in Lipogems compared with crude lipoaspirate.[27,40,44] MSCs were defined as CD105+/CD90+/CD73+,[40] CD90+/CD29+/CD34−,[27] or solely on CD105 expression.[44]

Banyard et al demonstrated a 3-fold increase in ASCs (CD45−/CD31−/CD13+/CD73+) in SVF derived from SMD (crude lipoaspirate further subjected to intersyringe processing) compared to SVF derived from crude lipoaspirate ($P = 0.024$).[45] He et al demonstrated that an SMF and SMD derivative (scissored fat further processed using intersyringe shifting) had a significantly higher ratio of ASCs (CD29+/CD31−/CD34+) in its total SVF cell population compared to

		Crude Lipoaspirate	Centrifuged Lipoaspirate	SMF	SMD				SMF Derived from SMD	
				Lipogems	SVF gel	PureG	Shifted	Micro-fat	Micronized	Adipose Matrix Complex
Crude Lipoaspirate		–	N/A	MSCs + + + (31, 43, 47) Pericytes + + + + (31, 55-57) EPCs + (56) VEGF + + (31, 43) HGF + + (31, 43) bFGF + (42)	N/A	N/A	ASCs + (48) Pericytes + (58) EPCs + + (48, 58)	N/A	N/A	N/A
Centrifuged Lipoaspirate		N/A	–	N/A	EPCs + (53) ASCs + (52)	ASCs + (56)	N/A	VEGF + (44) IGF + (44)	N/A	Pericytes + (49) EPCs + (49) Fat Graft Retention + (49)
SMF	SF	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ASCs + (46) HGF + (46)	N/A

FIGURE 3. A visual summary of all the statistically significant vasculogenic factors demonstrated with fat derivatives examined in this review. Each "+" in the table represents a single study that reports a statistically significant beneficial effect on the primary and surrogate markers of vasculogenic potential seen with a certain fat derivative compared to another. Derivatives who have not yet been compared are labeled as "N/A." Results in red font favor the derivative on the *x*-axis; results in blue font favor the derivative on the *y*-axis. (i) S*hifted:* crude lipoaspirate repeatedly transferred through a fixed aperture. (ii) *PureG*: adipose processed with the Puregraft (Bimini Technologies, LLC) device. (iii) *Adipose matrix complex*: filtered lipoaspirate that has been cut into pieces. (iv) *Micronized*: scissored fat that has been syringe shifted. (v) *SVF gel*: Coleman fat further subjected to intersyringe processing and centrifugation as described by Yao and colleagues.[37] (vi) *Microfat*: lipoaspirate harvested with a microfat cannula with apertures 1 mm or less. (vii) Lipogems: crude lipoaspirate processed with the Lipogems (Lipogems International SpA) device. (viii) *SF*: scissored fat. Harvested fat cut into 1-mm-diameter samples using scissors.

		Crude Lipoaspirate	Centrifuged Lipoaspirate	SMF		SMD				
				Lipogems	M-Fat	LipiVage	SVF Gel	Micro-fat	PureG	Filtered
Crude Lipoaspirate		–	N/A	DMD Model + (66) Metabolomic Analysis + (67) Sepsis Model + (68)	N/A	N/A	N/A	N/A	N/A	N/A
Centrifuged Lipoaspirate		N/A	–	Cartilage Regeneration + (64)	N/A	Adipocyte Function + (30)	Fat Graft Retention + + (52, 53) Facial Rejuvenation + (62) Wound Healing + (52)	Fat Graft Retention + (24)	N/A	N/A
SMF	Lipogems	N/A	Regenerative Gene Expression + (42)	–	N/A	N/A	N/A	N/A	Cartilage Regeneration + (64)	N/A
	M-Fat	N/A	Osteoarthritis Model + (65)	N/A		N/A	N/A	N/A	N/A	Osteoarthritis Model + (65)

FIGURE 4. A visual summary of all the statistically significant positive biological effects demonstrated with fat derivatives examined in this review. Each "+" in the table represents a single study that reports a statistically significant beneficial biological effect seen with a certain fat derivative compared to another. Derivatives whose biological effects have not yet been demonstrated are labeled as "N/A." Results in red font favor the derivative on the *x*-axis; results in blue font favor the derivative on the *y*-axis. The only significant human trial finding is in italics. (i) *LipiVage:* crude lipoaspirate that has been processed using the LipiVage (Genesis Biosystems Inc) system. (ii) *M-Fat:* crude lipoaspirate mechanically fragmented with metal spheres. (iii) *Lipogems*: crude lipoaspirate processed with the Lipogems (Lipogems International SpA) device. (iv) *PureG*: adipose processed with the Puregraft (Bimini Technologies, LLC) device. (v) *DMD model*: Duchenne muscular dystrophy model. (vi) *Filtered*: crude lipoaspirate washed with Ringer lactate and then passed through a filtering membrane.

