HHS Public Access

Author manuscript

*Nano Today*. Author manuscript; available in PMC 2022 August 01.

Published in final edited form as:

*Nano Today*. 2021 August ; 39: . doi:10.1016/j.nantod.2021.101159.

# Extracellular vesicle therapeutics from plasma and adipose tissue

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## Abstract

Extracellular vesicles (EVs) are cell-released lipid-bilayer nanoparticles that contain biologically active cargo involved in physiological and pathological intercellular communication. In recent years, the therapeutic potential of EVs has been explored in various disease models. In particular, mesenchymal stromal cell-derived EVs have been shown to exert anti-inflammatory, anti-oxidant, anti-apoptotic, and pro-angiogenic properties in cardiovascular, metabolic and orthopedic conditions. However, a major drawback of EV-based therapeutics is scale-up issues due to extensive cell culture requirements and inefficient isolation protocols. An emerging alternative approach to time-consuming and costly cell culture expansion is to obtain therapeutic EVs directly from the body, for example, from plasma and adipose tissue. This review discusses isolation methods and therapeutic applications of plasma and adipose tissue-derived EVs, highlighting advantages and disadvantages compared to cell culture-derived ones.

## Graphical abstract

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CRediT authorship contribution statement

Conceptualization DI, MY, and JW; writing, reviewing, and editing all authors; visualization, DI; supervision JW, LDM, CC; project administartion JW; funding acquisition JW, LDM, DI, and MY;

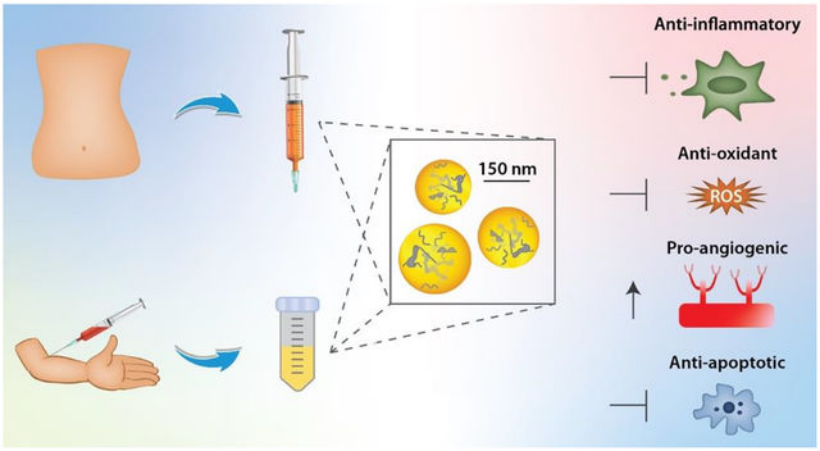


COMPETING INTERESTS

The authors declare no competing interest.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.




**Keywords**

Adipose tissue; extracellular vesicles; isolation methods; plasma; therapeutic

## 1. Introduction

The term extracellular vesicle (EV) has been used in the literature since the 1970s to refer to membranous structures that are released extracellularly [1]. EVs consist of an external bilayer and internal core that are composed of a wide variety of lipids [2], carbohydrates [3], proteins [4], and/or nucleic acids [5]. EVs are released by all cells and can be divided into two main subclasses based on biogenesis: exosomes usually have a size range of 30–100 nm and are derived from multivesicular bodies that fuse with the cell membrane, while microvesicles have a size range of around 50–1000 nm and bud directly from the cell membrane [6]. In addition to biogenesis, there is considerable heterogeneity in terms of EV content and function depending on the cell source, condition, and microenvironment [6]. Current isolation methods and molecular assays are incapable of separating and distinguishing exosomes from microvesicles due to overlapping size and biomolecular content [7].

EVs have important intercellular communication roles in several physiological and pathological processes, and are also capable of crossing biological barriers, such as the blood-brain barrier [8–13]. EVs from a wide variety of cell types, including mesenchymal stromal cells (MSC) [14] and dendritic cells (DC) [15], and organs, such as the placenta [16], display anti-inflammatory and reparative properties. Specifically, various EV-associated proteins and microRNAs (miRNAs) have been shown to suppress inflammatory, oxidative, and apoptotic pathways, while promoting angiogenesis and tissue repair [17–19]. Early-stage clinical trials involving DC-derived EVs (cancer immunotherapy) and MSC-derived EVs (anti-inflammatory and regenerative therapy) have been conducted in a small number of patients [20]. However, the production of cell culture-derived EVs is challenging, as large-scale cell propagation is time-consuming and requires expensive, equipment, reagents, and facilities. Additionally, cells in culture produce low quantities of EVs and

conventional isolation methods are unable to consistently recover EVs from conditioned media without causing EV damage and co-isolation of various contaminants.

To overcome scale up issues and enable large quantities of EVs to be produced, EV isolation directly from the human body is emerging as a promising alternative to cell culture-based approaches. Specifically, therapeutic EVs have been isolated from human plasma and lipoaspirate, which are abundant and easily accessible sources of biological material.

### 1.1. Comparison of cell culture and bodily fluids as EV sources

The isolation of EVs directly from the body is more time efficient (hours compared to months), cost-efficient (cell culture facilities are not required), and results in substantially higher yields than cell culture-derived EVs [21]. Additionally, obtaining EVs directly from the body eliminates the need for exogenous cell culture media products, for example, fetal bovine serum, which is not suitable for clinical-grade manufacturing. The therapeutic use of EVs directly from the human body is not limited to autologous applications, as is evident from the wide-spread practice of transfusion medicine. Plasma contains large quantities of EVs (up to $10^{10}$/mL) released from all cells in the body [22] yet blood and plasma transfusions seldom cause adverse reactions [23]. Other allogeneic nanoparticles derived from plasma, such as lipoproteins, are in large-scale clinical trials, demonstrating the feasibility of such approaches. For example, CSL-112, a high-density lipoprotein (HDL) mimetic consisting of apolipoprotein (apo)A-1 purified from human plasma and reconstituted with phospholipids, is currently in a phase III clinical trial involving 17,400 patients with acute coronary syndrome [24, 25].

In comparison to cell culture, plasma and adipose-tissue also have certain disadvantages as EV sources, including being more heterogenous and containing high levels of contaminants that co-isolate with EVs. Another disadvantage of obtaining EVs directly from the body is that genetic engineering of cells to obtain EVs with specific protein or RNA-based therapeutics or targeting ligands is not possible. Nevertheless, several approaches exist to load EVs with drugs post-isolation by temporarily creating pores in the lipid bilayer to enable the entry of water soluble drugs into the aquatic EV core [26]. These approaches include the use of electrical fields (electroporation), ultrasound (sonication), thermal energy (freeze-thaw cycled), pressure (extrusion), or chemical agents (detergents). Non-water soluble drugs can be loaded into the EV bilayer with simple mixing protocols [26].

