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Review

Engineered Extracellular Vesicles as a New Class of Nanomedicine

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Cite This: *Chem Bio Eng.* 2025, 2, 3–22

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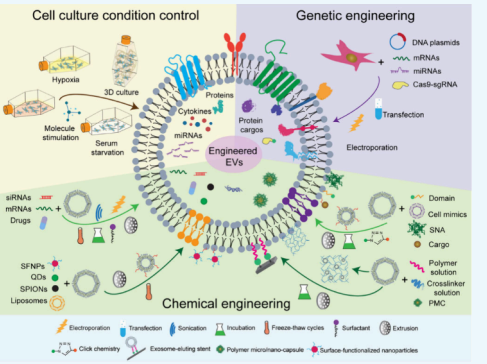
ABSTRACT: Extracellular vesicles (EVs) are secreted from biological cells and contain many molecules with diagnostic values or therapeutic functions. There has been great interest in academic and industrial communities to utilize EVs as tools for diagnosis or therapeutics. In addition, EVs can also serve as delivery vehicles for therapeutic molecules. An indicator of the enormous interest in EVs is the large number of review articles published on EVs, with the focus ranging from their biology to their applications. An emerging trend in EV research is to produce and utilize "engineered EVs", which are essentially the enhanced version of EVs. EV engineering can be conducted by cell culture condition control, genetic engineering, or chemical engineering. Given their nanometer-scale sizes and therapeutic potentials, engineered EVs are an emerging class of nanomedicines. So far, an overwhelming majority of the research on engineered EVs is preclinical studies; there are only a very small number of reported clinical trials. This Review focuses on engineered EVs, with a more specific focus being their applications in therapeutics. The various approaches to producing engineered EVs and their applications in various diseases are reviewed. Furthermore, *in vivo* imaging of EVs, the mechanistic understandings, and the clinical translation aspects are discussed. The discussion is primarily on preclinical studies while briefly mentioning the clinical trials. With continued interdisciplinary research efforts from biologists, pharmacists, physicians, bioengineers, and chemical engineers, engineered EVs could become a powerful solution for many major diseases such as neurological, immunological, and cardiovascular diseases.



KEYWORDS: *Cell therapy, exosome, extracellular vesicle, cell engineering, stem cell, immunotherapy, drug delivery*

1. INTRODUCTION

All biological cells release vesicles, namely, extracellular vesicles (EVs). These vesicles, which enclose their molecular contents within membranes, were initially thought to be merely a form of waste of the cells. In 2007, a groundbreaking paper from Jan Lötvall's group at Göteborg University showed that exosomes, a type of EVs, can transport *functional* nucleic acids between different cells.[1] Since then, numerous papers have been published demonstrating that this is in fact a general phenomenon.[2] It appears that EVs can work as mediators of communication between cells. Among different types of EVs, exosomes are of particular interest because their cellular generation involves a distinct intracellular regulatory process which likely dictates their composition and functions, after being released into the extracellular environment.[2,3] As fundamental research into the biology of EVs continues, efforts to apply EVs as tools to diagnose or treat diseases have also flourished. For disease diagnosis, exosomes are attractive because they could be used as minimally invasive liquid biopsies with the potential for longitudinal sampling to follow disease progression.[2,4] For disease treatment, exosomes and other EVs are being investigated as drug delivery vehicles or/and therapeutics themselves.[2,5,6] This review article focuses on the disease treatment aspect.

In the efforts to treat diseases with EVs, a new trend is emerging, i.e., modifying the structures of EVs to enhance certain functions or to integrate with additional functions. The resulting EVs, called engineered EVs here, are analogous to engineered organisms, e.g., genetically modified rice with vitamin enrichment or genetically modified corn with insect resistance. Yet, the sizes of engineered EVs are at nanometer-scale, which are much smaller than those of engineered organisms. Thus, engineered EVs can also be considered as a new class of nanomedicine (nanometer-scale matters as medicine). This article reviews the burgeoning field of engineered EVs as nanomedicine, discussing how to generate engineered EVs, their medical applications, probing their biological fates, and their clinical/industrial translation.

Received: July 13, 2024
Revised: October 19, 2024
Accepted: October 20, 2024
Published: October 28, 2024

https://doi.org/10.1021/cbe.4c00122
Chem Bio Eng. 2025, 2, 3–22

2. EVs

Cells release many different types of EVs. In general, these EVs can be classified into two major categories, namely, ectosomes and exosomes. Ectosomes are vesicles generated by the direct outward budding of the plasma membrane. Ectosomes include microvesicles, microparticles, and large vesicles in the size range of ∼50 nm to 1 mm in diameter. On the other hand, exosomes originated from endosomes in the cell and in a size range of ∼40 to 160 nm in diameter. Exosomes are of particular interest because of their generation involves a distinct intracellular process, which is thus tightly regulated and likely determines their composition and functions.[2,5,6] This Review focuses on exosomes, unless stated otherwise.

As mentioned above, the biogenesis of exosomes is linked to endosomal pathways. More specifically, the exosome biogenesis process involves the following steps: (1) Invagination of the plasma membrane forms a cup-shaped structure that includes cell-surface proteins and soluble proteins associated with the extracellular milieu. (2) This leads to *de novo* formation of an early sorting endosomes (ESE), which may merge with a preexisting ESE. The trans-Golgi network and endoplasmic reticulum can also contribute to the formation of the ESE. (3) ESEs mature into late-sorting endosomes (LSEs) and eventually generate multivesicular bodies (MVBs). This process gives rise to MVBs containing several intraluminal vesicles (ILVs), which are future exosomes. 4) The MVBs can either enter the degradation pathway (usually by fusing with lysosomes or autophagosomes), or fuse with the plasma membrane to release the ILVs as exosomes to the extracellular environment.[7−10] Interestingly, recent research has found that autophagy can also lead to exosome secretion, in a process termed secretary autophagy.[11] The exosome biogenesis pathway intersects with other molecular pathways associated with the trafficking of intracellular vesicles. Rab GTPases, a large family of small GTPases, play central roles in ensuring precise transport in these vesicles.[12] The ESCRT (endosomal sorting complexes required for transport) machinery plays a key role in the generation of ILVs in MVBs.[13] The fusion of MVBs with the plasma membrane to release exosomes is mainly mediated by SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein receptors).[14] Other important molecular players in the exosome biogenesis include Sytenin-1, TSG101 (tumor susceptibility gene 101), ALIX (apoptosis-linked gene 2-interacting protein X), syndecan-1, phospholipids, tetraspanins, ceramides, and sphingomyelinases.[7−10] Exosome surface proteins include tetraspanins (e.g., CD63, CD9, and CD81, commonly used as biomarkers of exosomes), integrins, immunomodulatory proteins, and others. Exosomes can contain different types of cell surface proteins, intracellular protein, RNA, DNA, amino acids, and metabolites.[15,16] Figure 1 shows a schematic illustration of the biogenesis process and molecular content of exosomes.

The fact that exosomes contain many molecular contents from the source cells gives rise to exosomes' diverse biological functions. The molecular contents can be further altered by environmental or innate (e.g., oncogenic) changes.[17,18] The inherent properties of exosomes in regulating complex intracellular pathways offers their potential utility as therapeutics of a wide range of diseases, such as immunogenic diseases, tissue damage, neurodegenerative diseases, and cancers.[2] For therapeutic purposes, exosomes are sometimes called "cell-free cell therapeutics", because they can impart the

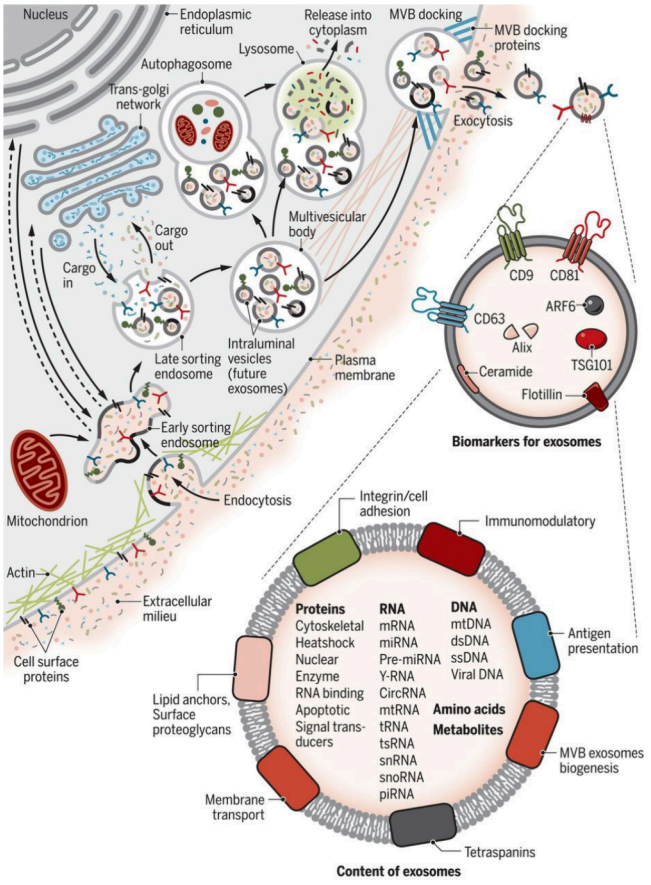


Figure 1. Biogenesis and identification of exosomes. Reproduced with permission from ref 2. Copyright 2024 The American Association for the Advancement of Science.

therapeutic functions of the source cells while bypassing the potential problems (e.g., tumorigenesis and mutagenesis risks) associated with using live cells.[19]

Exosomes have been used to regulate immune responses. Exosomes from distinct source cells, e.g., immune cells, epithelial cells, and mesenchymal stem cells (MSCs), contain molecular cargos that can influence the activities of recipient cells of both the innate and adaptive immune system. These molecular cargos include but are not limited to miRNAs, antigenic peptides, DNAs inducing cGAS-STING (cyclic GMP-AMP synthase stimulator of interferon genes) signaling, and surface ligands inducing various signaling pathways. For example, exosomes from antigen-presenting cells (APCs) carry p-MHC-II (major histocompatibility complex II with antigenic peptide) and costimulatory signals, and directly present the peptide antigen to specific T cells to induce their activation.[20] MSCs-derived exosomes can suppress the pro-inflammatory M2 macrophage phenotype, suppress pro-inflammatory Th17 cells, and cause T-regulatory cell polarization.[21,22] Thus, these exosomes have been explored as immunomodulatory therapeutic for inflammatory diseases, autoimmune diseases and graft-versus-host diseases.[23−25] In an asthma mouse model, EVs generated from adipose-derived MSCs (AMSCs) were found to enhance the FoxO1 signaling pathway, by inhibiting miR-183−5p, thereby inhibiting the M1 macrophage marker iNOS (inducible nitric oxide synthase).[26] In a mouse model of bacterial pneumonia and COVID-19 infection, MSCs-derived EVs promoted the polarization of macrophages to an anti-

https://doi.org/10.1021/cbe.4c00122
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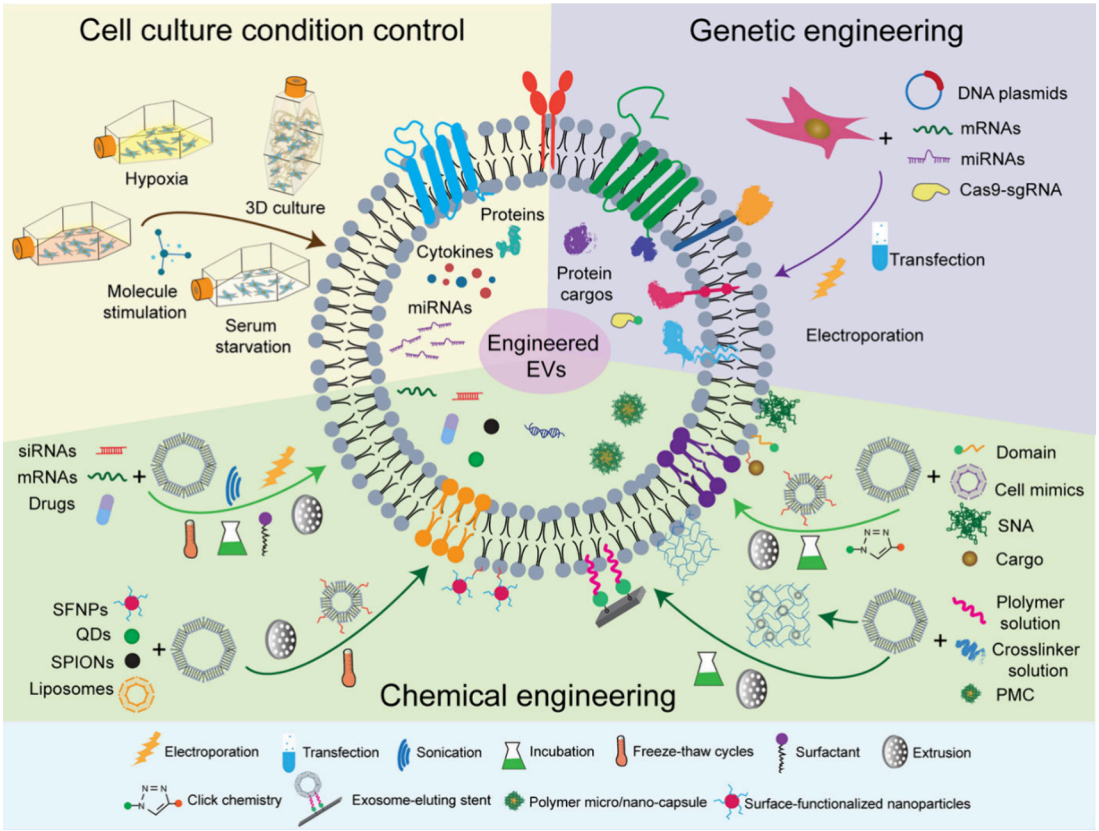

Figure 2. Schematic illustration of the various methods of modifying the content and structure of EVs, for enhanced and integrated and new functions.

inflammatory M2 phenotype when delivered via inhalation.[27] MSCs-derived EVs reduced the expression of pro-inflammatory cytokines, increased the expression of anti-inflammatory cytokines, and improved pathological scores in acute lung injury.

Exosomes have also been used for tissue repair and regeneration of various organs.[19] Exosomes can reduce cell apoptosis and tissue inflammation while increasing cell proliferation, survival, angiogenesis, and lineage specific differentiation. The most popular option as the cell source of exosomes is so far MSCs. Other reported cell sources of exosomes for tissue regeneration include embryonic induced pluripotent and tissue-specific stem cells, primary precursor cells and mature cells from the tissue to be regenerated or from immune/endothelial origin.[28−30] For example, intravitreal administration of MSCs-derived EVs 24 h after the ischemic event resulted in improved functional recovery, reduced neuroinflammation, and decreased apoptosis.[31] In another example, a phase II clinical trial of COVID-19-associated acute respiratory distress syndrome (ARDS) showed that intravenous administration of MSCs-derived EVs significantly reduced the 60 day mortality rate of critical patients. In this trial it was also reported that allogenic MSCs-derived EVs caused no significant increase in treatment-related serious adverse events compared to the placebo.[32]

In addition to functioning as therapeutics themselves, EVs have also been explored as delivery carriers for pharmaceuticals. Potentially, EVs can circumvent several key problems associated with synthetic nanoparticles (e.g., liposomes, lipid nanoparticles, and polymer nanoparticles) as drug delivery carriers. Compared with these synthetic nanoparticles, EVs are less immunogenic, less toxic, and are more capable of crossing biological barriers such as the blood-brain barrier and extracellular matrix.[33] The advantages of EVs in low immunogenicity and low toxicity largely originate from the fact that they are derived from biological cells.[34] Interestingly, it has been reported that CD47 on exosomes derived from normal fibroblast-like mesenchymal cells results in a "don't-eat-me" signal, protecting them from phagocytosis and limiting their clearance from circulation.[35] In another important mechanistic study, it has been shown that the reasons why EVs (from mouse mesenchymal stromal cells) can traverse dense extracellular matrix (with pore sizes smaller than those of EVs) are likely because (1) matrix stress relaxation allows EVs to overcome the confinement, and (2) water permeation through aquaporin-1 mediates the EV deformability.[36]

There are many unanswered questions regarding the biology of EVs. For example, the exact roles of EVs in communications between cells within the same organ and between organs remains unclear. Also, it is unclear whether the biogenesis and molecular content of EVs change with age. Answers to these questions would help guide the design of EVs-based therapies.

