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Research

A Systematic Review on Extracellular Vesicles-Enriched Fat Grafting: A Shifting Paradigm

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Mohammad Ghiasloo, MD; Laura De Wilde, BSc; Kashika Singh, MD; Patrick Tonnard, MD, PhD; Alexis Verpaele, MD, PhD; Olivier De Wever, Apr, PhD; and Phillip Blondeel, MD, PhD

Abstract

Background: Recent evidence confirms that mesenchymal stem cells (MSCs) facilitate angiogenesis mainly through paracrine function. Extracellular vesicles (EVs) are regarded as key components of the cell secretome, possessing functional properties of their source cells. Subsequently, MSC-EVs have emerged as a novel cell-free approach to improve fat graft retention rate.

Objectives: The authors sought to provide a systematic review of all studies reporting the utilization of MSC-EVs to improve graft retention rate.

Methods: A systematic search was undertaken employing the Embase, PubMed, and Cochrane Central Register of Controlled Trials databases. Outcome measures included donor/receptor organism of the fat graft, study model, intervention groups, evaluation intervals, EV research data, and in vitro and in vivo results.

Results: Of the total 1717 articles, 62 full texts were screened. Seven studies reporting on 294 mice were included. Overall, EV-treated groups showed higher graft retention rates compared with untreated groups. Notably, retention rate was similar following EV and MSC treatment. In addition to reduced inflammation, graft enrichment with EVs resulted in early revascularization and better graft integrity. Interestingly, hypoxic preconditioning of MSCs improved their beneficial paracrine effects and led to a more proangiogenic EV population, as observed by both in vitro and in vivo results.

Conclusions: MSC-EVs appear to offer an interesting cell-free alternative to improve fat graft survival. Although their clinical relevance remains to be determined, it is clear that not the cells but rather their secretome is essential for graft survival. Thus, a paradigm shift from cell-assisted lipotransfer towards "secretome-assisted lipotransfer" is well on its way.

Level of Evidence: 4

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Dr Ghiasloo is a fellow, Department of Plastic and Reconstructive Surgery, Ghent University Hospital, Ghent, Belgium. Ms De Wilde is a medical student, Faculty of Medicine, Ghent University, Ghent, Belgium. Dr Singh is a surgical resident, Department of General and Visceral Surgery, Evangelical Hospital Bethel, Bielefeld, Germany. Drs Tonnard and Verpaele are co-directors in private practice in Ghent, Belgium. Dr De Wever is a full professor, Laboratory of Experimental Cancer Research, Department of Human Structure and Repair, Ghent University, Ghent, Belgium. Dr Blondeel is the chairman, Department of Plastic and Reconstructive Surgery, Ghent University Hospital, Ghent, Belgium.

Corresponding Author:
Dr Phillip Blondeel, Department of Plastic and Reconstructive Surgery, Ghent University Hospital, Corneel Heymanslaan 10 Route 1281, Ghent B-9000, Belgium.
E-mail: phillip.blondeel@ugent.be

Adipose tissue (AT) is considered as the ideal soft tissue filler, giving rise to its widespread utilization in reconstructive and aesthetic surgery.[1,2] In addition to its biocompatibility, AT is abundant and easy to harvest through liposuction. However, fat grafting is highly unpredictable and associated with high rates of graft resorption over time.[3] Resorption is mainly caused by local ischemia and delayed revascularization following graft injection.[4] In addition, liposuction-induced membrane damage, devascularization, ischemic cell injury, and processing-associated factors must be considered as well.[5]

Attempts to improve graft survival have led to the introduction of the cell-assisted lipotransfer (CAL).[6] Although the CAL method was associated with better graft retention compared with conventional fat grafting, this effect was only significant for injected volumes under 100 cc.[7] Furthermore, patients treated with the CAL technique experienced more complications while undergoing a similar number of procedures compared with patients who underwent conventional fat grafting.[7] Although the mechanisms of CAL remain elusive, it is widely accepted that instead of differentiating towards any specific cell lineage, the mesenchymal stem cells (MSCs) rather exert a pro-survival secretome, stimulating neovascularization and tissue regeneration. In addition, their paracrine functions appear to be enhanced due to local ischemia and hypoxia.[8-10] Regardless, cell-based therapies are associated with many limitations, including senescence-induced genetic instability, undesirable long-term side effects, and uncontrolled differentiation.[11] Hence, cell-free alternatives appear to be on the rise.

