APPLIED SCIENCES AND ENGINEERING

Leveraging nature's nanocarriers: Translating insights from extracellular vesicles to biomimetic synthetic vesicles for biomedical applications

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Naturally occurring extracellular vesicles (EVs) and synthetic nanoparticles like liposomes have revolutionized precision diagnostics and medicine. EVs excel in biocompatibility and cell targeting, while liposomes offer enhanced drug loading capacity and scalability. The clinical translation of EVs is hindered by challenges including low yield and heterogeneity, whereas liposomes face rapid immune clearance and limited targeting efficiency. To bridge these gaps, biomimetic synthetic vesicles (SVs) have emerged as innovative platforms, combining the advantageous properties of EVs and liposomes. This review emphasizes critical aspects of EV biology, such as mechanisms of EV-cell interaction and source-dependent functionalities in targeting, immune modulation, and tissue regeneration, informing biomimetic SV engineering. We reviewed a broad array of biomimetic SVs, with a focus on lipid bilayered vesicles functionalized with proteins. These include cell-derived nanovesicles, protein-functionalized liposomes, and hybrid vesicles. By addressing current challenges and highlighting opportunities, this review aims to advance biomimetic SVs for transformative biomedical applications.

INTRODUCTION

Extracellular vesicles (EVs) are naturally released from cells, characterized by a lipid bilayer membrane that does not contain a nucleus and a size range from 30 nm to 10 μm in diameter (*1*, *2*). Highly heterogeneous, EVs can be categorized based on their content, size, and origin (*1*–*3*). A major function of EVs is to transport cargo such as RNA (*4*), DNA (*5*), lipids (*6*), and proteins (*3*), acting as crucial messengers in intercellular communication.

EVs have multiple inherent features that hold therapeutic value. Universally, EVs carry cargo representing the state of the parental cells, serving as informative biomarkers for disease diagnosis and monitoring (*7*, *8*). Their small size allows them to traverse various tissues, while their lipid bilayer structure and surface moieties facilitate efficient internalization by recipient cells, making EVs important candidates for therapeutic delivery (*9*, *10*). For instance, EVs have been exploited to deliver anticancer agents, enhancing immune responses (*11*). In treating neurological disorders, EVs have demonstrated their ability to transport therapeutic cargo across the blood-brain barrier (BBB) (*12*). In addition, the functional properties of EVs are fine-tuned based on the types and conditions of their producer cells. For example, EVs derived from mesenchymal stem cells (MSCs) show a remarkable capacity for tissue regeneration (*13*). Tumor cell–derived EVs exhibit homing capabilities to their cells of origin (*14*). Dendritic cell–derived EVs have immunomodulatory functions and can act as carriers for antigens, serving as effective vaccine carriers (*15*). The biocomplexity of EVs, which enables multifaceted biological functions, opens new avenues to address diseases that remain challenging for conventional therapies (*9*). However, critical challenges in EV heterogeneity, yield, and purity of EV isolation, hinder the standardized production of clinical-grade EV therapeutics. Advanced engineering methods are being developed to enhance EV therapeutic potential (*9*, *10*, *16*), including improvements in targeting specificity, drug loading capacity, and methods for scaling up cell culture and EV isolation. However, the impact of these modifications on EV function remains under investigation.

Parallel to EV research, nanoparticles (NPs) have emerged as promising delivery systems. NPs can be constructed from a variety of materials, such as polymers, metals, and lipids (*17*). Approximately 40% of approved drugs in the market and nearly 90% of molecules in the discovery pipeline have low water solubility (*18*). Using NPs as drug carriers improves the solubility and stability of drugs in blood circulation. In particular, lipid-based carriers stand out for their excellent drug encapsulation ability and compatibility with biological systems (*19*). Liposomes share great similarities with EVs, in terms of size range, lipid bilayer membrane, and capability of encapsulating both hydrophilic and hydrophobic drugs (*20*). The first clinically approved liposomal drug, Doxil, has substantially improved the pharmacokinetics and toxicity profile of doxorubicin (Dox) (*21*). Despite the advantages in scalability and homogeneity compared to EVs, conventional liposome-based drug delivery relies on passive diffusion via the enhanced permeability and retention effect with limited targeting efficiency (*22*).