3. PRODUCTION OF ENGINEERED EVs

Modification of the content and structure of EVs, for enhanced or integrated or new functions, can be achieved by many different methods. Here, these methods are broadly categorized into three classes, namely, cell culture condition control, genetic engineering, and chemical engineering. Figure 2 is a schematic summarizing the various methods under the three broad classes.

https://doi.org/10.1021/cbe.4c00122
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3.1. Cell Culture Condition Control. The content and secretion rate of EVs can be influenced by the external environment of the source cells. Culturing the source cells in stress-inducing conditions, e.g., hypoxia, serum starvation, or inflammation, has been widely used.[37] For example, a short-term hypoxia treatment on MSCs yielded EVs with increased levels of several therapeutically important miRNAs such as miR-103 (with apoptosis inhibition function), miR-210 (with pro-angiogenesis and cardio-protection functions), and miR-17 (with fibrosis inhibition, pro-proliferation and pro-angiogenesis functions).[38] Preconditioning adipose-derived stem cells with endothelial differential medium increased the secretion of microvesicles and enhanced the angiogenic effect of the secreted microvesicles *in vitro*.[39] Gorgun et al. conducted a study to dissect the effects of preconditioning with inflammatory cytokines and hypoxia on the two different portions of MSC secretome, namely soluble factors and EVs. It was found that the inflammation stimuli strongly inhibited the pro-angiogenic capacity of the soluble factors, but did not significantly affect that of the EV portion.[40]

Liao et al. showed that treating MSCs with metformin, a widely used oral antihyperglycemic drug for diabetes, promoted the release of EVs from MSCs.[41] Further mechanistic studies found that the increased EV secretion occurred via an autophagy-related pathway. Proteomics analysis revealed that metformin increased the protein content of EVs involved in cell growth. Yang et al. reported that heat shock treatment on bone marrow MSCs (BMSCs) led to upregulation of the 70 kDa heat shock protein (HSP70) in BMSC-derived exosomes, and alleviation of cisplatin-induced ototoxicity in mice.[42] Multiple reports indicated that 3D culture of the source cells could significantly change the contents (including miRNAs and proteins) and amount of EVs.[43−45]

3.2. Genetic Engineering of EVs. The source cells can be genetically modified to produce EVs with a specific content enriched or added. For example, Tao et al. transduced human synovial MSCs with lentivector miR-140−5p. The EVs derived from the thus-formed miR-140−5p-overexpressing MSCs were found to improve cartilage tissue regeneration and prevented osteoarthritis of the knee in a rat model.[46] The content of therapeutic proteins in EVs can be modified in a similar way as for therapeutic miroRNAs. Yu et al. transduced bone marrow MSCs with the protein GATA-4 by a retroviral expression system. GATA-4 is a transcription factor important for the regulation of angiogenesis and cell survival. EVs derived from these GATA-4-overexpressing MSCs were shown to reduce apoptosis, restored cardiac contractile functions and reduced infarct size in a regional myocardial ischemia/infarction rodent model.[47]

Yang et al. reported a modified electroporation treatment on the source cells (called "cellular nanoporation method" in the paper).[48] The cellular nanoporation was achieved with a custom-designed biochip, which permits a monolayer of source cells to be cultured above the chip surface containing an array of nanochannels (approximately 500 nm in diameter). These nanochannels enables the passage of focal and transient electric pulses to the source cells, to load nucleic acids (e.g., mRNA and DNA plasmids) into the cells. Remarkably, it was shown that, compared with conventional "bulk" electroporation treatment, the cellular nanoporation method yielded a large increase in production rate of exosomes containing the above-mentioned nucleic acids. Mechanistically, it was found that the increase in exosome biogenesis was the result of a stress response. More specifically, it was caused by thermal shock through increased production of heat-shock proteins via the p53−TSAP6 signaling pathway. The cellular nanoporation method was successfully applied for gliomas treatment[48] and for collagen replacement therapy in small animal models.[49]

Fusion of the genetic sequence of a selected protein with that of a protein abundantly present on an EV membrane has become a successful strategy to load the selected protein on an EV membrane. This strategy has been employed to achieve targeted delivery of EVs to difficult-to-reach tissues.[50−52] A limitation of this strategy is that usually only a fraction of the EV population is labeled by the selected protein, thus, limiting the targeting ability to just a subset of EVs. To overcome this limitation, Gupta et al. conducted a systematic screening of EV-loading protein moieties, which resulted in optimization of the joint display of two different therapeutically relevant protein receptors on EVs namely cytokine-binding domains derived from tumor necrosis factor receptor 1 (TNFR1) and interleukin-6 signal transducer (IL-6ST).[53] The optimization of surface display of EVs led to enhanced targeting ability and improved therapeutic efficacy of anti-inflammation in three different inflammatory mouse models.

In a sophisticated utilization of genetic engineering for EV production, Stranford et al. successfully integrated three complementary functional components into EVs, all by genetic engineering of the source cells of EVs.[54] This work addressed several problems in genome editing of T cells for immunotherapy, including lack of endocytosis in T cells, targeting to T cells, loading of Cas9-based genome editing tools, and release of molecular cargo to cytoplasm of T cells. The authors termed their platform technology GEMINI (genetically encoded multifunctional integrated nanovesicles). In this work, the source cells used for EV production were HEK293FT producer cells; multiple populations of EVs were collected and used for delivery of Cas9 ribonucleoproteins (RNPs) into T cells.

GEMINI incorporates the following three main components. First, to enhance targeted binding of EVs with T cells, antibody single-chain variable fragments (scFvs) were genetically engineered on the surface of EVs to facilitate specific interactions with CD2, a costimulatory receptor that is highly expressed on T cells. The authors optimized the coding sequence of the scFv display construct for expression in human cells using a sliding window algorithm. This optimization led to a >100-fold increase in EV binding to CD2+ Jurkat T cells over nontargeted EVs. Second, to both enhance cargo protein loading and increase the likelihood that a given EV will incorporate both a cytosolic cargo protein and the membrane-bound scFv, the authors designed a small molecule dimerization-based loading system. This loading system is based on the plant hormone abscisic acid (ABA)-inducible interaction between truncated versions of the abscisic acid insensitive 1 (ABI) and pyrabactin resistance-like (PYL) proteins. This loading system has several benefits: the association is rapid; the dimerization is reversible, presumably allowing for cargo release in recipient cells; ABA is inexpensive and nontoxic; and small-molecule-regulated loading is readily applicable to biomanufacturing. Third, to enhance EV uptake and cargo release in T cells, fusion proteins were genetically engineered on the EV surface. Two fusion proteins, namely vesicular stomatitis virus glycoprotein (VSV-G) and T cell-specific measles virus glycoproteins hemeagglutinin (H) and

https://doi.org/10.1021/cbe.4c00122
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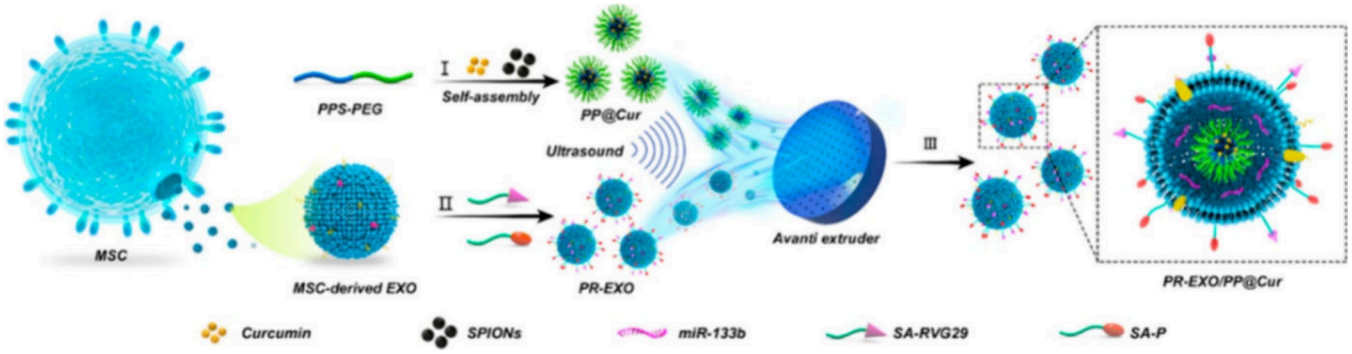

Figure 3. Multifunctional EVs, composed of MSC-derived exosomes incorporating curcumin-loaded SPIONs, for PD treatment. Reproduced with permission from ref 60. Copyright 2024 American Chemical Society.

fusion (F), showed positive outcome in enhancing EV fusion with T cells. By combining the above three genetic engineering methods, the authors achieved EV-mediated Cas9 editing of the CXCR4 locus (which encodes the HIV coreceptor CXCR4) in primary human T cells.

3.3. Chemical Engineering of EVs. In addition to treating the source cells, EV engineering can also be achieved by treating the EVs after they are generated from the source cells. These methods include loading therapeutic molecules into EVs, loading nanomaterials into EVs, surface modification of EVs, and the encapsulation of EVs into hydrogels.

3.3.1. Loading Therapeutic Molecules into EVs. The first report of exogenous drug loading into EVs was published in 2011, in which therapeutic siRNA was loaded by electroporation of exosomes.[55] Delivered by exosomes, the siRNA exhibited enhanced ability of crossing the blood-brain barrier and led to significant downregulation of an Alzheimer-associated gene in a mouse model. Electroporation was later also used for exogenous loading of small molecule drugs into EVs.[56] Fuhrmann et al. performed a systematic study on several different methods of exogenous loading of small molecule drugs, using hydrophobic porphyrins as the model drugs.[57] The examined drug loading methods included electroporation, surfactant, extrusion, and dialysis. These "active loading" methods were found to be up to 11-fold more efficient in drug loading than the passive loading method, i.e., simply incubating the drug with the EVs.

Dehghani et al. conducted another systematic study on different methods of exogenous loading of tofacitinib (TFC), a janus kinase (JAK) inhibitor for treating psoriasis (overactive immune system causing skin cells to overgrow).[58] The authors evaluated five different methods to load tofacitinib into exosomes of keratinocytes (the primary cell type found in the epidermis): (i) TFC incubation with donor cells of exosomes, (ii) TFC incubation with exosomes, (iii) freeze–thaw cycles of exosomes, (iv) probe sonication of exosomes, and (v) ultrasonic bath of exosomes. After particle size, zeta potential, drug loading efficiency, and release efficiency were compared, the probe sonication method was selected. The thus-prepared TFC-loaded exosomes showed a significant therapeutic effect in a mouse model of psoriasis.

In an effort to treat lung cancer and promote systemic immunity, Liu et al. loaded IL-12 mRNA into human embryonic kidney cell-derived exosomes (HEK-Exo) through electroporation, yielding IL-12-Exo.[59] IL-12 (interleukin-12) stimulates interferon-γ (IFNγ) production, turning "cold" tumors to "hot" ones to augment the cytolytic potentials of immune cells. Liposomes loaded with IL-12 mRNA (IL-12-Lipo) were used as a control for comparison. The authors delivered the exosomes by inhalation into mice with lung tumors. Remarkably, compared with IL-12-Lipo, IL-12-Exo showed superior performance in tumor microenvironment (TME) biodistribution and minimized toxicity. The inhaled IL-12-Exo promoted IFNγ-mediated immune activation, systemic immunity, and immune memory, culminating in lung tumor suppression and heightened resistance against tumor rechallenges.

3.3.2. Loading Nanomaterials. Nanomaterials, such as quantum dots (QDs), magnetic nanoparticles, liposomes, and polymer nanoparticles, possess functional or/and structural properties that can enhance the utility of native EVs. For example, QDs, if incorporated in EVs, can emit bright and stable fluorescence for the long-term tracking of EVs. On the other hand, polyester nanoparticles, with hydrophobic internal structure, can be used to load a large amount of hydrophobic drugs into EVs.

Peng et al. reported a multifunctional EVs, which incorporated curcumin-loaded superparamagnetic iron oxide nanoparticles (SPIONs) in MSC-derived exosomes, to treat Parkinson's disease (PD) in a mouse model.[60] MSC-derived exosomes have inherent ability to treat PD, given their miRNA and protein contents, particularly miR-188−3p, miR-106b, and miR-133b. The magnetism of SPIONs offered two new functions to the exosomes: first, enhanced delivery into the brain guided by a magnet; second, tracking by magnetic resonance imaging (MRI). The curcumin loaded on SPIONs helped PD treatment by reducing α-synuclein aggregates. The curcumin-loaded SPIONs were incorporated into the exosomes by passing both of the SPIONs and exosomes in an extruder (Figure 3). After treatment with these multifunctional EVs, PD model mice showed significant improvement in movement and coordination ability.

Rheumatoid arthritis is an autoimmune disease that involves several factors, namely, immune tolerance breakdown, local synovial inflammation, and tissue destruction, gradually leading to systemic and chronic disability. These factors act cooperatively, creating a vicious cycle. The current clinical treatment, which is based on anti-inflammatory drugs, can relieve only symptoms. MSCs-derived EVs could help to restore normal immune functionality but lack the ability to offer immediate suppression of inflammatory symptoms. Koo et al. developed a hybrid structure to provide both immune function normalization and immediate inflammatory suppression.[61] In this hybrid structure, ceria nanoparticles were

https://doi.org/10.1021/cbe.4c00122
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Figure 4. Metabolic tagging of EVs. Reproduced with permission from ref 66. Copyright 2024 Springer Nature.

conjugated to MSCs-derived vesicles by the thiol-maleimide reaction. The ceria nanoparticles (Ce NPs) can provide immediate inflammatory suppression, due to their ability to remove reactive oxygen species (ROS) by cycling between two reversible ionic states, Ce^{3+} and Ce^{4+}, to scavenge over-produced ROS in RA-inflicted knee joints, and to induce M1-to-M2 macrophage polarization. In a mouse model of collagen-induced arthritis, this hybrid structure was shown to successfully treat and prevent rheumatoid arthritis by both relieving the main symptoms and restoring the immune system through the induction of regulatory T cells.

Liposomes have been integrated with EVs to form hybrid structures in multiple reports.[62−64] Sato et al. used a freeze−thaw method to fuse liposomes with EV membrane, forming a hybrid nanoparticle.[63] The EVs were genetically engineered to overexpress HER2. By doing so, the authors showed that genetic modification can be combined with EV membrane engineering for rational design of engineered EVs. Ma et al. utilized dexamethasone-loaded liposomes (Dexlip) to tackle the limitations of MSCs in treating systemic lupus erythematosus (SLE).[64] Dexlip were coincubated with MSCs for 24 h. Dexlip was found to induce a shift in MSCs toward an anti-inflammatory phenotype by triggering the glucocorticoid receptor (GR) signaling pathway. It was further found that these MSCs released exosomes (Dex-MSC-EXOs) with elevated levels of expression of the anti-inflammatory protein CRISPLD2. In a mouse model of SLE, the combined method was shown to be more effective in treating SLE than Dexlip or MSC alone

3.3.3. Modifying the Surface of EVs. Wang et al. reported chemical conjugation of lung-derived exosomes to a recombinant SARS-CoV-2 receptor-binding domain (RBD), forming an inhalable COVID-19 vaccine.[65] The RBD antigen was first conjugated with (1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-poly(ethylene-glycol)-*N*-hydroxysuccinimide) (DSPE-PEG-NHS) to form RBD-PEG-DSPE. Then, RBD-PEG-DSPE was incubated with exosomes derived from lung spheroid cells (LSCs) for 24 h, resulting in RBD-decorated exosomes. In mice, the vaccine elicited RBD-specific IgG antibodies, mucosal IgA responses, and $CD4^+$ and $CD8^+$ T cells with a Th1-like cytokine expression profile in the animals' lungs and cleared them of SARS-CoV-2 pseudovirus after a challenge. In hamsters, two doses of the vaccine attenuated severe pneumonia and reduced inflammatory infiltrates after a challenge with live SARS-CoV-2.

Chemical conjugation to the functional groups of proteins in the EV membrane is a common strategy used for the surface modification of EVs. However, this strategy is limited by the low density of protein molecules in the EV membrane. Bhatta et al. developed a metabolic glycan tagging approach to address this issue (Figure 4).[66] A common metabolic labeling agent, tetraacetyl *N*-azidoacetylmannosamine (Ac_4ManAz), was synthesized and used for metabolic labeling of various types of cells. The cells were treated with Ac_4ManAz for 3 days and further incubated with DBCO-Cy5 for 30 min. EVs from Ac_4ManAz-treated cells showed significantly higher Cy5 fluorescence intensity, confirming the successful surface tagging of EVs (with Cy5 as the model here). The authors showed that this chemical tagging approach was applicable for many different cell types including cancer cells, MSCs, dendritic cells, and T cells. They also showed that, in the context of tumor vaccines, toll-like receptor 9 agonists could be conjugated onto EVs, thereby enabling timely activation of dendritic cells and generation of superior antitumor $CD8^+$ T cell response.

In the context of heart repair and regeneration post infarction, in order to improve the targeting ability of MSCs-derived EVs to ischemia-injured myocardium, Zhang et al. modified their surface by membrane fusion with monocyte mimics.[67] Taking advantage of the recruitment feature of monocytes after MI-RI (myocardial ischemia-reperfusion injury), the thus-formed modified EVs exhibited enhanced targeting efficiency to injured myocardium, and improved therapeutic outcome in a mouse MI/RI model

An interesting surface engineering method for EVs was reported by Chen et al., who modified the EV surface with spherical nucleic acid (SNA).[68] The authors mixed natural EVs with cholesterol-modified oligonucleotides; driven by hydrophobic interaction, the cholesterol-modified oligonucleotides coassembled with the natural EVs, forming an oligonucleotide shell on the EVs. The thus-formed new nanostructure, called extracellular vesicle spherical nucleic acid (EV-SNA), showed programmability: EV-SNA can respond to AND logic gates to achieve vesicle assembly manipulation. EV-SNA also displayed superior cellular delivery ability compared with liposome SNA.