One cell-free alternative are the soluble proteins and growth factors found in the MSC's secretome. However, concerns have been raised regarding the delivery, dose, cost, and safety of such therapies.[12,13] Another alternative includes the utilization of MSC-derived extracellular vesicles (EVs), a heterogenous population of nanosized membrane-enclosed vesicles involved in intercellular communication in both physiological and pathological conditions.[8,14] There are two main EV subtypes: the smaller exosomes (50-150 nm in diameter) originating from endosomes, and the generally larger cell membrane derived microvesicles (50-1000 nm in diameter, up to 10 µm in case of cancer-associated oncosomes).[14]

Increasingly, MSC-derived EVs (MSC-EVs) have demonstrated therapeutic potential as cell-free alternatives for treatment of various conditions.[15] Several reports have recently identified EVs as a promising alternative to increase fat graft retention rates.[16-22] The current study aims to systematically summarize the existing evidence regarding the effects of EV-enriched fat grafting.

METHODS

Protocol

A systematic review was performed according to the guidelines and recommendations of the preferred reporting items for systematic reviews and meta-analysis checklist (PRISMA).[23]

Literature Search

An electronic search strategy was designed and refined for the Embase, PubMed and the Cochrane Central Register of Controlled Trials databases. The search strategy is outlined in the Appendix. The systematic literature search was performed from date of inception until April 12, 2020.

Selection Criteria

Studies investigating the results of MSC-EV–enriched fat grafting were included. No language restrictions were applied a priori. When necessary, authors of non-English language studies were contacted to provide an English language summary of their findings. Failure to establish contact or to receive results in a timely manner resulted in exclusion. Non-MSC-EV sources, in vitro studies, reviews, letters, commentaries, correspondences, case reports, conference abstracts with insufficient information, expert opinions, and/or editorials were excluded.

Study Selection and Data Extraction

Studies were selected as follows: after pooling of all search results, titles and abstracts were independently reviewed by M.G. and L.D.W. In case of doubt, studies were included for full-text screening, which was conducted independently by M.G. and L.D.W. to identify studies fulfilling the aforementioned inclusion criteria. In case of disagreement, the senior authors were consulted and a decision was made in consensus. A final list of included articles was reviewed prior to data extraction.

Data extraction was performed independently by M.G. and L.D.W. utilizing a pre-approved form. Disagreements were discussed with senior authors until consensus was reached. The following outcomes were extracted: donor/receptor organism fat graft, study model (type, gender, number of models undergoing preclinical testing), intervention groups (including treatment scheme/dose), evaluation intervals, EV research data (including EV cell source, isolation method, and EV characterization methods), in vitro