Recognizing the challenges associated with both EVs and liposomes, researchers have developed semi- and fully synthetic biomimetic vesicles (biomimetic SVs) incorporated with biological components to replicate specific cell or EV-like functions (*16*, *22*–*25*). They are designed to have improved biocompatibility, tunability, and scalability. Current engineering strategies include the generation of cell-derived nanovesicles (CDNs) by rupturing producer cells, resulting in vesicles with EV-like sizes and biological functions while being produced at considerably higher yields. In contrast, a "bottom-up" approach involves the incorporation of recombinant or cell-derived proteins into liposomes to create protein-functionalized liposomes (PFLs) for biomedical applications. More recently, hybrid vesicles,

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created through membrane fusion or camouflaging combine the strengths of multiple vesicle types. These hybrid vesicles include EV-liposome, EV-CDN, and CDN-liposome vesicles, and fusion with other synthetic materials (*16*), with distinct configurations continuously being developed.

Despite the rapid advancements in biomimetic SV research, standardization has not been systematically implemented for their diverse classifications, synthesis methods, and quality control. Summarizing current methodologies could foster rigor and promote standardized practices across the field. Discussions on their bioinspired origins and corresponding biomedical applications could inspire innovative approaches, leveraging the current understanding of EV and cell biology to develop new therapeutic strategies. Last, highlighting the remaining technical challenges can help identify future directions for optimizing bioengineering techniques. On the other hand, while remarkable progress has been made in understanding EV biology, critical questions persist—particularly in unraveling the biocomplexity of EVs. This includes elucidating the mechanisms underlying cell or tissue targeting and endosomal escape, as well as clarifying their pharmacokinetic and biosafety profiles. Understanding these unsolved mechanisms could illuminate the direction for developing more effective and safer biomimetic SVs.

In this review, we explore the current understanding of the biology of EVs and the relevant insights to innovate biomimetic SVs as next-generation delivery systems. For the scope of discussion, we define biomimetic SVs as synthetic vesicles with lipid bilayer structures and contain at least one type of protein aimed to exert certain biological properties, which include but are not restricted to enhancement in circulation stability, immune modulation, disease targeting, intracellular endosomal escape, and controlled cargo release. These vesicles present high EV structural mimicry and thereby are suited to benefit from insights into EV biology compared to other NP classes made from other materials or structures. While not the focus of this review, we also mention engineered or programmable EVs, but these have been comprehensively reviewed previously (*9*, *10*, *16*, *22*, *26*).

Systematic review methodology

To ensure the comprehensiveness of the review, we conducted a systematic literature search (Fig. 1). A combination of keywords "(((biomimetic) OR (bioinspired)) OR (hybrid)) AND (((vesicles) OR (nanovesicles)) OR (liposomes))" derived from Medical Subject Headings (MeSH) and non-MeSH terms were searched in the title of research articles on PubMed and Web of Science from January 1980 until October 2024, with 240 and 618 articles found, respectively. Duplicate articles were removed. This resulted in 640 articles, which were all subsequently screened to identify whether one of the three types of biomimetic SVs (CDNs, PFLs, and hybrid vesicles—defined in Fig. 1) were developed in the studies. In addition, we have searched in reviews on relevant topics (*19*, *22*, *23*, *25*) to include impactful studies that did not pass this literature search criteria but play a fundamental role in this field. In total, 186 studies passed the selection and 112 innovative and high-impact studies are included in this review.

EV CLASSIFICATIONS AND FUNCTIONS

Notable advances have been made in elucidating the diversity of EV types and the biogenesis pathways that contribute to their highly heterogeneous composition and functions. In addition, ongoing research is shedding light on the precise mechanisms underlying EV-cell interactions, cell or tissue tropism, cellular uptake, and cytosolic cargo release. These characterizations position EVs as promising candidates for a wide range of biomedical applications.