3.3.4. Encapsulation in a Hydrogel. The native environment of EVs is often an extracellular matrix (ECM), which has a hydrogel structure. Cells receive feedback from the ECM and use intracellular processes to regulate the biogenesis of EVs. After secretion, various biochemical and biophysical factors determine whether EVs are locally incorporated in the matrix or transported out of the matrix. In principle, understandings of how EVs and ECM interact could be used to develop engineered hydrogels in which EV production, retention and release can be precisely controlled to yield desirable therapeutic outcomes.[69]

Lenzini et al. examined the transport of EVs, in comparison with synthetic nanoparticles, in ECM. It was found that EVs can readily transport through nanoporous ECM, in contrast to synthetic nanoparticles. The mechanisms of this transport phenomenon were revealed to have two aspects.[70] First, matrix stress relaxation allows EVs to overcome the confinement of pores in the ECM. Second, water permeation through aquaporin-1 (AQP1) mediates the deformability of EVs, which further supports EV transport in the ECM.

https://doi.org/10.1021/cbe.4c00122
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Encapsulating EVs in a hydrogel, forming an EVs-In-Gel structure, can be beneficial for the applications of EVs in several different ways. First, after local injection, EVs are often washed out, resulting in a large loss of the therapeutic agents. Encapsulating EVs in a hydrogel can help to immobilize the EVs in a desired site.[71−73] Second, a main limiting factor of the current EV-mediated therapies is the need for the prolonged presence of EVs at a specific location. This translates to multiple injections over the course of several weeks, which not only is disadvantageous in terms of patient compliance but also causes varying concentration over time. Encapsulating EVs in a hydrogel can offer sustained release of EVs, thereby yielding desirable efficacy, minimized side effects and improved patient compliance.[74−76] Third, the stability of EVs is of concern; loss of EV functionality occurs fairly quickly upon storage, likely due to membrane instability or degradation of proteins and RNAs in EVs. Encapsulating EVs in hydrogel can enhance the EV stability, as indicated in several reports.[77−79]

The polymers used for the hydrogel often include natural biopolymers, such as hyaluronic acid, alginate, chitosan, collagen, and amphiphilic peptides.[80−88] The considerations for the design of the polymer composition include the biomedical history, degradation, gelation kinetics, mechanical properties, and EV release kinetics. The pore size of a hydrogel is crucial to EV release. Because the diameter of exosomes is between 50 and 150 nm, the hydrogel needs to be nanoporous or microporous to allow efficient exosome release. On the other hand, if the applications of EVs are tissue regeneration, the pores of hydrogel need to be large enough to allow for ingrowth of cells.

In general, there are two different ways to encapsulate EVs into a hydrogel, as illustrated in Figure 5.[74] In the first approach, the polymers and EVs are mixed together; subsequently gelation is initiated by addition of a cross-linker or by activating an external trigger. In the second approach, polymers, cross-linkers and EVs are mixed together in solution, which is injected by a syringe (often a dual chamber syringe) at a specific site (e.g., the site of injury); subsequently *in situ* gelation occurs. Compared with the first approach, the second approach (injectable gel) is preferred in terms of the noninvasiveness of treatment.[89] The driving forces of cross-linking for injectable gels usually involve intermolecular forces (rather than covalent bonds), including guest−host interactions, ionic interactions, and hydrophobic interactions.

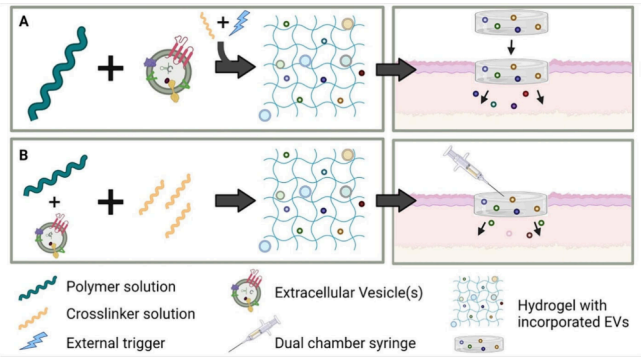

Figure 5. Overview of two different general approaches for encapsulating EVs into a hydrogel. (A) EVs and polymers are mixed, after which a cross-linker and/or an external trigger (e.g., heat, UV light) starts the gelation process. (B) Polymers, cross-linker and EVs are added simultaneously in a dual chamber syringe to achieve in situ gelation at the target site. Reproduced with permission from ref 74. Copyright 2024 Elsevier.

In addition to hydrogel encapsulation, other methods have been used to achieve a sustained release of EVs for improved therapeutic outcomes. In one example, Hu et al. coated stents with MSCs-derived exosomes, forming exosomes-eluting stents, to overcome limitations of drug-eluting stents as implants after ischemic injury.[90] Through a series of reactions, the surface of the stents was conjugated with 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (DSPE). Further incubation with exosomes led to insertion of DSPE into the lipid membrane of exosomes via hydrophobic interaction, yielding exosomes-coated stents. The exosomes showed a sustained release profile over several days. The release was accelerated by ROS accumulated in the ischemic site due to a ROS-responsive linker used in the above-mentioned surface conjugation. In rats with renal ischemia-reperfusion injury, compared with drug-eluting stents, the exosome-eluting stents showed more rapid reendothelialization and reduced in-stent restenosis 28 days after implantation. In another example, Bao et al. encapsulated EVs with microcapsules made of the common biodegradable polymer poly(lactic-*co*-glycolic acid) (PLGA), for treatment of vitreoretinal diseases.[91] In a mouse model of retinal ischemia-reperfusion injury, intravitreal injection of MSCs-exosomes-encapsulated PLGA microcapsules gave rise to prolonged release of exosomes for over one month and restoration of retinal thickness to nearly that of the healthy retina.

4. IMAGING AND MECHANISTIC UNDERSTANDING OF BIOLOGICAL FATE OF EVs

Many of the beneficial features of EVs for therapeutic efficacy bring challenges to characterization and understanding of how EVs work in a therapeutic context. EVs are heterogeneous, multicomponent, and dynamic. These features render the following analytical techniques particularly useful: dynamic bioimaging (tracking), single-particle/single-molecule/single-cell/single-vesicle analysis, and multiplexed bioanalysis (omics and others). With these techniques, researchers attempt to answer the following fundamental questions: (1) What are the trajectories, modes, and rates of transport of EVs at organ, tissue, cell, and subcellular levels? (2) What are the molecular mechanisms behind these motions? (3) What are the molecular contents of EVs? (4) What are the molecular mechanisms of the therapeutic effects of EVs? (5) What are the molecular mechanisms of the undesired side effects of EVs? Answers to these questions are imperative for guiding the development of EVs (including engineered EVs) in the clinic.

Animal imaging is typically used to understand the transport of EVs at the organ and tissue levels. *In vivo* animal imaging techniques of nanomedicines usually include fluorescence imaging, bioluminescence imaging, magnetic resonance imaging (MRI), X-ray computed tomography (X-ray CT), ultrasound imaging, positron emission tomography (PET), and single-photon emission computed tomography (SPECT). Virtually all of these imaging techniques have been adopted for imaging and tracking EVs in live animals. Each of these techniques has its unique benefits and limitations. For example, fluorescence and bioluminescence imaging are easy to perform and have good spatial resolution; but they do not offer high sensitivity and absolute quantification. On the other end of the spectrum is nuclear imaging techniques such as PET and SPECT. These techniques provide high sensitivity with

https://doi.org/10.1021/cbe.4c00122
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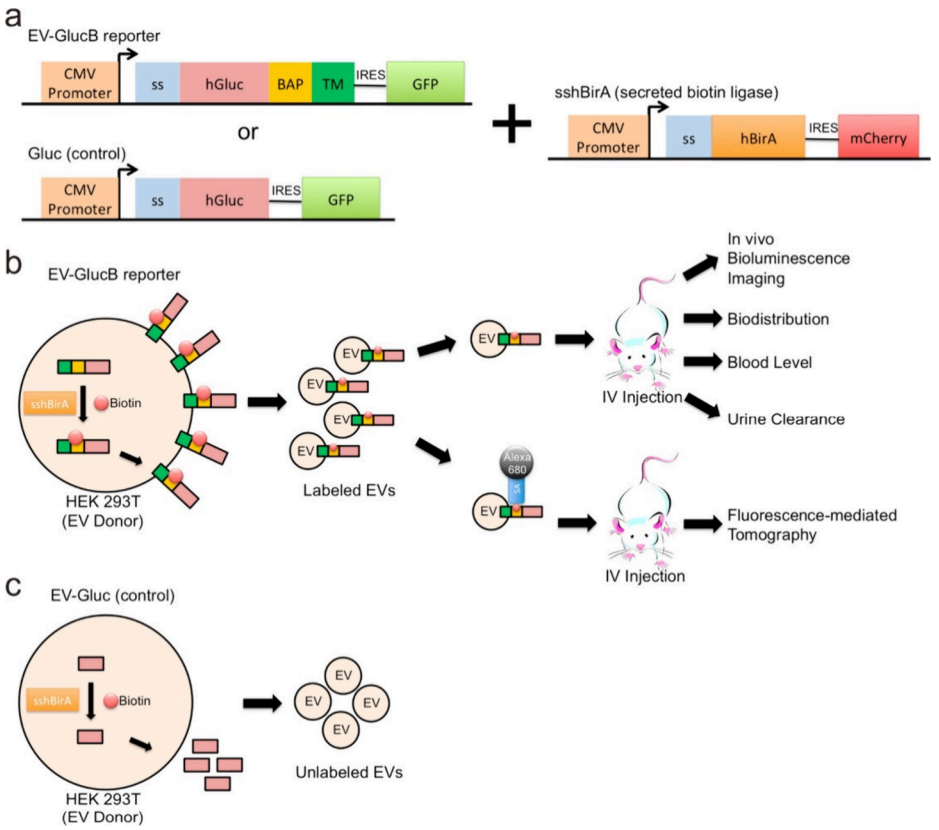

Figure 6. Schematic diagram of developing EV-GlucB reporter for *in vivo* multimodal imaging of EVs. (A) Membrane-bound Gluc (GlucB) or Gluc (control) and the secreted form of humanized bacterial biotin ligase (sshBirA) were delivered via lentivectors to HEK 293T cells for stable expression. (B) Upon expression and EV production by the cells, the sshBirA tags the BAP sequence of GlucB with a single biotin moiety at a specific lysine residue, which is then displayed on the cell surface as well as on the EV surface. EVs were isolated from conditioned medium of cells and injected intravenously (iv) via tail or retro-orbital veins into nude mice for bioluminescence and fluorescence-mediated tomography (FMT) imaging. For bioluminescence imaging, coelentrazine, a Gluc substrate, was iv-administered immediately prior to imaging. For FMT imaging, isolated EVs were conjugated with streptavidin-Alexa680 prior to administration into nude mice. (C) EVs derived from cells synthesizing naturally secreted Gluc were used as controls as Gluc is not present in the EVs. Abbreviations: BAP, biotin acceptor peptide; CMV, cytomegalovirus; GFP, green fluorescent protein; hBirA, humanized biotin ligase; hGluc, humanized Gaussia luciferase; IRES, internal ribosome entry site; SA, streptavidin; ss, signal peptide; TM, transmembrane domain of platelet-derived growth factor receptor. Reproduced with permission from ref 92. Copyright 2024 American Chemical Society.

minimal background noise and ability of absolute quantification; but they are very expensive and their availability is often limited by lack of access to instrument or/and lack of expertise. Bimodal/multimodal imaging strategies have been employed to combine the benefits and overcome the limitations. Below are several representative examples from the literature of applying imaging techniques to the study of EVs in live animals.

Lai et al. designed a sensitive and versatile probe that enables bimodal EV imaging in live animals, and tracking EV biodistribution and clearance over time (Figure 6).[92] The authors genetically engineered EVs to display a membrane reporter called EV-GlucB, consisting of Gluc (Gaussia luciferase) fused to a biotin acceptor domain, which is metabolically biotinylated when expressed in mammalian cells in the presence of biotin ligase. When incubated with the Gluc substrate coelentrazine (CTZ), these engineered EVs exhibit a strong bioluminescence signal. Furthermore, biotin on the surface allows EVs to be conjugated to any labeled streptavidin, which can then be imaged *in vivo* using different techniques, including but not limited to fluorescence-mediated tomography (FMT). *Ex vivo* analysis of tissue, blood, and urine with the Gluc assay permits evaluation of biodistribution and clearance of EVs. The bimodal imaging capacity originates from the combination of Gluc and biotin. Gluc emits flash bioluminescence (480 nm peak); meanwhile biotin allows another imaging modality such as MRI, SPECT/PET, and FMT.

A PET/MRI platform was reported by Banerjee et al. to track EVs *in vivo*.[93] Cu^{2+} was selected as the radiotracer because it can be produced on a large scale with high specific activity. Cu^{2+} was conjugated to EVs in two steps. First, the EV surface was conjugated with the metal chelator 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), using the free thiol groups in the EV membrane. Second, DOTA was complexed with Cu^{2+}. The stability of the EV-DOTA-Cu complex was evaluated in serum, PBS, and animal blood to be stable for at least 24 h. The potential influence of Cu labeling on the bioactivity of EVs was also examined. It was found that the prosurvival activity of EVs with Cu labeling against ischemic endothelial cells was higher than those without Cu labeling.

Nanoparticles have also been loaded to EVs for *in vivo* imaging, as shown in a study by Betzer et al.[94] For X-ray CT, exosomes were incubated with glucose-coated gold nanoparticles for 3 h at 37 °C, achieving labeling of exosomes with

https://doi.org/10.1021/cbe.4c00122
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gold nanoparticles (contrast agent for X-ray imaging). In a mouse model of stroke, the gold nanoparticle-labeled exosomes were delivered into the brain by intranasal administration. The gold nanoparticles permitted X-ray CT imaging and tracking of the exosomes in the mouse brain. *In vivo* CT imaging revealed the selective accumulation of exosomes in the stroke region.

A comprehensive comparative study was conducted by Lázaro-Ibáñez et al. to examine whether imaging probes would alter the biodistribution of EVs.[95] The following imaging probes (and the associated imaging modalities) were investigated: a noncovalently labeled fluorescent dye DiR, a covalently labeled nuclear imaging probe ^{111}Indium-DTPA, a genetically engineered fluorescent label mCherry, and genetically engineered bioluminescent label Firefly (Fluc) and NanoLuc (Nluc) luciferases. It was found that ^{111}Indium-DTPA and DiR provided the most sensitive *in vivo* imaging of the EVs. The biodistribution study revealed that NanoLuc fused to CD63 altered the EV biodistribution, resulting in high accumulation in the lungs.

Visualizing the behaviors of EVs at the cellular and subcellular level is usually conducted by fluorescence/bioluminescence microscopy. The results in the literature so far appear to indicate that the cellular uptake mechanisms of EVs are usually endocytosis, and a large portion of the endocytosed EVs is colocalized in intracellular vesicles such as lysosomes.[96−100] In addition to endocytosis, membrane fusion was also found to be involved in some studies.[101] EVs have been found to cross the blood-brain barrier by transcytosis.[102] More detailed and systematic studies are clearly needed. It is challenging to image both the EVs and their molecular contents (e.g., mRNAs, miRNAs) simultaneously. It is also challenging to image the biological impacts of EVs at the molecular level (e.g., translation of the mRNA cargos of EVs in the recipient cells). Lai et al. developed a genetically engineered probes-based imaging platform technology to address these challenges.[103] To generate fluorescent reporters of EVs (including all the subpopulations), a palmitoylation signal was genetically fused in-frame to the N terminus of fluorescent proteins (EGFP or tdTomato), forming PalmGFP or PalmtdTomato, respectively. The palmitoylation enables association of proteins with cellular membranes and thus all EV membranes. These reporters permitted multiphoton intravital microscopy of the EV dynamics in tumor tissue. It was observed that the lowest EV densities were typically in the areas of the highest tumor cell density at the core of the tumor parenchyma; in contrast, more peripheral regions, where the tumor parenchyma interfaces with the tumor stroma, showed the highest densities of EVs. In order to simultaneously visualize EV-packaged mRNAs and EVs, a dual-function reporter was designed. In this reporter scheme, PalmtdTomato protein was used for EV visualization, while its transcript was tagged with a repeated MS2 RNA binding sequence in the 30 UTR (PalmtdTomato-MS24X) for EV-RNA detection by bacteriophage MS2 coat protein fused with EGFP (MS2CP-GFP) expressed in the same cells. In order to detect and monitor translation of EV-delivered mRNA in parallel with EV uptake, fluorescent (PalmtdTomato) and bioluminescent (EV-GlucB) reporters were multiplexed. If the recipient cells translate EV-delivered GlucB mRNA, an increase in the GlucB signal would be observed, whereas treatment with cycloheximide (a protein translation inhibitor) would prevent GlucB mRNA translation and reduce any increase in the signal. Notably, an increase in the intensity of the GluB signal was observed as early as 1 h post-EV exposure, indicating that translation of EV-delivered GlucB mRNA started shortly after EV uptake by the recipient cells.

In an effort to address the challenge of distinguishing EVs-generating cells and EVs-receiving cells, Zomer et al. developed a Cre/LoxP-based imaging scheme, in which EV uptake led to Cre-induced red-to-green color conversion.[104,105] This color switch occurred specifically in reporter-expressing cells that take up EVs released from cells expressing Cre recombinase (Cre+ cells). With this EV imaging scheme, the authors were able to identify tumor cells that take up EVs *in vitro* and *in vivo* in a mouse model (using intravital fluorescence microscopy). It was found that EVs released by malignant tumor cells were taken up by less malignant tumor cells located within the same and within distant tumors and that these EVs carried mRNAs involved in migration and metastasis. It was further shown that the less malignant tumor cells that take up EVs displayed an enhanced migratory behavior and metastatic capacity.

CD63 has often been used to label and track exosomes and MVBs. However, it is challenging to observe the fusion events of MVBs with the plasma membrane, because the fluorescence emitted by the endosomes and MVBs labeled by CD63-fluorescent proteins is extremely bright, leading to poor signal-to-noise ratio during the fusion events. To solve this challenge, Sung et al. employed a pH-sensitive GFP derivative, pHluorin, to image the vesicle fusion events.[106] pHluorin is virtually nonfluorescent under acidic conditions but fluoresces at neutral pH, making it an ideal reporter to observe the fusion of acidic late endosomal MVBs with the plasma membrane. The authors developed a pHluorin-tagged CD63 reporter and used it to show that MVB fusion precedes adhesion formation in spreading cells by 1−2 min. Later, the same research group further improved the stability and brightness of pHluorin-CD63 by incorporating a single amino acid mutation, M153R.[107] Using this construct, they were able to track multiple aspects of the exosome lifecycle including MVB motion within cells before fusion, endocytosis of extracellular exosome deposits, and acidification of exosome-containing endocytic compartments.

