

REVIEW

Open Access



Targeted delivery of extracellular vesicles: the mechanisms, techniques and therapeutic applications

Shuang Zhao¹, Yunfeng Di¹, Huilan Fan¹, Chengyan Xu¹, Haijing Li¹, Yong Wang^{2,3}, Wei Wang^{3,4}, Chun Li^{3,4,5} and Jingyu Wang^{1*} 

Abstract

Extracellular vesicles (EVs) are cell-derived vesicles with a phospholipid bilayer measuring 50–150 nm in diameter with demonstrated therapeutic potentials. Limitations such as the natural biodistribution (mainly concentrated in the liver and spleen) and short plasma half-life of EVs present significant challenges to their clinical translation. In recent years, growing research indicated that engineered EVs with enhanced targeting to lesion sites have markedly promoted therapeutic efficacy. However, there is a dearth of systematic knowledge on the recent advances in engineering EVs for targeted delivery. Herein, we provide an overview of the targeting mechanisms, engineering techniques, and clinical translations of natural and engineered EVs in therapeutic applications. Enrichment of EVs at lesion sites may be achieved through the recognition of tissue markers, pathological changes, and the circumvention of mononuclear phagocyte system (MPS). Alternatively, external stimuli, including magnetic fields and ultrasound, may also be employed. EV engineering techniques that fulfill targeting functions includes genetic engineering, membrane fusion, chemical modification and physical modification. A comparative statistical analysis was conducted to elucidate the discrepancies between the diverse techniques on size, morphology, stability, targeting and therapeutic efficacy in vitro and in vivo. Additionally, a summary of the registered clinical trials utilizing EVs from 2010 to 2023 has been provided, with a full discussion on the perspectives. This review provides a comprehensive overview of the mechanisms and techniques associated with targeted delivery of EVs in therapeutic applications to advocate further explorations of engineered EVs and accelerate their clinical applications.

Keywords Extracellular vesicles, Exosome, Targeted delivery, Engineered extracellular vesicles, Targeting mechanism, EV engineering technique, Clinical translation

Introduction

Extracellular vesicles (EVs) are cell-derived vesicles with a 50–150 nm diameter, which carry proteins, nucleic acids, lipids and other biomolecules. Endogenous EVs play an important role in the initiation and progression of disease [1–5]. Breast cancer cell-derived EVs have been shown to promote proliferation, migration, epithelial-mesenchymal transition (EMT) and angiogenesis by inducing tumor-associated macrophage polarization [6]. While exogenous EVs have excellent therapeutic potential in the treatment of a variety of diseases (Fig. 1) [7–10]. For the treatment of a plethora of diseases, a multitude of

*Correspondence:

Jingyu Wang
wangjingyu@bucm.edu.cn

¹ College of Traditional Chinese Medicine, Beijing University of Chinese Medicine, Beijing 100029, China

² Dongzhimen Hospital, Beijing University of Chinese Medicine, Beijing 100029, China

³ Key Laboratory of Traditional Chinese Medicine Syndrome and Formula, Ministry of Education, Beijing 100029, China

⁴ State Key Laboratory of Traditional Chinese Medicine Syndrome, Guangzhou University of Chinese Medicine, Guangzhou 510006, China

⁵ Modern Research Center for Traditional Chinese Medicine, Beijing University of Chinese Medicine, Beijing 100029, China

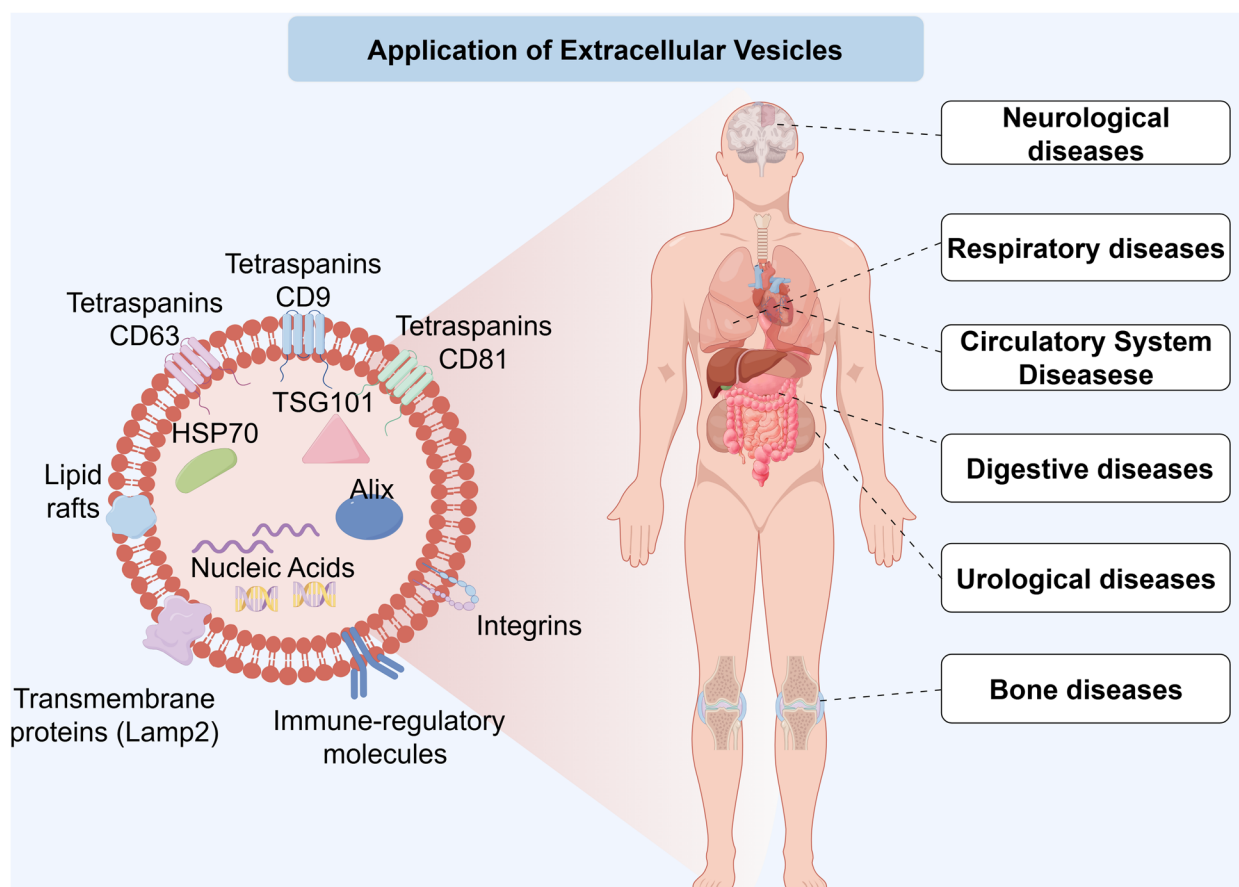


Fig. 1 Overview of engineered EVs applications. Engineered EVs can be used for treating variety of diseases. Elements were obtained from Figdraw

nanoparticles and nanoscale drug delivery vehicles have been developed based on natural and artificial polymers, for example, dextran nanoparticles [11]. However, in comparison to EVs, nanoparticles indicated poor capacity to carry multiple active ingredients in consideration of inner space, which limits their utility for drug delivery. The safety and stability issue of nanoparticles are also a sustaining concern associated with their artificial origin. EVs are of cellular origin with a vesicular structure, exhibit good biocompatibility and delivery ability, and thus display great potential for therapeutic applications with further developments [12–15].

EVs indicated significant advantages in the treatment of neurological disorders by increasing the capability of passing the blood-brain barrier (BBB). The BBB represents a protective physiological obstacle in the treatment of neurological disorders, as exogenous components are limited to traversing this barrier. Natural EVs possess favorable biocompatibility and are capable of crossing the BBB, thereby offering a promising avenue for the treatment of neurological diseases [16]. It has also been demonstrated that targeted modification serves

to augment the therapeutic effects of EVs derived from induced neural stem cells (iNSCs) on inflammatory response, apoptosis and neural repair after spinal cord injury [17]. Moreover, EVs represent a novel therapeutic tool with significant potential for the treatment of cardiovascular disease [18]. Their mechanisms of action encompass a range of processes, including promotion of angiogenesis, inhibition of myocardial fibrosis, reduction of apoptosis, and modulation of immune responses. EVs from hypoxia-preconditioned mesenchymal stem cells have been demonstrated to promote angiogenesis after myocardial infarction [19]. EVs have been observed therapeutic effects via various administration routes. The intravenous administration of EVs, the most often used route, has been demonstrated to facilitate neurological recovery, attenuate inflammation and promote cerebral remodeling following distal middle cerebral artery occlusion in rats [20]. Local injection, for example, intracardiac injection of stem cell-derived EVs has been shown to promote angiogenesis after myocardial infarction [21]. Inhalable stem cell EVs have been demonstrated to facilitate cardiac repair following myocardial infarction [22].

Furthermore, studies have employed hydrogels in conjunction with EVs to facilitate dermal administration of pharmaceuticals to enhance diabetic wound healing [23].

Currently, the clinical translation of EVs is still facing significant challenges. Firstly, most EVs used in clinical trials are derived from conditional cell culture medium and there is a lack of mature technology for large-scale production, which results in low yields and a high cost of EVs [24]. Secondly, the targeting of natural EVs to specific organs is ineffective. Following intravenous injection, natural EVs are primarily accumulated in the liver and spleen, with a half-life in the blood of approximately 10–30 min for most types [25–28]. Finally, the in vivo tracking of EVs remains a challenge, and the lack of clarity on the pharmacokinetics makes it hard to eliminate concerns about potential adverse effects. It is therefore of great urgency to develop engineering technologies for EVs to improve their accumulation at the lesion site [29, 30].

This review presents an overview of the prevailing engineering methods and challenges associated with the development of targeted delivery of EVs. Additionally, further statistical analysis are provided concerning the impact of disparate modification techniques and molecules on targeting and treatment efficacy in the context of ischemic heart disease. Moreover, an overview of the registered clinical trials related to EVs is provided, accompanied by a discussion of their findings on safety. Finally, the article concludes with an overview of potential future developments of applying EVs in targeted delivery from our perspectives.

Targeting mechanisms in EV engineering

Tissue-specific binding or bypassing phagocytosis capacity could facilitate the accumulation of EVs on lesion sites. Tissue-specific binding of EVs could be achieved by modifications of EVs according to tissue markers, pathological changes or physical interactions. In addition, ligands could be added to the EV membrane to decrease mononuclear phagocyte system (MPS) phagocytosis in the liver and spleen, the primary accumulation sites of EVs following oral or intravenous administration, and thereby extend the circulation time [31]. The targeting molecules that have been exploited to deliver EVs or cargo-bearing EVs as potential new therapies in recent publications are reviewed in Fig. 2.

Tissue markers as targets

Markers expressed in the membranes of specific cell types, such as neurons and endothelial cells, have been employed as binding objects in engineered EVs.

Acetylcholine receptors (AChR) are mainly synthesized and released by neuronal cells located in the central

regions of the brain and the kidney [32, 33]. Rabies virus glycoprotein (RVG) peptides have been developed for the selective binding of AChR [34]. In a model of traumatic brain injury, the accumulation of RVG modified EVs in the brain was increased approximately 2.8-fold, as that in the mouse brain of the cerebral ischemia model, Machado-Joseph model, and chronic unpredictable stress model [35–38]. There have also been studies using RVG-EVs to treat renal fibrosis, which resulted in a 1.38-fold increase in EV accumulation in the kidney, thereby improving renal tubulointerstitial fibrosis [39, 40].

Stromal cell-derived factor-1 (SDF-1), also known as chemokine C-X-C motif ligand 12 (CXCL12), is mainly expressed in bone marrow stromal cells and bone marrow endothelial cells and can be recognized by the CXC motif chemokine receptor type 4 (CXCR4) [41]. CXCR4 overexpressing EVs (CXCR4-EVs) derived from *E. coli* or 3T3 cells showed high homing potency to the bone marrow, thus providing a promising avenue for the treatment of bone related diseases. CXCR4-EVs have been used for the treatment of osteoporosis with improved bone retention and efficacy [42–44]. SDF-1 is also highly expressed in inflammatory tissue. CXCR4-EVs have been employed in treating acute myocardial infarction, cerebral ischemia-reperfusion injury, ulcerative colitis, periodontitis, and glioblastomas to target inflammatory lesions and improve treatment efficacy [45–55].

Pathological changes as targets

The pathological alterations observed at the site of a lesion represent a significant source of binding sites for the targeted delivery of EVs. It is frequently observed that pathological tissues exhibit expression changes of specific proteins, including cell adhesion molecules, glycoproteins, growth factor receptors, etc. In response to these changes, peptides and nucleic acids are designed to enable targeted delivery of EVs to lesion sites.

The expression of integrin $\alpha\beta3$, a major component of the integrin family of transmembrane adhesion receptors, is elevated at neovascular sites and in tumors (e.g., osteosarcoma) [56]. Arginine-glycine-aspartic acid (RGD) peptide consists of three amino acids with a linear or cyclic structure and binds specifically to $\alpha\beta3$. Consequently, engineering EVs using RGD improves the targeting of EVs. The cyclic structure of RGD (cyclo(RGDyK)) is more stable and has a higher binding affinity to the integrin receptor [57]. Cyclo(RGDyK) peptide-engineered EVs exhibited superior aggregation to the lesion area, with an 11-fold increase [58]. Other RGD-based peptides, including c(RGD-^DPhe-C) (an RGD peptide with an amino acid Phe in an unnatural D-conformation), iRGD (an RGD peptide with a cryptic CendR motif and cell-penetrating activity) [59], and RGD-4 C (an RGD

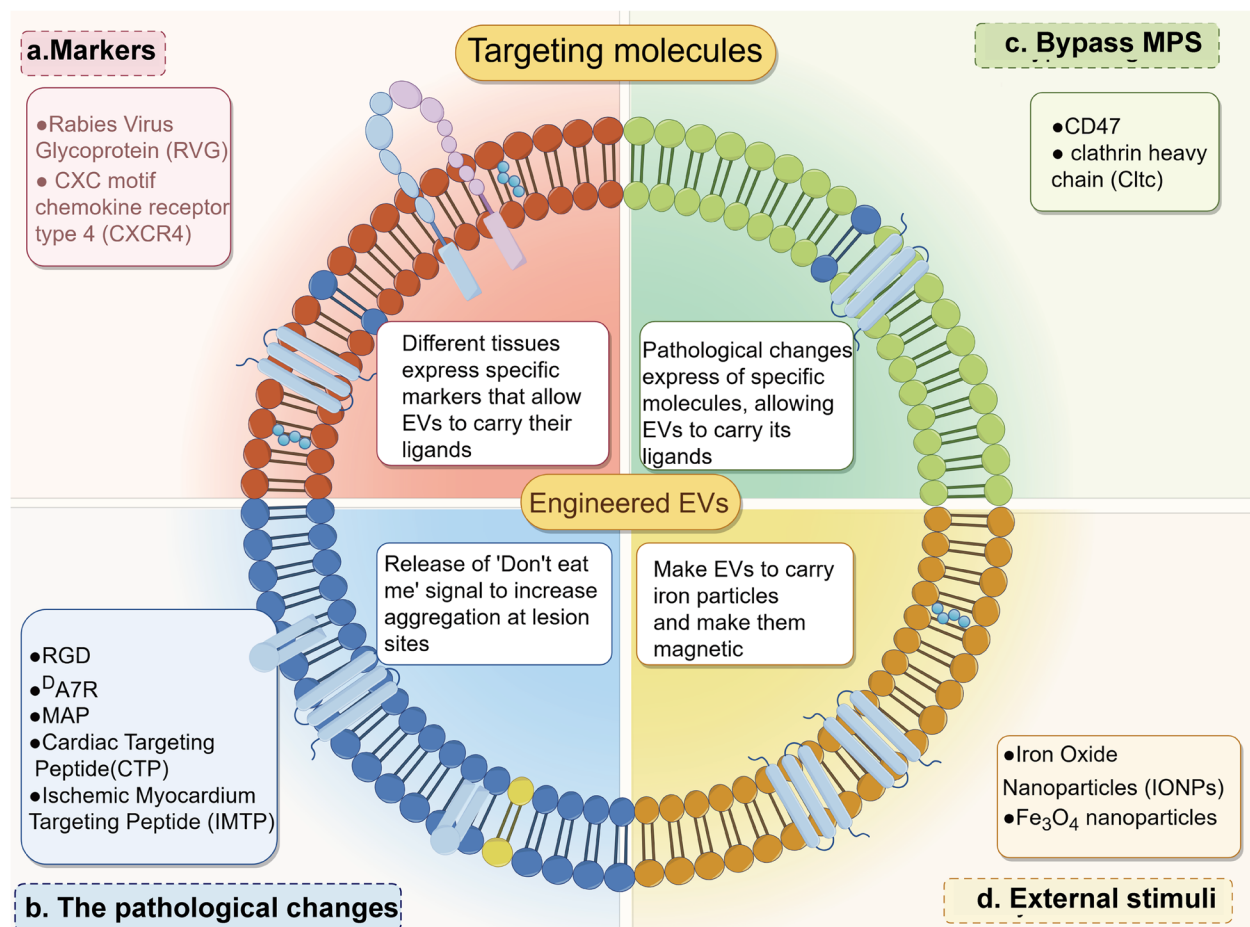


Fig. 2 The classification for targeting molecules. Targeting molecules are classified according to the principles on which they are based, as follows: marker (a), pathological changes (b), bypassing MPS (c) and external stimuli (d). Elements were obtained from Figdraw

peptide with a lactadherin C1C2 domain) [60], have been employed for EV modifications to achieve specific targeting to tumors. The modification of EVs to enable selective targeting represents a further potential avenue for their use as delivery vehicles, with the capacity to transport a range of payloads, including small molecules, nucleic acids and proteins [61]. Bone marrow mesenchymal stem cell (BMSC)-derived EVs modified with an integrin α_5 -targeting peptide were found to enhance the efficacy of therapeutic regimens targeting pancreatic cancer by promoting the reprogramming of cancer-associated fibroblasts (CAFs) while inhibiting the pro-carcinogenic effects [62]. A study utilizing iRGD peptide-modified EVs for targeted delivery of doxorubicin (DOX) demonstrated that the modified DOX@EVs markedly inhibited tumor growth while exhibiting increased biosafety and reduced adverse effects [63].