EV types and biogenesis

The most recent MISEV2023 guidelines define EVs as particles released from cells, delimited by a lipid bilayer, and that cannot replicate on their own (*1*). Compared to the definition in MISEV2018, the word "naturally" was removed to not exclude engineered EVs, as well as cell culture–derived EVs (*1*, *27*). On the basis of the size and biogenesis pathway, native EVs are classified into exosomes [30 to 150 nm (*28*)], microvesicles [40 nm to up to a few micrometers

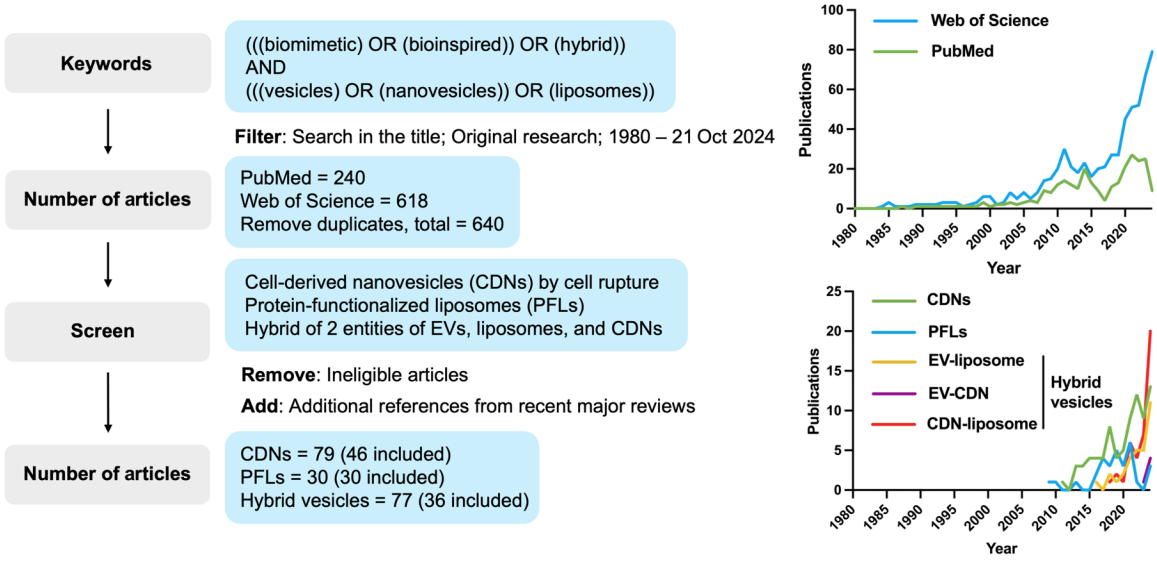

Fig. 1. Literature search methodology. Search methods, selection criteria, and the number of reviewed studies and their categorization.

(*29*)], and apoptotic bodies [500 nm to up to a few micrometers (*30*)] (Fig. 2). According to the recommendations of MISEV2023, these conventional terms should be used with caution and based on strong evidence of biogenesis pathways (*1*).

The term "exosomes" was introduced by Johnstone *et al.* in 1987 to describe small membrane-bound vesicles originating from the endosomal system and secreted by exocytosis (*28*, *31*). These processes typically include the inward budding of the plasma membrane, the formation of an early endosome, the formation of multivesicular bodies (MVBs), cargo sorting and loading into intraluminal vesicles within MVBs, and subsequent routing toward either degradation or exosome release via fusion with the plasma membrane. This pathway is regulated by specific molecular machinery. The endosomal sorting complexes required for transport (ESCRT) play a key role in protein sorting and determining the fate of endocytosed cargo. Alternatively, exosomes can be generated through an ESCRT-independent pathway, primarily involving lipids, such as sphingolipid ceramide, which triggers the inward budding of membranes within MVBs (*32*). Rab guanosine triphosphatases (GTPases) play an important role in determining whether late endosomes fuse with lysosomes for degradation or with the plasma membrane to secrete exosomes (*28*). CD63, CD81, and CD9 are widely recognized as exosome markers due to their enrichment on exosomes (*29*). However, controversies exist in terms of their roles in the endocytosis pathway in exosome biogenesis. A recent study suggests that these tetraspanins are primarily secreted through an endocytosis-independent pathway (*33*), challenging the conventional view of exosome formation. This finding reflects the need for further studies to clarify the molecular mechanisms involved.

In contrast to the endosomal origin of exosomes, microvesicles are formed by direct outward budding of the plasma membrane, a process regulated by key proteins, including ARF1, ARF6, Rab22a, and RhoA, reviewed in (*34*). This process involves the activation of contractile machinery, cargo sorting, and cytoskeletal rearrangements. These unique biogenesis mechanisms of microvesicles contribute to their distinct cargo composition, lipid profiles, timing of release, and physiological and pathological functions (*35*).