scissored lipoaspirate.[43] Flow cytometry conducted by Li et al demonstrated that SMF derived from SMD (adipose matrix complex) contained significantly lower populations of CD90+ cells than Coleman adipose tissue.[46]

Osinga et al compared immunohistochemical (IHC) staining of the cellular composition of centrifuged lipoaspirate to SMD (centrifuged lipoaspirate further subjected to intersyringe processing), observing no statistically significant difference in the cell types, the mean or maximum diameter of vessels, or the mean or maximum length of vessels between the groups.[47] Alharbi et al found no significant difference in ASC yield between centrifuged lipoaspirate and SMD (microfat).[41] Domenis et al used FACS to demonstrate that SMD [lipoaspirate processed with the Fastem device (CORIOS Soc.Coop.)] did not significantly increase the CD34+/CD45−/CD31− stromal cell population compared to lipoaspirate processed with centrifugation alone.[48] Feng et al showed that ASCs in SMD (fresh SVF gel) grew significantly fewer colonies than those in fresh Coleman fat ($P = 0.0186$).[49] Contrastingly, Ye et al described a significant increase in the proportion of ASCs (CD45−/CD31−/CD13+/CD73+) in SMD (SVF gel) compared to Coleman fat ($P < 0.05$).[50] Streit et al demonstrated that the number and percentage of ASCs in the pellet of centrifuged grafts was significantly lower than all other centrifuged fractions, decanted fat, and SMD [lipoaspirate processed with the Puregraft device (Bimini Technologies, LLC)] ($P < 0.05$).[51] No other significant differences in ASC number and percentage were seen between SMD and the other derivatives.

Perivascular Cell Population

Four studies reported on the pericyte populations between Lipogems and unprocessed lipoaspirate using FACS analysis, but no other fat groups were included in these studies. Both Vezzani et al and "Bianchi et al used the widely recognized CD146+/CD34− phenotype to identify pericytes, with Bianchi et al also using CD90+.[27,52] Both papers demonstrated a significant increase in the pericyte population in Lipogems compared to unprocessed lipoaspirate.[27,52] IHC staining by Bianchi et al demonstrated a statistically significant increase in CD146 and αSMA expression in Lipogems compared to unprocessed lipoaspirate ($P < 0.05$). Contrastingly, Polancec et al, using CD31−/CD34−/CD73±/CD90+/CD105−/CD146+ found no significant difference in the direct comparison of pericyte population between unprocessed lipoaspirate and Lipogems.[53] However, both Polancec et al and Vezzani et al demonstrated an enrichment of SVF pericytes, with a higher ratio of SVF pericytes over adipose-derived stromal cells in Lipogems when compared with unprocessed lipoaspirate ($P < 0.05$).

IHC undertaken by Ceserani et al reported an increase in the microvascular pericyte marker, NG2, staining in Lipogems compared to unprocessed lipoaspirate, but "more diffuse and intense" CD146 expression in unprocessed lipoaspirate, without any quantitative analysis and statistical testing.[54]

Banyard et al demonstrated that there was no significant difference between the number of CD45−/CD31−/CD34−/CD146+ pericytes between SVF derived from crude lipoaspirate and SVF derived from SMD (crude lipoaspirate further subjected to intersyringe processing).[45]

Finally, Zenic et al found a significantly higher percentage of CD31−/CD34−/CD73±/CD90+/CD105−/CD146+ pericytes in SMD (crude lipoaspirate repeatedly transferred through a 1.4-mm aperture) ($P = 0.0001$) and SMD subjected to further centrifugation ($P = 0.0001$) compared to crude lipoaspirate, but no difference between the 2 SMD derivatives.[55]

Endothelial Cells and Endothelial Progenitor Cells

Nava et al described a significantly higher percentage of endothelial cells (ECs) (CD31+) cells in Lipogems compared to crude lipoaspirate ($P < 0.05$).[40] Polancec and colleagues demonstrated that SVF isolated from Lipogems demonstrated an enrichment of endothelial progenitor cells (EPCs) (CD31+/CD34+/CD73±/CD90±/CD105±/CD146±).[53] Banyard et al demonstrated a 3-fold increase in EPCs (CD45−/CD34+/CD31+/CD146+) in SVF derived from SMD (crude lipoaspirate further subjected to intersyringe processing) compared to SVF derived from crude lipoaspirate ($P = 0.025$).[45] Li et al reported that SMF derived from SMD (adipose matrix complex) contained significantly lower populations of CD31+ ($P < 0.05$) and CD34+ ($P < 0.01$) cells than centrifuged Coleman adipose tissue.[46]