Each EV contains numerous molecular species, and this is a main reason why EV therapy can mimic cell therapy as a conventional drug only contains one (or a very small number of) molecular species. Given this feature of EVs, omics-based analysis has become invaluable for understanding molecular compositions of EVs and molecular mechanisms of the physiological functions of EVs.[108−111] Transcriptomics has been widely used in studying EVs, offering comprehensive information about mRNAs, miRNAs, lncRNAs, and circRNAs. Meanwhile, proteomics permits the comprehensive identification and quantification of proteins. Primarily due to the heterogeneity of EVs and limited detection sensitivity of omics, reproducibility (biological reproducibility or technical reproducibility) is currently a major challenge in using omics for studying EVs.[112,113] Thus, multiomics analysis and validation by other analytical methods are often needed.[112,113]

EVs are heterogeneous. Techniques that can analyze single vesicles are thus highly valuable in unraveling the heterogeneity of EVs. Some of these techniques are label-free methods, while others need labeling (e.g., fluorescence labeling) on the vesicles. Label-free methods can circumvent the potentially undesirable influence of labels on EVs, but these methods often produce weak signals. The most commonly used single-

https://doi.org/10.1021/cbe.4c00122
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Table 1. Engineered EVs for Therapeutics in Preclinical Studies[a]

Disease category	Specific disease	Engineered EVs	In vitro model and findings	In vivo model and findings	Reference
Cardiovascular diseases	Ischemic disease	Hypoxic OM-MSC-EVs	HBMECs. Improved proliferation, migration and angiogenic activities via miR-612 promoting HIF-1α-VEGF signaling.	N/A	146
	Ischemic myocardium	Lamp2b fused with IMTP MSC-exosomes	H9C2 cells. Increased internalization.	Acute myocardial infarction mice model. Attenuated inflammation, apoptosis and fibrosis; enhanced vasculogenesis and cardiac function.	149
	Ischemic Reperfusion	SαV-NVs MSCs-Exos and PLT-NVs	H9C2 cells and HUVECs. alleviated inflammatory responses.	I/R mice model. Enhancement in the left ventricular ejection fraction.	128
	Myocardial infarction	ISL1-MSCs- exosomes incorporated into Ang-1 gel	Endothelial cells. Enhanced antiapoptosis, proliferation and angiogenic capacity.	Ischemic heart mice model. Retained ISL1-MSCs-Exo at ischemic sites with improved survival and angiogenesis of endothelial cells, and accelerated the recovery of myocardial infarction.	150
		EPC-EVs incorporated into shear-thinning gel	N/A	Myocardial infarction mice model. Increased peri-infarct vascular proliferation, preservation of ventricular geometry, and improved hemodynamic function.	151
		GDF15- H9C2-EVs	H_2O_2-injured H9C2 cells. Inhibited apoptosis and promoted autophagy	Acute myocardial infarction mice model. Decreased the infarct area and enhanced the cardiac function by activating the AMPK signaling pathway.	129
Neurological diseases	Parkinson's disease	Dopamine-loaded blood exosomes	bEnd.3 cells. Increased internalized blood exosomes localized to the cytoplasm and nuclei.	6-OHDA-lesioned Parkinson's disease mice model. Improved dopaminergic neurons and ameliorated disease phenotype.	155
		siRNA loaded murine dendritic cell-exosomes	SH-SY5Y cells. Decreased α-synuclein mRNA and protein levels	S129D α-synuclein transgenic mice model. Decreased α-synuclein mRNA and protein levels.	156
		Catalase-loaded Raw 264.7 macrophage exosomes	PC12 neuronal cells. Significant neuroprotective effects	6-OHDA-lesioned Parkinson's disease mice model. Decreased neuroinflammation and provided potent neuroprotection.	157
	Ischemic stroke	M2pep-MSC-exosomes	Primary microglia. Decreased the susceptibility of M2 microglia to ferroptosis, suppressed inflammatory microenvironment and promoted neuronal survival.	Middle cerebral artery occlusion mice model. Targeted specifically for M2 microglia, inhibited M2 microglia ferroptosis and improved neurological function	130
	Alzheimer's disease	siBACE1 and TREM2 loaded biomimetic exosome-liposome hybrid nanovesicles	Microglia. Reprogramed M1 phenotype to M2 phenotype, restored nerve repair function and reduced the production of Aβ plaques by knocking out the BACE1.	APP/PS1 mice model. Ameliorated cognitive impairment by regulating the activated microglial phenotype, reducing the accumulation of Aβ, and preventing the retriggering of neuroinflammation.	131
		Fe65-hippocampus neuron cell-exosomes loaded with Cory-B	HT22 and N2a cells. Enhanced autophagy.	5xFAD mouse. Augmented crossing the blood-brain barrier, improved cognitive and locomotor behavior.	132
Cancers	Pancreatic cancer	siRNA or short hairpin RNA loaded fibroblast-like mesenchymal cell- exosomes	Panc-1 cells. Target oncogenic KRAS with an enhanced efficacy.	Panc-1 tumor mice model. Increased overall survival.	35
	PDAC	RGD and CD47$^{p110-130}$ HEK 293 cell- exosomes	PANC1, Mia PaCq2, AsPC3, Capan2, PDCL5, PDCL15 and HPDE cells. Enhanced active PDAC targeting and cellular uptake, inhibited macrophage phagocytosis.	Nude mice bearing orthotopic xenografted PDCL5 tumor. Decreased susceptibility to phagocytosis and prolonged circulation times.	133
	Breast cancer	PTX and SBC loaded HEK-293T cell-exosomes	MCF-7 cells. Improved cellular uptake and enhanced sonodynamic effects.	Breast tumor-bearing mice. Suppressed tumor growth without systemic toxicity.	159
		miR-125a and DTX loaded hUCBMSC-exosomes	4T1 cells. Reduced invasiveness along with prominent cytoskeletal degradation and nuclear deformation.	N/A	160
		miR-CVB3/DoxApt loaded 4T1 cell-exosomes	MCF-7 cells. Reduced cell viability.	4T1 tumor-bearing mice. Improved tumor inhibition and prolonged survival via recruiting a greater number of immune cells to the tumor microenvironment.	134
		PFTBA and IR780 loaded AApt-Lips integrated with M1-exosomes	4T1 cells. Enhanced in situ generation of ROS and repolarized TAMs toward an antitumor phenotype.	4T1 tumor-bearing mice. Overcoming hypoxic and immunosuppressive TME by targeted TAM reprogramming and enhanced tumor photodynamic immunotherapy.	135
	Liver cancer	Silicon nanoparticles/DOX loaded Bel7402 cell - exosomes	H22 CSCs tumor spheroids. Enhanced cellular uptake and intracellular retention with an excellent cytotoxicity against CSCs.	H22 tumor-bearing mice. Enhanced tumor accumulation, extravasation from blood vessels and penetration into deep tumor parenchyma.	161
Dermatology	Diabetic wound healing	miR-21−5p loaded MSC-exosomes	HaCaT cells. Promoted proliferation and migration via Wnt/β-catenin signaling.	Diabetic mice wound model. Accelerated diabetic wound healing by increasing re-epithelialization, collagen remodeling, angiogenesis, and vessel maturation.	165
		Pioglitazone loaded MSC-exosomes	HUVECs with high glucose treatment. Enhanced migration, tube formation, wound repair and VEGF expression through inhibition of the PI3K/AKT/eNOS pathway.	Diabetic rat wound model. Enhanced angiogenesis, promoted collagen deposition, ECM remodeling and VEGF and CD31 expression.	166
		MSC-exosomes incorporated into extracellular matrix hydrogel	HUVECs and HaCaT cells. Enhanced proliferation, migration, and tube formation.	Diabetic mice wound model. Reduced inflammation, promoted angiogenesis, collagen deposition, cell proliferation and migration.	170

https://doi.org/10.1021/cbe.4c00122
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Table 1. continued

Disease category	Specific disease	Engineered EVs	In vitro model and findings	In vivo model and findings	Reference
	Senile wound healing	HAMA/PVA MNP coated by young fibroblast- exosomes	HUVECs. Improved wound healing of aged skin with active A-FBs, more deposition of collagen and less production of IL-17A.	Senile mice wound model. Antiaging and anti-inflammation.	136
Immunological diseases	Autoimmune skin diseases	CX546-grapefruit-exosomes fused with CCR6+-MSC-nanovesicles	CD4+T cells. Inhibited T cell proliferation, activation and inflammatory cytokines production via influence the T cell receptor pathway and JAK-STAT pathway.	Imiquimod-induced psoriasis mice model. Mitigated psoriasis disease severity and suppressed inflammatory response by reducing immune cell infiltration.	137
	Rheumatoid arthritis	Triptolide loaded fibroblast-exosomes	Human RA fibroblasts. Reduced apoptosis and inflammatory factors.	Collagen-induced arthritis mice model. Ameliorated ankle joint swelling and decreased the number of inflammatory cells.	138
Reproductive system	Premature ovarian failure	miR-126−3p-hucMSC-exosomes	Primary rat ovarian granulosa cells. Promoted proliferation and inhibited apoptosis via PI3K/AKT/mTOR signaling pathway.	Premature ovarian failure rat model. Increased E2 and AMH levels, promoted ovarian angiogenesis and inhabited apoptosis.	175
		miR-21-MSC-exosomes	Rat granulosa cells. Decreased apoptosis by downregulating PTEN and PDCD4.	Premature ovarian failure rat model. Increased ovarian weight, follicle counts and E2 levels decreased apoptosis.	175
	Intrauterine adhesions	Collagen scaffold loaded with MSC-exosomes	Human endometrial stromal cells. Increased proliferation and inhibited apoptosis.	Intrauterine adhesion mice model. Promoted endometrial regeneration and fertility restoration through macrophage immunomodulation.	176, 177
		MSC-exosome loaded with poloxamer hydrogel	Human HESCs and endometrial glandular cells. Increased proliferation and rescued TGF-β1 induced HESC fibrosis.	Intrauterine adhesion mice model. Restored functional endometrium by downregulating fibrotic progression markers.	139
Respiratory diseases	Asthma	miR-511−3p loaded HEK-293 T-exosomes decorated with nanoparticles	N/A	miR-511−3p knockout mice. Reversed the increased airway inflammation by inhibiting complement C3.	178
	COVID-19	ACE2-MCF 7 cell -EVs fused with S-palmitoylation-dependent PM	SARS-CoV-2 pseudovirus. Greater ability to bind with the pseudotyped virus.	hACE2 mice model. Protected against SARS-CoV-2 infections by suppressing viral association and attachment to cell surface ACE2.	179
	Acute lung injury	let-7a-5p loaded MSC-EVs	TGF-β-induced LL29 cell model. Regulated M2-like macrophage activation in an inflammatory microenvironment and significantly induced interleukin-10 secretion.	Hyperoxia-induced rat model. Improved lung function by reducing macrophage infiltration and collagen deposition, and increasing IL-10 expression.	140
	Pulmonary fibrosis	CTS-loaded liposome-exosome hybrid vesicles	Human MRC-5 lung fibroblasts. Reduced the expression of fibrosis-related proteins.	Bleomycin-induced SD rats. Improved lung function and reduced fibrosis-related protein expressions.	141
Ophthalmic diseases	Corneal damage	iPSC-MSCs-exosomes combined with thermosensitive hydrogel	HCEs. Enhanced the proliferation, upregulated the expression of keratocyte genes and downregulated the expression of collagen genes.	Anterior lamellar damage rat model. Promoted the repair of corneal epithelium and stroma by downregulating collagen and vimentin.	181
		MSC exosomes-aT loaded microneedles	HCEs and M1 macrophages. Alleviated inflammation levels by promoting M2 polarization of macrophages.	Corneal alkali burns mice model. Mitigated corneal injuries, enhanced wound healing and alleviated inflammation.	142
	Dry eye disease	Liposome encapsulating anti-NFKBIZ siRNAs with CEC-exosomes	CECs. Reduced pro-inflammatory cytokine secretions.	Dry eye disease mouse model. Reduced pro-inflammatory cytokine secretions and reshaped inflammatory microenvironment	143
Kidney diseases	Acute kidney injury	IL-10 loaded macrophage-exosomes	N/A	Ischemia/reperfusion injury mice model. Ameliorated renal tubular injury and inflammation, drove M2 macrophage polarization.	183
		MSC-EVs loaded RGD hydrogels	Tubular epithelial cells. Reduced cell apoptosis and elevated cell autophagy	Mice model. Rescued renal function, attenuated histopathological damage, decreased tubular injury and promoted cell proliferation.	184
	Renal interstitial fibrosis	SPION decorated MSC-EVs with CHIP	Renal tubular cells NRK-52E. Alleviated Smad2/3 activation-mediated fibrosis-like changes and collagen deposition.	Unilateral ureteral obstruction rat model. Accumulated at the injury renal sites under an external magnetic field, ameliorated inflammatory infiltration	144

[a]OM: olfactory mucosa; SBC: sodium bicarbonate; hUCBMSCs: human umbilical cord blood-mesenchymal stem cell; PDAC: pancreatic ductal adenocarcinoma; DoxApt: AS1411 aptamer and doxorubicin; AApt-Lips: AS1411 aptamer-conjugated liposomes; HUVECs: Human umbilical vein vascular endothelial cells; HAMA/PVA MNP: hyaluronic acid methacrylate/poly(vinyl alcohol) microneedle patch; HBMECs: human brain microvascular endothelial cells; MSC: mesenchymal stem cell; 6-OHDA: 6-hydroxydopamine; PTX: paclitaxel; DTX: docetaxel; ECM: extracellular matrix; HESC: human embryonic stem cell; ACE2: angiotensin converting enzyme; PM: plasma membrane; CTS: cyptotanshinone; aT: antitumor necrosis factor-α antibodies; CEC: corneal epithelium cell; SPION: superparamagnetic iron oxide nanoparticles; CHIP: carboxyl terminus of Hsc70-interacting protein.

https://doi.org/10.1021/cbe.4c00122
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Table 2. Engineered EVs for Therapeutics in Clinical Trials[a]

Category of diseases	Specific disease	NCT number	Source of EVs	Modification on EVs	Quantity of engineered EVs	Gender and age	Phase and enrollment	Results posted	Country
Respiratory tract diseases	COVID-19	NCT04747574	T-REx-293 cells	CD24	Each patient receives 1×10^8, 5×10^8, 1×10^9, 1×10^{10} vesicles, in 2 mL saline	All, 18−85	1, 35	Unknown status	Israel
		NCT04969172	T-REx-293 cells	CD24	Each patient receives 1×10^{10} vesicles, in 4 mL saline	All, 18−88	2, 155	Unknown status	Israel
		NCT04902183	CovenD24	CD24	Each patient receives 1×10^9, 1×10^{10} vesicles	All, 18−80	2, 90	Unknown status	Greece
	ARDS	NCT05947747	T-REx-293 cells	CD24	Each patient receives 1×10^{10} vesicles	All, >18	2, 90	Recruiting	Israel
Musculoskeletal diseases	Bone graft	NCT04998058	Human AMSCs	Synthetic bone substitute	4 to 5 g of a synthetic bone substitute mixed with 10 to 15 mL of conditioned medium	All, >35	1/2, 20	Not recruiting	Brazil
Neoplasms	Pancreas cancer	NCT03608631	Fibroblast-like MSCs	KrasG12D siRNA	Not reported	All, >18	1, 15	Not recruiting	USA
	Colon cancer	NCT01294072	Plant	Curcumin	Not reported	All, >20	N/A, 35	Recruiting	USA
Nutritional and metabolic diseases	FH	NCT05043181	Human AMSCs	LDLR	0.044/0.088/0.145/0.220/0.295/0.394 mg/kg	All, 18−45	1, 30	Not recruiting	China

[a]Data obtained from ClinicalTrials.gov using "engineered exosome", "engineering exosome", and "exosome" as search keyword. The categorization of diseases follows the system by Clinical Trials.gov. ARDS: acute respiratory distress syndrome; FH: familial hypercholesterolemia; IBS: inflammatory bowel disease; N/A: not applicable; LDLR: low density lipoprotein receptor.

vesicle analysis techniques for EVs are nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM). In NTA, individual vesicles are detected by scattering the light of a laser beam; the trajectories of diffusing vesicles are tracked and analyzed to yield the hydrodynamic sizes of individual vesicles. In TEM, individual vesicles are directly visualized, producing information about morphology and size. Raman tweezer microspectroscopy (RTM), also known as laser tweezer Raman spectroscopy (LTRS), can be employed to investigate the chemical content of single EVs. RTM utilizes a tightly focused laser beam for both optical trapping of single (or a very small number of) vesicles in water and excitation for subsequent Raman scattering, which offers a vibrational chemical fingerprint from the trapped constituent biomolecules. This technique can be used to analyze both the surface and the interior of single EVs, revealing specific molecular signatures of proteins, lipids, nucleic acids, and carotenoids.[114,115] Other less frequently used, label-free methods include atom force microscopy (AFM) and single-particle interferometric reflectance imaging sensor (SP-IRIS).[116,117]

Label-based methods are dependent on the detection of signals from fluorescent molecules or signal-enhancing nanoparticles. High-resolution flow cytometry (hrFC) has been extensively used to analyze single EVs. This technique permits the quantification of the size distribution and diversity of EV populations by detecting multiparametric scattered light and fluorescence emitted by the labeled EVs. Using antibody-fluorophore conjugates, this technique can be used to profile the protein or nucleic acid content of a EV population at single vesicle level.[118] Fluorescence microscopy has been widely used for localizing fluorescently labeled targets. In particular, total internal reflection fluorescent microscopy (TIRF) is now commonly used for single vesicle analysis. This technique can be used in an aqueous environment to image fluorescent molecules located near a highly refractive solid substance. TIRF allows for fluorescence tracking of single EVs in live cells. It should be noted that in TIRF the fluorophores can be excited only within a few hundred nanometers from the solid substrate.[119] Another particularly useful fluorescence-based imaging technique is fluorescence (or Förster) resonance energy transfer (FRET) imaging. The phenomenon of FRET occurs via resonance energy transfer at distances <10 nm. FRET imaging is capable of producing single-vesicle fluorescence information very fast. This imaging technique offers unique abilities for assessing kinetic and structural dynamics of EVs (e.g., lipid-mixing process).[120−122] Super-resolution microscopy (SRM) is a group of advanced microscopy techniques that enables visualization of biological features smaller than the optical diffraction limit (typically ∼250 nm axial and ∼500 nm lateral resolution). This feature is especially useful for imaging single EVs and investigating their biological functions.[123−125] Finally, the applications of microfluidics have great potential in single EV analysis.[126,127]

5. APPLICATIONS OF ENGINEERED EVs IN THERAPEUTICS

The therapeutic applications of engineered EVs are being pursued in a large number of diseases and the number is growing. Thus, this section is not meant to be exhaustive but rather aims to provide representative examples, in which key methods and main findings are highlighted. Tables 1 and 2 briefly summarize reported preclinical studies and clinical studies of engineered EVs, respectively.