CD44 is a cell surface adhesion molecule overexpressed on cancer stem cells [64], thus applied in surface modification of Doxorubicin (DOX) loaded EVs for cancer

therapy. Meanwhile, the pH-responsive 3-(diethylamino) propylamine (DEAP) was modified on functional EV for the local release of DOX in response to the acid tumor microenvironment [65–67]. CD44 is also over-expressed in the context of fibrosis while at a relatively low expression level in the normal liver. CD44 could bind specifically to hyaluronic acid (HA), a biocompatible carbohydrate polymer that has been approved by the U.S. Food and Drug Administration (FDA) for use in drug delivery systems. Therefore, HA-engineered EVs facilitate interaction with CD44-specific ligand receptors and targeted delivery of coniferin A, the anti-hepatic fibrosis compound, to fibrotic tissue. The anti-hepatic fibrosis effect of coniferin A was thus enhanced through increased EV accumulation at the lesion site [68].

Glycoprotein P-selectin is a biomarker of ischemic acute kidney injury (AKI) in endothelial cells [69]. Consequently, a P-selectin binding peptide (PBP) was added to the membrane of EVs (PBP-EVs) through hydrophobic interactions. The results demonstrated that PBP-EVs

exhibited a selective targeting tendency to damaged kidneys as visualized using molecular imaging [70]. $^D A7R$ ($^D R^D P^D P^D L^D W^D T^D A$) peptide has a high binding affinity to vascular endothelial growth factor receptor 2 (VEGFR2) and neuropilin-1 (NRP-1) in damaged blood vessels. EVs with $^D A7R$ peptides on the surface showed a 1.57-fold increase in the ischemic cerebral regions after intravenous injection, which improved the differentiation of NSCs into neurons after stroke [58, 71–79].

Some of the targeted peptides are capable of penetrating cellular membranes, hence their designation as cell-penetrating peptides (CPPs), also known as protein transduction structural domains (PTDs). These peptides exhibit cell-penetrating properties as a consequence of the presence of basic amino acids [80]. Furthermore, engineered arginine-rich CPPs on EV membranes leads to activation of the giant cytosolic drinking pathway, and the number of arginine residues in the peptide sequence influences the efficiency of cellular EV uptake [81]. CPPs include those with targeting properties, such as iRGD, MAP, etc., and those without targeting properties, such as Trans-activator of transcription (TAT) peptide [82], which only have membrane-penetrating properties, could be integrated with other targeting peptides without membrane-penetrating properties to reduce the effect of receptor saturation [83].

Metal abstraction peptide (MAP) is a cell-penetrating peptide targeting matrix metalloproteinases (MMP) [84]. MMP-9 levels are elevated in the brain tissue of patients with ischemic and hemorrhagic stroke. Engineered EVs by MAP via hydrophobic interactions (MAP-EV) were developed for ameliorating ischemic stroke. After 12 h of intravenous injection, MAP-EVs were significantly enriched in the ischemic zone than in the control group, and this difference persisted up to 48 h [85, 86]. The dual tumor-penetrating peptides iRGD and tLyp1-modified EVs demonstrated a markedly enhanced uptake of doxorubicin (Dox) by MCF-7 and MDA-MB-231, the breast cancer cell lines, compared to single iRGD or tLyp1-engineered EVs or EVs alone [87].

However, the application of CPP presents a number of challenges, the first of which is toxicity. The toxicity of CPP is primarily contingent upon the peptide concentration, the specific cargo molecule, and the employed coupling strategy [88]. Amphiphilic CPPs tend to exhibit heightened toxicity relative to their cationic counterparts. The second challenge is stability [89]. Extracellular and intracellular CPPs could be degraded by proteases and lose the capacity for delivery. The potential strategy to improve metabolic stability is the end-to-end cyclisation of CPPs [90]. Thirdly, some CPPs have non-specific tissue and cell permeability, which limits the application in vivo [91]. Regarding this issue, CPPs are

integrated with peptides that are designed with tissue-targeting properties for engineering EVs. TAT peptide is able to penetrate the plasma membrane as well as the nuclear envelope of most living cells, while Angiopep-2 is an active target peptide with a high affinity for LRP1 in brain. Combining Ang and TAT, the dual peptide-engineered EVs not only exploited the efficient TAT-mediated cell membrane penetration and Ang-mediated targeting ability against gliomas, but also overcame Ang receptor saturation [92]. The targeting ability of RGD peptides and the cell-penetrating ability of Angiopep-2 peptides engineered EVs have shown that dual-engineered EVs significantly enhance transcellular permeability across the BBB in vivo. The engineered EVs presented targeting to ischemic blood vessels after intravenous administration, and also achieved rapid accumulation in the ischemic lesion area [93].

Besides known markers and pathological changes, Moreover, the targeting capacity could be achieved through peptide screening. The utilization of phage display technology enables the discovery of hitherto targeting peptides before identifying the unknown target peptides. This is achieved by employing target proteins (or target, cells or tissues) as bait, incubating them with a phage peptide library, and subsequently screening out the peptide sequences that interact with the target after rounds of binding, elution and amplification [94]. The resulting peptides have proved their effectiveness for EVs engineering, such as iRGD (internalizing-RGD) [72], CTP (cardiac targeting peptide, peptide sequences: APWHLSSQYSRT) [92, 93, 95, 96], IMTP (ischemic myocardium-targeting peptide, peptide sequences: CST-SMLKAC) [18, 97–104], HHP (heart homing peptide, peptide sequences: CRPPR) [105], CMP (cardiomyocyte specific peptide, peptide sequence: WLSEAGPVVT-VRALRGTSW) [106]. The CTP modification resulted in an approximate 16% improvement in the uptake of EVs by cardiomyocytes, while no significant enhancement was observed in the uptake by other cells. Furthermore, CTP modification resulted in a 2.44-fold increase in the accumulation of EVs in the heart at 24 h post-intravenous injection [96]. Compared to EVs, CTP-engineered EVs have presented a more therapeutics on cardiac repair following viral myocarditis by modulating the immune microenvironment, such as regulating macrophage polarization [107]. Concurrently, IMTP-EV has been observed to stimulate cell proliferation and angiogenesis by enhancing targeting to the infarcted heart, while simultaneously reducing fibrosis and scar formation [104]. HHP engineered EV localized in mouse cardiac tissue via selective binding to cysteine-rich proteoglycans. Consequently, HHP modification resulted in an increase in the accumulation of EVs in the heart [108].

In addition to proteins, nucleic acids can also be used for targeted modifications such as aptamers. Aptamers are oligonucleotides that are capable of binding to multiple ligands with high affinity and specificity, representing a novel class of EVs modification molecules [109]. AS1411 is a stable G-quadruplex-forming DNA sequence that binds to nuciferine (NCL) with high affinity and specificity, acting as a target protein [110]. NCL is overexpressed in the cytoplasm and cell membrane of a variety of tumors. To increase the EV binding to NCL, the 5-terminal carboxylic acid group of the AS1411 aptamer was converted to an amine-responsive NHS ester by EDC/NHS amide-coupled chemistry, which was then coupled to an amine group on the surface of the EVs. This resulted in an increased accumulation of AS1411 adaptor-modified EVs at tumor sites [111, 112].

Bypassing MPS for elongated circulation time

EVs in circulation were eliminated quickly through phagocytosis by the mononuclear phagocyte system (MPS) in the liver or spleen. Reduced phagocytosis and prolonged cycling time of EVs could be achieved by adding protein or peptides, such as CD47, clathrin heavy chain 1 (Cltc) and so on, by releasing the 'don't eat me' signal.

CD47 is an integrin-associated glycoprotein and could be recognized by signal regulatory protein α (SIRP α) of macrophages, reducing phagocytosis in the liver or spleen [113–115]. The membrane expression of CD47 of EVs has prevented their clearance by circulating monocytes [116, 117]. CD47 overexpressed EVs (CD47-EVs) prolong the circulation time of EVs from 30 to 120 min post-injection [118]. In addition, CD47 is highly expressed on cardiac resident macrophages (cMacs), so modification of EVs with cMacs membrane can also increase the immune evasion capacity, allowing for a longer circulation time from 12 to 24 h [119]. Cltc (clathrin heavy chain) has also been shown to play an important role in mediating exocytosis in the liver and spleen. By knocking down Cltc, the endocytosis of MPS into exocytosis in the spleen and liver can be significantly and specifically blocked, thus facilitating the efficient arrival of EVs to other tissues such as the heart. It has been shown in DOX-induced cardiomyopathy (DIC) model that Cltc@CTP-EVs led to a reduction in the internalization of the MPS, resulting in a reduction of 24.64% in the liver and 36.74% in the spleen [120, 121]. In addition to direct modification of EVs to reduce phagocytosis, it is also possible to prolong circulation time by preemptively blocking phagocytic receptors. Commonly used receptors include Phosphatidylserine (PS), Galactose lectin-5 (Gal-5), and Scavenger Receptor Class A family (SR-A). PS exposed to the surface of EVs and recognized by macrophages may also

affect macrophage clearance. Previous studies have found that pretreatment with negatively charged PS liposomes reduced the fluorescence intensity of EVs from 40 to 26% in liver [122]. Gal-5 is a predominantly cytoplasmic fraction of the lectin superfamily, and it has been found that Gal-5 binds to the surface of the EVs of rat reticulocytes. The addition of Gal-5 has reduced vesicle uptake by macrophages [123]. SR-A recognizes a variety of negatively charged ligands on EVs. A using dextran sulfate significantly reduced EVs clearance by mouse liver in vivo, while tumor uptake increased approximately 3-fold over 24 h [124].

External stimuli enabled targeted delivery of EVs

Tissue accumulation of EVs can be achieved not only by the recognition of specific molecules, termed as "intrinsic- stimuli", but also by external stimuli such as magnetism, ultrasound and light. The utilization of magnetism is facilitated by the incorporation of Iron Oxide Nanoparticles (IONPs), which can be enriched at the lesion site in the presence of an external magnetic field [125]. The IONPs are biocompatible and easy to be incorporated into the membrane and inner space of EVs. Using membrane filters and a mini-extruder, IONPs were encapsulated into MSC derived EV mimetics (magnetic EM, M-EM). EMs are EV-like vesicles that are produced through direct artificial manipulation with similar size, morphology, marker (such as tetraspanins CD9, CD63 and CD81) and delivery capacity [126]. The M-EM improved the targeting to ischemic foci by 2.9-fold and a significant enhancement in the treatment efficacy in ischemic stroke [127, 128]. In addition, IONP-engineered EVs have good potential in cancer therapy by enhancing the targeting and sacrifice of tumors. The ionization of IONPs at the acidic tumor microenvironment would release metal ions Fe^{2+} that undergo a Fenton or Fenton-like reaction to convert excessive H_2O_2 to $\cdot\text{OH}$. IONPs@EMs have shown enhanced uptake by ovarian cancer cells in a magnetically guided manner, exhibiting superior tumor targeting and suppression without significant side effects [129, 130].

Ultrasound-targeted microbubble disruption (UTMD) technology represents a promising approach for targeted EV delivery [131]. By emitting ultrasound waves of varying intensities at a specific site, microbubbles in the blood are ruptured, resulting in the release of energy that can increase the permeability of cell membranes and blood vessels. This facilitates the delivery of the therapeutic agent to the desired site, thereby enabling targeted therapy [132]. Utilizing this principle, targeted delivery of EVs was enhanced to heart, liver, spleen and adipose tissue when the EVs were irradiated [133]. The enhanced accumulation of EVs in the heart were achieved after an

injection of EVs in six minutes after UTMD [134]. The focused ultrasound and microbubble technology was utilized to achieve brain-targeted EV delivery by temporarily open the BBB. This approach enables EVs to cross the BBB more efficiently and reach the brain, thereby facilitating the prevention of amyloid beta plaques and providing neuroprotection [135].

Techniques in EV modification

In modifying EVs with various molecules, the most commonly used methods include genetic engineering, membrane fusion chemical modifications, and physical modification. These techniques helped to prolong circulation time and increase targeting by binding peptides or proteins on EVs membranes (Fig. 3).

Genetic engineering

Genetic engineering technology constructed plasmids for fusion proteins comprising EV membrane surface proteins, such as CD63 and Lamp2b (lysosomal

associated membrane protein 2b), in conjunction with specific targeting peptides, including IMTP, CTP, c(RGDyK), RVG and TAT, and ligands such as CXCR4, Angiopep-2(Ang-II) and CD47. Such plasmids are transfected to the parental cells, thereby integrating the targeting capacity to secreted EVs [104, 136, 137].

By genetic engineering, the newly constructed parental cells express fusion proteins stably. However, this method was limited by time-consuming operations with complex procedures, and varied transfection efficiency depending on the parent cell types [138]. In addition, targeting peptides on the membrane surface of engineered EVs may be degraded during EV maturation and secretion. A potential option is adding glycosylation sequences to the plasmid of the fusion protein to prevent degradation [139, 140]. In addition, the biological functions and stability of fusion proteins on EVs require careful experimental examinations *in vitro* and *in vivo* before clinical applications in the face of ethical and safety challenges.

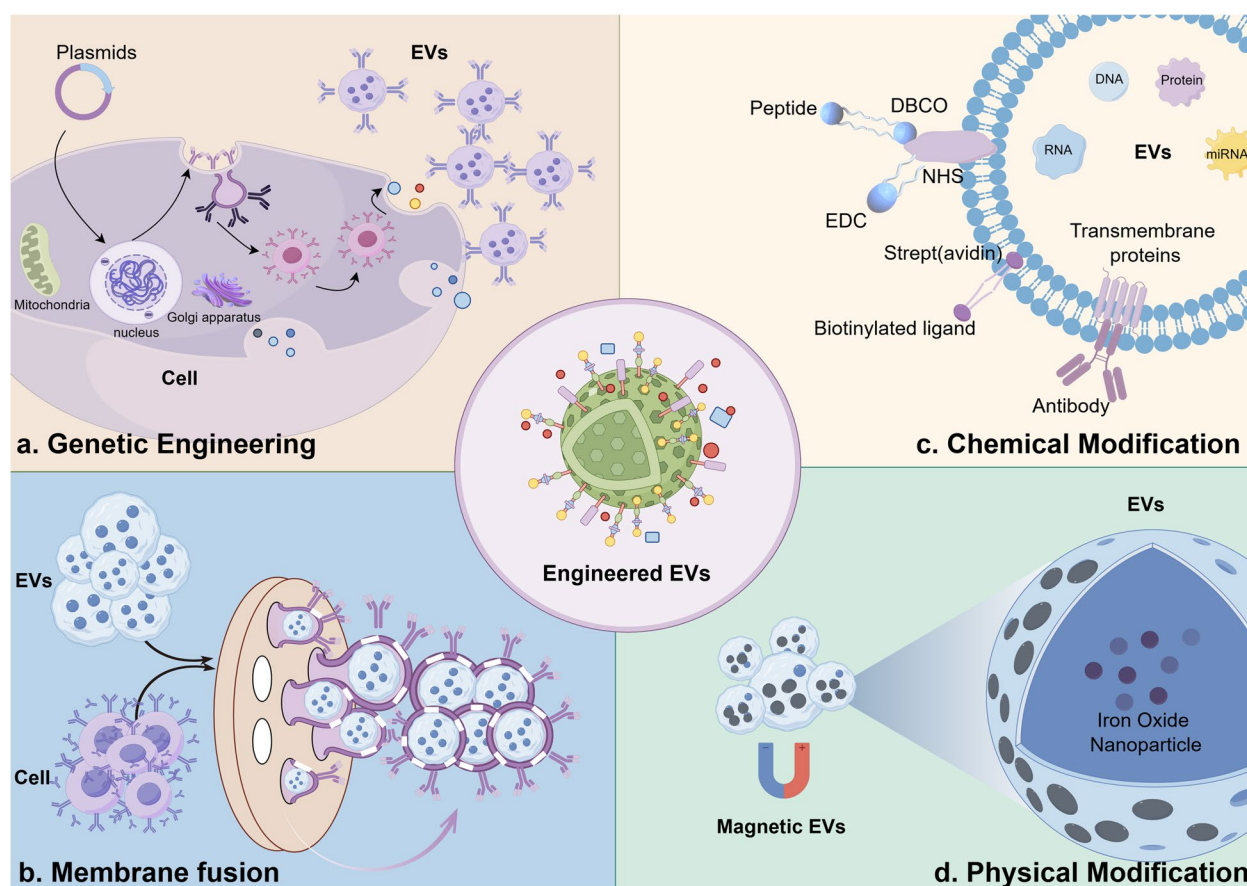


Fig. 3 The classification for engineering techniques. Commonly used engineering methods of EVs include genetic engineering (a), membrane fusion (b), chemical modification (c) and magnetism (d). Elements were obtained from Figdraw

Membrane fusion

Membrane fusion is to fuse the membrane of therapeutic EVs with another cell membrane containing the targeting molecules through the application of extrusion or repeated freeze-thawing. Membrane of platelet and monocyte are most widely used as source of membranes that contain targeting molecules since their natural response to recruitment of the inflammatory tissue [141–147].