A challenge for both cell culture and bodily fluid-derived EVs is maintaining consistent characteristics of the source material. Bioreactors can be used for cell culture scale-up and variability can be minimized by using immortalized cell lines. However, incorporation of immortalization agents in EVs may pose safety risks [27]. Scale up of both bodily fluid and non-immortalized cell-derived therapeutic products is likely to require several donors and the implementation of potency release criteria to ensure desired effects. Major advantages and disadvantages of cell culture versus biological tissue and fluid-derived EVs are summarized in table 1.

## 2. EV isolation methods

### 2.1. Conventional isolation methods

One of the main challenges with EV-based studies is a lack of efficient and reliable isolation methods. Compared to conditioned media, bodily fluids usually contain a more complex mixture of components, including various nanosized particles with similar size and density as EVs [28], making it even more challenging to obtain pure samples. Various methods have been used to isolate EVs from plasma and lipoaspirate, including differential ultracentrifugation, density gradient ultracentrifugation, size-exclusion chromatography, and precipitation-based isolation (Figure 1). Ultracentrifugation is based on high centrifugal forces (100 000 × g) that enable separation of various components according to size. A relatively large input volume (up to 1.5 L) is possible, but the process requires time-consuming steps and the EV yield is low with abundant contaminants [29–32]. In particular, ultracentrifugation leads to co-pelleting of albumin [29, 32], which is a major source of contamination in plasma-derived EV samples. Several studies have also showed that ultracentrifugation can lead to EV aggregation and morphological changes that can damage the surface and internal cargo [33–35]. An iodixanol gradient, which enables separation of compounds based on flotation density, has been used to reduce protein contaminants in serum-derived EVs isolated by ultracentrifugation [36]. Despite improved purity, density gradient ultracentrifugation has many disadvantages, including a small input volume, time consuming steps, and a low EV yield [30]. Size-exclusion chromatography is based on separation of components based on size and interactions with a porous material, where smaller molecules have longer retention times [37]. Similarly, to density gradient ultracentrifugation, this method requires a small input volume but produces plasma/serum-derived EV with high purity [36, 38]. Precipitation kits are time-efficient, cheap, and easy to use but result in EV samples with low purity that are contaminated with other biological components as well as synthetic polymers [26]. Various immunoaffinity-based methods have been described for the isolation of specific EV subpopulations from plasma, such as those expressing cluster of differentiation (CD)63 [39] and chondroitin sulphate peptidoglycan 4 [40]. A major challenge of these methods is the difficulty of detaching EVs from the captured surface without compromising functional properties. An immunoaffinity-based microfluidic platform incorporating desthiobiotin-conjugated antibodies was designed to enable EV release after capture. Specifically, EVs expressing CD9, CD63, and CD81 were isolated and shown to retain cellular uptake levels following release [41]. Although many of the aforementioned isolation methods may be suitable for *in vitro* and diagnostic clinical studies, they have low yields, are challenging to perform under sterile conditions, and/or lack scale-up capabilities (10–100 L) for clinical translation.

### 2.2. Emerging EV isolation methods for scale-up

An emerging method for EV isolation is tangential flow filtration (TFF). This method is based on fluid that flows tangentially (horizontally) across a filter, leading to reduced pore clogging and filter cake formation, which is common with conventional dead-end filtration methods (Figure 1) [29]. TFF protocols implement controlled flow and low shear rates that enable separation of different sized components without causing damage. These protocols usually rely on the use of two filters, one with a pore size that separates EVs and smaller

contaminants from larger cellular debris and a second filter with pore sizes that separate EVs from smaller contaminants (for example, free biomolecules, such as proteins, carbohydrates, lipids, and nucleic acids). TFF techniques also include diafiltration and concentration steps, enabling large input volumes of source material (for example, 1 L) to be purified and concentrated to smaller volumes (for example, 5 mL) in only a few hours [29]. Therefore, TFF is especially suitable for isolating EVs from large volumes of bodily fluids that are usually obtained by a single collection process and are at risk of degradation unless rapidly processed. The EV amount obtained from TFF can often be used to perform many functional cell culture assays and therapeutic animal studies [29]. Notably, the EV yield obtained by TFF is about five-fold higher than ultracentrifugation using the same source material [29]. Moreover, the ability to remove albumin, a common protein contaminant in plasma, is 40-fold higher by TFF than ultracentrifugation, making it particular suitable for EV isolation from bodily fluids. EV batches from the same starting material processed by TFF also display more consistent size distribution profiles compared to ones processed by ultracentrifugation, indicating that the former method has less technical variability [29]. Moreover, assays that detect mycoplasma, endotoxin, and bacterial contaminants have revealed that samples do not become contaminated during the sterile isolation process [29].

## 3. Plasma-derived EVs

Plasma has been used as a therapy for several decades [42–44]. For example, therapeutic plasma exchange is performed for a variety of conditions, while convalescent plasma has recently been used for the treatment of coronavirus disease 2019 (COVID-19) [45, 46]. Additionally, the therapeutic properties of individual components in plasma, such as EVs, have been explored. Plasma-derived EVs are released from various cells in the body and have been studied as therapeutics for many conditions, such as cardiac damage, osteoarthritis, wounds, aging, and muscle injuries (Table 2). In addition to regular plasma, various variations have been used as a source for therapeutic EVs, including plasma containing additional blood components (platelets), plasma from certain tissues (umbilical cord), plasma from donors who have undergone specific procedures (ischemic pre-conditioning or exercise), and plasma from donors with distinct characteristics (young age). In one study, it was demonstrated that human and/or rat plasma-derived EVs isolated by differential ultracentrifugation had protective effects in cardiomyocytes subjected to hypoxia and reoxygenation *in vitro* and in a rat model of myocardial ischemia-reperfusion injury [47]. These plasma-derived EVs were found to activate the extracellular signal−regulated protein kinase (ERK1/2) and heat shock protein 27 (HSP27) cardioprotective pathway [47, 48]. In addition to cardioprotective effects, plasma-derived EVs isolated by filtration and centrifugation have been shown to promote *in vitro* and *ex vivo* canine tendon repair by increasing tenocyte proliferation, decreasing tenocyte apoptosis, enhancing collagen formation, suppressing inflammatory pathways, improving failure strength, and reducing gap formation [49, 50].