5.1. Cardiovascular Diseases. EVs have been reported to be involved in many physiological and pathological processes in cardiovascular system, such as angiogenesis regulation, blood pressure control, cardiomyocyte hypertrophy, apoptosis, survival, and cardiac fibrosis.[145] In preclinical research, therapeutic potential of EVs has been demonstrated in cardiovascular regeneration and protection.[145]

The therapeutic functions of EVs have been enhanced by EV engineering in many studies in the literature. Hypoxia preconditioning of olfactory mucosa MSCs was reported to produce EVs with improved ability of angiogenesis promotion, via enrichment of miR-612.[146] Forced overexpression is another commonly used approach for improving therapeutic efficacy. In one study, forced Tcf21 overexpression suppressed transforming growth factor-β signaling and myofibroblast differentiation, in the context of myocardial fibrosis treat-

https://doi.org/10.1021/cbe.4c00122
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ment.[147] In another study, miR-126, as a critical regulator of angiogenesis, was overexpressed to promote vascular endothelial cell repair.[148]

Surface modification of EVs with targeting ligands has been used to enhance the targeting ability of EVs for improved efficacy in cardiovascular diseases. For example, Wang et al. engineered exosomes with ischemic myocardium-targeting (IMT) peptide on the surface, and showed that the IMT peptide significantly increased the accumulation of exosomes in ischemic heart area, and improved the therapeutic efficacy.[149] Embedding EVs in hydrogels has been used to enhance the efficacy of treating cardiovascular diseases. For example, Hu et al. found that incorporating MSC-derived exosomes in a class of angiogenin-1 hydrogel stents increased the efficacy, by prolonging the residence time of exosomes at the site of cardiac damage in the ischemic microenvironment.[150] Chen et al. showed that incorporating endothelial progenitor cell-derived EVs into shear-thinning hydrogels improved angiogenesis and promoted function after myocardial infarction, by sustained release of the EVs from the hydrogel.[151]

5.2. Neurological Diseases. Neuronal cells-derived EVs have been identified as vehicles for transferring misfolded proteins or coding materials between neurons. Transporting these disease-associated cargos may transform healthy cells into dysfunctional cells, contributing to the progression of neurological diseases such as Alzheimer's dementia (AD), PD, Huntington's disease, and prion diseases.[152,153] Because of the inherent ability of EVs to cross the blood-brain barrier, they have been employed as a delivery vehicle or/and a therapeutic agent to treat diseases in the brain.[50,154] For example, EVs isolated from human blood and loaded with dopamine have been shown to deliver dopamine to the central nervous system through the transferrin receptor.[155] EVs derived from murine dendritic cells containing siRNAs targeting α-synuclein have resulted in reduced levels of mRNA and protein of α-synuclein in the brains of mouse models of Huntington's disease.[156] EVs also showed protective capability for the encapsulated biomolecules, such as enzymes.[157] Surface modification of EVs with the brain-tumor-targeting cyclic RGDyK peptide has been shown to enhance drug delivery across the blood-brain barrier.[158] Finally, MSCs-derived EVs have been shown to promote neuronal repair and regeneration; engineering these EVs (e.g., by loading a drug) could further enhance the functions.[60]

5.3. Cancers. A landmark paper of using engineered EVs for cancer treatment was published in 2017.[35] In this article, clinical-grade exosomes derived from normal fibroblast-like mesenchymal cells were engineered to carry siRNA or shRNA specific to oncogenic KRASG12D, a common mutation in pancreatic cancer. The engineered exosomes were found to target oncogenic Kras with an enhanced efficacy (compared with liposomes) that is dependent on CD47. In multiple mouse models of pancreatic cancer, the engineered exosome treatment suppressed cancer and significantly improved overall survival. In other studies, chemotherapeutic drugs, sonosensitizers, and photoacoustic imaging agents were loaded to EVs for enhanced anticancer therapeutic efficacy.[159−161] It is worth noting that tumor cells-generated exosomes can be a cause of cancer metastasis.[162−164]

5.4. Dermatology. In a recent report, human dermal fibroblasts-derived EVs were loaded with mRNA encoding for extracellular-matrix α1 type-I collagen (COL1A1), and induced the formation of collagen-protein grafts and reduced wrinkle formation in the collagen-depleted dermal tissue of mice with photoaged skin.[49] In another study, human adipose MSCs-derived EVs were loaded with miR-21−5p, and exhibited potent promotion effect on diabetic wound healing.[165] Similarly, MSCs-derived EVs loaded with pioglitazone promoted collagen deposition and ECM remodeling by regulating P13K/AKT/eNOS pathway and enhancing neovascularization, thereby accelerating wound healing in diabetes.[166] In employing EVs for treating diabetic wound healing, silk fibroin patches[167] or hydrogels[168−170] have been utilized for EV immobilization and prolonged release at the wound site.

5.5. Immunological Diseases. EVs can be functional in immune regulation, likely due to the transfer and presentation of antigenic peptides, delivery of DNA-inducing cGAS-STING (cyclic GMP-AMP synthase stimulator of interferon genes) signaling in recipient cells, gene-expression manipulation by exosomal miRNA, and induction of different signaling pathways by surface ligands present on the EVs.[2,171] Engineered EVs have been successfully used to elicit adaptive and innate immune reactions, suggesting their utility as therapeutics. In one example, a hybrid nanoparticle of ceria and vesicles was prepared to modulate both innate and adaptive immunity in a collagen-induced arthritis model. The individual components of the hybrid structure worked synergistically to alleviate inflammation and modulate the tissue environment into an immunotolerant-favorable state, by bridging innate and adaptive immunity.[61]

5.6. Reproductive System. Engineered EVs are being investigated to treat reproductive disorders including premature ovarian insufficiency (POI), polycystic ovarian syndrome (PCOS), recurrent spontaneous abortion (RSA), intrauterine adhesion (IUA), and endometriosis (EMS).[172] The molecular mechanisms of the therapeutic efficacy have been attributed to the molecular cargos of the EVs, including miRNAs, circRNAs, lncRNAs, proteins, and small molecules.[173−175] Combining EVs with a scaffold has been shown to enhance the therapeutic functions. In one study, a collagen scaffold was loaded with human umbilical cord-derived MSCs (CS/UC-MSCs) and was applied for treating IUA. CS/UC-MSCs showed EVs-mediated endometrial regeneration in a model of endometrial damage, by promoting endometrial stromal cell proliferation and apoptosis inhibition.[176] In another study, a collagen scaffold was laden with MSCs-derived exosomes for treating IUA. In a rat endometrium-damage model, the treatment was found to promote endometrium regeneration and restore fertility through macrophage immunomodulation.[177]

5.7. Respiratory Diseases. Multiple studies have been reported on applying engineered EVs for treating respiratory diseases. In one study, Tu et al. constructed an engineered EV system by loading miR-511−3p into exosomes derived from HEK293T cells, and then decorating the exosome surface with the RNA nanoparticle PRNA-3WJ (three-way junction of the bacteriophage phi29 motor packaging RNA) with mannose surface modification.[178] The mannose decorated EVs were designed to target macrophages through the mannose receptor Mrc1. In a mouse model of asthma, intratracheal inhalation of these engineered EVs effectively penetrated the airway mucus barrier, delivered functional miR-511−3p to lung macrophages, and successfully reversed the increased airway inflammation. Complement C3 (C3) was identified as a major target of miR-511−3p. In another study, Xie et al.

https://doi.org/10.1021/cbe.4c00122
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developed an engineered EV system as a COVID-19 therapy.[179] By fusing the S-palmitoylation-dependent plasma membrane targeting sequence with ACE2 (a key cell surface receptor interacting with the viral spike protein), we engineered EVs enriched with ACE2 on their surface were engineered. The engineered EVs showed neutralization potency against SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2) in human ACE2 transgenic mice and efficiently blocked viral load of SARS-CoV-2, thereby protecting the host against SARS-CoV-2-induced lung inflammation.

5.8. Ophthalmic Diseases. Engineered EVs have shown remarkable potential in treating ophthalmic diseases, including both anterior (front-of-the-eye) and posterior (back-of-the-eye) diseases. In order to treat corneal damage (a major anterior eye disease), Tang et al. used a thermosensitive chitosan-based hydrogel to encapsulate exosomes generated by induced pluripotent stem cell-derived MSCs (iPSC-MSCs).[180] The hydrogel permitted sustained release of exosomes, which effectively promoted repair of damaged corneal epithelium and stromal layer, downregulating expression of mRNAs coding for the three most enriched collagens in the corneal stroma and reducing scar formation in vivo. Mechanistically, it was found that the therapeutic effect was via miR-432−5p in the exosomes, which suppresses translocation-associated membrane protein 2 (TRAM2), a vital modulator of the collagen biosynthesis in the corneal stromal stem cells, to avert the deposition of extracellular matrix (ECM). In order to treat retinal ischemia-reperfusion injury (IRI) (a major posterior eye disease), Yu et al. studied the effects of exosomes derived from gingival MSCs.[181] Exosomes from gingival MSCs were isolated by ultracentrifugation and were injected into the vitreous of mice. It was found that TNF-α stimulation of the source cells enhanced the neuroprotective effects of exosomes in IRI. Mechanistic studies revealed that the enhancement effect was due to enrichment of miR-21−5p in the exosomes.

5.9. Kidney Diseases. Increasing evidence in preclinical models demonstrates the potential of using EVs, especially those derived from stem cells, to treat kidney diseases such as acute kidney injury (AKI) and chronic kidney diseases (CKD).[182] Engineered EVs can further augment therapeutic performance, as suggested by multiple reports. Tang et al. developed interleukin-10 (IL-10)-loaded EVs by engineering macrophages for treating ischemic AKI.[183] The utilization of EVs enhanced not only the stability of IL-10, but also the targeting to the kidney due to adhesive components on the EV surface. In a mouse model, the treatment with IL-10-loaded EVs ameliorated renal tubular injury and inflammation caused by ischemia/reperfusion injury, and prevented transition to CKD. In an effort to use hydrogel encapsulation to enhance therapeutic efficacy of EVs, Zhang et al. developed a hydrogel based on RGD (Arg-Gly-Asp) peptide.[184] RGD peptide binds strongly to integrins, which are present on the membrane surface of MSC-derived EVs. The EVs-encapsulated hydrogel increased the retention and stability of the EVs. After intrarenal injection into a mouse AKI model, the EVs-encapsulated hydrogel showed superior performance in rescuing renal function in early stages of AKI and in antifibrosis in chronic stages.

6. TRANSLATION ASPECTS

As a new class of nanomedicine as well as a new class of cell therapy, EVs (native EVs and engineered EVs) face important challenges in translation to the clinic and industry. A major challenge comes from the manufacturing of EVs on a large scale with satisfactory quality control to meet the needs of clinical trials and industry production. This is due to the inherent complexity of EVs, size heterogeneity, and natural batch-to-batch variations in the production. In the source cell culture step, the methods can include multilayered culture flasks, bioreactors, and hollow fiber cartridges. Small-scale manufacturing can be performed in shake flasks, spinners, roller bottles, wave bags, and bioreactors. Large-scale cell culture can be conducted in stainless steel bioreactors (up to 20,000 L scale), platform-rocker wave bags (up to 500 L scale), or disposable bioreactors (up to 2000 L scale). Genetic drift and contamination need to be monitored closely. In the EV harvesting and isolation step, the commonly used method, namely, ultracentrifugation, poses difficulties for large-scale production. Many other methods are being examined such as tangential flow filtration, size exclusion chromatography, affinity chromatography, and magnetic isolation. In the drug loading step, sonication, surfactant, *etc.* have been used to facilitate the diffusion of drug molecules into EVs. The EV products are often stored at 4 °C or −80 °C. Lyophilization has been tested for long-term storage of EVs; but its impact on EV integrity needs to be carefully studied. Quality control by characterizing the critical quality attributes (CQAs) is needed. These include but are not limited to viability and surface marker expression of the source cells, quantity, size, and surface marker expression of the EVs, microbial contamination (for example, detection of endotoxin and mycoplasma), and functional activities specific to the applications.

Further, for clinical translation of EVs as therapeutics, "biological unknowns" and "pharmacological unknowns" need to addressed.[185] Addressing the biological unknowns includes resolving the functional ambiguity of EV action and elucidating the subpopulations and payloads of EVs. Addressing the pharmacological unknowns includes mapping out the optimal dose, mode of administration, systemic distribution, and pharmacokinetic, pharmacodynamics, and other properties of EVs. To address these issues, developing advanced characterization techniques (e.g., multiomics analysis, single-cell analysis, dynamic imaging, and super-resolution microscopy) for EVs is essential. Finally, standardization efforts are highly important. An example of such efforts is the standardization of EV isolation and characterizations by the International Society for Extracellular Vesicles (ISEV), resulting in the publication of "MISEV" (Minimal information for studies of extracellular vesicles). So far three editions of MISEV have been published (publication year: 2014, 2018, 2023).[186]

7. CONCLUSIONS AND PERSPECTIVES

Many difficult-to-treat diseases, such as neurological diseases and immunological diseases, lack single targets to effectively treat by conventional drugs. EVs, with a large number of molecular species, could potentially be an ideal solution to these diseases. Furthermore, EVs exhibit an excellent capacity to cross delivery barriers. To realize the full potential of EVs, many techniques have been developed to modify native EVs, forming engineered EVs. These engineered EVs have shown great efficacy and minimal toxicity in treating an array of major diseases, demonstrating potential as both a new class of nanomedicine and a new class of cell therapy.

Looking forward, interdisciplinary and collaborative work is needed to tackle challenges in the following aspects to translate

https://doi.org/10.1021/cbe.4c00122
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this potential to the clinic and industry. First, quality control and scale up of production are challenging for all nanomedicines and especially so for engineered EVs. In addition to the complexity and heterogeneity of engineered EVs, these challenges arise from the fact that the production process involves steps that are difficult to control. In the upstream of the production process, EV biogenesis is difficult to control. In the downstream of the production process, isolation of EVs based on ultracentrifugation (the dominant method used in the current practice) is difficult to control and scale up. Second, clinical translation requires systematic and deep understanding of the transport behaviors and molecular mechanisms underlying the efficacies and toxicities of engineered EVs. Gaining this understanding needs application-specific development of advanced analytical techniques.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors acknowledge the financial support from Jiangsu Natural Science Foundation (No. BK20171259), Nantong Natural Science Foundation (No. JC2019045, No. JC2019049), and XJTLU Research Development Funding-RDF-21-02-007.

REFERENCES

(1) Valadi, H.; Ekström, K.; Bossios, A.; Sjöstrand, M.; Lee, J. J.; Lötvall, J. O. Exosome-Mediated Transfer of mRNAs and MicroRNAs Is a Novel Mechanism of Genetic Exchange Between Cells. *Nat. Cell. Bio.* **2007**, *9*, 654−659.

(2) Kalluri, R.; LeBleu, V. S. The biology, Function, and Biomedical Applications of Exosomes. *Science* **2020**, *367* (6478), No. eaau6977.

(3) Mathieu, M.; Martin-Jaular, L.; Lavieu, G.; Théry, C. Specificities of Secretion and Uptake of Exosomes and Other Extracellular Vesicles for Cell-To-Cell Communication. *Nat. Cell Biol.* **2019**, *21*, 9−17.

(4) Skog, J.; Würdinger, T.; van Rijn, S.; Meijer, D. H.; Gainche, L.; Curry, W. T., Jr; Carter, B. S.; Krichevsky, A. M.; Breakefield, X. O. Glioblastoma Microvesicles Transport RNA and Proteins that Promote Tumour Growth and Provide Diagnostic Biomarkers. *Nat. Cell. Bio.* **2008**, *10*, 1470−1476.

(5) Herrmann, I. K.; Wood, M. J. A.; Fuhrmann, G. Extracellular Vesicles As a Next-Generation Drug Delivery Platform. *Nat. Nanotechnol.* **2021**, *16*, 748−759.