Platelets were used to modify MSC derived EVs since their natural targeting properties to atherosclerotic plaques. Platelet membranes modified EVs (P-EVs) aggregated at injured endothelial cells *in vitro*, and atherosclerotic plaques in the aorta or heart *in vivo*, indicating improved atherosclerotic treatment [148–150].

The advantage of membrane fusion is that it is easy to follow and requires only physical manipulation. However, there are also shortcomings. It is difficult to ascertain whether membrane proteins are affected during extrusion, and whether active substances such as miRNA are affected. The membrane fusion is easy to follow with only physical manipulations. However, further research is required to elucidate the optimal methods for maintaining the functionality of cell membranes during the modification of EVs. Given that cell membranes constitute a vital component of living organisms, they are susceptible to a multitude of environmental factors during the modification process, including temperature, the specific reagents employed, and substrate concentration. It is difficult to ascertain the distribution, portion and biological activity of membrane proteins in fusion. The loss of therapeutic contents, such as miRNA, in the process of membrane disruption required further optimizations on the physical manipulations. The development of effective techniques for verifying the success of membrane fusion and quantifying the proportion of each membrane in the synthesized new membrane represents a crucial avenue for further investigation. Moreover, the supply and regulation of mammalian platelets or monocytes as membrane sources in EV engineering also raise concerns in clinical applications [151].

Chemical modification

Chemical modification is connecting the targeting peptide or ligands on the surface of EVs membranes to enhance their targeting properties by click chemistry reactions, copper-free click chemistry reactions, carbodiimide chemistry, antigen and antibody interactions, and biotin-avidin binding.

Click chemistry reactions are based on copper-catalyzed azide-alkyne cyclisation to bind the target peptide or ligand to the EV surface [152]. Click chemistry reaction is used to bind glioma-specific ligand RGERPPR

peptide (RGE) on the surface of EVs, improving the targeting of EVs to gliomas [153]. Canonical click chemistry is catalyzed by copper ions. However, the copper ions are cytotoxic and the resident ions in the system may bring risks in clinical applications [154]. Thus copper-free click chemistry was developed using the reactive dibenzylcyclooctyne (DBCO) with amine-containing molecules on the membrane of EVs (DBCO-EVs). Peptides comprising an azide group undergo a click-chemical reaction with the cyclooctyne on DBCO, thereby being attached to the surface of EVs [155]. The copper-free click chemistry reaction was utilized to link c(RGDyK) [58, 156], ¹²⁵I-A7R [79], and CTP [157] peptides to the surface of EVs. The enrichment of modified EVs at lesion sites increased by approximately 3-fold, 1.58-fold and 2-fold.

The carbodiimide chemistry is also commonly used for engineered EVs using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide (EDC/NHS) to covalently couple the target to amine-containing molecules on the EVs surface. AS1411 aptamer-engineered EVs using EDC/NHS displayed the highest fluorescence intensity at tumor sites after 48 h, while the fluorescence of natural EVs decreased to near-baseline levels [111, 158]. Similarly, LJM-3064 aptamer was linked to MSC derived EVs through EDC/NHS for improved treatment of multiple sclerosis.

Biotin-avidin exhibits high affinity between proteins and ligands, enabling the maintenance of interactions under harsh conditions *in vivo*, including low pH, high temperature and enzymatic degradation [159]. Studies have employed the phospholipids of EVs to combine with biotin to generate bio-EVs, in addition to the synthesis of biotin-modified GelMA (BioGelMA). Furthermore, affinities have been added to bio-EVs and BioGelMA to obtain complexes, which has resulted in the most potent sustained-release efficacy of the EVs and prolonged the duration of action of the EVs up to 28 days [160].

Chemical modification represents a direct alteration of the EVs membrane, a process that is relatively straightforward and requires a modest investment of time. For instance, the copper-free click chemistry method necessitates about 12–16 h [102, 156], with minimal impact on the contents in comparison to membrane fusion. Moreover, the advantages of click chemistry include high specificity and good compatibility of reaction solvents, as well as the ability to be performed in a wide range of solvents. Copper-free click chemistry is highly biocompatible, exhibiting no significant cytotoxicity [161], and is not limited by the type of parental cell of EVs. However, it should be noted that the chemical modification method utilizes specific chemical reactions such as amide bond formation and click chemistry to link the target molecules to proteins or lipids on the EVs membrane [162].

It is possible that this approach may have an impact on the natural structure and function of EVs. In addition, detailed characterization and functional verification of engineered EVs are required to ensure their safety and efficacy.

Physical modification

The targeting of EVs may be enhanced by utilizing physical interactions, including magnetic attraction, hydrophobic interactions, or electrostatic interactions.

Magnetism targeting can be achieved by loading the EV with iron oxide nanoparticles. The loading of nanoparticles can be achieved through two main methods: extrusion and ligand-receptor interactions of transferrin. Extrusion is a frequently employed technique for the loading of iron nanoparticles. This is achieved through the utilization of a polycarbonate membrane filter with pores at nano sizes, which enables the disruption of cellular membranes at varying sizes. Extrusion facilitates the loading of iron particles into the EV, thereby response to magnetic field and achieve targeted delivery [163, 164].

The transferrin receptor (TfR) is a membrane protein that is highly abundant in EVs. The COO⁻ groups of carboxylated arboxylated chitosan (CS) could react with the NH₃⁺ groups of transferrin (Tf) to produce amido bonds in the presence of EDC and NHS sodium salt to produce Tf-SPIONs. SPION can be attached to the surface of EVs via a process known as transferrin-transferrin receptor interactions. In the presence of a magnetic field, the engineered EVs can target the lesion sites as indicated by the increased fluorescence intensity of EVs in pancreatic islets with approximately 9-fold at 10 min and 20-fold at 30 min [165].

Hydrophobicity can be employed to anchor the targeting molecules with the EV transmembrane. Since the targeting molecules assume a transmembrane α -helical configuration within the phosphatidylcholine bilayer which permits them to migrate towards the membrane surface and enter the aqueous phase, thus enabling hydrophobic insertion [166–169]. DOPE (1,2-dioleoyl-sn-glycerol-3-phosphoethanolamine) is a helper phospholipid which enhances the stability of liposomes and their capacity to merge cellular membranes [170]. It can be inserted into the surface of EV membranes through hydrophobic interactions. Furthermore, DOPE has the potential to bind to target molecules, subsequently attaching targeting molecules to the surface of EVs. The modification of MSC-derived EVs (RVG-EVs) with CNS-specific rabies virus glycoprotein (RVG) peptides, based on the aforementioned principle, resulted in improved targeting of EVs to the Alzheimer's disease (AD) mouse cortex and hippocampus. This further enhanced the therapeutic effects of EVs on learning and memory, while

significantly reducing plaque deposition and A β levels [171]. This principle was employed to couple IMTP to EVs, thereby demonstrating that the targeting of IMTP-EVs to the heart can also be enhanced [98, 172]. The IL4RPep-1 peptide, which targets the IL4R, was attached to the surface of EVs via hydrophobic interactions. The findings suggest that IL4RPep-1-EVs enhance the capacity to target tumors [173]. Electrostatic interaction can be employed for the targeted modification of EVs. The decrease of surface charge of citroconjugated EVs (cit-EVs) was observed from the normal -15 mV to -50 mV. In the absence of serum proteins, EVs with a high negative charge are more likely to be taken up by macrophages [174].

Characterization of engineered EVs

The impact of distinct engineered methods on EVs was assessed in four key areas: size, morphology, stability, targeting and therapeutic efficacy. Subsequently, a statistical analysis was conducted on 30 research articles on the engineering of EVs for the treatment of neurological and cardiovascular disorders.

Size

Size of EVs were usually analyzed using nanoscale flow cytometry, atomic force microscopy (AFM), dynamic light scattering (DLS), electron microscopy, nanoparticle tracking analysis (NTA), and single-particle interferometric reflectance imaging sensor [175]. The impact of disparate modification techniques on the size of EVs was evaluated through statistical analysis, which revealed no statistically significant differences. The small variation coefficient (CV) value showed that genetically engineered EVs were more stable among publications (Table 1). However, a wide range of EV sizes was observed across publications, from 79.78 to 162.7 nm. This may be due to the variation of EV isolation, measuring principles and instruments, which requires full consideration of EV size detection (Fig. 4a). The MISEV2023 guidelines indicate that EVs are typically defined as nano- to micrometer-sized particles confined in lipid bilayers. The size range of these particles is typically between 30 and 150 nm. However, it should be noted that the size of EVs may vary depending on the cell type of origin and the biological conditions under which they are produced [176].

Morphology

Mostly, EV morphology was imaged using transmission electron microscopy (TEM) and AFM in publications to visualize the appearance of EVs and E-EVs (Fig. 4a). TEM revealed two dimensional images in nanoscale, and the machine is more accessible than AFM.

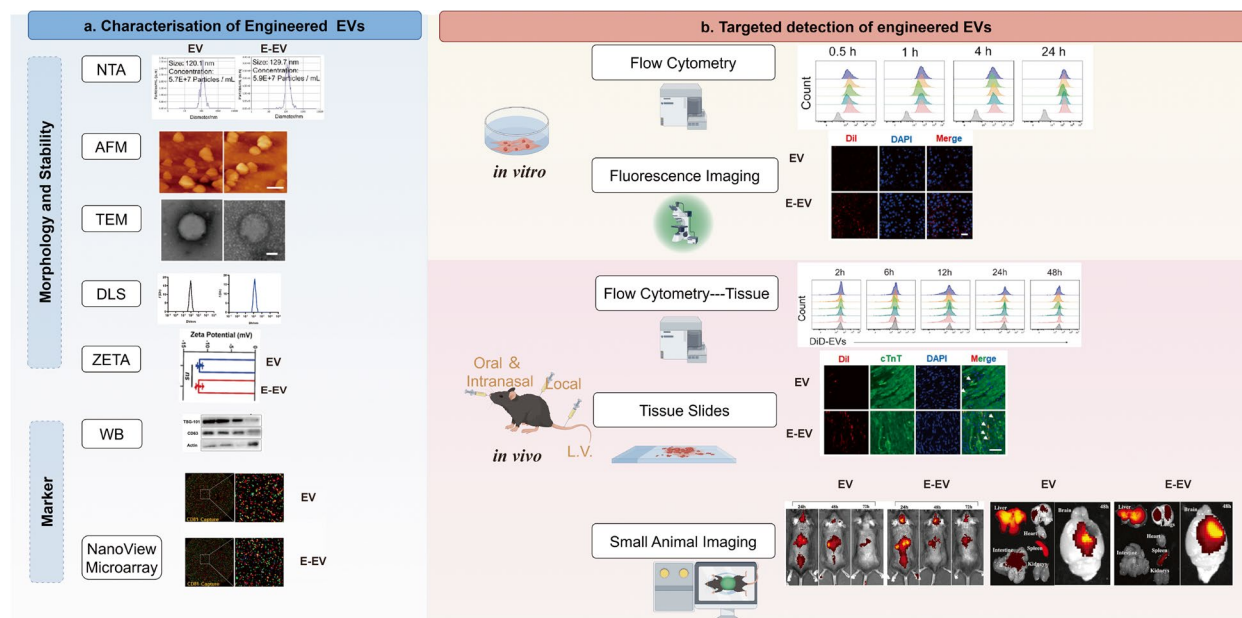


Fig. 4 Methods for characterizing engineered EVs. Characterizations are divided into two distinct categories: EVs characterization and targeted characterization. EVs characterization includes morphological (NTA, AFM, TEM, DLS,), stability (ZETA) and protein (WB, Nanoview microarray) characterization (a). Targeted characterization including in vitro (flow cytometry, fluorescence imaging) and in vivo (flow cytometry, tissue slides staining and small animal imaging) (b). Copyright Permission were obtained from reference [38, 107, 121]

Table 1 Size of Engineered EVs

Article	EV Modification	EV/nm	E-EV/nm	E-EV/EV	CV
Kang, J.Y [96]	Genetic engineering—CTP	162.70	157.30	0.97	0.03
Wang, X [104]	Genetic engineering—IMTP	134.00	135.00	1.01	
Mao, L [108]	Genetic engineering—HHP	103.00	101.00	0.98	
Li, Q [141]	Membrane fusion—platelet membrane	138.80	139.90	1.01	0.12
Hu, S [142]		79.78	114.70	1.44	
Li, Q [143]		117.50	129.20	1.10	
Zhang, N [144]	Membrane fusion—monocyte membrane	92.76	109.76	1.18	
Jiang, J [145]	Membrane fusion—Platelet membrane	91.10	111.40	1.22	
Meng, W.T [101]	Michael addition reaction—IMTP	142.00	169.00	1.19	0.16
Zhu, L.P [102]	Copper-free click chemistry reactions—IMTP	126.00	139.00	1.10	
Ruan, H [79]	Copper-free click chemistry reactions —DA7R and SDF-1	120.00	120.00	1.00	
Zhang, H [73]	Copper-free click chemistry reactions —c(RGDyK)	115.00	135.00	1.17	
Tian, T [58]	Copper click chemical modification—c(RGDyK)	92.00	131.00	1.42	
Chen, M [121]	Hydrophobic fractions—CTP	129.00	124.50	0.97	
Guo, L [177]	Carbodiimide method	135.10	137.50	1.02	
Kim, H.Y [128]	Magnetism—IIONP	168.30	194.20	1.15	-

1. E-EV Engineered EV

Images from an AFM represent data in 3D so that it is possible to measure the height of the nano vesicles

quantitatively. Both TEM and AFM images revealed that the modification made no significant changes on

the morphology of E-EVs, which were typical round cup-shaped structures.

Stability

The stability characterization of engineered EVs can be evaluated through the composition of proteins and nucleic acids, the zeta potential and particle number (Fig. 4a). The expression of the EV markers, including transmembrane or lipid-binding proteins (e.g., CD9, CD63, CD81, etc.) and cytoplasmic proteins (e.g., TSG101, Alix, etc.) were characterized using WB and NanoView microarray methods. The RNA of EVs plays a pivotal role in the functionality of EVs, thus offering a promising avenue for detecting the stability of EVs. The total RNA content is usually purified and quantified using Ultraviolet–visible spectroscopy (UV-Vis) spectroscopy, while a specific mRNA or miRNA could be quantified using PCR [178]. In addition to protein and nucleic acid characterization of stability, zeta potential analysis can also detect vesicle stability [179]. The zeta value is typically negative (Fig. 4a).

The stability of EVs is contingent upon a number of factors, including buffer concentration and pH value [180]. It has been demonstrated that the solution has a considerable effect on the storage of EVs, and that PBS is unsuitable for the storage. A comparison of EVs stored in PBS at +4 °C, −20 °C and −80 °C demonstrated a progressive decline in particle concentration, accompanied by a notable reduction in RNA levels, which decreased to approximately 50% of the initial value at +4 °C. EVs in human albumin and trehalose to phosphate-buffered saline (PBS-HAT) was found to be 3.5 times higher than PBS, which observed by imaging flow cytometry (IFCM) [181].

Targeting efficiency

Targeted detection was conducted in vitro and in vivo (Fig. 4b). Targeting specificity of engineered EVs were detected in vitro using flow cytometry or fluorescence imaging in examination of their uptake by targeted cells. Fluorescence imaging allows for better in situ observation. In comparison to fluorescence imaging, flow cytometry offers a more quantitative and intuitive approach to visualizing the uptake of EVs by specific cells. Co-culture of multiple kinds of cell enables a more accurate evaluation of the targeting of specific cells in comparison with that in cell culture with mono kind [182]. Targeting of engineered EV in vivo was identified through small animal imaging systems, fluorescence imaging of tissue sections, or enzymatic digestion of tissues to obtain single cell suspensions for flow cytometry fluorescence detection [107]. Small animal imaging systems allows for direct continuous detection of living subjects, but

has low resolution and requires highly fluorescent dyes, and individual organs are usually separated to assess the change in fluorescence intensity of each organ for a more accurate and intuitive assessment of the targeting effect [33, 44, 125]. Tissue sections intended for fluorescence imaging detection are characterized by high resolution, but suffer from a lack of continuous observation potential and susceptible to fluorescence quenching during the preparation process. While enzymatic tissue lysis for flow cytometry can quantify fluorescence, the process of lysing cells into single cells may result in a loss of fluorescence signal and is more complicated to perform.

The labelling of EVs with fluorescent dye facilitates the tracking of EV distribution. Fluorescent dyes are available in a range of labelling types, including fluorescent, bioluminescent, radioisotope labelling and so on. Fluorescent dyes such as PKH, MemBright, DiI, DiO, DiR [183]. Lipid fluorescent dye is relatively simple and straight forward, however, it is susceptible to fluorescence quenching and photobleaching, and it lacks specific labelling [184]. Radiotracing is the most accurate method for tracking EVs, enabling the comprehensive and quantitative assessment of their biodistribution, including the analysis of pharmacokinetic processes. One study has shown that the distribution of labelling methods may vary from different label. Consequently, it is essential to exercise caution when selecting an appropriate labelling method. They assessed the impact of five distinct optical and nuclear tracers on the biodistribution of EVs in vivo, including lipid fluorescent dye DiR, the radioisotope ¹¹¹indium-DTPA, and either fluorescent proteins fused to the EV marker CD63 (mCherry) or bioluminescent proteins (firefly and NanoLuc luciferase) to label Expi293F EVs. The study revealed that ¹¹¹In and DiR were the most sensitive tracers for in vivo imaging, providing the most accurate quantification of vesicle biodistribution by ex vivo organ imaging and tissue lysate analysis. Furthermore, the investigation compared EVs derived from the same cellular sources and administered via the same route and dose to homozygous BALB/c mice, with the aim of specifically studying the impact of the tracers. It was observed that the genetic modification of EVs for tracing purposes, in this case through the fusion of NanoLuc to CD63, resulted in a notable alteration in their biodistribution, with a significant accumulation in the lungs [26].