Another source for therapeutic EVs is platelet-enriched plasma (PRP), which has a platelet concentration that is up to six times higher than that of blood [61]. PRP is thought to have reparative properties due to various growth factors that are released during platelet activation [61]. Platelet activation can be achieved during PRP preparation by, for example, adding

calcium chloride and/or thrombin, or PRP can be spontaneously activated post-administration [62]. It is important to note that many PRP preparation methods also enrich for leukocytes, which may have beneficial or detrimental effects [61]. PRP has been widely used in dental [63] musculoskeletal [64], wound healing [65], and orthopedic applications [64, 66]. Guo *et al.* reported that PRP-EVs isolated by differential ultracentrifugation, contained higher levels of platelet-derived growth factor-BB (PDGF-BB), transforming growth factor beta 1 (TGF-β1), and basic fibroblast growth factor (bFGF) compared to the platelet-depleted fraction of PRP (normalized for protein content) (Figure 2) [52], suggesting that EVs may be a critical bioactive component in PRP. Recently, PRP-derived EVs have been reported to have beneficial effects in various biological processes, including collagen synthesis, angiogenesis, and re-epithelialization [51–55]. For example, PRP-EVs were superior to PRP in increasing fibroblast and endothelial cell migration and proliferation and promoting re-epithelialization and angiogenesis in a diabetic rat wound model (Figure 3) [52]. Similarly, PRP-EVs isolated with a membrane affinity kit displayed increased therapeutic efficacy compared to platelet-depleted PRP in cell culture and in an osteoarthritis rabbit model [55]. In addition to regular plasma and PRP, umbilical cord plasma has been explored as a source for therapeutic EVs. Hu and colleagues demonstrated that umbilical cord plasma-derived EVs obtained by differential ultracentrifugation accelerated wound repair through miR-21–3p-mediated inhibition of the phosphatase and tensin homolog (PTEN) and sprouty homolog 1 (SPRY1) pathways [56].

Strategies to increase the therapeutic efficacy of plasma-derived EVs by exposing donors to certain conditions have also been explored. It has previously been shown that brief episodes of ischemia (hypoxia) and reperfusion (reoxygenation) confer local and remote protection against longer periods of ischemia [67]. Cardioprotection through remote ischemic preconditioning was found to be dependent on EVs [68], highlighting that this phenomenon could potentially be exploited to improve the therapeutic effects of plasma-derived EVs. A study demonstrated that plasma EVs (polymer-based precipitation) obtained from donor rats that were subjected transient limb ischemia, had increased cardioprotective effects in a model of myocardial ischemia-reperfusion injury compared to regular plasma EVs [57]. Similarly, EVs isolated by polymer-based precipitation from the plasma of human subjects undergoing ischemic preconditioning protected cardiomyoblasts in culture from hypoxia-induced apoptosis, while regular plasma EVs failed to exert therapeutic effects [58].

Another type of plasma EV that has been explored for cardioprotection is derived from donors exposed to exercise training [59]. Exercise is thought to induce both immediate and gradual cardioprotective effects through various mechanisms, including increased levels of antioxidants, mitochondrial adaptions, and structural changes in coronary blood vessels [69]. Myokines, which are cytokines secreted by skeletal muscle tissue, are thought to play a major role in mediating the beneficial effects of physical exercise [70]. A recent study demonstrated that EVs isolated by differential ultracentrifugation from the plasma of rats and humans that underwent a long-term exercise regimen protected cardiomyocytes from hypoxia/reoxygenation injury, while plasma EVs from control subjects failed to do so [59]. Similarly, intramyocardially injected plasma EVs from exercised rats had protective effects when administered prior to ischemia reperfusion injury in a rat model. Therapeutic effects were shown to be primarily mediated by miR-342-5p, which was contained within the EVs,

and caused the inhibition of apoptotic mediators, such as caspase 9 and c-Jun N-terminal kinase 2 (JNK 2), and the activation of pro-survival signals, such as alpha serine/threonine-protein kinase (Akt) [59]. Notably, miR-342-5p in plasma-derived EVs was primarily thought to originate from endothelial cells as opposed to skeletal muscle cells, which have previously been postulated to play major role in exercise-induced cardioprotection [59]. The authors demonstrated that cultured endothelial cells exposed to laminar shear stress released increased levels of EVs with miR-342-5p, indicating the exercise-induced changes in blood flow may mediate the release of cardioprotective EVs [59].

The effect of donor age on the therapeutic properties of plasma-derived EVs has also been explored. In the past century, circulating factors have been shown to play important roles in aging and in other multiple studies where the circulatory systems of young and old animals have been connected through heterochronic parabiosis [71–73]. A recent study demonstrated that plasma EVs from young mice, isolated by differential ultracentrifugation or polymer-based precipitation, contain higher levels of extracellular nicotinamide phosphoribosyltransferase (eNAMPT) compared to those from old mice [60]. eNAMPT is an enzyme involved in the biosynthesis of nicotinamide adenine dinucleotide ($NAD^+$), which is associated with anti-aging effects [60]. Intracellular levels of $NAD^+$ in primary hypothalamic neurons was shown to be higher following treatment with plasma EVs from young mice than old mice [60]. Additionally, intraperitoneal administration of plasma EVs from young mice in old mice led to a substantial improvement in wheel-running activity and lifespan (10% extension) [60]. Notably, these improvements were more evident in female mice, indicating the presence of sex differences in plasma EV-mediated anti-aging effects [60]. Adipocytes are known to secrete eNAMPT-containing EVs, and adipose tissue is thought to be a major source of such EVs in the circulation [60]. The important role of eNAMPT in EV-mediated increases in wheel-running activity was demonstrated by comparing mice administered with EVs from control and *Nampt*-knock down adipocytes [60]. It is plausible that similar anti-aging effects would be seen in humans, as the levels of circulating eNAMPT declines in both aging mice and humans, and eNAMPT was found to be present in human plasma-derived EVs [60].