Ye et al reported a lower proportion of EPCs (CD45−/CD34+/CD31+/CD146+) in SMD (SVF gel) compared to centrifuged Coleman grafts.[50] Mashiko et al showed that SMD [intersyringe processed residual tissue of emulsification (RTEF)] demonstrated a higher composition of CD45−/CD31+/CD34+ ECs (1.8×) compared to lipoaspirate only processed using centrifugation without statistical testing.[23] However, when normalized to 1 mL of the source centrifuged fat, the number of ECs were similar between the 2 groups.

Zenic et al demonstrated that SMD (crude lipoaspirate repeatedly transferred through a 1.4-mm aperture) ($P = 0.0001$) and SMD subjected to further centrifugation ($P = 0.0001$) contained a significantly higher percentage of EPCs compared to crude lipoaspirate.[55]

Comparison of Outcomes With In Vivo Applications

Fat Graft Retention

Two studies compared fat graft retention between SMD fat (SVF gel) and Coleman fat grafts by injecting an equal volume of each into the flanks of nude mice. Both fresh and 1-month cryopreserved SVF gel demonstrated a significantly increased graft weight retention compared with fresh and 1-month cryopreserved Coleman grafts, respectively ($P < 0.05$, $P = 0.016$, respectively).[49] In a separate study, SVF gel exhibited a statistically significant increase in volume retention at 3, 14, 28, and 60 days compared to Coleman fat grafts ($P < 0.001$).[50] The retention of microfat was also compared against the Coleman method using a semiquantitative rating system in a murine model of scleroderma with better graft retention seen in the microfat population.[56] Contrastingly, Nguyen and colleagues reported that the Coleman technique demonstrated a graft persistence of 78% compared with 70% with SMD (microfat technique) in a murine fat grafting model after 12 weeks.[20] Over the same time period, Smith et al demonstrated no significant difference in weight retention between centrifuged grafts and crude lipoaspirate injected into immunodeficient mice.[57]

Finally, Li et al reported that the volume retention rate of SMF derived from SMD (adipose matrix complex) was significantly higher than Coleman fat 90 days postimplantation into a murine model ($P < 0.05$).[46]

Wound Healing

Feng et al demonstrated that the injection of 1 month cryopreserved SMD (SVF cryo-gel) in mice resulted in a significantly reduced wound size on days 8 ($P = 0.0375$) and 10 ($P < 0.05$) compared with 1 month cryo-preserved centrifuged fat.[49] SVF gel derivatives were also seen, unlike their centrifuged counterparts, to achieve complete wound healing by day 14.[49]

Wu and colleagues compared centrifuged lipoaspirate to SMF [micronized cellular adipose matrix (MCAM)] in the healing of irradiated punched skin defects in mice.[58] Both MCAM ($P = 0.021$) and centrifuged lipoaspirate ($P = 0.021$) significantly accelerated the reduction in wound size compared to a Dulbecco's Modified Eagle Medium (DMEM) control.

Comparison of Outcomes With Clinical Applications

Facial Rejuvenation

Patients undergoing Coleman fat transfer showed statistically significant increased injection volumes ($P = 0.021$) and rates of second treatment ($P < 0.001$) compared to the SMD (SVF gel) group. SVF gel

patients reported significantly increased overall satisfaction compared to the Coleman group ($P < 0.001$).[59]

Breast Reconstruction

Both the Breast-Q questionnaire and BCCT.core software revealed no significant differences in outcomes between patients receiving Coleman (n = 15) and SMD [Puregraft (Bimini Technologies, LLC)] (n = 15) lipoaspirate.[60] There was no significant difference in subcutaneous breast thicknesses in those receiving Fastem (CORIOS Soc.Coop.) processed grafts (n = 3) compared to those receiving centrifuged grafts (n = 16).[48]

Biological effects from other included studies such as adipocyte function,[26] cartilage regeneration,[61] osteoarthritis,[62] Duchenne muscular dystrophy,[63] metabolomics analysis,[64] sepsis,[65] and regenerative gene expression[39] are detailed in Supplementary Table 4 (http://links.lww.com/SAP/B17). All statistically significant beneficial biological effects demonstrated with fat derivatives examined in this review are summarized in Fig. 4.