In order to assess the targeting efficacy of the various modification methods, the fluorescence intensity ratio was quantified before and after the engineering. One-way ANOVA was conducted to analyze the impact of the four modification techniques, namely genetic engineering, membrane fusion, chemical modification and magnetism, on targeting. The results demonstrated that these modification methods did not have a significant effect on

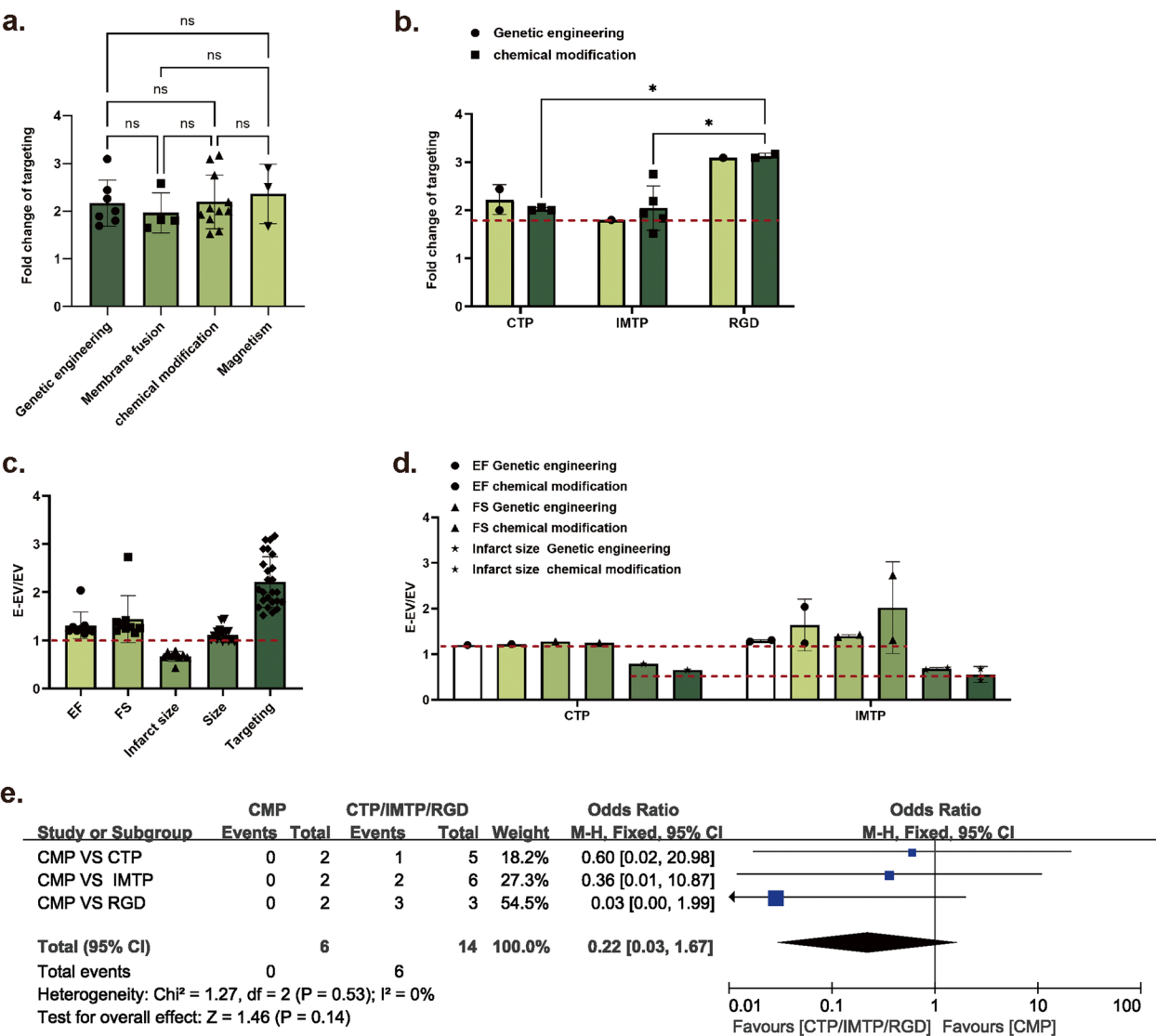


Fig. 5 Effect of different engineering methods and molecules on targeting. **a** Effect of different engineering methods on targeting for the same targeting molecule (CTP). **b** Effect of different targeting molecules on targeting. Original data of a and b were obtained from Table 2. **c** Engineered effects on Cardiac Function (EF, FS), Infarct Size, Size of EV and Targeting. **d** Different peptide on therapeutic effect. Original data of c and d were obtained from Table 3. **a-d** were drawn by GraphPad Prism 9 and analyzed by One-way ANOVA. **e** Forest plot for the correlation between cardiac targeting modification molecules and targeting efficiency. Original data of e were obtained from Table 2. **e** was drawn by Review Manager 5.4 and analyzed by dichotomous variables

the targeting (Fig. 5a). In addition, there was also no statistically significant difference in the impact of different engineering methods based on the modified molecules. However, the targeting molecules were found to be significantly affected by the type of modification molecules (Fig. 5b). Furthermore, additional mean values were calculated for the targeting fold change, with values over the mean representing the effective time and values below the mean defined as the ineffective time. All studies modified with this peptide was counted as total events.

Statistical analyses were conducted using dichotomous variables, and the forest plot results demonstrated differences in the targeting effect of the three cardiac-targeting peptides. It was concluded that RGD, IMTP and CTP were more effective than CMP in targeting. Consequently, greater emphasis should be placed on targeting molecules (Fig. 5e). Subsequent statistical analyses were conducted to evaluate the consistency of the diverse modification techniques, as reflected by the coefficient of variation. The results demonstrated that there were

Table 2 Targeting efficiency of engineered EVs

Article	EV Modification	Measurement	E-EV/EV	Time/h	CV
Kang, J.Y [96] ^a	Genetic engineering—CTP	Small animal imaging	2.44	24	0.22
Kim, H [185]			2.00	24	
Wang, X [104] ^a			1.80	72	
Mentkowski, K.I. [106]	Genetic engineering—CMP		1.69	24	
Mentkowski, K.I. [186]			2.26	2	
Mao, L [108] ^a	Genetic engineering—HHP		1.89	24	
Tian, T [77] ^a	Genetic engineering—RGD-4Cpeptide		3.09	24	
Li, Q [141] ^a	Membrane fusion—platelet membrane		1.80	24	0.21
Hu, S [142] ^a			1.82	72	
Li, Q [143] ^a			2.58	72	
Zhang, N [144]	Membrane fusion—monocyte membrane		1.65	2	
Zhu, L.P [102] ^a	Copper-free click chemistry reactions—IMTP	In vivo fluoresce imaging	1.83	24	0.26
Meng, W.T [101] ^a	Michael addition reaction—IMTP	Small animal imaging	2.79	24	
Mun, D [93] ^a	Copper-free click chemistry reactions—CTP		2.00	24	
Ruan, H [79] ^a	Copper-free click chemistry reactions—DA7R and SDF-1		1.58	2	
Zhang, H [73] ^a	Copper-free click chemistry reactions—c(RGDyK)		3.09	6	
Tian, T [58] ^a	Copper click chemical modification—c(RGDyK)		3.17	6	
Vandergriff, A. [98] ^a	Hydrophobic fractions—IMTP	Fluorescence imaging of Cardiac slices	1.52	24	
Lee, J.R. [100] ^a	Copper-free click chemistry reactions—IMTP	In vivo fluoresce imaging	2.26	24	
Kang, J.Y [157] ^a	Copper-free click chemistry reactions—CTP	Small animal imaging	2.00	24	
Chen, M [121]	Hydrophobic fractions—CTP		2.06	6	
Zhang, J. [18] ^a	Covalent modification—IMTP		2.90	24	
Kim, H.Y [128] ^a	Magnetism—IONP		2.90	24	0.26
Lee, J.R [164] ^a	Magnetism—IONP	In vivo imaging	1.68	24	
Liu, S [187] ^a	Magnetic -Antibody—Fe3O4@SiO2-PEG-CHO and MLC, CD63	ex vivo fluorescence imaging	2.50	0.17	

1. E-EV Engineered EV

2. ^ano data in the original research article, extraction of data by Origin 2021 software

notable discrepancies in the modification techniques employed by different researchers, encompassing genetic engineering, chemical modification, membrane extrusion, and magnetic (Table 2).

Therapeutic efficiency

Engineered EVs, whether genetic engineering, chemical modification, membrane fusion, or physical, have the highly promising therapeutic for a range of diseases, including cancer, neurological disorders, and cardiovascular diseases. In this review, treatment efficacy was analyzed as a representative of acute myocardial infarction, with the main analyses being cardiac function and infarct size.

Cardiac function was primarily assessed in terms of left ventricular ejection fraction (EF) and left ventricular shortening fraction (FS). Due to the considerable discrepancy in the values observed across different studies, we sought to eliminate this discrepancy by comparing the values of engineered EVs (E-EVs) after treatment with those of natural EVs. The approach with $EF_{E-EV}/$

$EF_{EV} > 1$, and $FS_{E-EV}/FS_{EV} > 1$ was assigned as an effective treatment. While in analyzing the infarct size (IS), the approach with a ratio of $IS_{E-EV}/IS_{EV} < 1$ indicates effective treatment. The statistical results demonstrated that E-EVs exhibited remarkable efficacy in enhancing cardiac function and reducing myocardial infarct size. Additional statistical analyses of the impact of modifying molecules on the therapeutic efficacy demonstrated that the same modifying molecule did not exert a notable influence on the therapeutic efficacy (Table 3; Fig. 5c, d). Furthermore, studies have demonstrated that there was no significant toxicity on other organs by hematoxylin and eosin and enzyme-linked immunosorbent assay, which substantiates the safety of engineered EVs [185, 188].

Besides treatment of cardiovascular diseases. Engineered EVs can also be recognized as an optimal drug delivery vehicle, particularly for nucleic acids, compounds and drugs with poor water solubility, poor biocompatibility. EVs engineered by Her2, a specific tumor homing peptide, and loaded with miR-21i or 5-FU exhibited a notable effect when used in conjunction with the

Table 3 Therapeutic efficiency of engineered EVs

Article	EV Modification	Ejection Fractions (EF)			Fraction Shorting (FS)			Infarct size (IS)		
		E-EV/%	EV/%	E-EV/EV	E-EV/%	EV %	E-EV/EV	Measure	E-EV/%	EV %
Kang, J.Y. [96]	Genetic engineering—CTP	53.65	44.64	1.20	30.7	23.91	1.28	TTC	30.20	38.25
Wang, X [104]	Genetic engineering—IMTP	54.28	41.48	1.31	24.74	17.97	1.38	Masson	31.80	47.90
Wang, Y [99]		50.76	39.58	1.28	36.15	25.37	1.42	Masson	30.14	42.83
Hu, S [142]	Membrane fusion	56.22	49.41	1.14	29.54	24.59	1.20	Masson	16.29	25.08
Li, Q. [143]		55.54	46.80	1.19	29.6	25.51	1.16	Masson	16.20	21.23
Li, Q [141]		52.88	44.62	1.20	29.15	23.22	1.26	Masson	14.10	20.10
Zhu, L.P [102]	Copper-free click chemistry reactions —IMTP	47.67	38.33	1.24	32.33	24.67	1.31	Masson	8.14	18.83
Lee, J.R. [100]a		26.70	54.58	2.04	7.70	21.01	2.73	Masson	20.69	30.24
Mun, D [93]a	Copper-free click chemistry reactions —CTP	42.18	51.33	1.22	20.12	25.19	1.25	Masson	21.82	33.38

1. E-EV Engineered EV
2. a:no data in the original research article, extraction of data byOrigin 2021 software

respective monotherapy. This was achieved by the reversal of drug resistance and the considerable enhancement of cytotoxicity in 5-FU-resistant colon cancer cells [189]. Engineered EVs with a bone-targeting peptide (AspSerSer, DSS)₆ by means of click chemistry and then loaded them with SRT2104, a SIRT1 (silent mating-type information regulation 2 homologue 1) agonist, and the MRI contrast agent MnB NPs, utilizing electroporation. This demonstrated that the engineered EVs have excellent bone repair properties, which inhibit bone resorption and stimulate bone formation [190]. Engineered EVs have also been conducted in the delivery of compounds. In the middle cerebral artery occlusion/reperfusion model, the expression of GAP43 was increased in damaged neurons in the ischemic penumbra. Consequently, the EVs were modified with a monoclonal antibody to GAP43 (mAb GAP43), enabling the mAb GAP43-EVs to be targeted to damaged neurons with a high expression of GAP43 in the ischemic penumbra. This was subsequently employed for the targeted delivery of quercetin, a chemical found naturally in a number of foods. The findings demonstrated that the targeted delivery of mAb GAP43-EVs augmented the neuroprotective impact of quercetin [177].

Besides nucleic acids, compounds and drugs, which have proved their efficiency, new pathological findings could also be applied in developing EV-based therapy. A study employed circSCMH1 screening from the plasma of subjects diagnosed with acute ischemic stroke through the utilization of circRNA microarray. The findings indicated a notable decrease in plasma levels of circSCMH1 among stroke patients relative to the control cohort. Furthermore, this reduction was identified as exhibiting a significant correlation with the predictive capability of stroke outcomes. Based on this finding, researchers achieved targeted delivery of circSCMH1 to the brain

using RVG-engineered EVs. This modified EVs were able to selectively deliver circSCMH1 to the brain, and the results suggest that engineered EVs in rodent and non-human primate models of ischemic stroke have a promising effect in promoting functional recovery after stroke. This study provides a compelling rationale for the continued exploration of EVs as a diagnostic tool in clinical settings [191].

From bench to bedside

EVs have demonstrated a considerable therapy in cell and animal experiments, the number of registered clinical trials base on EV is growing. A search of the keywords “exosome” and “extracellular vesicle” on ClinicalTrials.gov (<https://clinicaltrials.gov/>) revealed 235 clinical studies involving EVs, as listed in supplementary Table 1. The number of clinical studies has increased significantly over the past five years, with a particular focus on the use of EVs for diagnostic, therapeutic and drug delivery purposes across a range of research areas (Fig. 6).

EVs are derived from a range of biological fluids, including urine and plasma. A current study seeks to evaluate the potential of plasma. Plasma EVs-derived miRNAs have been demonstrated to function as a biomarker for oesophageal squamous cell carcinoma [192]. A multicenter clinical study has demonstrated that EVs-based microRNAs exhibit superior performance in the diagnosis of pancreatic ductal adenocarcinoma in comparison to liquid biopsy tests that are commonly employed in clinical practice for the measurement of carbohydrate antigen 19–9 (CA19-9) levels [193]. The results of this study indicate that miRNAs packaged in EVs play a significant role in the pathogenesis of the disease. EVs are also an early indicator of poor prognosis in

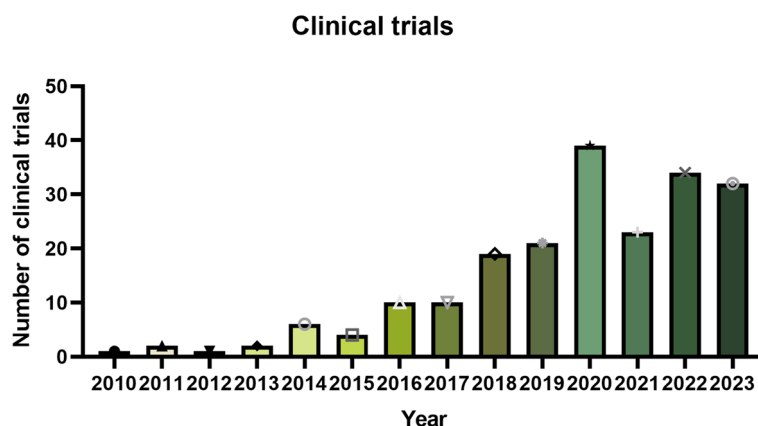


Fig. 6 Number of clinical trials from 2010 to 2023. Original data of Fig. 6 were obtained from ClinicalTrials.gov (<https://clinicaltrials.gov/>), and drawn by GraphPad Prism 9

patients with ST-segment elevation myocardial infarction (NCT06070974).

In therapeutic clinical trials of EVs includes cancer, respiratory diseases, graft-versus-host disease, neurological disorders, orofacial and dermatological conditions, and aesthetic medicine. Additionally, studies have explored the potential for plant-derived EV delivery of curcumin using an oral approach to treat colon cancer (NCT01294072).

The sources of EVs in therapeutic primarily encompass both cellular and plant. The cells are predominantly of MSC origin, the MSC-EV has favorable application prospects, mainly due to MSC itself has efficacious therapeutic effects in the treatment of disease and less side effects [194]. A study on the tolerance of human adipose-derived MSC-EVs (haMSC-EVs) nebulized in all volunteers demonstrated that no serious adverse events were observed from the commencement of nebulization to day 7 post-nebulization [195]. Furthermore, a phase 2 clinical trial has demonstrated the safety and efficacy of MSC-derived EVs in the treatment of refractory perianal fistulas in patients with Crohn's disease [196]. Studies have employed MSC-derived EVs to address atrophic acne scars. This was done in a 12-week prospective, double-blind, randomized, split-face trial of 25 patients. The findings also demonstrated that EVs are safe, in addition, it is an accessible modality for the treatment of atrophic acne scars [197]. Plant sources of EVs include ginger source, aloe source, and grape source. A completed study demonstrated the efficacy of ginger-derived EVs in the treatment of inflammatory bowel disease (IBD) and colitis-associated cancers. The study revealed that these vesicles contain high levels of lipids, low levels of proteins, approximately 125 kinds of microRNAs (miRNAs), and ginger's bioactive constituents, including 6-gingerol and 6-gingerol [198].