Taken together, various types of plasma-derived EVs show therapeutic effects in a wide variety of disease models (Table 2). While some studies report that regular plasma-derived EVs have beneficial effects, others demonstrate the need to isolate plasma EVs from specific fractions, tissues, or donors in order to observe therapeutic activity (Table 2). In general, the mechanisms by which plasma-derived EVs impact the tissue environment and recipient cells remain largely unknown, although specific EV-contained miRNAs or proteins have been implicated in select studies. However, therapeutic effects are likely to be mediated by a combination of EV-contained biomolecules, including lipids and glycans that have been less studied. In addition to activating signaling pathways in recipient cells, it is possible that EVs bind unbeneficial biomolecules in the circulation or tissue environment, preventing them from exerting detrimental effects. The contribution of hundreds of cell types to the EV population in plasma is likely to make mechanistic studies more challenging than those involving cell culture-derived EVs. Additionally, most of the isolation methods that have been used in the reported studies are not capable of completely removing lipoproteins, which is the most abundant population of nanoparticles in plasma (six orders of magnitude

higher concentration than EVs [22]), raising the possibility that therapeutic effects are partially or primarily mediated by lipoproteins.

## 4. Adipose tissue-derived EVs

Many preclinical and clinical studies have explored the use of MSC therapies to treat inflammation and tissue damage, and therapeutic effects have primarily been attributed to cell-released EVs [14, 74–78]. For example, EVs derived from adipose-derived MSCs (AMSCs) were shown to be therapeutically active in various inflammatory disease models, including myocardial ischemia-reperfusion injury, liver fibrosis, and obesity [79–84]. Specifically, AMSC-derived EVs have been shown to decrease inflammatory, fibrotic, and apoptotic pathways, while promoting anti-inflammatory, antioxidant, pro-survival, and angiogenic signaling [79–84]. Compared to cell-based therapies the use of EVs has many benefits as a non-living therapeutic modality, including easier handling and storage, as well as the inability to proliferate and form malignant lesions. Additionally, therapeutic EVs are usually in the 50–300 nm size range, making it unlikely for vascular obstructions to occur, which can be an issue with cells (15000 – 30000 nm) [11].

In order to exploit the aforementioned benefits of AMSC-derived EVs, while circumventing cell culture requirements, adipose tissue has been explored as a scalable alternative source (Table 3). In addition to AMSCs, adipose tissue contains many other cell types, such as adipocytes, endothelial cells, fibroblasts, pericytes, smooth muscle cells, and immune cells [85], which release EVs that may strengthen or counteract therapeutic effects. A study demonstrated that the secretome of adipose tissue that was cultured intact, that is not enzymatically processed to remove specific cells, displayed greater anti-inflammatory effects than the secretome of the cultured enzymatically digested stromal vascular fraction, which lacks adipocytes [86]. These data suggest that intact heterogenous adipose tissue may have a superior secretome in terms of therapeutic effects compared to adipose tissue that has been processed and separated into different fractions. Additionally, it is possible that important properties are lost upon transfer of adipose tissue from a native milieu to a laboratory-expanded environment.

Adipose tissue is usually abundant and easily accessible through liposuction. Notably, the isolation of EVs from human lipoaspirate is more time efficient (hours compared to months of cell culture), cost-efficient (cell culture facilities are not required), and results in substantially higher yields than cell culture-derived EVs (Figure 4) [21]. Tian *et al.*, used TFF as an isolation method to obtain donor-matched AMSC-EVs and lipoaspirate EVs. Lipoaspirate was obtained from non-obese patients undergoing liposuction and processed via mechanical disruption to obtain micro-fragmented fat that was used for AMSC culture and an acellular fraction that was processed directly by TFF. The EV yield was 30-fold higher from lipoaspirate compared to the AMSC-derived conditioned media [21]. The protein levels were much higher in the AMSC EVs than lipoaspirate EVs, and the lipid composition varied depending on the donor. Fatty acids were the main lipid component in both AMSC EVs and lipoaspirate EVs, while the latter contained a higher content of glycerolipids [21]. EVs from both sources had a spherical structure with unilamellar or multilamellar lipid bilayers as was apparent by cryogenic transmission electron microscopy

[21]. Notably, the lipoaspirate samples also contained another nanoparticle population consisting of lipoprotein-like spherical structures (Figure 4). Lipoaspirate EV samples also had much higher miRNAs levels than AMSC-EVs, which may be attributable to lipoprotein in these samples, which are known to bind to miRNAs [21]. Further processing of the TFF-isolated lipoaspirate samples by SEC led to removal of a large portion of lipoproteins (reduction in apoE), which was estimated to account for approximately 53% of the nanoparticles isolated by TFF (Figure 4) [21]. Notably, AMSC-EVs and lipoaspirate nanoparticles displayed similar anti-oxidative, anti-apoptotic, and anti-inflammatory effects in various macrophage cell lines, indicating that separation of AMSCs from adipose tissue is not necessary to obtain a therapeutic secretome.

The therapeutic effect of lipoaspirate EVs was further improved by loading guanabenz, a small molecule drug, in the lipid bilayer using a simple mixing protocol (44% encapsulation efficiency) [21]. Guanabenz is clinically approved to treat hypertension but has also been shown to decrease lipopolysaccharide (LPS)-induced inflammatory cascades in macrophages [88]. Lipoaspirate EVs loaded with guanabenz caused a greater reduction in LPS-induced inflammatory cytokines, including interleukin (IL)-6 and IL-1β compared to lipoaspirate EVs alone. Additionally, while lipoaspirate EVs were unable to decrease nitric oxide synthase-2 (NOS2), cyclooxygenase 2 (COX2), and granulocyte macrophage colony-stimulating factor (GM-CSF) in inflamed macrophages, the addition of guanabenz in the EVs caused a reduction in these factors. Encapsulation of small molecule drugs, such as guanabenz, in nanocarriers can also provide several advantages compared to administration of free drugs, including longer circulation times, improved site-specific delivery, decreased side effects, and co-delivery with other cargo [89–93]. Additionally, drug delivery systems can protect RNA and protein-based drugs from degradation and promote their cellular uptake and lysosomal escape [90, 94]. Notably, EVs were recently shown to outperform synthetic nanoparticles in terms of delivering small-interfering RNA (siRNA) to target cells *in vivo* [95].

In another study, Bellei *et al.* collected the adipose tissue secretome, including EVs, by dead-end filtration of human lipoaspirate [87]. The lipoaspirate secretome was capable of increasing keratinocyte, fibroblast, and melanocyte proliferation compared with non-treated cells [87]. Notably, donor-matched plasma increased fibroblast proliferation to a similar or greater extent as the lipoaspirate secretome but decreased proliferation of keratinocytes and melanocytes, which was speculated to be due to cell differentiation induced by the presence of high levels of calcium in plasma. The proliferative effects of the lipoaspirate secretome was limited to normal skin cells, as the proliferation of malignant skin cells decreased or remained unchanged in response to treatment [87]. The lipoaspirate secretome also stimulated fibroblast cell migration by increasing the expression of proteins involved in wound repair, for example, vascular endothelial grow factor (VEGF), fibronectin, CD44, and N-cadherin, and decreasing the levels of connexin 43, a gap junction protein [87]. Moreover, both plasma and the lipoaspirate secretome displayed antioxidant effects, and the latter had higher levels of grow factors associated with skin regeneration, such as bFGF and VEGF [87].