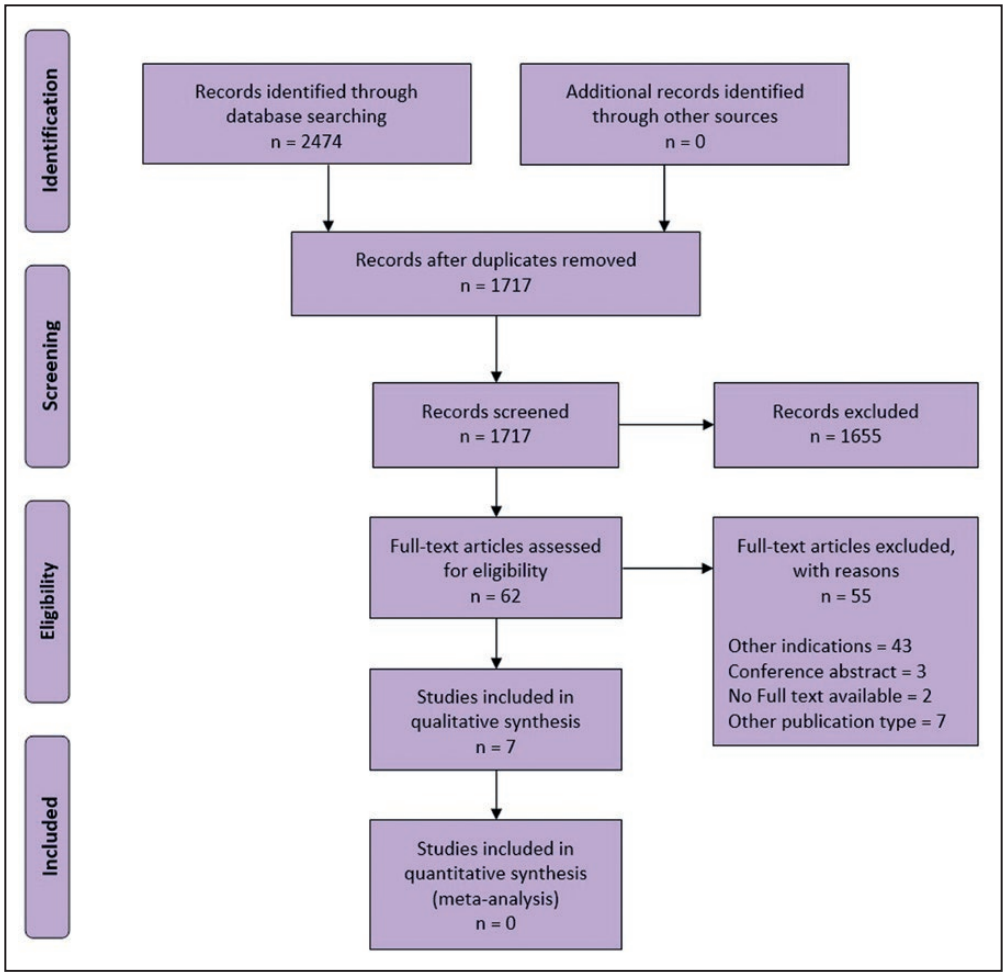

Figure 1. PRISMA flow chart of search strategy results.

results (including tube formation and migration/scratch assay), and in vivo results (including graft retention rate, graft integrity, neovascularization, adipogenesis, inflammatory profile, and extracellular matrix properties).

Assessment of Quality of Studies

All studies were submitted to the EV-TRACK knowledgebase (www.evtrack.org), producing the EV-METRIC of the experiments within the individual studies.[24] The EV-METRIC is expressed as a percentage of fulfilled components from a list of nine, with each component deemed as indispensable for unambiguous interpretation and independent replication of EV experiments. Furthermore, the risk of bias of the included studies was independently evaluated by M.G. and L.D.W. using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) risk of bias assessment tool.[25]

RESULTS

Overall, 2474 articles were retrieved from the aforementioned databases (Figure 1). After deduplication, 1717 articles were screened based on title and abstract. Sixty-two articles were included for full-text screening, of which 55 were excluded. Reasons for exclusion were other indications (43 studies), conference abstracts (3), no available full text (2), and other publications types (eg, review, editorial) (7). Finally, seven studies were included[16-22] (Table 1). EVs were isolated from bone marrow mesenchymal stem cells (BMSCs)[16] and adipose derived stem cells (ADSCs).[17-22] Hypoxic preconditioning was performed under 1%[16] and 5% oxygen.[17,18,21] Four studies evaluated the effects of EVs isolated from hypoxia preconditioned MSCs (hypEVs),[16-18,21] of which only three compared the effects of hypEVs to EVs isolated from normoxic preconditioned MSCs (nEVs).[17,18,21] One study evaluated different EV doses to a control group

Table 1. Basic Characteristics of the Included Studies

Study	Donor organism fat graft	Receptor organism fat graft	Study model (no.)	Intervention groups including application scheme/dose	Measurement interval(s)
Chen et al, (2019)[20]	Mouse (inguinal)	Mouse (back)	8-wk-old ♂ C57/BL6 mice (48)	3 groups: control: 0.200 cc fat + 0.02 cc PBS; MSC: 0.200 cc fat + 1 x 10^4 MSC + 0.02 cc PBS; EV: 0.200 cc fat + 40 µg EV + 0.02 cc PBS. EV-groups received supplementary EV injection at day 7 and day 14 (40 µg EV + 0.02 cc PBS), whereas the control and MSC group received 0.02 cc PBS injections	3 and 10 wk
Han et al, (2018)[17]	Human (abdomen)	Mouse (back)	6-wk-old ♀ BALB/c nude mice (20)	4 groups: control 1: 1 cc fat + 0.1 cc PBS; control 2: 1 cc fat + 0.1 cc PBS; hypEV: 1 cc fat + 50 µg/0.1 cc hypEV; nEV: 1 cc fat + 50 µg/0.1cc nEV. EV-groups received supplementary EV injections at 1-week intervals	2, 4, 6, and 8 wk
Han et al, (2019)[18]	Human (abdomen)	Mouse (back)	6-wk-old ♀ BALB/c nude mice (15)	3 groups: control: 1 cc fat + 0.1 cc PBS; hypEV: 1 cc fat + 50 µg/0.1cc hypEV; nEV: 1 cc fat + 50 µg/0.1 cc nEV. EV-groups received supplementary EV injections at 1-week intervals	2 and 15 days, 4 weeks
Huang et al, (2017)[16]	Human (abdomen)	Mouse (scalp)	6-wk-old ♀ BALB/c nude mice (27)	3 groups: control: 1 cc fat + 0.2 cc PBS; low dose hypEV: 1 cc fat + 50µg EV/0.2 cc PBS; high dose hypEV: 1 cc fat + 100µg EV/0.2 cc PBS	12 wk
Mou et al, (2019)[19]	Human (abdomen)	Mouse	6-wk-old ♂ BALB/c nude mice (16)	2 groups: control: 0.35 cc fat + 0.02 cc PBS; EV: 0.35 cc fat + 7 µg of EV in 0.02 cc PBS	1 and 3 mo
Zhu et al, (2020)[21]	Human (abdomen)	Mouse (scalp)	6-wk-old ♀ BALB/c nude mice (108)	3 groups: control: 0.300 cc fat + 0.100 cc PBS; nEV: 0.300 cc fat + 0.100 cc nEV (50 µg/cc); hypEV: 0.300 cc fat + 0.100 cc hypEV (50 µg/cc)	3 d, 4 and 12 wk
Zhu et al, (2020)[22]	Mouse (inguinal)	Mouse (scalp)	8-wk-old ♀ C57/BL6 mice (60)	2 groups: control: 150 mg fat + 0.200 cc PBS once per week for 12 weeks; EV: 150 mg fat + 0.200 cc EV (100 µg/cc) once per week for 12 weeks	2, 4, and 12 wk