Despite the existence of numerous clinical studies on EVs, the clinical trial of engineered EVs have progressed at a relatively slow pace. Only one clinical study employs focused transcranial ultrasound to enhance the delivery of growth factors and anti-inflammatory agents to localized targets, with the aim of treating depression, anxiety and dementia (NCT04202770). However, no results have been published in this ongoing trial.

Multiple modes of administration have been used in clinical trials, encompassing oral, nasal, intraperitoneal, intradermal, topical application and nebulization. Grape-derived EVs in oral administration were used in the prevention and treatment of oral mucositis associated with radiotherapy for head and neck cancer (NCT01668849). Using nasal drops, EVs derived from adipose mesenchymal stem cells have utilized in treatment of neurological disorders (NCT04388982). A recent study conducted

at Tehran University of Medical Sciences in August 2024. Through intraperitoneal injection, EVs was investigated the effect of EVs in preventing early leakage in rectal cancer patients undergoing low anterior resection (NCT06536712). Furthermore, intradermal EVs is conducted for the treatment of androgenetic alopecia (NCT06239207). In addition, studies have also been conducted to investigate the impact EVs containing liquid dressings on the recovery of patients after Nd: YAG laser 532 treatment (NCT06279039). Nebulized inhalation represents a potential avenue for the treatment of lung disease. This approach has been explored in the context of lung infections caused by carbapenem-resistant gram-negative bacilli, with the use of allogeneic human adipose mesenchymal progenitor cells (haMPC) EVs. (NCT04544215).

The initial clinical trials have yielded encouraging outcomes in a multitude of ailments. Nevertheless, there are still hurdles to overcome before these findings can be translated into clinical applications. One such challenge is stability. The optimal temperature for the preservation of EVs is -80°C , which is challenging to achieve in clinical settings and transportation [199]. Furthermore, previous studies have demonstrated that EVs are prone to aggregation in vehicle leading to blockage. At last, it is costly to produce [200]. It is necessary for further research to address issues related to preservation, transport, and monodispersity.

Conclusions and perspectives

Engineered EVs have been demonstrated to be highly efficacy in the treatment of disease. This is achieved through the introduction of engineered modifications that facilitate precise targeting of the diseased area, while simultaneously reducing the impact on surrounding normal tissues. This approach has the potential to minimize toxicity and side effects. Furthermore, the therapeutic effect can be enhanced by increasing the concentration of EVs at the lesion site and amplifying the therapeutic effect through the loading of different therapeutic molecules. Furthermore, the half-life can be extended by enhancing the stability of the vesicles in the bloodstream, thus ensuring that a greater quantity of active ingredients reach the target site.

Furthermore, our statistical analysis of engineered EVs indicates that there is no notable impact of these on the overall size of the EVs. Additionally, the TEM results demonstrate that the morphology of the EVs remains largely unaffected by the introduction of engineered molecules.

However, engineered EVs technology needs further development. Firstly, further investigation is required to ascertain the pharmacodynamic characters of engineered

EVs. In particular, the circulating metabolic pathways of EVs after entering the body require elucidation. There is a need for further development of tracking techniques *in vivo*. Secondly, it is recommended that further investigation be conducted into the reduced possibility of circumventing MPS and immunogenicity in order to achieve precise control of MPS while minimizing adverse effects on the innate immune system. Thirdly, it is necessary to investigate the engineering effect on structure and function of EVs. Finally, it is essential to enhance reproducibility and efficiency on modifications engineering EVs in manufacturing large scale for clinical translation.

Given the low production and high cost of EVs, which severely limit their clinical translation, it is imperative to explore EVs from different sources, such as plants, milk, bacteria, and etc. Evidence indicates that ginseng-derived EVs can effectively inhibit glioma progression and modulate tumor-associated macrophages (TAMs) [201]. Groundnut-derived EVs has been demonstrated to alleviate lipopolysaccharide-induced acute lung injury and intestinal dysbiosis [202]. *Rhodiola rosea* vesicles contain sRNAHJT-sRNA-m7, which directly targets the fibrotic proteins α -SMA, the ECM component fibronectin, and COL3A1, thereby improving the symptoms of pulmonary fibrosis. Furthermore, the formation of liposomes by phosphatidylcholine facilitates the entry of small RNAs into the alveoli, thereby exerting a therapeutic effect [203]. Due to their highly resistant to harsh gastrointestinal tract environments, milk-derived EVs have been used as an oral delivery vehicle of miR-31-5p to accelerates diabetic wound healing through promoting angiogenesis [204]. EVs derived from Gram-negative bacteria contain a variety of pathogen-associated molecular patterns (PAMPs), including peptidoglycan, lipopolysaccharide, and flagellin. These molecules possess a robust capacity to stimulate intrinsic immune signaling pathways, rendering them a valuable resource for the development of adjuvants and vaccine carriers. Bacterial-derived EVs were used to enhance the anti-tumor activity produced by a trained immunity-related vaccines (TIRV) [205]. Nevertheless, the intricate nature of the constituents of plant-derived or bacterial-derived EVs calls for a more comprehensive assessment of their pharmacokinetics, pharmacodynamics, safety, stability, and efficacy. Consequently, engineering various derived EVs presents a promising avenue for further advancement.

In conclusion, the therapeutic potential of engineered EVs has been significantly augmented by the introduction of targeted molecules through diverse modification techniques, thereby conferring them with extensive prospective applications in the disease management. With the ongoing advancement of research and technological

developments, engineered EVs were rendered as a promising cell-free therapeutic modality.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s43556-024-00230-x>.

Supplementary Material 1.

Acknowledgements

The authors would like to acknowledge the Figdraw platform in drawing Figs. 1, 2 and 3.

Authors' contributions

Shuang Zhao and Jingyu Wang designed the study. Shuang Zhao, Yunfeng Di, Huilan Fan, and Chengyan Xu prepared materials, collected data, and conducted analysis. Shuang Zhao drafted the manuscript. Yanli Liu, Jialing Bei, Haijing Li, Yong Wang, Wei Wang, Chun Li and Jingyu Wang revised the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by the Starting Grants for Young Talents of Beijing University of Chinese Medicine.

Data availability

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Received: 14 August 2024 Revised: 12 November 2024 Accepted: 13 November 2024

Published online: 21 November 2024

References

1. Lu M, DiBernardo E, Parks E, Fox H, Zheng SY, Wayne E. The role of extracellular vesicles in the pathogenesis and treatment of autoimmune disorders. *Front Immunol*. 2021;12: 566299. <https://doi.org/10.3389/fimmu.2021.566299>.
2. Han C, Yang J, Sun J, Qin G. Extracellular vesicles in cardiovascular disease: biological functions and therapeutic implications. *Pharmacol Ther*. 2022;233: 108025. <https://doi.org/10.1016/j.pharmthera.2021.108025>.
3. Chen M, Li Y, Zhang M, Ge S, Feng T, Chen R, et al. Histone deacetylase inhibition enhances extracellular vesicles from muscle to promote osteogenesis via miR-873-3p. *Signal Transduct Target Ther*. 2024;9(1):256. <https://doi.org/10.1038/s41392-024-01976-0>.
4. Zhang X, Xu Y, Ma L, Yu K, Niu Y, Xu X, et al. Essential roles of exosome and circRNA_101093 on ferroptosis desensitization in lung adenocarcinoma. *Cancer Commun (Lond)*. 2022;42(4):287–313. <https://doi.org/10.1002/cac2.12275>.
5. Yuan Y, Mei Z, Qu Z, Li G, Yu S, Liu Y, et al. Exosomes secreted from cardiomyocytes suppress the sensitivity of tumor ferroptosis in ischemic heart failure. *Signal Transduct Target Ther*. 2023;8(1):121. <https://doi.org/10.1038/s41392-023-01336-4>.
6. Meng Z, Zhang R, Wu X, Piao Z, Zhang M, Jin T. LncRNA HAGLROS promotes breast cancer evolution through miR-135b-3p/COL10A1 axis and exosome-mediated macrophage M2 polarization. *Cell Death Dis*. 2024;15(8):633. <https://doi.org/10.1038/s41419-024-07020-x>.

7. Du Y, Wu L, Wang L, Reiter RJ, Lip GYH, Ren J. Extracellular vesicles in cardiovascular diseases: from pathophysiology to diagnosis and therapy. *Cytokine Growth Factor Rev.* 2023;74:40–55. <https://doi.org/10.1016/j.cytogr.2023.09.006>.
8. Xiong YY, Gong ZT, Tang RJ, Yang YJ. The pivotal roles of exosomes derived from endogenous immune cells and exogenous stem cells in myocardial repair after acute myocardial infarction. *Theranostics.* 2021;11(3):1046–58. <https://doi.org/10.7150/thno.53326>.
9. Singh G, Mehra A, Arora S, Gugulothu D, Vora LK, Prasad R, et al. Exosome-mediated delivery and regulation in neurological disease progression. *Int J Biol Macromol.* 2024;264(Pt 2):130728. <https://doi.org/10.1016/j.jbiomac.2024.130728>.
10. Weng J, Xiang X, Ding L, Wong AL, Zeng Q, Sethi G, et al. Extracellular vesicles, the cornerstone of next-generation cancer diagnosis? *Semin Cancer Biol.* 2021;74:105–20. <https://doi.org/10.1016/j.semcancer.2021.05.011>.
11. Yang H, Zhang LM. Hollow organic/inorganic hybrid nanoparticles from dextran-block-polypeptide copolymer: double click reaction synthesis and properties. *Int J Biol Macromol.* 2021;181:1243–53. <https://doi.org/10.1016/j.jbiomac.2021.05.101>.
12. Di Y, Zhao S, Fan H, Li W, Jiang G, Wang Y, et al. Mass production of Rg1-Loaded small extracellular vesicles using a 3D Bioreactor System for enhanced cardioprotective efficacy of Doxorubicin-Induced Cardiotoxicity. *Pharmaceutics.* 2024;16(5):593. <https://doi.org/10.3390/pharmaceutics16050593>.
13. Verma ML, Dhanya BS, Sukriti, Rani V, Thakur M, Jeslin J, et al. Carbohydrate and protein based biopolymeric nanoparticles: current status and biotechnological applications. *Int J Biol Macromol.* 2020;154:390–412. <https://doi.org/10.1016/j.jbiomac.2020.03.105>.
14. Chamundeeswari M, Jeslin J, Verma ML. Nanocarriers for drug delivery applications. *Environ Chem Lett.* 2019;17(2):849–65. <https://doi.org/10.1007/s10311-018-00841-1>.
15. Lin SW, Yu CP, Tsai JC, Shyong YJ. Delivery of extracellular vesicles loaded with immune checkpoint inhibitors for immunotherapeutic management of glioma. *Mater Today Bio.* 2024;28: 101244. <https://doi.org/10.1016/j.mtbio.2024.101244>.
16. Rehman FU, Liu Y, Zheng M, Shi B. Exosomes based strategies for brain drug delivery. *Biomaterials.* 2023;293:121949. <https://doi.org/10.1016/j.biomaterials.2022.121949>.
17. Rong Y, Wang Z, Tang P, Wang J, Ji C, Chang J, et al. Engineered extracellular vesicles for delivery of siRNA promoting targeted repair of traumatic spinal cord injury. *Bioact Mater.* 2023;23:328–42. <https://doi.org/10.1016/j.bioactmat.2022.11.011>.
18. Zhang J, Zhang B, Zhang L, Xu X, Cheng Q, Wang Y, et al. Engineered nanovesicles mediated cardiomyocyte survival and neovascularization for the therapy of myocardial infarction. *Colloids Surf B Biointerfaces.* 2024;243: 114135. <https://doi.org/10.1016/j.colsurfb.2024.114135>.
19. Liao Z, Chen Y, Duan C, Zhu K, Huang R, Zhao H, et al. Cardiac telocytes inhibit cardiac microvascular endothelial cell apoptosis through exosomal miRNA-21-5p-targeted cldp1 silencing to improve angiogenesis following myocardial infarction. *Theranostics.* 2021;11(1):268–91. <https://doi.org/10.7150/thno.47021>.
20. Abuzan M, Surugiu R, Wang C, Mohamud-Yusuf A, Tertel T, Catalin B, et al. Extracellular vesicles obtained from hypoxic mesenchymal stromal cells induce neurological recovery, anti-inflammation, and brain remodeling after distal middle cerebral artery occlusion in rats. *Transl Stroke Res.* 2024. <https://doi.org/10.1007/s12975-024-01266-5>.
21. Li Q, Xu Y, Lv K, Wang Y, Zhong Z, Xiao C, et al. Small extracellular vesicles containing mir-486-5p promote angiogenesis after myocardial infarction in mice and nonhuman primates. *Sci Transl Med.* 2021;13(584):eabb0202. <https://doi.org/10.1126/scitranslmed.abb0202>.
22. Li J, Sun S, Zhu D, Mei X, Lyu Y, Huang K, et al. Inhalable stem cell exosomes promote Heart Repair after myocardial infarction. *Circulation.* 2024;150(9):710–23. <https://doi.org/10.1161/circulationaha.123.065005>.
23. Cao Y, Chen B, Liu Q, Mao Y, He Y, Liu X, et al. Dissolvable microneedle-based wound dressing transdermally and continuously delivers anti-inflammatory and pro-angiogenic exosomes for diabetic wound treatment. *Bioact Mater.* 2024;42:32–51. <https://doi.org/10.1016/j.bioactmat.2024.08.016>.
24. Vestuto V, Conte M, Vietri M, Mensitieri F, Santoro V, Di Muro A, et al. Multiomic profiling and neuroprotective bioactivity of saliva hairy root-derived extracellular vesicles in a cellular model of parkinson's disease. *Int J Nanomed.* 2024;19:9373–93. <https://doi.org/10.2147/ijn.5479959>.
25. Morishita M, Takahashi Y, Nishikawa M, Sano K, Kato K, Yamashita T, et al. Quantitative analysis of tissue distribution of the B16BL6-derived exosomes using a streptavidin-lactadherin fusion protein and iodine-125-labeled biotin derivative after intravenous injection in mice. *J Pharm Sci.* 2015;104(2):705–13. <https://doi.org/10.1002/jps.24251>.
26. Lázaro-Ibáñez E, Faruqi FN, Saleh AF, Silva AM, Tzu-Wen Wang J, Rak J, et al. Selection of fluorescent, bioluminescent, and Radioactive tracers to accurately reflect extracellular vesicle biodistribution in vivo. *ACS Nano.* 2021;15(2):3212–27. <https://doi.org/10.1021/acsnano.0c09873>.
27. Aimaletdinov AM, Gomzikova MO. Tracking of extracellular vesicles' biodistribution: new methods and approaches. *Int J Mol Sci.* 2022;23(19):11312. <https://doi.org/10.3390/ijms231911312>.
28. Wiklander OP, Nordin JZ, O'Loughlin A, Gustafsson Y, Corso G, Mäger I, et al. Extracellular vesicle in vivo biodistribution is determined by cell source, route of administration and targeting. *J Extracell Vesicles.* 2015;4:26316. <https://doi.org/10.3402/jev.v4.26316>.
29. Barreca V, Boussadia Z, Polignano D, Galli L, Tirelli V, Sanchez M, et al. Metabolic labelling of a subpopulation of small extracellular vesicles using a fluorescent palmitic acid analogue. *J Extracell Vesicles.* 2023;12(12): e12392. <https://doi.org/10.1002/jev.2.12392>.
30. Linnemannstöns K, Karuna MP, Witte L, Choezon D, Honemann-Capito M, Lagurin AS, et al. Microscopic and biochemical monitoring of endosomal trafficking and extracellular vesicle secretion in an endogenous in vivo model. *J Extracell Vesicles.* 2022;11(9): e12263. <https://doi.org/10.1002/jev.2.12263>.
31. Manca S, Upadhyaya B, Mutai E, Desaulniers AT, Cederberg RA, White BR, et al. Milk exosomes are bioavailable and distinct microRNA cargos have unique tissue distribution patterns. *Sci Rep.* 2018;8(1):11321. <https://doi.org/10.1038/s41598-018-29780-1>.
32. Sine SM. End-plate acetylcholine receptor: structure, mechanism, pharmacology, and disease. *Physiol Rev.* 2012;92(3):1189–234. <https://doi.org/10.1152/physrev.00015.2011>.
33. Wang F, Nelson ME, Kuryatov A, Olale F, Cooper J, Keyser K, et al. Chronic nicotine treatment up-regulates human alpha3 beta2 but not alpha3 beta4 acetylcholine receptors stably transfected in human embryonic kidney cells. *J Biol Chem.* 1998;273(44):28721–32. <https://doi.org/10.1074/jbc.273.44.28721>.
34. Kim SS, Ye C, Kumar P, Chiu I, Subramanya S, Wu H, et al. Targeted delivery of siRNA to macrophages for anti-inflammatory treatment. *Mol Ther.* 2010;18(5):993–1001. <https://doi.org/10.1038/mt.2010.27>.
35. Yang J, Wu S, Hou L, Zhu D, Yin S, Yang G, et al. Therapeutic effects of simultaneous delivery of nerve growth factor mRNA and protein via Exosomes on cerebral ischemia. *Mol Ther Nucleic Acids.* 2020;21:512–22. <https://doi.org/10.1016/j.mtn.2020.06.013>.
36. Yu X, Bai Y, Han B, Ju M, Tang T, Shen L, et al. Extracellular vesicle-mediated delivery of circDYM alleviates CUS-induced depressive-like behaviours. *J Extracell Vesicles.* 2022;11(1): e12185. <https://doi.org/10.1002/jev.2.12185>.
37. Rufino-Ramos D, Albuquerque PR, Leandro K, Carmona V, Martins IM, Fernandes R, et al. Extracellular vesicle-based delivery of silencing sequences for the treatment of Machado-Joseph disease/spinocerebellar ataxia type 3. *Mol Ther.* 2023;31(5):1275–92. <https://doi.org/10.1016/j.ymthe.2023.04.001>.
38. Haroon K, Zheng H, Wu S, Liu Z, Tang Y, Yang GY, et al. Engineered exosomes mediated targeted delivery of neuroprotective peptide NR2B9c for the treatment of traumatic brain injury. *Int J Pharm.* 2024;649: 123656. <https://doi.org/10.1016/j.ijpharm.2023.123656>.
39. Wang H, Wang B, Zhang A, Hassounah F, Seow Y, Wood M, et al. Exosome-mediated miR-29 transfer reduces muscle atrophy and kidney fibrosis in mice. *Mol Ther.* 2019;27(3):571–83. <https://doi.org/10.1016/j.ymthe.2019.01.008>.
40. Ji JL, Shi HM, Li ZL, Jin R, Qu GT, Zheng H, et al. Satellite cell-derived exosome-mediated delivery of microRNA-23a/27a/26a cluster ameliorates the renal tubulointerstitial fibrosis in mouse diabetic nephropathy. *Acta Pharmacol Sin.* 2023;44(12):2455–68. <https://doi.org/10.1038/s41401-023-01140-4>.