In another study, it was demonstrated that EVs derived from cultured mouse adipose tissue had protective effects in pulmonary microvascular endothelial cells and in a mouse model of ventilator-induced lung injury [77]. Specifically, intravenously administered adipose tissue EVs suppressed the expression of the mechanical stress receptor, transient receptor potential vanilloid 4 (TRPV4), and increased the expression of adherens junctions, β-catenin and vascular endothelial (VE)-cadherin in lung tissue, leading to reduced pulmonary microvascular hyperpermeability and attenuated tissue injury. Additionally, adipose tissue EVs decreased inflammatory cytokines, such as IL-6, and suppressed myeloperoxidase (MPO) activity [77]. Notably, EV-depleted conditioned medium from cultured adipose tissue failed to display therapeutic effects, highlighting the importance of EVs as a bioactive component in the adipose tissue secretome [77]. The authors also demonstrated that serum and AMSC-derived EVs had therapeutic effects in ventilator-induced lung injury, being less and more effective than adipose tissue-derived EVs, respectively [77]. The authors speculated that the observed therapeutic effects could be related to the obesity paradox, where increased body mass index is counterintuitively associated with improved outcomes [96]. For example, obese patients with acute lung injury that undergo mechanical ventilation have better outcomes than non-obese ones [97–99]. Mouse studies have also demonstrated that a high-fat diet confers protection against ventilator-induced lung injury [77, 100]. It has been speculated that obesity-induced protection is partially due to the body having higher levels of beneficial factors secreted from adipose tissue [96]. In contrast, other studies have shown that individuals with obesity and/or metabolic disorders can display adipose tissue with proinflammatory factors [101] and EVs that mediate metabolic dysfunction [102]. Such contradictory findings highlight the complex association between obesity and the adipose tissue secretome, which may be partially explained by the existence of different forms of obesity (for example, healthy versus unhealthy) as well as differences in adipose tissue types [96]. For example, subcutaneous and visceral white adipose tissue display considerable difference in fat uptake capacity, lipolytic activity, and immune cell populations [103]. A study demonstrated that subcutaneous adipose tissue-derived AMSCs had greater immunosuppressive effects on macrophages compared to visceral adipose tissue-derived ones [104], suggesting that similar differences may be seen with EVs.

Taken together, adipose tissue-derived EVs have been shown to display anti-inflammatory, proliferative, pro-survival, and angiogenic effects. Compared to plasma-derived EVs, less studies have focused on the therapeutic effects of adipose tissue-derived EVs. From a practical standpoint plasma is more easily accessible than adipose tissue and blood banks have been using standardized and optimized protocols for plasma safety screening for decades. However, adipose tissue displays unique metabolic and immunological properties that could generate EVs with superior properties to plasma-derived EVs. Indeed, initial studies that have compared regular plasma and adipose tissue-derived EVs/secretome indicate that the latter is more therapeutically active [77, 87]. Further studies are required to understand the impact of adipose tissue type as well as donor characteristics on EV properties. For example, sex hormones are known to impact adipose tissue characteristics and several studies have shown that female adipose tissue displays greater anti-inflammatory properties compared to male adipose tissue [105–108].

## CONCLUSION

In recent years, EVs have gained considerable attention as therapeutic agents due to their involvement in intercellular communication, especially in terms of immunomodulation and tissue repair. However, the field has experienced challenges in clinical translation due to manufacturing and scale-up issues. The vast majority of preclinical and early-stage clinical studies have focused on cell culture-derived EVs. Despite expensive and time-consuming protocols, EV isolation from cell culture usually results in low yields. Isolation of EVs directly from the body is emerging as a promising cost and time-efficient alternative. For example, plasma and adipose tissue-derived EVs can be obtained by TFF in a few hours resulting in much higher yields compared to cell culture-derived EVs. Such EVs have anti-inflammatory and tissue-protective properties that can be further enhanced by loading therapeutic agents in the lipid bilayer or aqueous core. Suitable potency and physicochemical release-criteria that confirm efficacy, safety, and consistency will need to be developed to overcome regulatory challenges related to the complexity and heterogeneity of plasma and lipoaspirate.

## ACKNOWLEDGEMENTS

This work is partially supported by the Mayo Clinic Center for Regenerative Medicine in Florida (JW), USA, the National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA, under award number R21AI152318 (JW), the American Heart Association, USA, under award number 20TPA35490415 (JW), the Italian Ministry of Education, University and Research, Italy, under the national project Programma Operativo Nazionale Ricerca e Innovazione (PON) 2014-2020 (CCI 2014IT16M2OP005) Fondo Sociale Europeo, Azione I.1, Dottorati Innovativi con Caratterizzazione Industriale (DI, JW, LDM), and China Scholarship Council, China (MY). The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies. Figure 3a was made in ©BioRender - biorender.com.

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## Highlights

- Extracellular vesicles (EVs) have immunomodulatory and tissue repair properties
- Scale-up issues limit the therapeutic potential of cell culture-derived EVs
- Plasma and adipose tissue EVs provide a cost and time-efficient alternative
- Therapeutic effects are seen in cardiovascular, skeletal, and metabolic diseases