(6) Nagelkerke, A.; Ojansivu, M.; van der Koog, L.; Whittaker, T. E.; Cunnane, E. M.; Silva, A. M.; Dekker, N.; Stevens, M. M. Extracellular Vesicles For Tissue Repair And Regeneration: Evidence, Challenges And Opportunities. *Adv. Drug Delivery Rev.* **2021**, *175*, 113775.

(7) van Niel, G.; D'Angelo, G.; Raposo, G. Shedding light on the cell biology of extracellular vesicles. *Nat. Rev. Mol. Cell Biol.* **2018**, *19*, 213−228.

(8) Mathieu, M.; Martin-Jaular, L.; Lavieu, G.; Théry, C. Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication. *Nat. Cell Biol.* **2019**, *21*, 9−17.

(9) McAndrews, K. M.; Kalluri, R. Mechanisms associated with biogenesis of exosomes in cancer. *Mol. Cancer* **2019**, *18*, 52.

(10) Kalluri, R. The biology and function of exosomes in cancer. *J. Clin. Invest.* **2016**, *126*, 1208−1215.

(11) Leidal, A. M.; Huang, H. H.; Marsh, T.; Solvik, T.; Zhang, D.; Ye, J.; Kai, F.; Goldsmith, J.; Liu, J. Y.; Huang, Y. H.; Monkkonen, T.; Vlahakis, A.; Huang, E. J.; Goodarzi, H.; Yu, L.; Wiita, A. P.; Debnath, J. The LC3-Conjugation Machinery Specifies the Loading of RNA-Binding Proteins into Extracellular Vesicles. *Nat. Cell Biol.* **2020**, *22*, 187−199.

(12) Stenmark, H. Rab GTPases as Coordinators of Vesicle Traffic. *Nat. Rev. Mol. Cell Biol.* **2009**, *10*, 513−525.

(13) Vietri, M.; Radulovic, M.; Stenmark, H. The Many Functions of ESCRTs. *Nat. Rev. Mol. Cell Biol.* **2020**, *21*, 25−42.

(14) Jahn, R.; Cafiso, D. C.; Tamm, L. K. Mechanisms of SNARE proteins in membrane fusion. *Nat. Rev. Mol. Cell Biol.* **2024**, *25*, 101−118.
(15) Keerthikumar, S.; et al. ExoCarta: A web-based compendium of exosomal cargo. *J. Mol. Biol.* **2016**, *428*, 688−692.
(16) Pathan, M.; et al. Vesiclepedia 2019: A compendium of RNA, proteins, lipids and metabolites in extracellular vesicles. *Nucleic Acids Res.* **2019**, *47*, D516−D519.
(17) Choi, D.; et al. The impact of oncogenic EGFRvIII on the proteome of extracellular vesicles released from glioblastoma cells. *Mol. Cell. Proteomics* **2018**, *17*, 1948−1964.
(18) Cossetti, C.; et al. Extracellular vesicles from neural stem cells transfer IFN-g via Ifngr1 to activate Stat1 signaling in target cells. *Mol. Cell* **2014**, *56*, 193−204.
(19) Nagelkerke, A.; Ojansivu, M.; van der Koog, L.; Whittaker, T. E.; Cunnane, E. M.; Silva, A. M.; Dekker, N.; Stevens, M. M. Extracellular vesicles for tissue repair and regeneration: evidence, challenges and opportunities. *Adv. Drug Delivery Rev.* **2021**, *175*, 113775.
(20) Zitvogel, L.; et al. Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. *Nat. Med.* **1998**, *4*, 594−600.
(21) Zhang, B.; Yin, Y.; Lai, R. C.; Tan, S. S.; Choo, A. B.; Lim, S. K. Mesenchymal stem cells secrete immunologically active exosomes. *Stem Cells Dev.* **2014**, *23*, 1233−1244.
(22) Lai, P.; Chen, X.; Guo, L.; Wang, Y.; Liu, X.; Liu, Y.; Zhou, T.; Huang, T.; Geng, S.; Luo, C.; Huang, X.; Wu, S.; Ling, W.; Du, X.; He, C.; Weng, J. A potent immunomodulatory role of exosomes derived from mesenchymal stromal cells in preventing cGVHD. *J. Hematol. Oncol.* **2018**, *11*, 135.
(23) Giebel, B.; Kordelas, L.; Borger, V. Clinical potential of mesenchymal stem/stromal cell-derived extracellular vesicles. *Stem Cell Investig.* **2017**, *4*, 84.
(24) Forbes, G. M.; Sturm, M. J.; Leong, R. W.; Sparrow, M. P.; Segarajasingam, D.; Cummins, A. G.; Phillips, M.; Herrmann, R. P. A phase 2 study of allogeneic mesenchymal stromal cells for luminal Crohn's disease refractory to biologic therapy. *Clin. Gastroenterol. Hepatol.* **2014**, *12*, 64−71.
(25) Gonzalez, M. A.; Gonzalez-Rey, E.; Rico, L.; Buscher, D.; Delgado, M. Adipose-derived mesenchymal stem cells alleviate experimental colitis by inhibiting inflammatory and autoimmune responses. *Gastroenterology* **2009**, *136*, 978−989.
(26) Shang, Y.; Sun, Y.; Xu, J.; Ge, X.; Hu, Z.; Xiao, J.; Ning, Y.; Dong, Y.; Bai, C. Exosomes from Mmu_circ_0001359-Modified ADSCs Attenuate Airway Remodeling by Enhancing FoxO1 Signaling-Mediated M2-like Macrophage Activation. *Mol. Ther. Nucleic Acids* **2020**, *19*, 951−960.
(27) Zhao, R.; Wang, L.; Wang, T.; Xian, P.; Wang, H.; Long, Q. Inhalation of MSC-EVs Is a Noninvasive Strategy for Ameliorating Acute Lung Injury. *J. Controlled Release* **2022**, *345*, 214−230.
(28) Lopez-Verrilli, M. A.; Caviedes, A.; Cabrera, A.; Sandoval, S.; Wyneken, U.; Khoury, M. Mesenchymal stem cell-derived exosomes from different sources selectively promote neuritic outgrowth. *Neuroscience* **2016**, *320*, 129−139.
(29) Weilner, S.; Keider, V.; Winter, M.; Harreither, E.; Salzer, B.; Weiss, F.; Schraml, E.; Messner, P.; Pietschmann, P.; Hildner, F.; Gabriel, C.; Redl, H.; Grillari-Voglauer, R.; Grillari, J. Vesicular Galectin-3 levels decrease with donor age and contribute to the reduced osteo-inductive potential of human plasma derived extracellular vesicles. *Aging* **2016**, *8*, 16−33.
(30) Xu, H.; Wang, Z.; Liu, L.; Zhang, B.; Li, B. Exosomes derived from adipose tissue, bone marrow, and umbilical cord blood for cardioprotection after myocardial infarction. *J. Cell Biochem.* **2020**, *121*, 2089−2102.
(31) Mathew, B.; Ravindran, S.; Liu, X.; Torres, L.; Chennakesavalu, M.; Huang, C. C.; Feng, L.; Zelka, R.; Lopez, J.; Sharma, M.; Roth, S. Mesenchymal Stem Cell-Derived Extracellular Vesicles and Retinal Ischemia-Reperfusion. *Biomaterials* **2019**, *197*, 146−160.
(32) Direct Biologics, L.L.C. Bone Marrow Mesenchymal Stem Cell Derived Extracellular Vesicles Infusion Treatment for COVID-19 Associated Acute Respiratory Distress Syndrome (ARDS): A Phase II Clinical Trial. Clinical Trials.gov Identifier: NCT04493242, Last Update Posted: April 11, 2023.
(33) Cully, M. Exosome-based candidates move into the clinic. *Nat. Rev. Drug Discovery* **2021**, *20*, 6−7.
(34) Elsharkasy, O. M.; Nordin, J. Z.; Hagey, D. W.; de Jong, O. G.; Schiffelers, R. M.; Andaloussi, S. E. L.; Vader, P. Extracellular vesicles as drug delivery systems: why and how? *Adv. Drug Deliv Rev.* **2020**, *159*, 332−343.
(35) Kamerkar, S.; et al. Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer. *Nature* **2017**, *546*, 498−503.
(36) Lenzini, S.; Bargi, R.; Chung, G.; Shin, J. W. Matrix mechanics and water permeation regulate extracellular vesicle transport. *Nat. Nanotechnol.* **2020**, *15*, 217−223.
(37) de Abreu, R. C.; Fernandes, H.; da Costa Martins, P. A.; Sahoo, S.; Emanueli, C.; Ferreira, L. Native and bioengineered extracellular vesicles for cardiovascular therapeutics. *Nat. Rev. Cardiol.* **2020**, *17*, 685−697.
(38) Gray, W. D.; French, K. M.; Ghosh-Choudhary, S.; Maxwell, J. T.; Brown, M. E.; Platt, M. O.; Searles, C. D.; Davis, M. E. Identification of Therapeutic Covariant microRNA Clusters in Hypoxia Treated Cardiac Progenitor Cell Exosomes Using Systems Biology. *Circ. Res.* **2015**, *116*, 255−263.
(39) Kang, T.; Jones, T. M.; Naddell, C.; Bacanamwo, M.; Calvert, J. W.; Thompson, W. E.; Bond, V. C.; Chen, Y. E.; Liu, D. Adipose-Derived Stem Cells Induce Angiogenesis via Microvesicle Transport of miRNA-31. *Stem Cells Transl. Med.* **2016**, *5*, 440−450.
(40) Gorgun, C.; Ceresa, D.; Lesage, R.; Villa, F.; Reverberi, D.; Balbi, C.; Santamaria, S.; Cortese, K.; Malatesta, P.; Geris, L.; Quarto, R.; Tasso, R. Dissecting the effects of preconditioning with inflammatory cytokines and hypoxia on the angiogenic potential of mesenchymal stromal cell (MSC)-derived soluble proteins and extracellular vesicles (EVs). *Biomaterials* **2021**, *269*, 120633.
(41) Liao, Z.; Li, S.; Lu, S.; Liu, H.; Li, G.; Ma, L.; Luo, R.; Ke, W.; Wang, B.; Xiang, Q.; Song, Y.; Feng, X.; Zhang, Y.; Wu, X.; Hua, W.; Yang, C. Metformin facilitates mesenchymal stem cell-derived extracellular nanovesicles release and optimizes therapeutic efficacy in intervertebral disc degeneration. *Biomaterials* **2021**, *274*, 120850.
(42) Yang, Ta.; Li, W.; Peng, A.; Wang, Q. Exosomes Derived from Heat Shock Preconditioned Bone Marrow Mesenchymal Stem Cells Alleviate Cisplatin-Induced Ototoxicity in Mice. *J. Biol. Eng.* **2022**, *16*, 24.
(43) Rocha, S.; Carvalho, J.; Oliveira, P.; Voglstaetter, M.; Schvartz, D.; Thomsen, A. R.; Walter, N.; Khanduri, R.; Sanchez, J.; Keller, A.; Oliveira, C.; Nazarenko, I. 3D Cellular Architecture Affects MicroRNA and Protein Cargo of Extracellular Vesicles. *Adv. Sci.* **2019**, *6*, 1800948.
(44) Yan, L.; Wu, X. Exosomes produced from 3D cultures of umbilical cord mesenchymal stem cells in a hollow-fiber bioreactor show improved osteochondral regeneration activity. *Cell Biol. Toxicol.* **2020**, *36*, 165.
(45) Cao, J.; et al. Three-dimensional culture of MSCs produces exosomes with improved yield and enhanced therapeutic efficacy for cisplatin-induced acute kidney injury. *Stem Cell Res. Ther.* **2020**, *11*, 206.
(46) Tao, S. C.; Yuan, T.; Zhang, Y. L.; Yin, W. J.; Guo, S. C.; Zhang, C. Q. Exosomes derived from miR-140−5p-overexpressing human synovial mesenchymal stem cells enhance cartilage tissue regeneration and prevent osteoarthritis of the knee in a rat model. *Theranostics* **2017**, *7*, 180−195.
(47) Yu, B.; Kim, H. W.; Gong, M.; Wang, J.; Millard, R. W.; Wang, Y.; Ashraf, M.; Xu, M. Exosomes secreted from GATA-4 overexpressing mesenchymal stem cells serve as a reservoir of anti-apoptotic microRNAs for cardioprotection. *Int. J. Cardiology* **2015**, *182*, 349−360.

(48) Yang, Z.; et al. Large-scale generation of functional mRNA-encapsulating exosomes via cellular nanoporation. *Nat. Biomed. Eng.* **2020**, *4*, 69−83.
(49) You, Y.; et al. Intradermally delivered mRNA-encapsulating extracellular vesicles for collagen-replacement therapy. *Nat. Biomed. Eng.* **2023**, *7*, 887−900.
(50) El-Andaloussi, S.; et al. Exosome-mediated delivery of siRNA in vitro and in vivo. *Nat. Protoc.* **2012**, *7*, 2112−2126.
(51) Koppers-Lalic, D.; Hogenboom, M. M.; Middeldorp, J. M.; Pegtel, D. M. Virus-modified exosomes for targeted RNA delivery; A new approach in nanomedicine. *Adv. Drug Deliv Rev.* **2013**, *65*, 348−356.
(52) Mentkowski, K. I.; Lang, J. K. Exosomes engineered to express a cardiomyocyte binding peptide demonstrate improved cardiac retention in vivo. *Sci. Rep.* **2019**, *9*, 10041.
(53) Gupta, D.; et al. Amelioration of systemic inflammation via the display of two different decoy protein receptors on extracellular vesicles. *Nat. Biomed. Eng.* **2021**, *5*, 1084−1098.
(54) Stranford, D. M.; Simons, L. M.; Berman, K. E.; Cheng, L.; DiBiase, B. N.; Hung, M. E.; Lucks, J. B.; Hultquist, J. F.; Leonard, J. N. Genetically encoding multiple functionalities into extracellular vesicles for the targeted delivery of biologics to T cells. *Nat. Biomed. Eng.* **2024**, *8*, 397−414.
(55) Alvarez-Erviti, L.; Seow, Y.; Yin, H.; Betts, C.; Lakhal, S.; Wood, M. J. A. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat. Biotechnol.* **2011**, *29*, 341−345.
(56) Kooijmans, S. A. A.; Stremersch, S.; Braeckmans, K.; de Smedt, S. C.; Hendrix, A.; Wood, M. J. A.; Schiffelers, R. M.; Raemdonck, K.; Vader, P. Electroporation-induced siRNA precipitation obscures the efficiency of siRNA loading into extracellular vesicles. *J. Controlled Release* **2013**, *172*, 229−238.
(57) Fuhrmann, G.; Serio, A.; Mazo, M.; Nair, R.; Stevens, M. M. Active loading into extracellular vesicles significantly improves the cellular uptake and photodynamic effect of porphyrins. *J. Controlled Release* **2015**, *205*, 35−44.
(58) Dehghani, P.; Varshosaz, J.; Mirian, M.; Minaiyan, M.; Kazemi, M.; Bodaghi, M. Keratinocyte Exosomes for Topical Delivery of Tofacitinib in Treatment of Psoriasis: an In Vitro/ In Vivo Study in Animal Model of Psoriasis. *Pharm. Res.* **2024**, *41*, 263−279.
(59) Liu, M.; Hu, S.; Yan, N.; Popowski, K. D.; Cheng, K. Inhalable extracellular vesicle delivery of IL-12 mRNA to treat lung cancer and promote systemic immunity. *Nat. Nanotechnol.* **2024**, *19*, 565−575.
(60) Peng, H.; Li, Y.; Ji, W.; Zhao, R.; Lu, Z.; Shen, J.; Wu, Y.; Wang, J.; Hao, Q.; Wang, J.; Wang, W.; Yang, J.; Zhang, X. Intranasal Administration of Self-Oriented Nanocarriers Based on Therapeutic Exosomes for Synergistic Treatment of Parkinson's Disease. *ACS Nano* **2022**, *16*, 869−884.
(61) Koo, S.; et al. Ceria-vesicle nanohybrid therapeutic for modulation of innate and adaptive immunity in a collagen-induced arthritis model. *Nat. Nanotechnol.* **2023**, *18*, 1502−1514.
(62) Evers, M. J. W.; van de Wakker, S.; de Groot, E. M.; de Jong, O. G.; Gitz-Francois, J. J. J.; Seinen, C. S.; Sluijter, J. P. G.; Schiffelers, R. M.; Vader, P. Functional siRNA Delivery by Extracellular Vesicle-Liposome Hybrid Nanoparticles. *Adv. Healthc. Mater.* **2022**, *11*, No. e2101202.
(63) Sato, Y. T.; Umezaki, K.; Sawada, S.; Mukai, S. A.; Sasaki, Y.; Harada, N.; Shiku, H.; Akiyoshi, K. Engineering hybrid exosomes by membrane fusion with liposomes. *Sci. Report.* **2016**, *6*, 1−11.
(64) Ma, W.; Che, J.; Chen, W.; Wang, D.; Zhang, H.; Zhao, Y. Dexamethasone-Integrated Mesenchymal Stem Cells for Systemic Lupus Erythematosus Treatment via Multiple Immunomodulatory Mechanisms. *ACS Nano* **2024**, *18*, 13249−13265.
(65) Wang, Z.; et al. Exosomes decorated with a recombinant SARS-CoV-2 receptor-binding domain as an inhalable COVID-19 vaccine. *Nat. Biomed. Eng.* **2022**, *6*, 791−805.
(66) Bhatta, R.; et al. Metabolic tagging of extracellular vesicles and development of enhanced extracellular vesicle based cancer vaccines. *Nat. Commun.* **2023**, *14*, 8047.
(67) Zhang.; et al. Monocyte mimics improve mesenchymal stem cell-derived extracellular vesicle homing in a mouse MI/RI model. *Biomaterials* **2020**, *255*, 120168.
(68) Chen, H.; Ding, Q.; Li, L.; Wei, P.; Niu, Z.; Kong, T.; Fu, P.; Wang, Y.; Li, J.; Wang, K.; Zheng, J. Extracellular Vesicle Spherical Nucleic Acids. *JACS Au* **2024**, *4*, 2381.
(69) Debnath, K.; Las Heras, K.; Rivera, A.; Lenzini, S.; Shin, J.-W. Extracellular vesicle-matrix interactions. *Nat. Rev. Mater.* **2023**, *8*, 390−402.
(70) Lenzini, S.; Bargi, R.; Chung, G.; Shin, J. Matrix mechanics and water permeation regulate extracellular vesicle transport. *Nat. Nanotechnol.* **2020**, *15*, 217−223.
(71) Gallet, R.; et al. Exosomes secreted by cardiosphere- derived cells reduce scarring, attenuate adverse remodelling, and improve function in acute and chronic porcine myocardial infarction. *Eur. Heart J.* **2016**, *38*, 201−211.
(72) Mathiyalagan, P.; et al. Angiogenic mechanisms of human CD34+ stem cell exosomes in the repair of ischemic hindlimb. *Circ. Res.* **2017**, *120*, 1466−1476.
(73) Henriques-Antunes, H.; et al. The kinetics of small extracellular vesicle delivery impacts skin tissue regeneration. *ACS Nano* **2019**, *13*, 8694−8707.
(74) van de Looij, S. M.; de Jong, O. G.; Vermonden, T.; Lorenowicz, M. J. Injectable hydrogels for sustained delivery of extracellular vesicles in cartilage regeneration. *J. Controlled Release* **2023**, *355*, 685−708.
(75) Takahashi, Y.; Nishikawa, M.; Shinotsuka, H.; Matsui, Y.; Ohara, S.; Imai, T.; Takakura, Y. Visualization and in vivo tracking of the exosomes of murine melanoma B16-BL6 cells in mice after intravenous injection. *J. Biotechnol.* **2013**, *165*, 77−84.
(76) Saunderson, S. C.; Dunn, A. C.; Crocker, P. R.; McLellan, A. D. CD169 mediates the capture of exosomes in spleen and lymph node. *Blood* **2014**, *123*, 208−216.
(77) Chabria, Y.; Duffy, G. P.; Lowery, A. J.; Dwyer, R. M. Hydrogels: 3D drug delivery systems for nanoparticles and extracellular vesicles. *Biomedicines* **2021**, *9*, 1694.
(78) Görgens, A. Identification of storage conditions stabilizing extracellular vesicles preparations. *J. Extracell. Vesicles* **2022**, *11*, e12238.
(79) Zhang, K.; et al. Enhanced therapeutic effects of mesenchymal stem cell-derived exosomes with an injectable hydrogel for hindlimb ischemia treatment. *ACS Appl. Mater. Interfaces* **2018**, *10*, 30081−30091.
(80) Henriques-Antunes, H.; et al. The kinetics of small extracellular vesicle delivery impacts skin tissue regeneration. *ACS Nano* **2019**, *13*, 8694−8707.
(81) Liu, B.; et al. Cardiac recovery via extended cell- free delivery of extracellular vesicles secreted by cardiomyocytes derived from induced pluripotent stem cells. *Nat. Biomed. Eng.* **2018**, *2*, 293−303.
(82) Chen, C. W.; et al. Sustained release of endothelial progenitor cell- derived extracellular vesicles from shear- thinning hydrogels improves angiogenesis and promotes function after myocardial infarction. *Cardiovasc. Res.* **2018**, *114*, 1029−1040.
(83) Chung, J. J.; et al. Delayed delivery of endothelial progenitor cell- derived extracellular vesicles via shear thinning gel improves postinfarct hemodynamics. *J. Thorac. Cardiovasc. Surg.* **2020**, *159*, 1825−1835.
(84) Guo, S. C.; et al. Exosomes derived from platelet- rich plasma promote the re- epithelization of chronic cutaneous wounds via activation of YAP in a diabetic rat model. *Theranostics* **2017**, *7*, 81−96.
(85) Lv, K.; et al. Incorporation of small extracellular vesicles in sodium alginate hydrogel as a novel therapeutic strategy for myocardial infarction. *Theranostics* **2019**, *9*, 7403−7416.
(86) Tao, S. C.; et al. Chitosan wound dressings incorporating exosomes derived from microRNA-126-overexpressing synovium mesenchymal stem cells provide sustained release of exosomes and heal full- thickness skin defects in a diabetic rat model. *Stem Cell Transl Med.* **2017**, *6*, 736−747.