41. Teicher BA, Fricker SP. CXCL12 (SDF-1)/CXCR4 pathway in cancer. *Clin Cancer Res.* 2010;16(11):2927–31. <https://doi.org/10.1158/1078-0432.CCR-09-2329>.
42. Liu H, Song P, Zhang H, Zhou F, Ji N, Wang M, et al. Synthetic biology-based bacterial extracellular vesicles displaying BMP-2 and CXCR4 to ameliorate osteoporosis. *J Extracell Vesicles.* 2024;13(4): e12429. <https://doi.org/10.1002/jev2.12429>.
43. Hu Y, Li X, Zhang Q, Gu Z, Luo Y, Guo J, et al. Exosome-guided bone targeted delivery of Antagomir-188 as an anabolic therapy for bone loss. *Bioact Mater.* 2021;6(9):2905–13. <https://doi.org/10.1016/j.bioactmat.2021.02.014>.
44. Liu H, Zhang H, Wang S, Cui J, Weng W, Liu X, et al. Bone-targeted bioengineered bacterial extracellular vesicles delivering siRNA to ameliorate osteoporosis. *Compos Part B: Eng.* 2023;255:110610. <https://doi.org/10.1016/j.compositesb.2023.110610>.
45. Suratt BT, Petty JM, Young SK, Malcolm KC, Lieber JG, Nick JA, et al. Role of the CXCR4/SDF-1 chemokine axis in circulating neutrophil homeostasis. *Blood.* 2004;104(2):565–71. <https://doi.org/10.1182/blood-2003-10-3638>.
46. De Filippo K, Rankin SM. CXCR4, the master regulator of neutrophil trafficking in homeostasis and disease. *Eur J Clin Invest.* 2018;48 Suppl 2(Suppl Suppl 2):e12949. <https://doi.org/10.1111/eci.12949>.
47. Bogoslovsky T, Spatz M, Chaudhry A, Maric D, Luby M, Frank J, et al. Stromal-derived factor-1[alpha] correlates with circulating endothelial progenitor cells and with acute lesion volume in stroke patients. *Stroke.* 2011;42(3):618–25. <https://doi.org/10.1161/strokeaha.110.596007>.
48. Uematsu M, Yoshizaki T, Shimizu T, Obata JE, Nakamura T, Fujioka D, et al. Sustained myocardial production of stromal cell-derived factor-1 α was associated with left ventricular adverse remodeling in patients with myocardial infarction. *Am J Physiol Heart Circ Physiol.* 2015;309(10):H1764–71. <https://doi.org/10.1152/ajpheart.00493.2015>.
49. Ciullo A, Biemmi V, Milano G, Bolis S, Cervio E, Fertig ET, et al. Exosomal expression of CXCR4 targets cardioprotective vesicles to myocardial infarction and improves outcome after systemic administration. *Int J Mol Sci.* 2019;20(3). <https://doi.org/10.3390/ijms20030468>.
50. Li X, Zhang Y, Wang Y, Zhao D, Sun C, Zhou S, et al. Exosomes Derived from CXCR4-Overexpressing BMSC promoted activation of microvascular endothelial cells in cerebral Ischemia/Reperfusion Injury. *Neural Plast.* 2020;2020:8814239. <https://doi.org/10.1155/2020/8814239>.
51. Wang D, Jiang S, Zhang F, Ma S, Heng BC, Wang Y, et al. Cell membrane vesicles with enriched CXCR4 display enhances their targeted delivery as drug carriers to inflammatory sites. *Adv Sci (Weinh).* 2021;8(23): e2101562. <https://doi.org/10.1002/advs.202101562>.
52. Luo H, Chen D, Li R, Li R, Teng Y, Cao Y, et al. Genetically engineered CXCR4-modified exosomes for delivery of miR-126 mimics to macrophages alleviate periodontitis. *J Nanobiotechnol.* 2023;21(1):116. <https://doi.org/10.1186/s12951-023-01863-w>.
53. Luo L, Zang G, Liu B, Qin X, Zhang Y, Chen Y, et al. Bioengineering CXCR4-overexpressing cell membrane functionalized ROS-responsive nanotherapeutics for targeting cerebral ischemia-reperfusion injury. *Theranostics.* 2021;11(16):8043–56. <https://doi.org/10.7150/thno.60785>.
54. Du Y, Chen Y, Li F, Mao Z, Ding Y, Wang W. Genetically engineered cellular nanovesicle as targeted DNase I delivery system for the clearance of neutrophil extracellular traps in acute lung injury. *Adv Sci (Weinh).* 2023;10(32):e2303053. <https://doi.org/10.1002/advs.202303053>.
55. Wang K, Kumar US, Sadeghipour N, Massoud TF, Paulmurugan RA. Microfluidics-based scalable approach to generate extracellular vesicles with enhanced therapeutic MicroRNA loading for intranasal delivery to mouse glioblastomas. *ACS Nano.* 2021;15(11):18327–46. <https://doi.org/10.1021/acsnano.1c07587>.
56. Tsai HC, Lai YY, Hsu HC, Fong YC, Lien MY, Tang CH. CCL4 stimulates cell migration in human osteosarcoma via the mir-3927-3p/Integrin α v β 3 Axis. *Int J Mol Sci.* 2021;22(23):12737. <https://doi.org/10.3390/ijms22312737>.
57. Li N, Qiu S, Fang Y, Wu J, Li Q. Comparison of linear vs. cyclic RGD pentapeptide interactions with integrin α (v) β (3) by molecular dynamics simulations. *Biology (Basel).* 2021;10(7). <https://doi.org/10.3390/biology10070688>.
58. Tian T, Zhang HX, He CP, Fan S, Zhu YL, Qi C, et al. Surface functionalized exosomes as targeted drug delivery vehicles for cerebral ischemia therapy. *Biomaterials.* 2018;150:137–49. <https://doi.org/10.1016/j.biomaterials.2017.10.012>.
59. Xia T, Liu Z, Du Y, Zhang J, Liu X, Ouyang J, et al. Bifunctional iRGD-Exo-DOX crosses the blood-brain barrier to target central nervous system lymphoma. *Biochem Pharmacol.* 2024;223:116138. <https://doi.org/10.1016/j.bcp.2024.116138>.
60. Roefs MT, Heusermann W, Brans MAD, Snijders Blok C, Lei Z, Vader P, et al. Evaluation and manipulation of tissue and cellular distribution of cardiac progenitor cell-derived extracellular vesicles. *Front Pharmacol.* 2022;13: 1052091. <https://doi.org/10.3389/fphar.2022.1052091>.
61. Elsharkasy OM, Nordin JZ, Hagey DW, de Jong OG, Schiffelers RM, Andaloussi SE, et al. Extracellular vesicles as drug delivery systems: why and how? *Adv Drug Deliv Rev.* 2020;159:332–43. <https://doi.org/10.1016/j.addr.2020.04.004>.
62. Zhou P, Du X, Jia W, Feng K, Zhang Y. Engineered extracellular vesicles for targeted reprogramming of cancer-associated fibroblasts to potentiate therapy of pancreatic cancer. *Signal Transduct Target Ther.* 2024;9(1):151. <https://doi.org/10.1038/s41392-024-01872-7>.
63. Wang C, Li N, Li Y, Hou S, Zhang W, Meng Z, et al. Engineering a HEK-293T exosome-based delivery platform for efficient tumor-targeting chemotherapy/internal irradiation combination therapy. *J Nanobiotechnol.* 2022;20(1):247. <https://doi.org/10.1186/s12951-022-01462-1>.
64. Chen C, Zhao S, Karnad A, Freeman JW. The biology and role of CD44 in cancer progression: therapeutic implications. *J Hematol Oncol.* 2018;11(1):64. <https://doi.org/10.1186/s13045-018-0605-5>.
65. Lee H, Park H, Noh GJ, Lee ES. pH-responsive hyaluronate-anchored extracellular vesicles to promote tumor-targeted drug delivery. *Carbohydr Polym.* 2018;202:323–33. <https://doi.org/10.1016/j.carbpol.2018.08.141>.
66. Li D, Gong L, Lin H, Yao S, Yin Y, Zhou Z, et al. Hyaluronic acid-coated bovine milk exosomes for achieving tumor-specific intracellular delivery of miRNA-204. *Cells.* 2022;11(19):3065. <https://doi.org/10.3390/cells11193065>.
67. Li D, Yao S, Zhou Z, Shi J, Huang Z, Wu Z. Hyaluronan decoration of milk exosomes directs tumor-specific delivery of doxorubicin. *Carbohydr Res.* 2020;493: 108032. <https://doi.org/10.1016/j.carres.2020.108032>.
68. Gong L, Zhou H, Zhang S, Wang C, Fu K, Ma C, et al. CD44-targeting drug delivery system of exosomes loading Forsythiaside A combats liver fibrosis via regulating NLRP3-Mediated pyroptosis. *Adv Healthc Mater.* 2023;12(11):e2202228. <https://doi.org/10.1002/adhm.202202228>.
69. Ma W, Wu D, Long C, Liu J, Xu L, Zhou L, et al. Neutrophil-derived nanovesicles deliver IL-37 to mitigate renal ischemia-reperfusion injury via endothelial cell targeting. *J Control Release.* 2024;370:66–81. <https://doi.org/10.1016/j.jconrel.2024.04.025>.
70. Zhang K, Li R, Chen X, Yan H, Li H, Zhao X, et al. Renal endothelial cell-targeted extracellular vesicles protect the kidney from ischemic injury. *Adv Sci (Weinh).* 2023;10(3):e2204626. <https://doi.org/10.1002/advs.202204626>.
71. Ying M, Shen Q, Zhan C, Wei X, Gao J, Xie C, et al. A stabilized peptide ligand for multifunctional glioma targeted drug delivery. *J Control Release.* 2016;243:86–98. <https://doi.org/10.1016/j.jconrel.2016.09.035>.
72. Sugahara KN, Teesalu T, Karmali PP, Kotamraju VR, Agemy L, Girard OM, et al. Tissue-penetrating delivery of compounds and nanoparticles into tumors. *Cancer Cell.* 2009;16(6):510–20. <https://doi.org/10.1016/j.ccr.2009.10.013>.
73. Zhang H, Wu J, Wu J, Fan Q, Zhou J, Wu J, et al. Exosome-mediated targeted delivery of miR-210 for angiogenic therapy after cerebral ischemia in mice. *J Nanobiotechnol.* 2019;17(1):29. <https://doi.org/10.1186/s12951-019-0461-7>.
74. Fang Z, Sun Y, Xiao H, Li P, Liu M, Ding F, et al. Targeted osteosarcoma chemotherapy using RGD peptide-installed doxorubicin-loaded biodegradable polymeric micelle. *Biomed Pharmacother.* 2017;85:160–8. <https://doi.org/10.1016/j.biopha.2016.11.132>.
75. Abumiya T, Lucero J, Heo JH, Tagaya M, Koziol JA, Copeland BR, et al. Activated microvessels express vascular endothelial growth factor and integrin α (v) β 3 during focal cerebral ischemia. *J Cereb Blood Flow Metab.* 1999;19(9):1038–50. <https://doi.org/10.1097/00004647-199909000-00012>.
76. Yuan Z, Gui L, Zheng J, Chen Y, Qu S, Shen Y, et al. GSH-activated light-up near-infrared fluorescent probe with high affinity to α (v) β (3) Integrin