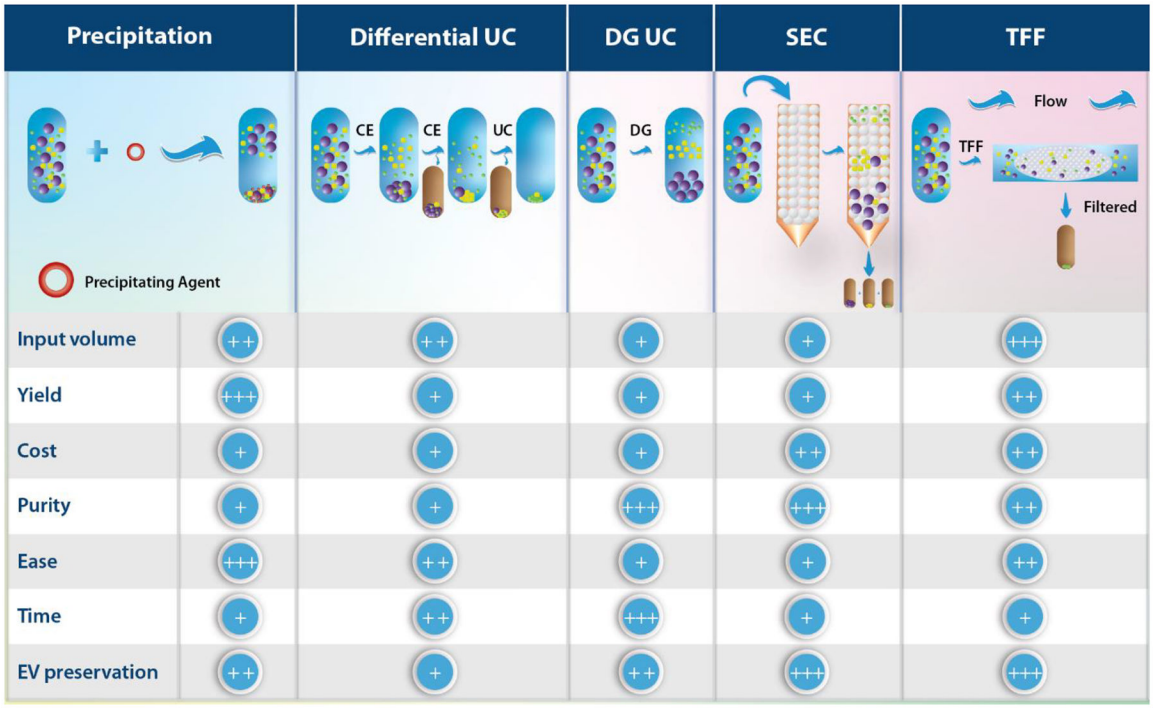


**Figure 1:**

Schematic representation of common extracellular vesicle (EV) isolation methods. Low (+), medium (++), high (+++). CE, centrifuge; DG, density gradient; SEC, size-exclusion chromatography; TFF, tangential flow filtration; UC, ultracentrifugation.

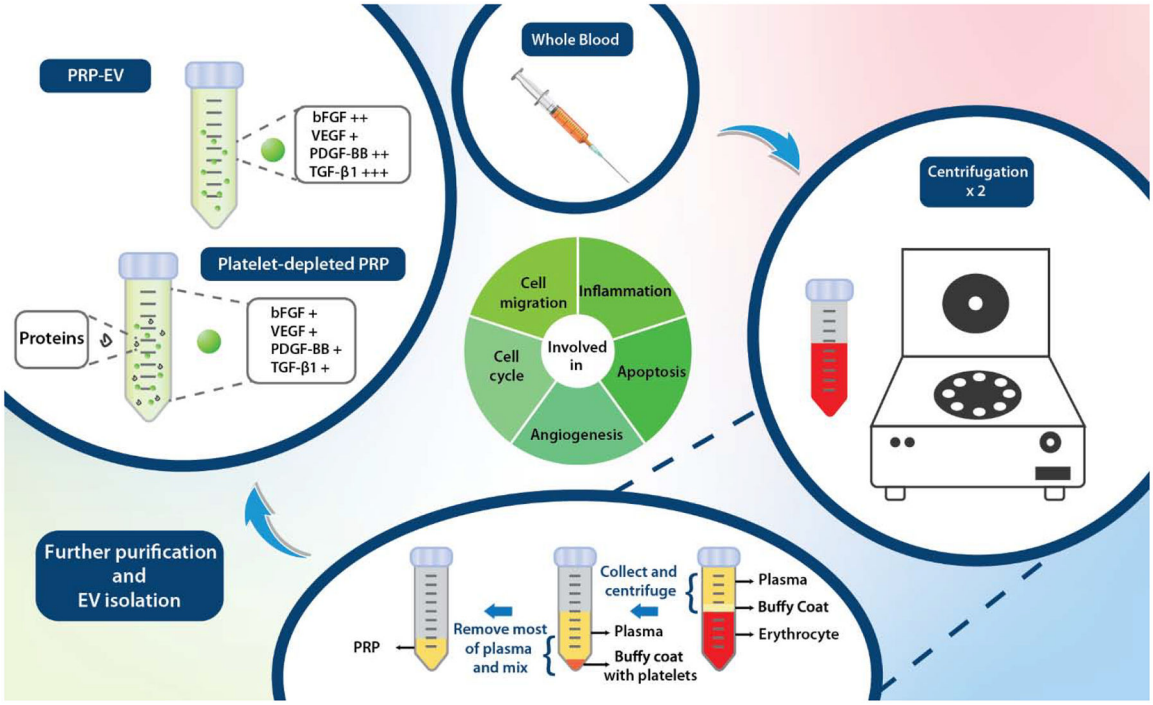


**Figure 2:**

Example of a platelet-rich plasma (PRP) preparation method and comparison of growth factors in platelet-depleted PRP and PRP-derived EVs [52]. bFGF, basic fibroblast growth factor; EVs, extracellular vesicles; PDGF-BB, platelet-derived growth factor BB; TGF-β, transforming growth factor-β, VEGF, vascular endothelial growth factor.