EVs, extracellular vesicles; hypEVs, extracellular vesicles derived from hypoxic preconditioned mesenchymal stem cells; MSC, mesenchymal stem cell; nEVs, extracellular vesicles derived from normoxic conditioned mesenchymal stem cells; PBS, phosphate-buffered solution.

(ie, conventional fat grafting),[16] whereas another one compared the effects of the EVs to their source MSCs.[20] Three studies investigated possible mechanisms of EV-enhanced graft retention.[18,21,22] Detailed assessment of risk of bias of the individual studies is outlined in Table 2.

In Vitro

EV Research Methodology and Characterization

Twelve different EV experiments (ie, EVs harvested from different conditions) were observed in seven studies, with EV-METRIC of individual studies ranging between 15-38% (Table 3, Figure 2). Six different EV separation methods were used, mostly based on differential ultracentrifugation—separating EVs based on size and density using sequentially increasing centrifugal force.[16-18,21,22]. Following separation, EVs were characterized most frequently using EV-enriched markers CD9 (71%, 5/7), CD63 (71%, 5/7), TSG101 (57%, 4/7) and CD81 (43%, 3/7). Conversely, only two studies evaluated the presence of contaminants using non-EV enriched markers. While most studies included qualitative particle analysis methods (eg, nanoparticle tracking analysis, dynamic light scattering, electron microscopy), quantitative data such as EV concentration/density and total protein count were rarely or not reported. In addition, none of the studies reported information regarding lysate preparation. Evaluation of EV sizes revealed broad ranges across all studies, suggesting the isolated EV mixtures to comprise of both exosomes and microvesicles, or rather, small and large EVs.[16-22] Compared to nEVs, Han and colleagues reported hypEVs to be larger and more enriched in angiogenic proteins, suggesting hypoxia preconditioning to result in a higher EV cargo capacity.[18] However, this difference in size was not observed by others.[17,21]

Table 2. Risk of Bias Assessed With SYRCLE Risk of Bias Assessment Tool

Study	Selection bias			Performance bias		Detection bias		Attribution bias	Reporting bias	Other
	Sequence generation	Baseline characteristics	Allocation concealment	Random housing	Blinding	Random outcome assessment	Blinding	Incomplete outcome data	Selective outcome reporting	Other sources of bias
Chen et al, (2019)[20]	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk
Han et al, (2018)[17]	Unclear risk	Low risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk
Han et al, (2019)[18]	Unclear risk	Low risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk
Huang et al, (2017)[16]	Unclear risk	Low risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk	Low risk
Mou et al, (2019)[19]	Unclear risk	Low risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	Low risk
Zhu et al, (2020)[21]	Unclear risk	Low risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk
Zhu et al, (2020)[22]	Unclear risk	Low risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk

Table 3. EV Isolation and Characterization

Study	EV cell source	EV isolation method	Biophysical characterization	Biochemical characterization	EV-METRIC (%)
Chen et al, (2019)[20]	Mouse AT; normoxic ADSCs	(Differential) (ultra)centrifugation + filtration + ultrafiltration	NTA TEM	WB	38
Han et al, (2018)[17]	Human AT; normoxic (20% oxygen) and hypoxic (5% oxygen) cultured ADSCs	(Differential) (ultra)centrifugation + filtration	NTA TEM	FC WB	15-34
Han et al, (2019)[18]	Human AT; normoxic (20% oxygen) and hypoxic (5% oxygen) cultured ADSCs	(Differential) (ultra)centrifugation + filtration	NTA TEM	FACS WB	23-34
Huang et al, (2017)[16]	Rat BM; hypoxic (1% oxygen) cultured BMSCs	(Differential) (ultra)centrifugation	DLS SEM	FC	38
Mou et al, (2019)[19]	Human AT; normoxic cultured ADSCs	(Differential) (ultra)centrifugation	DLS SEM	WB	38
Zhu et al, (2020)[21]	Human AT; normoxic (20% oxygen) and hypoxic (5% oxygen) cultured ADSCs	(Differential) (ultra)centrifugation + filtration	NTA TEM	WB	15-34
Zhu et al, (2020)[22]	Human AT; normoxic cultured ADSCs	(Differential) (ultra)centrifugation + filtration	NTA TEM	WB	22