- for Precise Early Tumor Identification. *ACS Appl Mater Interfaces*. 2018;10(37):30994–1007. <https://doi.org/10.1021/acsami.8b09841>.
77. Tian T, Cao L, He C, Ye Q, Liang R, You W, et al. Targeted delivery of neural progenitor cell-derived extracellular vesicles for anti-inflammation after cerebral ischemia. *Theranostics*. 2021;11(13):6507–21. <https://doi.org/10.7150/thno.56367>.
 78. Parrasia S, Rossa A, Roncaglia N, Mattarei A, Honisch C, Szabò I, et al. DA7R: a 7-Letter zip code to target PDAC. *Pharmaceutics*. 2023;15(5):1508. <https://doi.org/10.3390/pharmaceutics15051508>.
 79. Ruan H, Li Y, Wang C, Jiang Y, Han Y, Li Y, et al. Click chemistry extracellular vesicle/peptide/chemokine nanocarriers for treating central nervous system injuries. *Acta Pharm Sin B*. 2023;13(5):2202–18. <https://doi.org/10.1016/j.apsb.2022.06.007>.
 80. Bottens RA, Yamada T. Cell-penetrating peptides (CPPs) as therapeutic and diagnostic agents for Cancer. *Cancers (Basel)*. 2022;14(22). <https://doi.org/10.3390/cancers1422546>.
 81. Nakase I, Noguchi K, Aoki A, Takatani-Nakase T, Fujii I, Futaki. Arginine-rich cell-penetrating peptide-modified extracellular vesicles for active macropinocytosis induction and efficient intracellular delivery. *Sci Rep*. 2017;7(1):1991. <https://doi.org/10.1038/s41598-017-02014-6>.
 82. Zhao M, Weissleder R. Intracellular cargo delivery using tat peptide and derivatives. *Med Res Rev*. 2004;24(1):1–12. <https://doi.org/10.1002/med.10056>.
 83. Sharma G, Lakkadwala S, Modgil A, Singh J. The role of cell-penetrating peptide and transferrin on enhanced delivery of drug to Brain. *Int J Mol Sci*. 2016;17(6):806. <https://doi.org/10.3390/ijms17060806>.
 84. Dixit N, Settle JK, Ye Q, Berrie CL, Spencer P, Laurence JS. Grafting MAP peptide to dental polymer inhibits MMP-8 activity. *J Biomed Mater Res B Appl Biomater*. 2015;103(2):324–31. <https://doi.org/10.1002/jbm.b.33205>.
 85. Rosell A, Ortega-Aznar A, Alvarez-Sabín J, Fernández-Cadenas I, Ribó M, Molina CA, et al. Increased brain expression of matrix metalloproteinase-9 after ischemic and hemorrhagic human stroke. *Stroke*. 2006;37(6):1399–406. <https://doi.org/10.1161/01.STR.0000223001.06264.af>.
 86. You Y, Xu J, Liu Y, Li H, Xie L, Ma C, et al. Tailored apoptotic vesicle delivery platform for inflammatory regulation and tissue repair to ameliorate ischemic stroke. *ACS Nano*. 2023;17(9):8646–62. <https://doi.org/10.1021/acsnano.3c01497>.
 87. Tran NH, Nguyen DD, Nguyen NM, Tran C, Nguyen Thi NT, Ho DT, et al. Dual-targeting exosomes for improved drug delivery in breast cancer. *Nanomed (Lond)*. 2023;18(7):599–611. <https://doi.org/10.2217/nnm-2022-0328>.
 88. Lafarga V, Sirozh O, Díaz-López I, Galarreta A, Hisaoka M, Zarzuela E, et al. Widespread displacement of DNA- and RNA-binding factors underlies toxicity of arginine-rich cell-penetrating peptides. *Embo j*. 2021;40(13):e103311. <https://doi.org/10.15252/emboj.2019103311>.
 89. Zhou M, Zou X, Cheng K, Zhong S, Su Y, Wu T, et al. The role of cell-penetrating peptides in potential anti-cancer therapy. *Clin Transl Med*. 2022;12(5):e822. <https://doi.org/10.1002/ctm2.822>.
 90. Qian Z, Rhodes CA, McCroskey LC, Wen J, Appiah-Kubi G, Wang DJ, et al. Enhancing the cell permeability and metabolic stability of peptidyl drugs by reversible bicyclization. *Angew Chem Int Ed Engl*. 2017;56(6):1525–9. <https://doi.org/10.1002/anie.201610888>.
 91. Derakhshankhah H, Jafari S. Cell penetrating peptides: a concise review with emphasis on biomedical applications. *Biomed Pharmacother*. 2018;108:1090–6. <https://doi.org/10.1016/j.biopha.2018.09.097>.
 92. Kim H, Yun N, Mun D, Kang JY, Lee SH, Park H, et al. Cardiac-specific delivery by cardiac tissue-targeting peptide-expressing exosomes. *Biochem Biophys Res Commun*. 2018;499(4):803–8. <https://doi.org/10.1016/j.bbrc.2018.03.227>.
 93. Mun D, Kang JY, Kim H, Yun N, Joung B. Small extracellular vesicle-mediated CRISPR-Cas9 RNP delivery for cardiac-specific genome editing. *J Control Release*. 2024;370:798–810. <https://doi.org/10.1016/j.jconrel.2024.05.023>.
 94. Yang M, Liu C, Niu M, Hu Y, Guo M, Zhang J, et al. Phage-display library biopanning and bioinformatic analysis yielded a high-affinity peptide to inflamed vascular endothelium both in vitro and in vivo. *J Control Release*. 2014;174:72–80. <https://doi.org/10.1016/j.jconrel.2013.11.009>.
 95. Feldman KS, Pavlou MP, Zahid M. Cardiac targeting peptide: from identification to validation to mechanism of transduction. *Methods Mol Biol*. 2021;2211:97–112. https://doi.org/10.1007/978-1-0716-0943-9_8.
 96. Kang JY, Kim H, Mun D, Yun N, Joung B. Co-delivery of curcumin and miRNA-144-3p using heart-targeted extracellular vesicles enhances the therapeutic efficacy for myocardial infarction. *J Control Release*. 2021;331:62–73. <https://doi.org/10.1016/j.jconrel.2021.01.018>.
 97. Kanki S, Jaalouk DE, Lee S, Yu AY, Gannon J, Lee RT. Identification of targeting peptides for ischemic myocardium by in vivo phage display. *J Mol Cell Cardiol*. 2011;50(5):841–8. <https://doi.org/10.1016/j.jmcc.2011.02.003>.
 98. Vandergriff A, Huang K, Shen D, Hu S, Hensley MT, Caranasos TG, et al. Targeting regenerative exosomes to myocardial infarction using cardiac homing peptide. *Theranostics*. 2018;8(7):1869–78. <https://doi.org/10.7150/thno.20524>.
 99. Wang Y, Ding N, Guan G, Liu G, Huo D, Li Y, et al. Rapid delivery of Hsa-Mir-590-3p using targeted exosomes to treat Acute myocardial infarction through regulation of the cell cycle. *J Biomed Nanotechnol*. 2018;14(5):968–77. <https://doi.org/10.1166/jbn.2018.2493>.
 100. Lee J-R, Sim W-S, Park H-J, Park B-W, Joung YK. Targeted delivery of apoptotic cell-derived nanovesicles prevents cardiac remodeling and attenuates cardiac function exacerbation. *Adv Funct Mater*. 2023;33(23):2210864. <https://doi.org/10.1002/adfm.202210864>.
 101. Meng WT, Zhu J, Wang YC, Shao CL, Li XY, Lu PP, et al. Targeting delivery of miR-146a via IMTP modified milk exosomes exerted cardioprotective effects by inhibiting NF- κ B signaling pathway after myocardial ischemia-reperfusion injury. *J Nanobiotechnol*. 2024;22(1):382. <https://doi.org/10.1186/s12951-024-02631-0>.
 102. Zhu LP, Tian T, Wang JY, He JN, Chen T, Pan M, et al. Hypoxia-elicited mesenchymal stem cell-derived exosomes facilitates cardiac repair through miR-125b-mediated prevention of cell death in myocardial infarction. *Theranostics*. 2018;8(22):6163–77. <https://doi.org/10.7150/thno.28021>.
 103. Yin T, Wang N, Jia F, Wu Y, Gao L, Zhang J, et al. Exosome-based WTAP siRNA delivery ameliorates myocardial ischemia-reperfusion injury. *Eur J Pharm Biopharm*. 2024;197:114218. <https://doi.org/10.1016/j.ejpb.2024.114218>.
 104. Wang X, Chen Y, Zhao Z, Meng Q, Yu Y, Sun J, et al. Engineered exosomes with ischemic myocardium-targeting peptide for targeted therapy in myocardial infarction. *J Am Heart Assoc*. 2018;7(15):e008737. <https://doi.org/10.1161/jaha.118.008737>.
 105. Zhang L, Hoffman JA, Ruoslahti E. Molecular profiling of heart endothelial cells. *Circulation*. 2005;112(11):1601–11. <https://doi.org/10.1161/circulationaha.104.529537>.
 106. Mentkowski KI, Tarvirdizadeh T, Manzanero CA, Eagler LA, Lang JK. Surface engineering enhances the therapeutic potential of systemically delivered extracellular vesicles following acute myocardial infarction. *Faseb j*. 2024;38(18):e70070. <https://doi.org/10.1096/fj.202400828R>.
 107. Pei W, Zhang Y, Zhu X, Zhao C, Li X, Lü H, et al. Multitargeted immunomodulatory therapy for viral myocarditis by engineered extracellular vesicles. *ACS Nano*. 2024;18(4):2782–99. <https://doi.org/10.1021/acsnano.3c05847>.
 108. Mao L, Li YD, Chen RL, Li G, Zhou XX, Song F, et al. Heart-targeting exosomes from human cardiosphere-derived cells improve the therapeutic effect on cardiac hypertrophy. *J Nanobiotechnol*. 2022;20(1):435. <https://doi.org/10.1186/s12951-022-01630-3>.
 109. Nimjee SM, White RR, Becker RC, Sullenger BA. Aptamers as therapeutics. *Annu Rev Pharmacol Toxicol*. 2017;57:61–79. <https://doi.org/10.1146/annurev-pharmtox-010716-104558>.
 110. Yazdian-Robati R, Bayat P, Oroojalian F, Zargari M, Ramezani M, Taghdisi SM, et al. Therapeutic applications of AS1411 aptamer, an update review. *Int J Biol Macromol*. 2020;155:1420–31. <https://doi.org/10.1016/j.jbiomac.2019.11.118>.
 111. Hosseini NF, Amini R, Ramezani M, Saidijam M, Hashemi SM, Najafi R. AS1411 aptamer-functionalized exosomes in the targeted delivery of doxorubicin in fighting colorectal cancer. *Biomed Pharmacother*. 2022;155: 113690. <https://doi.org/10.1016/j.biopha.2022.113690>.
 112. Carvalho J, Mergny JL, Salgado GF, Queiroz JA, Cruz C. G-quadruplex, friend or foe: the role of the G-quartet in anticancer strategies. *Trends Mol Med*. 2020;26(9):848–61. <https://doi.org/10.1016/j.molmed.2020.05.002>.

113. van Duijn A, Van der Burg SH, Scheeren FA. CD47/SIRPα axis: bridging innate and adaptive immunity. *J Immunother Cancer*. 2022;10(7): e004589. <https://doi.org/10.1136/jitc-2022-004589>.
114. Jaiswal S, Jamieson CH, Pang WW, Park CY, Chao MP, Majeti R, et al. CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. *Cell*. 2009;138(2):271–85. <https://doi.org/10.1016/j.cell.2009.05.046>.
115. Kamekar S, LeBleu VS, Sugimoto H, Yang S, Ruivo CF, Melo SA, et al. Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer. *Nature*. 2017;546(7659):498–503. <https://doi.org/10.1038/nature22341>.
116. Gong L, Tian L, Cui K, Chen Y, Liu B, Li D, et al. An off-the-shelf small extracellular vesicle nanomedicine for tumor targeting therapy. *J Control Release*. 2023;364:672–86. <https://doi.org/10.1016/j.jconrel.2023.11.013>.
117. Cheng L, Zhang X, Tang J, Lv Q, Liu J. Gene-engineered exosomes-thermosensitive liposomes hybrid nanovesicles by the blockade of CD47 signal for combined photothermal therapy and cancer immunotherapy. *Biomaterials*. 2021;275: 120964. <https://doi.org/10.1016/j.biomaterials.2021.120964>.
118. Wei Z, Chen Z, Zhao Y, Fan F, Xiong W, Song S, et al. Mononuclear phagocyte system blockade using extracellular vesicles modified with CD47 on membrane surface for myocardial infarction reperfusion injury treatment. *Biomaterials*. 2021;275: 121000. <https://doi.org/10.1016/j.biomaterials.2021.121000>.
119. Cheng L, Zhang P, Liu Y, Liu Z, Tang J, Xu L, et al. Multifunctional hybrid exosomes enhanced cancer chemo-immunotherapy by activating the STING pathway. *Biomaterials*. 2023;301: 122259. <https://doi.org/10.1016/j.biomaterials.2023.122259>.
120. Wan Z, Zhao L, Lu F, Gao X, Dong Y, Zhao Y, et al. Mononuclear phagocyte system blockade improves therapeutic exosome delivery to the myocardium. *Theranostics*. 2020;10(1):218–30. <https://doi.org/10.7150/thno.38198>.
121. Chen M, Wang S, Chen Y, Shen H, Chen L, Ding L, et al. Precision cardiac targeting: empowering curcumin therapy through smart exosome-mediated drug delivery in myocardial infarction. *Regen Biomater*. 2024;11:rbad108doi. <https://doi.org/10.1093/rb/rbad108>.
122. Matsumoto A, Takahashi Y, Nishikawa M, Sano K, Morishita M, Charoenviriyakul C, et al. Role of phosphatidylserine-derived negative surface charges in the Recognition and Uptake of Intravenously injected B16BL6-Derived exosomes by macrophages. *J Pharm Sci*. 2017;106(1):168–75. <https://doi.org/10.1016/j.xphs.2016.07.022>.
123. Barrès C, Blanc L, Bette-Bobillo P, André S, Mamoun R, Gabius HJ, et al. Galectin-5 is bound onto the surface of rat reticulocyte exosomes and modulates vesicle uptake by macrophages. *Blood*. 2010;115(3):696–705. <https://doi.org/10.1182/blood-2009-07-231449>.
124. Watson DC, Bayik D, Srivatsan A, Bergamaschi C, Valentini A, Niu G, et al. Efficient production and enhanced tumor delivery of engineered extracellular vesicles. *Biomaterials*. 2016;105:195–205. <https://doi.org/10.1016/j.biomaterials.2016.07.003>.
125. Vangijzegem T, Stanicki D, Laurent S. Magnetic iron oxide nanoparticles for drug delivery: applications and characteristics. *Expert Opin Drug Deliv*. 2019;16(1):69–78. <https://doi.org/10.1080/17425247.2019.1554647>.
126. Lozano-Andrés E, Libregts SF, Toribio V, Royo F, Morales S, López-Martín S, et al. Tetraspanin-decorated extracellular vesicle-mimetics as a novel adaptable reference material. *J Extracell Vesicles*. 2019;8(1): 1573052. <https://doi.org/10.1080/20013078.2019.1573052>.
127. Kolosnjaj-Tabi J, Lartigue L, Javed Y, Luciani N, Pellegrino T, Wilhelm C, et al. Biotransformations of magnetic nanoparticles in the body. *Nano Today*. 2016;11(3):280–4. <https://doi.org/10.1016/j.nantod.2015.10.001>.
128. Kim HY, Kim TJ, Kang L, Kim YJ, Kang MK, Kim J, et al. Mesenchymal stem cell-derived magnetic extracellular nanovesicles for targeting and treatment of ischemic stroke. *Biomaterials*. 2020;243: 119942. <https://doi.org/10.1016/j.biomaterials.2020.119942>.
129. Fu LH, Wan Y, Qi C, He J, Li C, Yang C, et al. Nanocatalytic theranostics with glutathione depletion and enhanced reactive oxygen species generation for efficient cancer therapy. *Adv Mater*. 2021;33(7): e2006892. <https://doi.org/10.1002/adma.202006892>.
130. Wang S, Mao Y, Rong S, Liu G, Cao Y, Yang Z, et al. Engineering magnetic extracellular vesicles mimetics for enhanced targeting chemodynamic therapy to overcome ovary cancer. *ACS Appl Mater Interfaces*. 2024;16(30):39021–34. <https://doi.org/10.1021/acsami.4c06862>.
131. Nguyen Cao TG, Truong Hoang Q, Hong EJ, Kang SJ, Kang JH, Ravichandran V, et al. Mitochondria-targeting sonosensitizer-loaded extracellular vesicles for chemo-sonodynamic therapy. *J Control Release*. 2023;354:651–63. <https://doi.org/10.1016/j.jconrel.2023.01.044>.
132. Wang S, Chen K, Wang Y, Wang Z, Li Z, Guo J, et al. Cardiac-targeted delivery of nuclear receptor RORα via ultrasound targeted microbubble destruction optimizes the benefits of regular dose of melatonin on sepsis-induced cardiomyopathy. *Biomater Res*. 2023;27(1):41. <https://doi.org/10.1186/s40824-023-00377-8>.
133. Zheng J, Huang J, Zhang L, Wang M, Xu L, Dou X, et al. Drug-loaded microbubble delivery system to enhance PD-L1 blockade immunotherapy with remodeling immune microenvironment. *Biomater Res*. 2023;27(1):9. <https://doi.org/10.1186/s40824-023-00350-5>.
134. Chen J, Qiu S, Liu Y, Sun W, Zhou T, Zhao L, et al. Ultrasound targeted microbubble destruction assisted exosomal delivery of siHmx1 effectively inhibits doxorubicin-induced cardiomyocyte ferroptosis. *J Nanobiotechnol*. 2024;22(1):531. <https://doi.org/10.1186/s12951-024-02794-w>.
135. Deng Z, Wang J, Xiao Y, Li F, Niu L, Liu X, et al. Ultrasound-mediated augmented exosome release from astrocytes alleviates amyloid-β-induced neurotoxicity. *Theranostics*. 2021;11(9):4351–62. <https://doi.org/10.7150/thno.52436>.
136. Zhu Z, Zhai Y, Hao Y, Wang Q, Han F, Zheng W, et al. Specific anti-glioma targeted-delivery strategy of engineered small extracellular vesicles dual-functionalised by Angiopoietin-2 and TAT peptides. *J Extracell Vesicles*. 2022;11(8):e12255. <https://doi.org/10.1002/jev.12255>.
137. Peng W, Xie Y, Liu Y, Xu J, Yuan F, Li C, et al. Targeted delivery of CD163(+) macrophage-derived small extracellular vesicles via RGD peptides promote vascular regeneration and stabilization after spinal cord injury. *J Control Release*. 2023;361:750–65. <https://doi.org/10.1016/j.jconrel.2023.08.025>.
138. Li J, Chen X, Yi J, Liu Y, Li D, Wang J, et al. Identification and characterization of 293T cell-derived exosomes by profiling the protein, mRNA and MicroRNA Components. *PLoS ONE*. 2016;11(9):e0163043. <https://doi.org/10.1371/journal.pone.0163043>.
139. Hung ME, Leonard JN. Stabilization of exosome-targeting peptides via engineered glycosylation. *J Biol Chem*. 2015;290(13):8166–72. <https://doi.org/10.1074/jbc.M114.621383>.
140. Chen J, Zhang E, Wan Y, Huang T, Wang Y, Jiang H. A quick and innovative pipeline for producing chondrocyte-homing peptide-modified extracellular vesicles by three-dimensional dynamic culture of hADSCs spheroids to modulate the fate of remaining ear chondrocytes in the M1 macrophage-infiltrated microenvironment. *J Nanobiotechnol*. 2024;22(1):300. <https://doi.org/10.1186/s12951-024-02567-5>.
141. Li Q, Song Y, Wang Q, Chen J, Gao J, Tan H, et al. Engineering extracellular vesicles with platelet membranes fusion enhanced targeted therapeutic angiogenesis in a mouse model of myocardial ischemia reperfusion. *Theranostics*. 2021;11(8):3916–31. <https://doi.org/10.7150/thno.52496>.
142. Hu S, Wang X, Li Z, Zhu D, Cores J, Wang Z, et al. Platelet membrane and stem cell exosome hybrid enhances cellular uptake and targeting to heart injury. *Nano Today*. 2021;39:39. <https://doi.org/10.1016/j.nantod.2021.101210>.
143. Li Q, Huang Z, Wang Q, Gao J, Chen J, Tan H, et al. Targeted immunomodulation therapy for cardiac repair by platelet membrane engineering extracellular vesicles via hitching peripheral monocytes. *Biomaterials*. 2022;284:121529. <https://doi.org/10.1016/j.biomaterials.2022.121529>.
144. Zhang N, Song Y, Huang Z, Chen J, Tan H, Yang H, et al. Monocyte mimics improve mesenchymal stem cell-derived extracellular vesicle homing in a mouse MI/Rl model. *Biomaterials*. 2020;255: 120168. <https://doi.org/10.1016/j.biomaterials.2020.120168>.
145. Jiang J, Ni L, Zhang X, Wang H, Liu L, Wei M, et al. Platelet membrane-fused circulating Extracellular vesicles protect the heart from Ischemia/Reperfusion Injury. *Adv Healthc Mater*. 2023;e2300052. <https://doi.org/10.1002/adhm.202300052>.
146. Sato YT, Umekaki K, Sawada S, Mukai SA, Sasaki Y, Harada N, et al. Engineering hybrid exosomes by membrane fusion with liposomes. *Sci Rep*. 2016;6: 21933. <https://doi.org/10.1038/srep21933>.