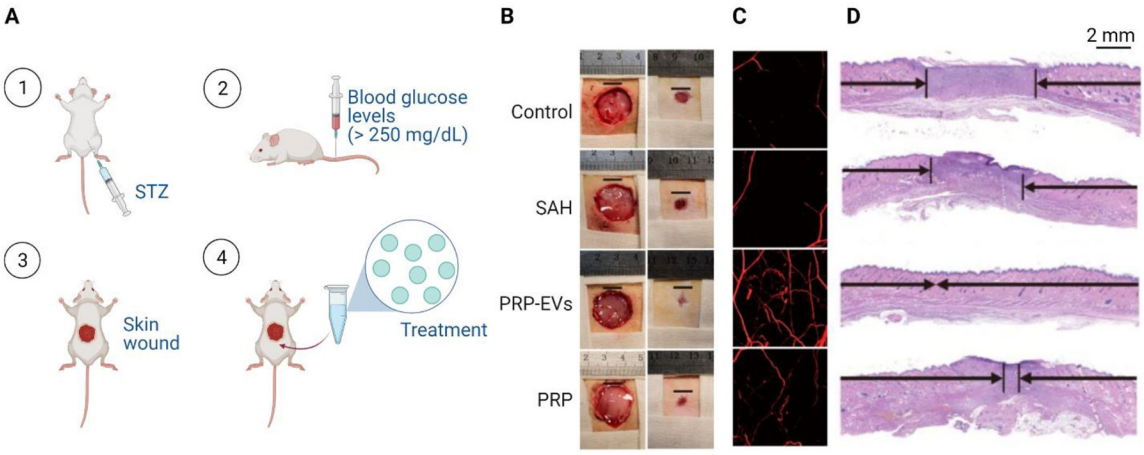


**Figure 3:**

(A) Schematic of chronic cutaneous wounds in a diabetic rat model described in [52]. Diabetes was induced with intraperitoneal administration of streptozotocin (STZ), with glucose levels > 250 mg/dL considered indicative of diabetes. Skin was excised from the back and wound beds were untreated (control) or treated with sodium alginate hydrogel (SAH), activated platelet-depleted PRP in SAH, or PRP-EVs in SAH. (B) Wound repair photos at day 0 (left) and day 14 (right) after skin excision. Scale bar, 1 cm. (C) Three-dimensional reconstructed images of blood vessels in the wounds obtained by micro computed tomography 14 days after skin excision. (D) Hematoxylin and eosin-stained sections of wounds 14 days after skin excision. Adapted with permission from [52].

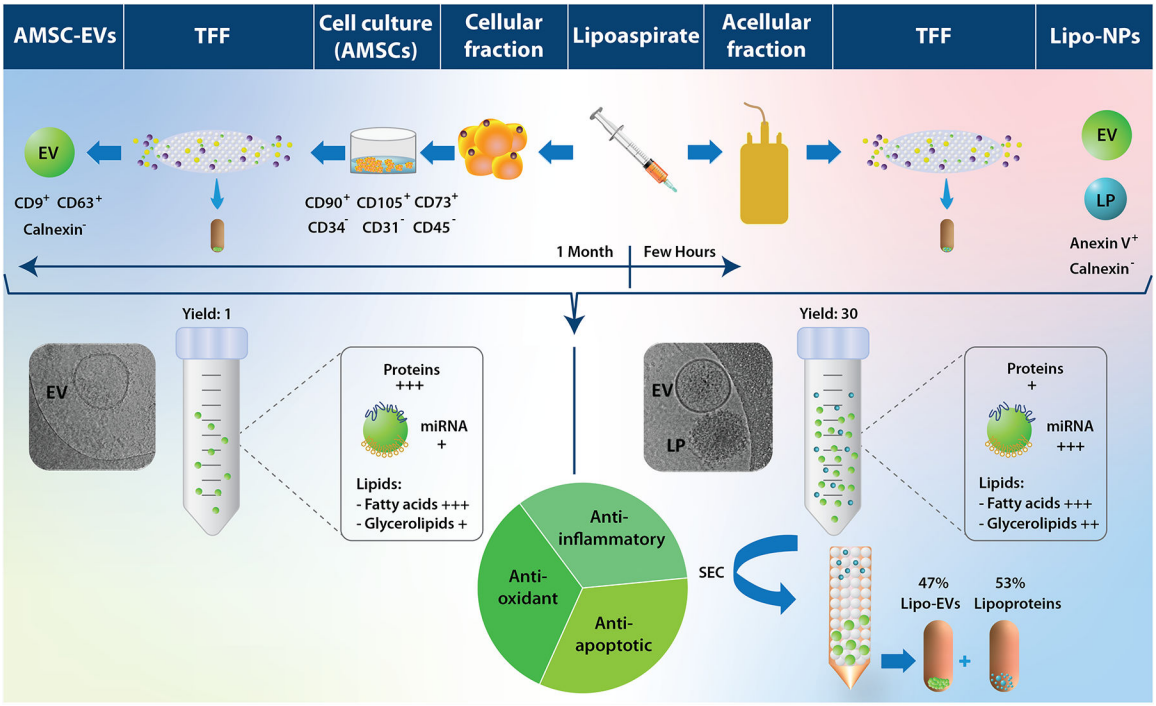


**Figure 4:**

Characteristics of adipose-derived mesenchymal sromal cell (AMSC) EVs and lipoaspirate nanoparticles (Lipo-NPs) isolated by TFF. Lipo-NPs are faster to obtain (few hours) and have higher yields (30-fold) compared to AMSCs-EVs (take one month to obtain). AMSC-EVs and Lipo-NP have similar size and shape (spherical), but the former has higher protein levels and lower glycerolipid and microRNA (miRNA) levels [21]. SEC-based processing of Lipo-NPs can remove lipoproteins (accounting for approximately 53% NPs isolated by TFF). Cryogenic transmission electron microscopy images are reproduced with permission from [21].

**Table 1:**

Comparison of extracellular vesicle (EV) therapeutics derived from cell culture or directly from the body.

| | EV therapeutics from cell culture | EV therapeutics from the body |
|---|---|---|
| Biocompatibility | High | High |
| Cost-efficiency | Low | High |
| Time-efficiency | Low | High |
| Yield | Low | High |
| Homogeneity | High | Low |
| Scale-up potential | Low | High |
| Potential to exploit genetic engineering approaches | High | Low |
| Use of exogenous products | High | Low |
| Presence of contaminants after isolation | Low | High |

**Table 2:**

Examples of plasma-derived EV therapeutics.