ADSCs, adipose-derived stem cells; AT, adipose tissue; BM, bone marrow; BMSC, bone marrow stem cells; DLS, dynamic light scattering; FACS, fluorescence-activated cell sorting; FC, flow cytometry; NTA, nanoparticle tracking analysis; SEM, scanning electron microscopy; TEM, transmission electron microscopy; WB, western blot.

Cell Migration and Tube Formation

Overall, EV-enriched human umbilical vein endothelial cells (HUVECs) showed more favorable tube formation and migration capabilities compared to non-treated HUVECs.[16-19,21] Interestingly, hypEVs were superior to nEVs, further enhancing HUVECs' migration and tube formation capacities.[18,19,22] In addition, EV-enhanced migration and tube formation was dose-dependent; the high-dose hypEV-treated HUVECs increased migration and tube formation by respectively ~1,39- and 1,34-fold compared to the control.[17]

Differentiation Assays

In ADSC adipogenic differentiation assays a higher percentage of adipocytes could be observed in the EV-enriched ADSC group compared to non-enriched

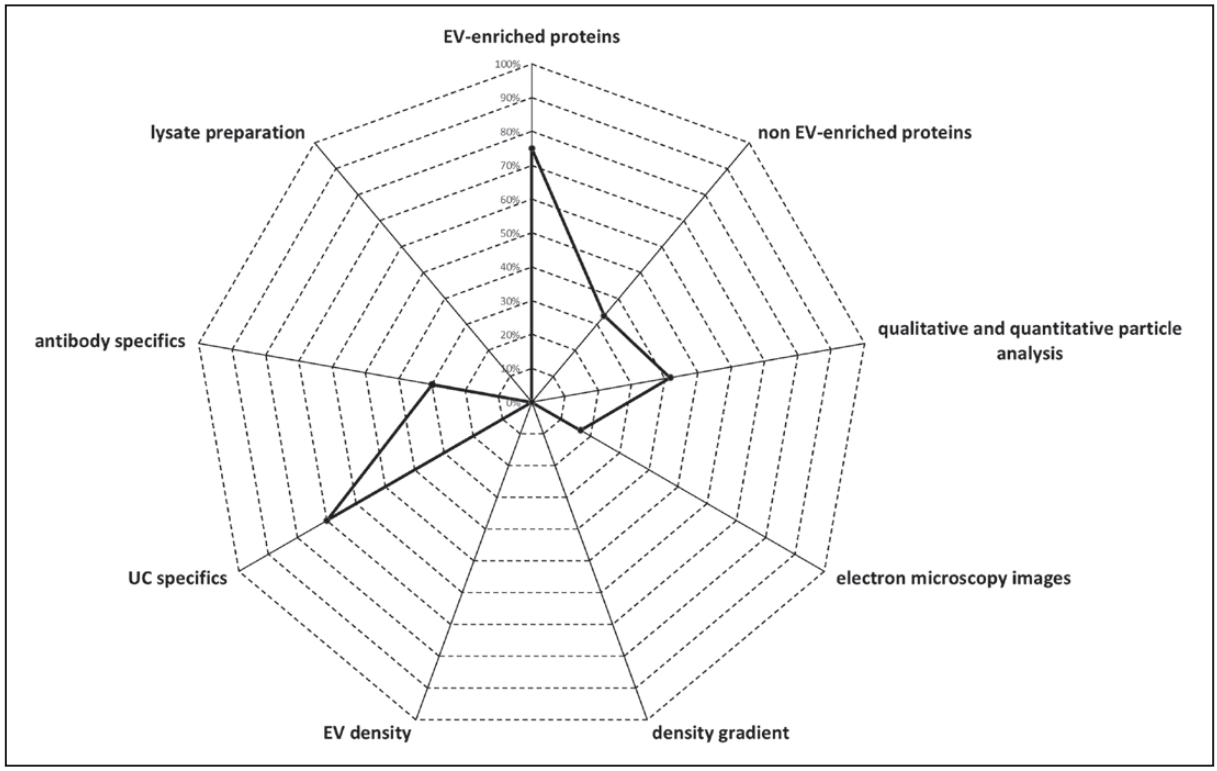

Figure 2. Spider chart representing the percentage of included studies that adhere to each of the respective EV-METRIC parameters.