147. Wen Y, Fu Q, Soliwoda A, Zhang S, Zheng M, Mao W, et al. Cell-derived nanovesicles prepared by membrane extrusion are good substitutes for natural extracellular vesicles. *Extracell Vesicle*. 2022;1:1. <https://doi.org/10.1016/j.vesic.2022.100004>.
148. Jiang Y, Yu M, Song ZF, Wei ZY, Huang J, Qian HY. Targeted delivery of mesenchymal stem cell-derived Bioinspired Exosome-Mimetic nanovesicles with platelet membrane Fusion for atherosclerotic treatment. *Int J Nanomed*. 2024;19:2553–71. <https://doi.org/10.2147/ijn.S452824>.
149. Jiang J, Ni L, Zhang X, Wang H, Liu L, Wei M, et al. Platelet membrane-fused circulating Extracellular vesicles protect the heart from Ischemia/Reperfusion Injury. *Adv Healthc Mater*. 2023;12(21). <https://doi.org/10.1002/adhm.202300052>.
150. Xie L, Chen J, Hu H, Zhu Y, Wang X, Zhou S, et al. Engineered M2 macrophage-derived extracellular vesicles with platelet membrane fusion for targeted therapy of atherosclerosis. *Bioact Mater*. 2024;35:447–60. <https://doi.org/10.1016/j.bioactmat.2024.02.015>.
151. Wu Z, Zhang H, Yan J, Wei Y, Su J. Engineered biomembrane-derived nanoparticles for nanoscale theranostics. *Theranostics*. 2023;13(1):20–39. <https://doi.org/10.7150/thno.76894>.
152. Smyth T, Petrova K, Payton NM, Persaud I, Redzic JS, Graner MW, et al. Surface functionalization of exosomes using click chemistry. *Bioconjug Chem*. 2014;25(10):1777–84. <https://doi.org/10.1021/bc500291r>.
153. Jia G, Han Y, An Y, Ding Y, He C, Wang X, et al. NRP-1 targeted and cargo-loaded exosomes facilitate simultaneous imaging and therapy of glioma in vitro and in vivo. *Biomaterials*. 2018;178:302–16. <https://doi.org/10.1016/j.biomaterials.2018.06.029>.
154. Xue Q, Kang R, Klionsky DJ, Tang D, Liu J, Chen X. Copper metabolism in cell death and autophagy. *Autophagy*. 2023;19(8):2175–95. <https://doi.org/10.1080/15548627.2023.2200554>.
155. Salunkhe S, Dheeraj, Basak M, Chitkara D, Mittal A. Surface functionalization of exosomes for target-specific delivery and in vivo imaging & tracking: strategies and significance. *J Control Release*. 2020;326:599–614. <https://doi.org/10.1016/j.jconrel.2020.07.042>.
156. Camardo SS, Dahal A, Ramamurthi A. Surface-functionalized stem cell-derived extracellular vesicles for Vascular Elastic Matrix Regenerative Repair. *Mol Pharm*. 2023;20(6):2801–13. <https://doi.org/10.1021/acs.molpharmaceut.2c00769>.
157. Kang JY, Mun D, Chun Y, Park DS, Kim H, Yun N, et al. Engineered small extracellular vesicle-mediated NOX4 siRNA delivery for targeted therapy of cardiac hypertrophy. *J Extracell Vesicles*. 2023;12(10):e12371 doi. <https://doi.org/10.1002/jev2.12371>.
158. Hosseini Shamili F, Alibolandi M, Rafatpanah H, Abnous K, Mahmoudi M, Kalantari M, et al. Immunomodulatory properties of MSC-derived exosomes armed with high affinity aptamer toward myelin as a platform for reducing multiple sclerosis clinical score. *J Control Release*. 2019;299:149–64. <https://doi.org/10.1016/j.jconrel.2019.02.032>.
159. Haugland RP, You WW. Coupling of antibodies with biotin. *Methods Mol Biol*. 2008;418:13–24. https://doi.org/10.1007/978-1-59745-579-4_2.
160. Deng D, Li X, Zhang JJ, Yin Y, Tian Y, Gan D, et al. Biotin-avidin system-based delivery enhances the therapeutic performance of MSC-Derived exosomes. *ACS Nano*. 2023;17(9):8530–50. <https://doi.org/10.1021/acsnano.3c00839>.
161. Yoon HY, Lee D, Lim DK, Koo H, Kim K. Copper-free click Chemistry: applications in drug delivery, cell tracking, and tissue engineering. *Adv Mater*. 2022;34(10):e2107192. <https://doi.org/10.1002/adma.202107192>.
162. Stump B. Click bioconjugation: modifying proteins using click-like chemistry. *ChemBioChem*. 2022;23(16): e202200016. <https://doi.org/10.1002/cbic.202200016>.
163. Zhang J, Ji C, Zhang H, Shi H, Mao F, Qian H, et al. Engineered neutrophil-derived exosome-like vesicles for targeted cancer therapy. *Sci Adv*. 2022;8(2):eabj8207. <https://doi.org/10.1126/sciadv.abj8207>.
164. Lee JR, Park BW, Kim J, Choo YW, Kim HY, Yoon JK, et al. Nanovesicles derived from iron oxide nanoparticles-incorporated mesenchymal stem cells for cardiac repair. *Sci Adv*. 2020;6(18):eaz0952doi. <https://doi.org/10.1126/sciadv.aaz0952>.
165. Zhuang M, Rao L, Chen Y, Xiao S, Xia H, Yang J, et al. Controlled SPION-Exosomes loaded with quercetin preserves pancreatic Beta cell survival and function in type 2 diabetes Mellitus. *Int J Nanomed*. 2023;18:5733–48. <https://doi.org/10.2147/ijn.S422416>.
166. Hatzakis NS, Bhatia VK, Larsen J, Madsen KL, Bolinger PY, Kunding AH, et al. How curved membranes recruit amphipathic helices and protein anchoring motifs. *Nat Chem Biol*. 2009;5(11):835–41. <https://doi.org/10.1038/nchembio.213>.
167. Liu L, Li Y, Peng H, Liu R, Ji W, Shi Z, et al. Targeted exosome coating gene-chem nanocomplex as nanoscavenger for clearing α -synuclein and immune activation of Parkinson's disease. *Sci Adv*. 2020;6(50). <https://doi.org/10.1126/sciadv.aba3967>.
168. Rath A, Tulumello DV, Deber CM. Peptide models of membrane protein folding. *Biochemistry*. 2009;48(14):3036–45. <https://doi.org/10.1021/bi900184j>.
169. Lewis RN, Zhang YP, Hodges RS, Subczynski WK, Kusumi A, Flach CR, et al. A polyaniline-based peptide cannot form a stable transmembrane α -helix in fully hydrated phospholipid bilayers. *Biochemistry*. 2001;40(40):12103–11. <https://doi.org/10.1021/bi010555m>.
170. Zoulikha M, Xiao Q, Boafu GF, Sallam MA, Chen Z, He W. Pulmonary delivery of siRNA against acute lung injury/acute respiratory distress syndrome. *Acta Pharm Sin B*. 2022;12(2):600–20. <https://doi.org/10.1016/j.apsb.2021.08.009>.
171. Cui GH, Guo HD, Li H, Zhai Y, Gong ZB, Wu J, et al. RVG-modified exosomes derived from mesenchymal stem cells rescue memory deficits by regulating inflammatory responses in a mouse model of Alzheimer's disease. *Immun Ageing*. 2019;16(10). <https://doi.org/10.1186/s12979-019-0150-2>.
172. Choi H, Choi Y, Yim HY, Mirzaaghasi A, Yoo JK, Choi C. Biodistribution of exosomes and Engineering Strategies for targeted delivery of therapeutic exosomes. *Tissue Eng Regen Med*. 2021;18(4):499–511. <https://doi.org/10.1007/s13770-021-00361-0>.
173. Gangadaran P, Gunassekaran GR, Rajendran RL, Oh JM, Vadevoo SMP, Lee HW, et al. Interleukin-4 receptor targeting peptide decorated extracellular vesicles as a platform for in vivo drug delivery to thyroid Cancer. *Biomedicine*. 2022;10(8):1978. <https://doi.org/10.3390/biomedicine10081978>.
174. Kim Y, Mok H. Citraconylated exosomes for improved internalization into macrophages. *Appl Biol Chem*. 2019;62(1):26. <https://doi.org/10.1186/s13765-019-0433-5>.
175. Szatanek R, Baj-Krzyworzeka M, Zimoch J, Lekka M, Siedlar M, Baran J. The methods of choice for Extracellular vesicles (EVs) characterization. *Int J Mol Sci*. 2017;18(6):1153. <https://doi.org/10.3390/ijms18061153>.
176. Welsh JA, Goberdhan DCI, O'Driscoll L, Buzas EI, Blenkiron C, Bus-solati B, et al. Minimal information for studies of extracellular vesicles (MISEV2023): from basic to advanced approaches. *J Extracell Vesicles*. 2024;13(2):e12404. <https://doi.org/10.1002/jev2.12404>.
177. Guo L, Huang Z, Huang L, Liang J, Wang P, Zhao L, et al. Surface-modified engineered exosomes attenuated cerebral ischemia/reperfusion injury by targeting the delivery of quercetin towards impaired neurons. *J Nanobiotechnol*. 2021;19(1):141. <https://doi.org/10.1186/s12951-021-00879-4>.
178. Mateescu B, Kowal EJ, van Balkom BW, Bartel S, Bhattacharyya SN, Buzás EI, et al. Obstacles and opportunities in the functional analysis of extracellular vesicle RNA - an ISEV position paper. *J Extracell Vesicles*. 2017;6(1):1286095. <https://doi.org/10.1080/20013078.2017.1286095>.
179. Zhu MZ, Xu HM, Liang YJ, Xu J, Yue NN, Zhang Y, et al. Edible exosome-like nanoparticles from portulaca oleracea L mitigate DSS-induced colitis via facilitating double-positive CD4(+)CD8(+)T cells expansion. *J Nanobiotechnol*. 2023;21(1):309. <https://doi.org/10.1186/s12951-023-02065-0>.
180. Midekessa G, Godakumara K, Ord J, Viil J, Lättetkivi F, Dissanayake K, et al. Zeta potential of extracellular vesicles: toward understanding the attributes that determine colloidal stability. *ACS Omega*. 2020;5(27):16701–10. <https://doi.org/10.1021/acsomega.0c01582>.
181. Görgens A, Corso G, Hagey DW, Jawad Wiklander R, Gustafsson MO, Fellidin U, et al. Identification of storage conditions stabilizing extracellular vesicles preparations. *J Extracell Vesicles*. 2022;11(6):e12238. <https://doi.org/10.1002/jev2.12238>.
182. Shimasaki T, Yamamoto S, Arisawa T. Exosome research and co-culture study. *Biol Pharm Bull*. 2018;41(9):1311–21. <https://doi.org/10.1248/bpb.b18-00223>.
183. Verweij FJ, Balaj L, Boulanger CM, Carter DRF, Compeer EB, D'Angelo G, et al. The power of imaging to understand extracellular vesicle biology in vivo. *Nat Methods*. 2021;18(9):1013–26. <https://doi.org/10.1038/s41592-021-01206-3>.

184. Liu Q, Huang J, Xia J, Liang Y, Li G. Tracking tools of extracellular vesicles for biomedical research. *Front Bioeng Biotechnol*. 2022;10: 943712. <https://doi.org/10.3389/fbioe.2022.943712>.
185. Kim H, Mun D, Kang JY, Lee SH, Yun N, Joung B. Improved cardiac-specific delivery of RAGE siRNA within small extracellular vesicles engineered to express intense cardiac targeting peptide attenuates myocarditis. *Mol Ther Nucleic Acids*. 2021;24:1024–32. <https://doi.org/10.1016/j.omtn.2021.04.018>.
186. Mentkowski KI, Lang JK. Exosomes engineered to express a cardiomyocyte binding peptide demonstrate improved cardiac retention in vivo. *Sci Rep*. 2019;9(1):10041. <https://doi.org/10.1038/s41598-019-46407-1>.
187. Liu S, Chen X, Bao L, Liu T, Yuan P, Yang X, et al. Treatment of infarcted heart tissue via the capture and local delivery of circulating exosomes through antibody-conjugated magnetic nanoparticles. *Nat Biomed Eng*. 2020;4(11):1063–75. <https://doi.org/10.1038/s41551-020-00637-1>.
188. Liu X, Cao Z, Wang W, Zou C, Wang Y, Pan L, et al. Engineered extracellular vesicle-delivered CRISPR/Cas9 for radiotherapy sensitization of glioblastoma. *ACS Nano*. 2023;17(17):16432–47. <https://doi.org/10.1021/acsnano.2c12857>.
189. Liang B, Zhu Y, Ali DJ, Tian T, Xu H, Si K, et al. Engineered exosomes for targeted co-delivery of miR-21 inhibitor and chemotherapeutics to reverse drug resistance in colon cancer. *J Nanobiotechnol*. 2020;18(1):10. <https://doi.org/10.1186/s12951-019-0563-2>.
190. Huang Q, Jiang Y, Cao Y, Ding Y, Cai J, Yang T, et al. Bone-targeting engineered milk-derived extracellular vesicles for MRI-assisted therapy of osteoporosis. *Regen Biomater*. 2024;11:rbae112doi. <https://doi.org/10.1093/rb/rbae112>.
191. Yang L, Han B, Zhang Z, Wang S, Bai Y, Zhang Y, et al. Extracellular vesicle-mediated delivery of circular RNA SCMH1 promotes functional recovery in rodent and nonhuman primate ischemic stroke models. *Circulation*. 2020;142(6):556–74. <https://doi.org/10.1161/circulationaha.120.045765>.
192. Wang Y, Li X, Wei X, Li L, Bai H, Yan X, et al. Identification of combinatorial miRNA panels derived from extracellular vesicles as biomarkers for esophageal squamous cell carcinoma. *MedComm*. 2023;4(5):e377. <https://doi.org/10.1002/mco2.377>.
193. Nakamura K, Zhu Z, Roy S, Jun E, Han H, Munoz RM, et al. An exosome-based transcriptomic signature for noninvasive, early detection of patients with pancreatic ductal adenocarcinoma: a multicenter cohort study. *Gastroenterology*. 2022;163(5):1252–e12662. <https://doi.org/10.1053/j.gastro.2022.06.090>.
194. Han HT, Jin WL, Li X. Mesenchymal stem cells-based therapy in liver diseases. *Mol Biomed*. 2022;3(1):23. <https://doi.org/10.1186/s43556-022-00088-x>.
195. Shi MM, Yang QY, Monsel A, Yan JY, Dai CX, Zhao JY, et al. Preclinical efficacy and clinical safety of clinical-grade nebulized allogenic adipose mesenchymal stromal cells-derived extracellular vesicles. *J Extracell Vesicles*. 2021;10(10): e12134. <https://doi.org/10.1002/jev2.12134>.
196. Hadizadeh A, Akbari-Asbagh R, Heirani-Tabasi A, Soleimani M, Gorovanchi P, Ebrahimi Daryani N, et al. Localized administration of mesenchymal stem cell-derived exosomes for the treatment of refractory perianal fistula in crohn's disease patients: a phase II clinical trial. *Dis Colon Rectum*. 2024. <https://doi.org/10.1097/dcr.00000000000003502>.
197. Kwon HH, Yang SH, Lee J, Park BC, Park KY, Jung JY, et al. Combination treatment with human adipose tissue stem cell-derived exosomes and fractional CO₂ laser for acne scars: a 12-week prospective, double-blind, randomized, split-face study. *Acta Derm Venereol*. 2020;100(18):adv00310. <https://doi.org/10.2340/00015555-3666>.
198. Zhang M, Viennois E, Prasad M, Zhang Y, Wang L, Zhang Z, et al. Edible ginger-derived nanoparticles: a novel therapeutic approach for the prevention and treatment of inflammatory bowel disease and colitis-associated cancer. *Biomaterials*. 2016;101:321–40. <https://doi.org/10.1016/j.biomaterials.2016.06.018>.
199. Zhang Y, Bi J, Huang J, Tang Y, Du S, Li P. Exosome: a review of its classification, isolation techniques, storage, diagnostic and targeted therapy applications. *Int J Nanomedicine*. 2020;15:6917–34. <https://doi.org/10.2147/ijn.S264498>.
200. Sunkara V, Park J, Han J, Rio D, Cho JS, Oh HJ. Exosome precipitation by ionic strength modulation: ExoPRISM. *ACS Appl Mater Interfaces*. 2023;15(49):56807–19. <https://doi.org/10.1021/acsaami.3c13527>.
201. Kim J, Zhu Y, Chen S, Wang D, Zhang S, Xia J, et al. Anti-glioma effect of ginseng-derived exosomes-like nanoparticles by active blood-brain-barrier penetration and tumor microenvironment modulation. *J Nanobiotechnol*. 2023;21(1):253. <https://doi.org/10.1186/s12951-023-02006-x>.
202. Qiu FS, Wang JF, Guo MY, Li XJ, Shi CY, Wu F, et al. Rgl-exomiR-7972, a novel plant exosomal microRNA derived from fresh *Rehmannia* Radix, ameliorated lipopolysaccharide-induced acute lung injury and gut dysbiosis. *Biomed Pharmacother*. 2023;165(115007):115007. <https://doi.org/10.1016/j.biopha.2023.115007>.
203. Du J, Liang Z, Xu J, Zhao Y, Li X, Zhang Y, et al. Plant-derived phosphocholine facilitates cellular uptake of anti-pulmonary fibrotic HJT-sRNA-m7. *Sci China Life Sci*. 2019;62(3):309–20. <https://doi.org/10.1007/s11427-017-9026-7>.
204. Yan C, Chen J, Wang C, Yuan M, Kang Y, Wu Z, et al. Milk exosomes-mediated mir-31-5p delivery accelerates diabetic wound healing through promoting angiogenesis. *Drug Deliv*. 2022;29(1):214–28. <https://doi.org/10.1080/10717544.2021.2023699>.
205. Liang J, Zhu F, Cheng K, Ma N, Ma X, Feng Q, et al. Outer membrane vesicle-based nanohybrids target tumor-associated macrophages to enhance trained immunity-related vaccine-generated antitumor activity. *Adv Mater*. 2023;35(46): e2306158. <https://doi.org/10.1002/adma.202306158>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.