https://doi.org/10.1021/cbe.4c00122
Chem Bio Eng. 2025, 2, 3−22

(87) Zhang, K.; et al. Enhanced therapeutic effects of mesenchymal stem cell- derived exosomes with an injectable hydrogel for hindlimb ischemia treatment. *ACS Appl. Mater. Interfaces* **2018**, *10*, 30081−30091.
(88) Han, C.; et al. Human umbilical cord mesenchymal stem cell derived exosomes encapsulated in functional peptide hydrogels promote cardiac repair. *Biomater. Sci.* **2019**, *7*, 2920−2933.
(89) Riau, A. K.; Ong, H. S.; Yam, G. H. F.; Mehta, J. S. Sustained delivery system for stem cell-derived exosomes. *Front. Pharmacol.* **2019**, *10*, 1368.
(90) Hu, S.; Li, Z.; Shen, D.; Zhu, D.; Huang, K.; Su, T.; Dinh, P.; Cores, J.; Cheng, K. Exosome-eluting stents for vascular healing after ischaemic injury. *Nat. Biomed. Eng.* **2021**, *5*, 1174−1188.
(91) Bao, H.; et al. Exosome-loaded degradable polymeric microcapsules for the treatment of vitreoretinal diseases. *Nat. Biomed. Eng.* **2023**, DOI: 10.1038/s41551-023-01112-3.
(92) Lai, C. P.; Mardini, O.; Ericsson, M.; Prabhakar, S.; Maguire, C.; Chen, J. W.; Tannous, B. A.; Breakefield, X. O. Dynamic biodistribution of extracellular vesicles in vivo using a multimodal imaging reporter. *ACS Nano* **2014**, *8*, 483−494.
(93) Banerjee, A.; Alves, V.; Rondão, T.; Sereno, J.; Neves, A.; Lino, M.; Ribeiro, A.; Abrunhosa, A. J.; Ferreira, L. S. A positron-emission tomography (PET)/magnetic resonance imaging (MRI) platform to track *in vivo* small extracellular vesicles. *Nanoscale* **2019**, *11*, 13243.
(94) Betzer, O.; Perets, N.; Angel, A.; Motiei, M.; Sadan, T.; Yadid, G.; Offen, D.; Popovtzer, R. In Vivo Neuroimaging of Exosomes Using Gold Nanoparticles. *ACS Nano* **2017**, *11*, 10883−10893.
(95) Lázaro-Ibáñez, E.; Faruqu, F. N.; Saleh, A. F.; Silva, A. M.; Wang, J. T.; Rak, J.; Al-Jamal, K.; Dekker, N. Selection of Fluorescent, Bioluminescent, and Radioactive Tracers to Accurately Reflect Extracellular Vesicle Biodistribution in Vivo. *ACS Nano* **2021**, *15*, 3212−3227.
(96) Mulcahy, L. A.; Pink, R. C.; Carter, D. R. Routes and mechanisms of extracellular vesicle uptake. *J. Extracell. Vesicles* **2014**, *3*, 24641.
(97) Tian, T.; et al. Dynamics of exosome internalization and trafficking. *J. Cell. Physiol.* **2013**, *228*, 1487−1495.
(98) Feng, D.; et al. Cellular internalization of exosomes occurs through phagocytosis. *Traffic* **2010**, *11*, 675−687.
(99) Mathieu, M.; Martin- Jaular, L.; Lavieu, G.; Thery, C. Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell- to- cell communication. *Nat. Cell Biol.* **2019**, *21*, 9−17.
(100) Heusermann, W.; et al. Exosomes surf on filopodia to enter cells at endocytic hot spots, traffic within endosomes, and are targeted to the ER. *J. Cell Biol.* **2016**, *213*, 173−184.
(101) Bonsergent, E.; Grisard, E.; Buchrieser, J.; Schwartz, O.; Théry, C.; Lavieu, G. Quantitative characterization of extracellular vesicle uptake and content delivery within mammalian cells. *Nat. Commun.* **2021**, *12*, 1864.
(102) Morad, G.; Carman, C. V.; Hagedorn, E. J.; Perlin, J. R.; Zon, L. I.; Mustafaoglu, N.; Park, T.; Ingber, D. E.; Daisy, C. C.; Moses, M. A. Tumor-Derived Extracellular Vesicles Breach the Intact Blood-Brain Barrier via Transcytosis. *ACS Nano* **2019**, *13*, 13853−13865.
(103) Lai, C. P.; Kim, E. Y.; Badr, C. E.; Weissleder, R.; Mempel, T. R.; Tannous, B. A.; Breakefield, X. O. Visualization and tracking of tumour extracellular vesicle delivery and RNA translation using multiplexed reporters. *Nat. Commun.* **2015**, *6*, 7029.
(104) Zomer, A.; et al. In vivo imaging reveals extracellular vesicle-mediated phenocopying of metastatic behavior. *Cell* **2015**, *161*, 1046−1057.
(105) Zomer, A.; Steenbeek, S. C.; Maynard, C.; van Rheenen, J. Studying extracellular vesicle transfer by a Cre-loxP method. *Nat. Protoc.* **2016**, *11*, 87−101.
(106) Sung, B. H.; Ketova, T.; Hoshino, D.; Zijlstra, A.; Weaver, A. M. Directional cell movement through tissues is controlled by exosome secretion. *Nat. Commun.* **2015**, *6*, 7164.
(107) Sung, B. H.; von Lersner, A.; Guerrero, J.; Krystofiak, E. S.; Inman, D.; Pelletier, R.; Zijlstra, A.; Ponik, S. M.; Weaver, A. M. A live cell reporter of exosome secretion and uptake reveals pathfinding behavior of migrating cells. *Nat. Commun.* **2020**, *11*, 2092.
(108) Jeppesen, D. K.; Fenix, A. M.; Franklin, J. L.; Higginbotham, J. N.; Zhang, Q.; Zimmerman, L. J.; Liebler, D. C.; Ping, J.; Liu, Q.; Evans, R.; Fissell, W. H.; Patton, J. G.; Rome, L. H.; Burnette, D. T.; Coffey, R. J. Reassessment of Exosome Composition. *Cell* **2019**, *177*, 428−445.
(109) Gongye, X.; Tian, M.; Xia, P.; Qu, C.; Chen, Z.; Wang, J.; Zhu, Q.; Li, Z.; Yuan, Y. Multi-Omics Analysis Revealed the Role of Extracellular Vesicles in Hepatobiliary & Pancreatic Tumor. *J. Controlled Release* **2022**, *350*, 11−25.
(110) Turchinovich, A.; Drapkina, O.; Tonevitsky, A. Transcriptome of Extracellular Vesicles: State-of-the-Art. *Front. Immunol.* **2019**, *10*, 202.
(111) You, Y.; Muraoka, S.; Jedrychowski, M. P.; Hu, J.; McQuade, A. K.; Young-Pearse, T.; Aslebagh, R.; Shaffer, S. A.; Gygi, S. P.; Blurton-Jones, M.; Poon, W. W.; Ikezu, T. Human Neural Cell Type-Specific Extracellular Vesicle Proteome Defines Disease-Related Molecules Associated with Activated Astrocytes in Alzheimer's Disease Brain. *J. Extracell. Vesicles* **2022**, *11*, No. e12183.
(112) Morales, R. T.; Ko, J. Future of Digital Assays to Resolve Clinical Heterogeneity of Single Extracellular Vesicles. *ACS Nano* **2022**, *16*, 11619−1164.
(113) Blandin, A.; Dugail, I.; Hilairet, G.; Ponnaiah, M.; Ghesquiere, V.; Froger, J.; Ducheix, S.; Fizanne, L.; Boursier, J.; Cariou, B.; Lhomme, M.; Le Lay, S. Lipidomic Analysis of Adipose-Derived Extracellular Vesicles Reveals Specific EV Lipid Sorting Informative of the Obesity Metabolic State. *Cell Rep.* **2023**, *42*, 112169.
(114) Tatischeff, I.; Larquet, E.; Falcon-Perez, J. M.; Turpin, P.-Y.; Kruglik, S. G. Fast characterisation of cell-derived extracellular vesicles by nanoparticles tracking analysis, cryo-electron microscopy, and raman tweezers microspectroscopy. *J. Extracell. Vesicles* **2012**, *1*, 19179.
(115) Carney, R. P.; et al. Multispectral optical tweezers for biochemical fingerprinting of CD9-positive exosome subpopulations. *Anal. Chem.* **2017**, *89*, 5357−5363.
(116) Sharma, S.; et al. Structural-mechanical characterization of nanoparticle exosomes in human saliva, using correlative AFM, FESEM, and force spectroscopy. *ACS Nano* **2010**, *4*, 1921−1926.
(117) Avci, O.; Ünlü, N. L.; Özkumur, A. Y.; Ünlü, M. S. Interferometric reflectance imaging sensor (IRIS)—a platform technology for multiplexed diagnostics and digital detection. *Sensors* **2015**, *15*, 17649−17665.
(118) van der Vlist, E. J.; Nolte-'t Hoen, E. N. M.; Stoorvogel, W.; Arkesteijn, G. J. A.; Wauben, M. H. M. Fluorescent labeling of nano-sized vesicles released by cells and subsequent quantitative and qualitative analysis by high-resolution flow cytometry. *Nat. Protoc.* **2012**, *7*, 1311−1326.
(119) Kudalkar, E. M.; Davis, T. N.; Asbury, C. L. Single-molecule total internal reflection fluorescence microscopy. *Cold Spring Harb. Protoc.* **2016**, *2016*, pdb.top077800.
(120) Maurel, D.; et al. Cell-surface protein-protein interaction analysis with time-resolved FRET and snap-tag technologies: application to GPCR oligomerization. *Nat. Methods* **2008**, *5*, 561−567.
(121) Hu, Y.; Tian, Z.; Diao, J. Single-molecule fluorescence measurement of SNARE-mediated vesicle fusion. *in SNAREs: Methods and Protocols (ed. Fratti, R.)* **2019**, *1860*, 335−344.
(122) Johnson, J. L.; et al. Munc13−4 Is a Rab11-binding protein that regulates Rab11-positive vesicle trafficking and docking at the plasma membrane. *J. Biol. Chem.* **2016**, *291*, 3423−3438.
(123) Willig, K. I.; Rizzoli, S. O.; Westphal, V.; Jahn, R.; Hell, S. W. STED microscopy reveals that synaptotagmin remains clustered after synaptic vesicle exocytosis. *Nature* **2006**, *440*, 935−939.
(124) Hell, S. W. Toward fluorescence nanoscopy. *Nat. Biotechnol.* **2003**, *21*, 1347−1355.
(125) Huang, B. Super-resolution optical microscopy: multiple choices. *Curr. Opin. Chem. Biol.* **2010**, *14*, 10−14.