MSCs and control.[20,22] Interestingly, ADSC-EV treatment of peritoneal macrophages increased the proportion of the CD206+ M2 macrophages—the anti-inflammatory "repair" subtype—compared to non-treated macrophages ($P = 0.02$). Furthermore, the conditioned medium derived from the EV-treated macrophages showed increased levels of catecholamines ($P = 0.04$).[22] Treatment of ADSCs with the conditioned medium of EV-treated macrophages showed a higher expression of uncoupling protein 1—marker of beige and brown adipose tissue—and higher oxygen consumption compared to control and EV-treated ADSCs, suggesting the macrophage secretome is responsible for adipose tissue beiging, instead of the ADSC-derived EVs.[22]

In Vivo

Fat Graft Retention

A total of 294 mice underwent fat grafting. Four studies performed supplementary injections post-transplantation.[17,18,20,22] The last evaluation date was at 4,[18] 8,[17] 10,[20] and 12 weeks.[16,19,21,22]

Overall, EV-treated grafts had higher retention rates compared with the control ($P < 0.05$).[16,17,19-22] At 12 weeks, the effects of EVs on fat graft retention were comparable with their source MSCs. However, the EV group received repeated EV injections at 7 and 14 days post-transplantation, whereas the MSC and control groups received phosphate-buffered saline (PBS) injections.[20] Furthermore, hypEV-treated grafts also had higher retention rates compared with the nEV-treated grafts ($P < 0.05$).[17,21] Notably, little difference could be observed between the high-dose hypEV-treated compared to the low-dose hypEV-treated group.[16]

Neovascularization

CD31 staining showed an increased capillary density in EV-treated grafts compared with the control ($P < 0.05$).[16-22] Additionally, hypEV treatment significantly improved neovascularization compared with the nEV treatment ($P < 0.05$).[17,18,21] The effects of EVs and their source stem cells on neovascularization were similar at 3 weeks post-transplantation.[20] Expression of proangiogenic factors was increased in the low-dose hypEV-treated grafts compared with the control.[16] Furthermore, hypEV treatment also elevated the expression levels of proangiogenic factors compared with nEV-treated grafts.[18] Overall, proangiogenic expression was similarly increased in both EV- and MSC-treated grafts. Notably, the expression of hepatocyte growth factor was significantly higher in the EV-treated grafts compared with the MSC-treated grafts.[20]

Interestingly, Zhu and colleagues[21] observed a distinct difference between graft and host revascularization. Whereas EV treatment appeared to improve vessel

formation within the graft, they appeared to have no influence on the host derived revascularization.[21] This difference in revascularization was ascribed to the utilization of human lipoaspirate and human MSC-EVs in a murine preclinical model; the co-transplanted human EVs were enriched with human miRNA, whereas the hosting organism was murine and subsequently unresponsive to the human miRNA molecules.

Integrity

Independent of dose, MSC- and EV-treated grafts showed less fibrosis and a higher structural integrity compared with the control.[16,18-20,22] Similarly, perilipin staining showed a higher tissue and adipocyte integrity in EV-treated grafts compared with the control,[17,19,20,22] and hypEVs could further enhance these effects ($P < 0.05$).[17] After 12 weeks, a smaller mean size of adipose cells was observed in EV-treated grafts compared to the control ($P < 0.05$).[22]

Inflammation

Histological analyses reported less infiltrations of inflammatory cells in EV-treated grafts compared with the control.[17,18] Fat grafts treated with EVs showed increased numbers of M2 macrophages compared with the control in both the peripheral region (after 2 and 4 weeks, both $P < 0.01$) and the central region (after 2 and 4 weeks, $P = 0.01$ and $P < 0.01$, respectively). The proportion of M2 macrophages was also increased after 12 weeks in the EV-treated grafts compared with the control ($P = 0.02$).[22]

Expression levels of inflammatory cytokines and chemokines were significantly higher in EV- and MSC-treated grafts compared with non-treated grafts.[20] Interestingly, the EV-treated groups—after receiving repeated injection at day 7 and 14—showed higher levels of cytokines and chemokines compared to the MSC-treated groups.[20]

Extracellular Matrix

Higher expression of collagen VI α3 and collagen III α1 could be observed in both EV- and MSC-treated grafts compared with the control at week 10 (all $P < 0.05$), indicating a more intact and increased adipose extracellular matrix structure.[20] This suggests that a more complete extracellular matrix structure may contribute to a higher fat graft retention rate.