Apoptotic bodies arise from cell disassembly during programmed cell death (*30*). Their generation can be triggered by intrinsic or extrinsic pathways, reviewed in (*36*). The intrinsic pathway is initiated by cellular stress, leading to increased mitochondrial permeability and the release of cytochrome c and the apoptosis-inducing factor. The extrinsic pathway involves the binding of the Fas ligand to the Fas receptor, which recruits the Fas-associated death domain protein. The ultimate result of both pathways is the activation of the caspase cascade reactions (caspase-3/6/7). During apoptosis, phosphatidylserine (PS) is translocated to the surface of the cell membrane, where it binds to annexin V. In addition, the surface molecules become oxidized and bind to thrombospondin or the complement protein C3b. Together, these interactions serve as "eat me" signals for engulfment by phagocytes (*36*). The apoptosis process does not trigger inflammatory reactions, representing a crucial homeostatic mechanism in maintaining cellular and tissue integrity.

In addition, distinct types of EVs have been identified. For example, large oncosomes (1 to 10 μm), produced specifically by cancer cells, are thought to contribute to tumor progression (*37*). Migrasomes are large EVs (500 nm to 3 μm) derived from retraction fibers during migracytosis, representing a recently identified mode of intercellular communication (*38*). Spherosomes (40 to 125 nm) exhibit

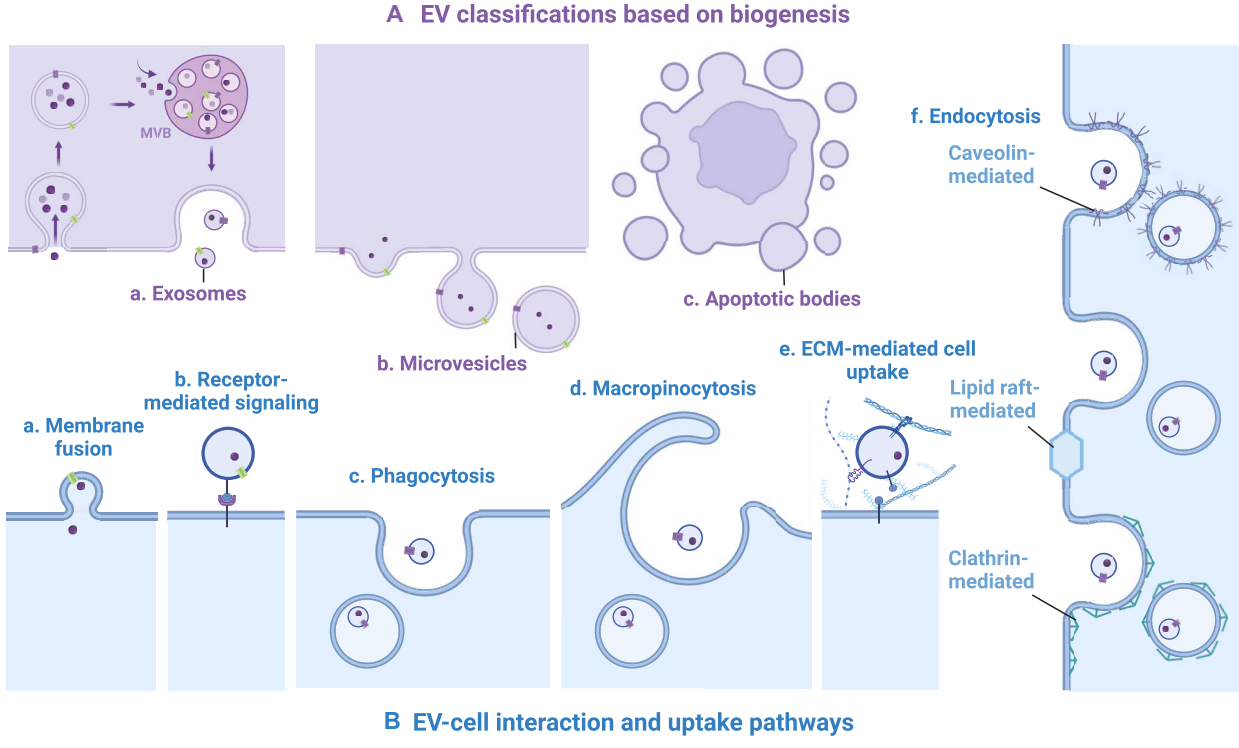

Fig. 2. Extracellular vesicle (EV) types and interaction mechanisms with cells. EV classifications based on biogenesis (**A**) and EV-cell interaction and uptake pathways (**B**). Created with www.biorender.com.

unique biogenesis, distinct from that of exosomes and microvesicles, as they are released as clusters enveloped within multivesicular spheres (*39*).