| Plasma source (species) | Isolation method | Condition | Experimental set up | Therapeutic effects | Proposed molecular mechanism | Ref |
|---|---|---|---|---|---|---|
| **Regular plasma EVs** | | | | | | |
| Human and rat | Differential ultracentrifugation | Diabetes-related myocardial ischemia-reperfusion injury | Primary rat cardiomyocytes | Improved cell survival during hypoxia and reoxygenation | Activation of extracellular signal-regulated protein kinase 1 and 2 (ERK1/2) and heat shock protein (HSP)27 | [48] |
| Human and rat | Differential ultracentrifugation | Myocardial ischemia-reperfusion injury | Rat model, mouse cardiomyocyte cells (HL-1), primary rat cardiomyocytes | Cardioprotection | EV-contained HSP70-mediated activation of Toll-like receptor 4 (TLR4), ERK1/2, and p38 mitogen-activated protein kinase (p38MAPK), leading to activation of HSP27 | [47] |
| Human | Filtration and centrifugation (details not specified) | Flexor tendon injury | Primary canine tenocytes | Increased proliferation, enhanced collagen deposition, decreased apoptosis | Unknown | [49] |
| Human | Filtration and centrifugation (details not specified) | Tendon injury | *Ex vivo* tendon model | Increased collagen formation, suppression of inflammatory pathways, increased failure strength, and reduction of gap formation | Increased expression of collagen type III alpha 1 chain (COL3A1) and decreased expression of transforming growth factor beta (TGF-β) and interleukin 6 (IL-6) | [50] |
| **Platelet-rich plasma EVs** | | | | | | |
| Human | Not mentioned | Wound | Diabetic rat model | Wound repair (collagen synthesis and angiogenesis) | Unknown | [51] |
| Human | Differential ultracentrifugation | Wound | Diabetic rat model, human microvascular endothelial cells (HMEC-1), primary human dermal fibroblasts | Wound repair (collagen synthesis and angiogenesis), cell proliferation and migration, endothelial cell tubule formation | Activation of hippo/yes-associated protein (YAP) pathway causing fibroblast proliferation and migration and activation of phosphatidylinositol 3-kinase (PI3K)/serine/threonine protein kinase B (PKB/Akt) and ERK1/2 causing endothelial cell proliferation and migration | [52] |
| Human | Differential ultracentrifugation | Osteonecrosis of the femoral head | Rat model, mouse osteoblastic cells (MC3T3-E1), human microvascular endothelial cells (HMEC-1), human bone marrow-derived mesenchymal stromal cells (BMSCs) | Prevention of osteonecrosis, enhanced cell proliferation, decreased apoptosis, increased angiogenesis, and maintenance of osteogenic differentiation upon exposure to glucocorticoid-associated endoplasmic reticulum stress | Activation of Act and EV-contained vascular endothelial growth factor A (VEGF-A)-mediated activation of ERK 1/2 | [53] |
| Rat | Differential ultracentrifugation | Muscle injury | Rat model | Accelerated muscle recovery and increase in centrally nucleated fibers | Increased myogenin | [54] |

| Plasma source (species) | Isolation method | Condition | Experimental set up | Therapeutic effects | Proposed molecular mechanism | Ref |
|---|---|---|---|---|---|---|
| Rabbit | Membrane affinity (exoEasy Maxi Kit) | Osteoarthritis | Rabbit model, primary rabbit chondrocytes | Delayed progression of osteoarthritis, increased cell proliferation, enhanced cell migration, suppression of apoptosis, and decreased inflammation upon exposure to interleukin (IL)-1β | Suppression of tumor necrosis factor-α (TNF-α) β-catenin, Wnt family member 5A (WNT5a), and runt-related transcription factor 2 (RUNX2) | [55] |
| **Umbilical cord plasma EVs** | | | | | | |
| Human | Differential ultracentrifugation | Wound | Mouse model, human skin fibroblasts, and human microvascular endothelial cells (HMEC-1) | Wound repair (accelerated re-epithelialization and angiogenesis), increased fibroblast proliferation and migration, and enhanced endothelial cell tubule formation | EV-contained miR-21-3p-mediated inhibition of phosphatase and tensin homolog (PTEN) and sprouty homolog 1 (SPRY1) | [56] |
| **Ischemic pre-conditioning plasma EVs** | | | | | | |
| Rat | Polymer-based precipitation (ExoQuick) | Myocardial ischemia-reperfusion injury | Rat model and rat cardiomyoblasts (H9c2) | Cardioprotection (suppression of cardiomyocyte apoptosis, reduction of infarct size, and improvement of heart function) and suppression of oxidative stress-mediated apoptosis | EV-contained miR-24-mediated suppression of cleaved caspase-3 and Bcl-2-like 11 (Bim) | [57] |
| Human | Polymer-based precipitation (ExoQuick) | Myocardial ischemia-reperfusion injury | Cardiomyoblasts (H9c2) | Suppression of hypoxia-induced apoptosis | Unknown | [58] |
| **Exercise plasma EVs** | | | | | | |
| Human and rat | Differential ultracentrifugation or polymer-based precipitation (ExoQuick) | Myocardial ischemia-reperfusion injury | Rat model and primary rat cardiomyocytes | Cardioprotection (reduced infarct size, decreased serum levels of lactate dehydrogenase, and increased cardiac function) and suppression of hypoxia/reoxy genation-induced cardiomyocyte apoptosis and lactate dehydrogenase secretion | EV-contained miR-342-5p-mediated inhibition of caspase 9, c-Jun N-terminal kinase 2 (JNK2), protein phosphatase Mg2+/Mn2+ dependent 1F (PPM1F) and activation of Act | [59] |
| **Young plasma EVs** | | | | | | |
| Mice | Differential ultracentrifugation or polymer-based precipitation (Total Exosome Isolation Reagent) | Aging | Mouse model and primary mouse hypothalamic neurons | Anti-aging (improved wheel-running activity and extended lifespan) | EV-contained extracellular nicotinamide phosphoribosyltransferase (eNAMPT)-mediated increase in nicotinamide adenine dinucleotide ($NAD^+$) | [60] |

**Table 3.**

Examples of adipose tissue-derived EV therapeutics.

| Adipose source (species) | Isolation method | Condition | Experimental set up | Therapeutic effects | Propose molecular mechanism | Ref |
|---|---|---|---|---|---|---|
| Human lipoaspirate | Tangential flow filtration | Macrophage-mediated inflammation | Mouse macrophages (Raw 264.7), rat Kupffer cells, primary human macrophages | Anti-inflammatory effects and delivery vehicle for exogenous therapeutic agents | Decreased Toll-like receptor 4-induced secretion of inflammatory cytokines in macrophages | [21] |
| Human lipoaspirate | Dead-end filtration | Skin damage | Primary human melanocytes, keratinocytes, and fibroblasts | Increased cell proliferation, fibroblast migration, anti-oxidative effects, and anti-senescent effects | Increased expression of VEGF, fibronectin, N-chaderin, superoxide dismutase (SOD-2), and catalase expression and decreased expression of senescent-induced insulinlike growth factor-binding protein 5 (IGFBP5), alpha smooth muscle actin (α-SMA), cyclin-dependent kinase inhibitor 1 (p21), and peroxisome proliferator-activated receptor gamma (PPAR-γ) | [87] |
| Mouse adipose tissue culture | Differential centrifugation | Ventilator-induced lung injury | Mouse model and murine pulmonary microvascular endothelial cells (PMVECs) | Reduced pulmonary endothelial barrier hyperpermeability, increase in adherens junctions, and suppression of inflammatory responses | Inhibition of transient receptor potential vanilloid 4 (TRPV4)/Ca2+ pathway and suppression of myeloperoxidase (MPO) activity | [77] |