Browning of Adipose Tissue

As indicated above, EV enrichment promoted the polarization of M2 macrophages. These macrophages—stimulated by EVs—appear to increase the secretion of catecholamines, in turn inducing browning of AT and increasing neovascularization in the peripheral region of EV-treated grafts compared with the control.[22]

DISCUSSION

To the best of our knowledge, this is the first systematic review describing the effects of EV-enriched fat grafting. Overall, EV-treated groups showed higher graft retention rates compared with untreated groups.[16-22] Notably, retention rate was similar following EV and MSC treatment.[20] In addition to reduced inflammation, graft enrichment with MSC-EVs resulted in early revascularization and subsequently better graft integrity.[16-22] Although angiogenesis pathways and their cellular and molecular motors have yet to be fully elucidated, the heterogenous cell populations are believed to contribute differently.[26] In this regard, cells from the endothelial lineage exclusively proliferate and differentiate into endothelial cells, providing building blocks for angiogenesis. Conversely, stem cells from the hematopoietic and mesenchymal lineage exert their funtion through paracrine signalling, creating a suitable micro-environment in which the endothelial cells could thrive. Only a small percentage of the MSCs differentiate into pericytes to stabilize the newly formed vessels.[26] Notably, stress stimuli—including ischemia, physiological, and pathological hypoxia as well as hypoxic preconditioning—push MSCs toward an upregulated and adapted secretome.[9,10,17,18,21,27-29] This shift towards a more prosurvival secretome was observable in hypEVs and their cargo, as the latter was shown to be adapted to the hypoxic micro-environment in early fat grafts.[17,18,21,27-29] In vitro HUVEC migration and tube formation was increased in hypEV-enriched groups compared to nEV groups, suggesting that EVs are at least partially contributing to vessel formation.[17,18,21,28] Presumably, graft enrichment with hypEVs will result in a supraphysiologic upregulation of the proangiogenic pathways as soon as hypEVs and fat graft are mixed, hence facilitating early revascularization within graft.

Although the field of EV-enriched fat grafting is still in its infancy and has yet to fully develop, the following considerations must be made at this point:

1. Only one study evaluated the effects of BMSC-EV,[16] whereas the other six used ADSC-EVs.[17-22] Indeed, ADSCs are easier to access, are more abundant—up to 500 times higher in concentration compared to BMSCs, have stronger immunosuppressive properties, secrete several proangiogenic and antiapoptotic cytokines, and display a higher phenotypic variety.[30-32] However, it is unknown how EVs from various MSC sources affect graft retention. Considering the differences in micro-environments between the various MSC types and their adaptive response to stress,[33-35] it is plausible for each to manifestate differently when exposed to graft hypoxia;

2. Enzymatic digestion has long been considered as the gold standard for stem cell isolation, and was the method of choice in the included studies. It is however unknown whether mechanically isolated stem cells secrete a similar EV profile as compared to enzymatically isolated MSCs;
3. In addition to their heterogeneity, EVs are carriers of a variety of miRNA and protein molecules, and their cargo is continuously fine-tuned depending on the direct micro-environment as well as systemic conditions.[8,14] As we try to improve our understanding of EVs, it is advisable to optimize existing separation protocols.[24,36-41] Despite the disadvantages of differential ultracentrifugation for EV separation,[39,42] it is still one of the most frequently used methods of EV separation.[24,43] To further advance the field of fat grafting and general EV research, novel EV separation protocols must be developed, aimed at reducing contamination with non-EV components, and where possible, increasing EV yield. In this regard, reference materials such as recombinant EVs (rEVs)[36,37,44,45] and orthogonal separation methods have become invaluable.[45,46] Compared to single technique protocols such as differential ultracentrifugation-based separation, orthogonal biophysical methods—combination of complementary techniques to separate EVs based on size and density—will allow for EV separation with higher specificity, offering a crucial advantage for downstream characterization and (pre)clinical application.[45,46] EV research is further faciliatated by the use of rEVs, biological reference materials with similar biochemical and biophysical characteristics as sample EVs, which function as an internal control throughout all steps of EV research, such as the development, optimization and assessment of separation methods and downstream EV characterization.[36,37,44,45] Implementation of rEVs and orthogonal biophysical methods, as well as consulting the EV-TRACK knowledgebase and the Minimal Information for Studies of Extracellular Vesicles 2018 (MISEV 2018) guidelines[41] will undoubtedly result in high specificity protocols, which in turn will allow for reproducibile and indisputable evaluation of in vivo effects, thus improving the quality of evidence of future studies[8,38];
4. Application of EVs from hypoxic preconditioned MSCs appears to be an interesting approach in improving ischemic conditions.[16-18,21,28] While the use of hypoxic EVs should be further incentivized, this should be performed under true hypoxic conditions, as characterized by an oxygen concentration between 0.5-2%.[47] Regardless, the impact of true hypoxic preconditioning, as well as suboptimal oxygenation of MSCs, their EVs and their subsequent role in fat grafting should be investigated;
5. Similar to MSC-enriched fat grafting, the ideal dose-response curve should be pursued in both in vitro and in vivo experiments.[48] While it is true that Huang and colleagues did not find any difference between their low- (50 µg EV/0.2 cc PBS) and high-dose (100 µg EV/0.2 cc PBS) group regarding graft retention at 12 weeks,[16] enrichment with varying doses should be further investigated to identify the optimal dose per grafted volume of lipoaspirate, as well as whether there is a dose limit after which adverse effects are to be expected;
6. As previously mentioned, EV treatment resulted in similar outcomes compared to source MSC[20]. However, the EV group received additional EV injections at day 7 and 14, compared to PBS-injection in the MSC group. While the results are exciting, further research is necessary to identify whether EV-enrichtment without repeated injections offers similar results compared to source MSCs. Furthermore, it should be emphasized that repeated injections of any enrichment solution in fat grafting are inefficient and even unrealistic in a clinical setting. Should repeated doses offer significant benefit compared to a single dose, then delayed release approaches should be integrated[49];
7. While it is generally agreed that adipogenesis stabilizes around the 3 month mark, graft stabilization will take up to a year post grafting.[50,51] It should therefore be encouraged to assess graft retention after a longer period of time (eg, 4 or 6 months). In the very least, further volumetric assessment (eg, magnetic resonance imaging, computer tomography) should be encouraged;
8. While tempting to implement EVs in clinical setting, it is evident that EVs are not yet primed for full-scale clinical implementation. While diagnostic testing[52] and occasional clinical applications are increasing,[53] our knowledge of EV-specific biomarkers—and potentially their cargo and destination—is still limited. When EV-specific biomarkers are available, cumbersome ultracentrifugation-based techniques can be exchanged by more easily implemented technologies (eg, enzyme-linked immunosorbent assay). Subsequently, one could potentially develop repeatable EV separation methods with high specificity and purity for extensive preclinical and clinical testing.