(126) Witters, D.; Knez, K.; Ceyssens, F.; Puers, R.; Lammertyn, J. Digital microfluidics-enabled single-molecule detection by printing and sealing single magnetic beads in femtoliter droplets. *Lab Chip* **2013**, *13*, 2047−2054.
(127) Friedrich, R.; et al. A nano flow cytometer for single lipid vesicle analysis. *Lab Chip* **2017**, *17*, 830−841.
(128) Lai, J.; et al. Triple hybrid cellular nanovesicles promote cardiac repair after ischemic reperfusion. *ACS Nano* **2024**, *18*, 4443−4455.
(129) Zou, A.; et al. Engineered exosomes with growth differentiation factor-15 overexpression enhance cardiac repair after myocardial injury. *Int. J. Nanomedicine* **2024**, *19*, 3295−3314.
(130) Wang, Y.; et al. Anti-ferroptosis exosomes engineered for targeting M2 microglia to improve neurological function in ischemic stroke. *J. Nanobiotechnology* **2024**, *22*, 291.
(131) Jiang, S.; et al. Biomimetic nanovesicles as a dual gene delivery system for the synergistic gene therapy of Alzheimer's Disease. *ACS Nano* **2024**, *18*, 11753−11768.
(132) Iyaswamy, A.; et al. Fe65-engineered neuronal exosomes encapsulating corynoxine-B ameliorate cognition and pathology of Alzheimer's disease. *Signal Transduct. Target Ther.* **2023**, *8*, 404.
(133) Creeden, J. F.; et al. Smart exosomes enhance PDAC targeted therapy. *J. Controlled Release* **2024**, *368*, 413−429.
(134) Bahreyni, A.; et al. Synergistic viro-chemoimmunotherapy in breast cancer enabled by bioengineered immunostimulatory exosomes and dual-targeted coxsackievirus B3. *ACS Nano* **2024**, *18*, 4241−4255.
(135) Zhen, X.; et al. Biointerface-engineered hybrid nanovesicles for targeted reprogramming of tumor microenvironment. *Adv. Mater.* **2024**, *8*, No. e2401495.
(136) Xu, J.; et al. Highly active frozen nanovesicles microneedles for senile wound healing via antibacteria, immunotherapy, and skin regeneration. *Adv. Healthc. Mater.* **2024**, *13*, No. e2304315.
(137) Huang, R.; et al. Plant exosomes fused with engineered mesenchymal stem cell-derived nanovesicles for synergistic therapy of autoimmune skin disorders. *J. Extracell. Vesicles* **2023**, *12*, No. e12361.
(138) Jiang, X.; et al. Engineered exosomes loaded with triptolide: an innovative approach to enhance therapeutic efficacy in rheumatoid arthritis. *Int. Immunopharmacol.* **2024**, *129*, 111677.
(139) Lin, Y.; et al. Exosome-based regimen rescues endometrial fibrosis in intrauterine adhesions via targeting clinical fibrosis biomarkers. *Stem Cells Transl. Med.* **2023**, *12*, 154−168.
(140) Chen, S.-Y.; et al. Engineered extracellular vesicles carrying let-7a-5p for alleviating inflammation in acute lung injury. *J. Biomed. Sci.* **2024**, *31*, 30.
(141) Wang, X.; et al. Inhalable FN-binding liposomes or liposome-exosome hybrid bionic vesicles encapsulated microparticles for enhanced pulmonary fibrosis therapy. *Int. J. Pharm.* **2024**, *656*, 124096.
(142) Yu, F. Engineered mesenchymal stromal cell exosomes-loaded microneedles improve corneal healing after chemical injury. *ACS Nano* **2024**, *18*, 20065.
(143) Xie, M.; et al. Membrane fusion-mediated loading of therapeutic siRNA into exosome for tissue-specific application. *Adv. Mater.* **2024**, *36*, No. e2403935.
(144) Ji, C.; et al. Engineered extracellular vesicle-encapsulated CHIP as novel nanotherapeutics for treatment of renal fibrosis. *NPJ. Regen. Med.* **2024**, *9*, 3.
(145) de Abreu, R. C.; Fernandes, H.; da Costa Martins, P. A.; Sahoo, S.; Emanueli, C.; Ferreira, L. Native and Bioengineered Extracellular Vesicles for Cardiovascular Therapeutics. *Nat. Rev. Cardiol.* **2020**, *17*, 685−697.
(146) Ge, L.; Xun, C.; Li, W.; Jin, S.; Liu, Z.; Zhuo, Y.; Duan, D.; Hu, Z.; Chen, P.; Lu, M. Extracellular Vesicles Derived from Hypoxia-Preconditioned Olfactory Mucosa Mesenchymal Stem Cells Enhance Angiogenesis via miR-612. *J. Nanobiotechnol.* **2021**, *19*, 380.
(147) Zhu, D.; Liu, S.; Huang, K.; Li, J.; Mei, X.; Li, Z.; Cheng, K. Intrapericardial Long Non-Coding RNA-Tcf21 Antisense RNA Inducing Demethylation Administration Promotes Cardiac Repair. *Eur. Heart J.* **2023**, *44*, 1748−1760.
(148) Jansen, F.; Yang, X.; Hoelscher, M.; Cattelan, A.; Schmitz, T.; Proebsting, S.; Wenzel, D.; Vosen, S.; Franklin, B. S.; Fleischmann, B. K.; Nickenig, G.; Werner, N. Endothelial Microparticle-Mediated Transfer of MicroRNA-126 Promotes Vascular Endothelial Cell Repair via SPRED1 and is Abrogated in Glucose-Damaged Endothelial Microparticles. *Circulation* **2013**, *128*, 2026−2038.
(149) Wang, X.; Chen, Y.; Zhao, Z.; Meng, Q.; Yu, Y.; Sun, J.; Yang, Z.; Chen, Y.; Li, J.; Ma, T.; Liu, H.; Li, Z.; Yang, J.; Shen, Z. Engineered Exosomes With Ischemic Myocardium-Targeting Peptide for Targeted Therapy in Myocardial Infarction. *J. Am. Heart Assoc.* **2018**, *7*, No. e008737.
(150) Hu, X.; Ning, X.; Zhao, Q.; Zhang, Z.; Zhang, C.; Xie, M.; Huang, W.; Cai, Y.; Xiang, Q.; Ou, C. Islet-1 mesenchymal stem cells-derived exosome-incorporated angiogenin- 1 hydrogel for enhanced acute myocardial infarction therapy. *ACS Appl. Mater. Interfaces* **2022**, *14*, 36289−36303.
(151) Chen, C. W.; Wang, L. L.; Zaman, S.; Gordon, J.; Arisi, M. F.; Venkataraman, C. M.; Chung, J. J.; Hung, G.; Gaffey, A. C.; Spruce, L. A.; Fazelinia, H.; Gorman, R. C.; Seeholzer, S. H.; Burdick, J. A.; Atluri, P. Sustained Release of Endothelial Progenitor Cell-Derived Extracellular Vesicles from Shear-Thinning Hydrogels Improves Angiogenesis and Promotes Function after Myocardial Infarction. *Cardiovasc. Res.* **2018**, *114*, 1029−1040.
(152) Soto, C.; Pritzkow, S. Protein Misfolding, Aggregation, and Conformational Strains in Neurodegenerative Diseases. *Nat. Neurosci.* **2018**, *21*, 1332−1340.
(153) You, Y.; Muraoka, S.; Jedrychowski, M. P.; Hu, J.; McQuade, A. K.; Young-Pearse, T.; Aslebagh, R.; Shaffer, S. A.; Gygi, S. P.; Blurton-Jones, M.; Poon, W. W.; Ikezu, T. Human Neural Cell Type-specific Extracellular Vesicle Proteome Defines Disease-related Molecules Associated with Activated Astrocytes in Alzheimer's Disease Brain. *J. Extracell. Vesicles* **2022**, *11*, No. e12183.
(154) Terstappen, G. C.; Meyer, A. H.; Bell, R. D.; Zhang, W. Strategies for Delivering Therapeutics Across the Blood-Brain Barrier. *Nat. Rev. Drug Discovery* **2021**, *20*, 362−383.
(155) Qu, M.; Lin, Q.; Huang, L.; Fu, Y.; Wang, L.; He, S.; Fu, Y.; Yang, S.; Zhang, Z.; Zhang, L.; Sun, X. Dopamine-Loaded Blood Exosomes Targeted to Brain for Better Treatment of Parkinson's Disease. *J. Controlled Release* **2018**, *287*, 156−166.
(156) Cooper, J. M.; Wiklander, P. B.; Nordin, J. Z.; Al-Shawi, R.; Wood, M. J.; Vithlani, M.; Schapira, A. H.; Simons, J. P.; El-Andaloussi, S.; Alvarez-Erviti, L. Systemic Exosomal siRNA Delivery Reduced Alpha-synuclein Aggregates in Brains of Transgenic Mice. *Mov. Disord.* **2014**, *29*, 1476−1485.
(157) Haney, M. J.; Klyachko, N. L.; Zhao, Y.; Gupta, R.; Plotnikova, E. G.; He, Z.; Patel, T.; Piroyan, A.; Sokolsky, M.; Kabanov, A. V.; Batrakova, E. V. Exosomes as Drug Delivery Vehicles for Parkinson's Disease Therapy. *J. Controlled Release* **2015**, *207*, 18−30.
(158) Tian, T.; Liang, R.; Erel-Akbaba, G.; Saad, L.; Obeid, P. J.; Gao, J.; Chiocca, E. A.; Weissleder, R.; Tannous, B. A. Immune Checkpoint Inhibition in GBM Primed with Radiation by Engineered Extracellular Vesicles. *ACS Nano* **2022**, *16*, 1940−1953.
(159) Nguyen Cao, T. G.; Kang, J. H.; Kim, W.; Lim, J.; Kang, S. J.; You, J. Y.; Truong Hoang, Q.; Kim, W. J.; Rhee, W. J.; Kim, C.; Ko, Y. T.; Shim, M. S. Engineered Extracellular Vesicle-Based Sonotheranostics for Dual Stimuli-Sensitive Drug Release and Photoacoustic Imaging-guided Chemo-Sonodynamic Cancer Therapy. *Theranostics* **2022**, *12*, 1247−1266.
(160) Basak, M.; Sahoo, B.; Chaudhary, D. K.; Narisepalli, S. B.; Tiwari, S.; Chitkara, D.; Mittal, A. Human Umbilical Cord Blood-Mesenchymal Stem Cell Derived Exosomes as an Efficient Nano-carrier for Docetaxel and MiR-125a: Formulation Optimization and Anti-Metastatic Behaviour. *Life Sci.* **2023**, *322*, 121621.
(161) Yong, T.; Zhang, X.; Bie, N.; Zhang, H.; Zhang, X.; Li, F.; Hakeem, A.; Hu, J.; Gan, L.; Santos, H. A.; Yang, X. Tumor Exosome-

Based Nanoparticles are Efficient Drug Carriers for Chemotherapy. *Nat. Commun.* **2019**, *10*, 3838.
(162) Kucharzewska, P.; et al. Exosomes reflect the hypoxic status of glioma cells and mediate hypoxia-dependent activation of vascular cells during tumor development. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, 7312−7317.
(163) Zhou, W.; et al. Cancer-secreted miR-105 destroys vascular endothelial barriers to promote metastasis. *Cancer Cell* **2014**, *25*, 501−505.
(164) Yokoi, A.; et al. Malignant extracellular vesicles carrying MMP1 mRNA facilitate peritoneal dissemination in ovarian cancer. *Nat. Commun.* **2017**, *8*, 14470.
(165) Lv, Q.; Deng, J.; Chen, Y.; Wang, Y.; Liu, B.; Liu, J. Engineered Human Adipose Stem-Cell-Derived Exosomes Loaded with miR-21−5p to Promote Diabetic Cutaneous Wound Healing. *Mol. Pharmaceutics* **2020**, *17*, 1723−1733.
(166) Hu, Y.; Tao, R.; Chen, L.; Xiong, Y.; Xue, H.; Hu, L.; Yan, C.; Xie, X.; Lin, Z.; Panayi, A. C.; Mi, B.; Liu, G. Exosomes Derived from Pioglitazone-Pretreated MSCs Accelerate Diabetic Wound Healing through Enhancing Angiogenesis. *J. Nanobiotechnol.* **2021**, *19*, 150.
(167) Li, Q.; Hu, W.; Huang, Q.; Yang, J.; Li, B.; Ma, K.; Wei, Q.; Wang, Y.; Su, J.; Sun, M.; Cui, S.; Yang, R.; Li, H.; Fu, X.; Zhang, C. MiR146a-loaded Engineered Exosomes Released from Silk Fibroin Patch Promote Diabetic Wound Healing by Targeting IRAK1. *Signal Transduct. Target. Ther.* **2023**, *8*, 62.
(168) Wang, Y.; Cao, Z.; Wei, Q.; Ma, K.; Hu, W.; Huang, Q.; Su, J.; Li, H.; Zhang, C.; Fu, X. VH298-Loaded Extracellular Vesicles Released from Gelatin Methacryloyl Hydrogel Facilitate Diabetic Wound Healing by HIF-1α-mediated Enhancement of Angiogenesis. *Acta Biomater.* **2022**, *147*, 342−355.
(169) Wang, C.; Wang, M.; Xu, T.; Zhang, X.; Lin, C.; Gao, W.; Xu, H.; Lei, B.; Mao, C. Engineering Bioactive Self-Healing Antibacterial Exosomes Hydrogel for Promoting Chronic Diabetic Wound Healing and Complete Skin Regeneration. *Theranostics* **2019**, *9*, 65−76.
(170) Song, Y.; You, Y.; Xu, X.; Lu, J.; Huang, X.; Zhang, J.; Zhu, L.; Hu, J.; Wu, X.; Xu, X.; Tan, W.; Du, Y. Adipose-Derived Mesenchymal Stem Cell-Derived Exosomes Biopotentiated Extracellular Matrix Hydrogels Accelerate Diabetic Wound Healing and Skin Regeneration. *Adv. Sci.* **2023**, *10*, No. e2304023.
(171) Marar, C.; Starich, B.; Wirtz, D. Extracellular vesicles in immunomodulation and tumor progression. *Nat. Immunol.* **2021**, *22*, 560−570.
(172) Zhang, M.; Xing, J.; Zhao, S.; Chen, H.; Yin, X.; Zhu, X. Engineered extracellular vesicles in female reproductive disorders. *Biomed. Pharmacother.* **2023**, *166*, 115284.
(173) Ding, C.; Zhu, L.; Shen, H.; Lu, J.; Zou, Q.; Huang, C.; Li, H.; Huang, B. Exosomal miRNA-17−5p Derived from Human Umbilical Cord Mesenchymal Stem Cells Improves Ovarian Function in Premature Ovarian Insufficiency by Regulating IRT7. *Stem Cells* **2020**, *38*, 1137−1148.
(174) Qu, Q.; Liu, L.; Cui, Y.; Liu, H.; Yi, J.; Bing, W.; Liu, C.; Jiang, D.; Bi, Y. miR-126−3p Containing Exosomes Derived from Human Umbilical Cord Mesenchymal Stem Cells Promote Angiogenesis and Attenuate Ovarian Granulosa Cell Apoptosis in a Preclinical Rat Model of Premature Ovarian Failure. *Stem Cell Res. Ther.* **2022**, *13*, 352.
(175) Fu, X.; He, Y.; Wang, X.; Peng, D.; Chen, X.; Li, X.; Wang, Q. Overexpression of miR-21 in Stem Cells Improves Ovarian Structure and Function in Rats with Chemotherapy-Induced Ovarian Damage by Targeting PDCD4 and PTEN to Inhibit Granulosa Cell Apoptosis. *Stem Cell Res. Ther.* **2017**, *8*, 187.
(176) Xin, L.; Lin, X.; Pan, Y.; Zheng, X.; Shi, L.; Zhang, Y.; Ma, L.; Gao, C.; Zhang, S. A Collagen Scaffold Loaded with Human Umbilical Cord-Derived Mesenchymal Stem Cells Facilitates Endometrial Regeneration and Restores Fertility. *Acta Biomater.* **2019**, *92*, 160−171.
(177) Xin, L.; Lin, X.; Zhou, F.; Li, C.; Wang, X.; Yu, H.; Pan, Y.; Fei, H.; Ma, L.; Zhang, S. A Scaffold Laden with Mesenchymal stem Cell-Derived Exosomes for Promoting Endometrium Regeneration and Fertility Restoration Through Macrophage Immunomodulation. *Acta Biomater.* **2020**, *113*, 252−266.
(178) Tu, W.; Hu, X.; Wan, R.; Xiao, X.; Shen, Y.; Srikaram, P.; Avvaru, S. N.; Yang, F.; Pi, F.; Zhou, Y.; Wan, M.; Gao, P. Effective Delivery of miR-511−3p With Mannose-Decorated Exosomes with RNA Nanoparticles Confers Protection Against Asthma. *J. Controlled Release* **2024**, *365*, 602−616.
(179) Xie, F.; Su, P.; Pan, T.; Zhou, X.; Li, H.; Huang, H.; Wang, A.; Wang, F.; Huang, J.; Yan, H.; Zeng, L.; Zhang, L.; Zhou, F. Engineering Extracellular Vesicles Enriched with Palmitoylated ACE2 as COVID-19 Therapy. *Adv. Mater.* **2021**, *33*, No. e2103471.
(180) Tang, Q.; Lu, B.; He, J.; Chen, X.; Fu, Q.; Han, H.; Luo, C.; Yin, H.; Qin, Z.; Lyu, D.; Zhang, L.; Zhou, M.; Yao, K. Exosomes-Loaded Thermosensitive Hydrogels for Corneal Epithelium and Stroma Regeneration. *Biomaterials* **2022**, *280*, 121320.
(181) Yu, Z.; Wen, Y.; Jiang, N.; Li, Z.; Guan, J.; Zhang, Y.; Deng, C.; Zhao, L.; Zheng, S. G.; Zhu, Y.; Su, W.; Zhuo, Y. TNF-α Stimulation Enhances the Neuroprotective Effects of Gingival MSCs Derived Exosomes in Retinal Ischemia-Reperfusion Injury via the MEG3/miR-21a-5p axis. *Biomaterials* **2022**, *284*, 121484.
(182) Grange, C.; Bussolati, B. Extracellular Vesicles in Kidney Disease. *Nat. Rev. Nephrol.* **2022**, *18*, 499−513.
(183) Tang, T. T.; Wang, B.; Wu, M.; Li, Z. L.; Feng, Y.; Cao, J. Y.; Yin, D.; Liu, H.; Tang, R. N.; Crowley, S. D.; Lv, L. L.; Liu, B. C. Extracellular Vesicle-Encapsulated IL-10 as Novel Nanotherapeutics Against Ischemic AKI. *Sci. Adv.* **2020**, *6*, No. eaaz0748.
(184) Zhang, C.; Shang, Y.; Chen, X.; Midgley, A. C.; Wang, Z.; Zhu, D.; Wu, J.; Chen, P.; Wu, L.; Wang, X.; Zhang, K.; Wang, H.; Kong, D.; Yang, Z.; Li, Z.; Chen, X. Supramolecular Nanofibers Containing Arginine-Glycine-Aspartate (RGD) Peptides Boost Therapeutic Efficacy of Extracellular Vesicles in Kidney Repair. *ACS Nano* **2020**, *14*, 12133−12147.
(185) Li, G.; et al. Current challenges and future directions for engineering extracellular vesicles for heart, lung, blood and sleep diseases. *J. Extracell. Vesicles* **2023**, *12*, No. e12305.
(186) Welsh, J. A. Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches. *J. Extracell. Vesicles* **2024**, *13*, No. e12404.

https://doi.org/10.1021/cbe.4c00122
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