Nonvesicular extracellular NPs (NVEPs) represent a diverse group of amembranous particles that have recently gained attention in the field of intercellular communication. NVEPs are identified through various isolation techniques. Exomeres (typically <50 nm) have been isolated from asymmetric-flow field-flow fraction (*40*) or ultracentrifugation (167,000*g*) (*41*). Exomeres lack markers of small EVs but are enriched in endoplasmic reticulum, mitochondria, extracellular matrix (ECM), proteasome accessory complex, and microtubule proteins, suggesting that they originate from distinct biogenesis pathways (*40*). Supermeres (supernatant of exomeres) (22 to 32 nm) require ultracentrifugation (367,000*g*) (*2*, *42*).

It is also acknowledged that EVs are distinct from lipoproteins, despite some overlap in size. Lipoproteins contain a central hydrophobic core of cholesterol esters and triglycerides, surrounded by free cholesterol, phospholipids, and apolipoproteins (*43*). They primarily transport lipids in the body, while EVs consist of a lipid bilayer membrane capable of carrying biomolecules like nucleic acids, lipids, and proteins. Given these differences, reconstituted lipoprotein-like NPs were not discussed in this review.

The ambiguous use of terms such as "exosomes" and "microvesicles" in certain scientific discourse reflects the challenges in precisely characterizing and classifying EVs. To mitigate potential confusion over nomenclature, the MISEV2023 guidelines recommend the use of the generic term "EV" along with operational extensions, to maintain consistency (*1*). By providing clear operational definitions, EVs can be classified based on their physical and biochemical characteristics. For example, EVs can be categorized by size into "small EVs" (typically described as <200 nm) and "large EVs" (typically described as >200 nm). Their density (low, medium, or high) serves as another distinguishing factor. In addition, the conditions and characteristics of the source from which EVs are isolated can be considered for their classification.

EV cargo

The growing body of knowledge on EV cargo, including proteins, nucleic acids, and lipids, has led to the creation of several databases, such as Vesiclepedia (*44*), ExoCarta (*45*), EVpedia (*46*), and EV-ADD (*47*). EVs contain proteins related to their mechanisms of biogenesis and secretion, such as tetraspanins (CD81, CD63, and CD9), syntenin-1, VAMP3, Rab GTPases, and integrins (*3*, *29*, *48*). Kowal *et al.* (*49*) showed that EV subpopulations isolated through differential centrifugation and immunoprecipitation exhibit protein heterogeneity depending on vesicle size. For example, GP96 was predominantly found in large EVs. Actinin-4 and mitofilin were present in both large and medium-sized EVs but were not detected in small EVs. Syntenin-1, TSG101, ADAM10, and EHD4 were exclusively found in small EVs. The heterogeneity in EV protein composition also reflects differences in tissue of origin and disease states. Hurwitz *et al.* performed proteomic profiling of EVs isolated by ultracentrifugation across 60 cell lines (*48*). In total, 6071 unique proteins were identified. A total of 213 proteins, including 3 EV markers (CD81, Alix, and HSC70), were commonly found in all isolates. CD63, CD9, TSG101, syntenin-1, and flotillin-1 were found in at least two-thirds of all isolates. Furthermore, clustering analysis revealed that EV samples tend to cluster based on tissue of origin and metastatic potential, underscoring the biological relevance of EV-associated cargo.