Discussion

Vasculogenic potential of fat grafts is difficult to quantify. In the absence of significant literature to directly measure graft vascularity such as vessel density, we included levels of angiogenic growth factors and quantification of mesenchymal or progenitor cell populations as the primary outcomes of this systematic review. Surrogate parameters of vasculogenic potential, such as fat graft retention and clinical outcomes, were also examined and contributed to the evaluation of each derivative.

Postulated mechanisms to explain the beneficial effect of SMD or SMF include enrichment in ASCs with minimal extracellular matrix fragmentation, providing an angiogenic microenvironment that serves as a biological scaffold for infiltrating cells and traumatized fat leading to an injury response and healing phenotype.[66,67] The role of mechanotransduction in angiogenesis and vascular signaling is still being elucidated, which may provide further insight into the increased pro-angiogenic properties of mechanically processed lipoaspirate. Fluid shear stress has been shown to mediate functional and proliferative changes in ASCs, with a cumulative nitrous oxide–mediated production of hepatocyte growth factor (HGF) by ASCs.[68] It has also been hypothesized that the shear stress from processing induces activation of pro-angiogenic genes and increases the expression of pluripotency genes such as Sox2, Nanog, and Oct4, which are expressed during tissue regeneration.[68–70] ECs similarly display a pro-angiogenic phenotype on exposure to shear stress with increased expression of VEGF A.[71]

The majority of evidence available suggests that there is an enriched population of pericytes, ASCs, and EPCs after processing with the Lipogems device (Lipogems International SpA), with increased vasculogenic growth factor secretion, despite using different cell markers.[27,40,52,53] Lipogems-processed lipoaspirate is also reported to have a beneficial anti-inflammatory and regenerative effect. There is mixed evidence on the enrichment of relevant cell populations in other SMD/SMF methods, with nonstandardized use of centrifugation regimes in intersyringing methods as well as different microcannula sizes used for harvesting, limiting the generalization of outcomes, as these factors have previously been shown to affect outcomes of fat grafting.[2] Furthermore, the majority of the high-quality in vitro studies in this review are in relation to the study of Lipogems, which are largely industry funded. Significant flaws in these studies are the inadequate reporting of methodology, statistical testing, and presentation of data in studies.

The enhanced presence of these cells supports suggestions that SMD transforms fat into a healing phenotype, explaining its improved performance in in vivo studies. CD146+ cells present in transplanted fat in vivo were shown to express increased angiogenic markers (angiopoietin-1, FGF-1, VEGF-A)[72] and enhance engraftment.[73] This is one potential explanation for the significantly improved graft retention and enhanced wound healing when using SMD, SVF, and microfat over lipoaspirate or centrifuged lipoaspirate in the majority studies in this review.[49,52,53,59,61]

As the number of human studies identified was limited, it is difficult to draw any substantial conclusions. Equally, there was only 1 randomized nonblinded single-center study comparing centrifugation vs SMD with the remaining publications in this review being either nonrandomized pilot trials, case series, preclinical animal studies, or reported results from in vitro experimentation. As such, the current evidence is somewhat limited.

Although no strong conclusion or clinical recommendation can be drawn due to the heterogeneity and low evidence level of many of the included studies, there is a suggestion in the literature that there is increased vasculogenic potential in fat grafts processed by mechanical fragmentation.

The next frontier in fat grafting is its clinical translation within existing regulatory constraints. Ideally, multicenter, large-scale randomized control trials comparing the clinical outcomes of using lipoaspirate, SMD, and SMF methods are needed to accurately evaluate any potential benefits. Existing literature often compares mechanical processing of lipoaspirate with enzymatic digestion, which does not fit within existing regulatory framework. By limiting our study to techniques that will fit the definition of "minimal manipulation," we highlight the existing benefits of using "minimally manipulated fat," which would allow us to harness its potential in the tissue engineering and eventual clinical translation.

CONCLUSION

Overall, this review concludes that whilst there is insufficient high-quality evidence to direct clinical practice, the literature examined suggests that mechanically manipulated lipoaspirate has significantly higher vasculogenic potential compared to centrifuged or crude lipoaspirate. Enhanced ASC, pericyte, EPC, and angiogenic growth factor levels explain the reported in vivo improvements in graft retention and wound healing in animal models; however, adequately powered human studies remain elusive. Further standardization of fat graft preparation techniques and larger scale studies are required to define the optimal manipulation process and fat grafting procedure.

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