Several limitations were encountered. First, only 7 articles were included. Although the evidence is still limited at this point, the timing of this work allows the introduction of EV research in general and specifically EV-enriched fat grafting to a broader audience. Furthermore, synthetizing of the current results allows to offer future direction (see supra). Second, not all predetermined data could be extracted, limiting the robustness of the current study and preventing heterogeneity assessment. Subsequently, no meta-analyses could be performed. Finally, the terminology

to describe certain EV subpopulations in the included reports was not followed in the current work. Instead, a general term, that is, EV, was used to report the observed effects. Although most mixtures appeared to mainly contain smaller EVs—often referred to as exosomes—they seemed to contain various EV populations, soluble proteins and cell debris among others. Following MISEV2018, specific terminology should only be used when the EV is caught in the act of release by live imaging techniques.[41] In any other circumstances, EVs should ideally be described either by their physical characteristics (eg, size, density), biochemical composition (eg, "CD63+/CD81+"-EVs), or by describing conditions or cell of origin (eg, normoxic/hypoxic MSC-EVs). If EV characterization cannot be achieved in accordance to the MISEV2018 guidelines, then general terminology such as "extracellular particle" might be more appropriate.

When considering possible cell competition mechanisms—where "ischemic" graft cells have to compete with "healthier" or even "empowered" CAL cells for survival[4,54-57]—as well as increasing cell-therapy limitations,[11,58] it appears that alternative cell-free approaches are gaining traction. In this regard, "cell-free fat extract"—the liquid fraction obtained after centrifugation of the mechanically isolated adipose stromal vascular fraction—has shown great potential for a variety of indications such as fat grafting, flap survival, and skin regeneration.[59-64] Combined with our improved understanding of the role of MSCs in angiogenesis and the benefits of EV enrichment, it is clear that not the cells but their secretome—that is, soluble proteins and EVs—is essential for graft survival.[65] While the clinical relevance of EVs has yet to be determined, they have formed the foundation of an emerging research niche regarding the crosstalk of fat cells in fat grafting and revascularization. Despite the many unanswered questions, a paradigm shift from CAL toward a "secretome-assisted lipotransfer" is well on its way. Future studies addressing aforementioned limitations should be encouraged and will undoubtedly advance the field.

CONCLUSIONS

MSC-EV-enriched AFG appears to be a novel approach in improving graft survival. Although the field is still in its infancy, it is crucial to identify knowledge gaps and steer the field towards standardized and reproducible results. Early adherence to the MISEV2018 guidelines and EV-TRACK knowledgebase should therefore be encouraged. While the field will surely mature and expand in the future, the next step towards a "secretome-assisted lipotransfer" is well on its way.

Supplemental Material

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Disclosures

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