The RNA cargo of EV encompasses a wide range of types and can be categorized into long and short RNA transcripts, or coding and noncoding RNA transcripts (*4*, *29*, *50*, *51*). MicroRNA (miRNAs) are one of the most studied types of short RNAs, due to their profound regulatory functions. RNA binding proteins, such as A2B1 (encoded by *hnRNPA2B1*), play an important role in the selective sorting of miRNAs into EVs by recognizing specific miRNA motifs (*52*). Besides EV-miRNA, progress has been made in the characterization of EV long RNA transcripts [messenger RNAs, long noncoding RNAs (lncRNAs), and circular RNAs] (*50*, *51*). Amorim *et al.* (*50*) developed a method by next-generation sequencing for transcriptome profiling of plasma-derived EVs. This approach enables unbiased characterization of the full RNA cargo, including small and long RNAs, in a single library preparation. Abundant small RNAs (e.g., transfer RNAs and miRNAs), protein-coding and lncRNAs, and circular RNAs were identified and experimentally validated. This method provides complete transcriptome data from samples of limited quantities, facilitating biomarker discovery.

Although research on EV-DNA is still in its early stages and subject to debate (*29*), growing evidence has shown DNA cargo (genomic DNA, mitochondrial DNA, and cytoplasmic chromatin fragments) associated with EVs isolated from various biofluids, as documented in the EV-ADD database (*47*). In the past decade, advancement has been made in revealing the localization of EV-DNA and its implications in various diseases, reviewed in (*5*). Recent research has shown that DNA is predominantly located on the surface of small EVs (*53*, *54*), while luminal DNA is mainly found in larger EVs (*55*, *56*). The DNA binding and histone-binding proteins on the surface of EVs were found to play an important role in loading DNA onto the crown of EVs (*57*). To advance the understanding of EV-DNA, current research is actively focusing on a few aspects, including optimizing techniques for EV-DNA isolation and characterization, identifying mechanisms of EV-DNA loading and emission, discovering the biomarker potential of EV-DNA for disease diagnosis, and elucidating the functions of EV-DNA in the recipient cells (*5*). A more detailed discussion on the functions and biomarker potential of EV-DNA is provided in the following sections.

In addition, EVs contain other molecules that are gaining research interest. The lipid-bilayer membranes of EVs typically consist of phosphatidylcholine, PS, phosphatidylethanolamine, sphingomyelin, and cholesterol, with the relative abundance of these lipids varying across EV subpopulations (*6*, *58*). Profiling of EV cargo continues to uncover biomarker and therapeutic targets across various disease contexts, offering valuable insights into diagnostic and therapeutic applications (*9*).

EV function

EVs were initially regarded as a waste disposal mechanism for cells. In the 1980s, their pivotal role in intercellular communication was recognized (*28*, *59*, *60*). One of the earliest discoveries was the selective enrichment of major histocompatibility complex (MHC) molecules on secreted exosomes. Raposo *et al.* (*59*) demonstrated by immunoelectron microscopy that MHC class II-containing vesicles secreted by B lymphocytes could induce antigen-specific T cell response. In contrast, tumor-derived EVs have been shown to exert anti-immune responses. Poutsiaka *et al.* (*60*) reported that EVs shed from murine melanoma cells selectively inhibit the expression of immune response region-associated antigens in macrophages.

EVs are now known to influence both innate and adaptive immunity, reviewed in (*61*). Bacteria-derived EVs carry pathogen-associated molecular patterns that can trigger inflammatory responses in the recipient cells. For example, membrane vesicles (MVs) produced by *Staphylococcus aureus* activate Toll-like receptor 2 (TLR2) located on the cell surface, and uptake of MV-associated RNA, DNA, and peptidoglycan cargo further activates endosomal TLR7, 8, and 9, triggering the intracellular degradation of MVs via autophagy (*62*). In mammals, red blood cell (RBC)–derived EVs up-regulate TLR4-MyD88-NF-κB-MAPK signaling, promoting macrophage cytokine production, including tumor necrosis factor–α (TNF-α), interleukin-6 (IL-6), and IL-1β (*63*). Beyond their role within the innate immune system, EVs are also critical mediators of communication between innate and adaptive immunity. For example, T cell–derived EVs containing genomic and mitochondrial DNA can trigger antiviral responses in dendritic cells via the cyclic GMP-AMP synthase (cGAS)–stimulator of interferon genes (STING) cytosolic DNA–sensing pathway (*64*). This interaction primes dendritic cells and enhances their resistance to subsequent viral infections. These studies highlight the important role of EVs in both innate and adaptive immune regulations.

As intercellular couriers, EVs exhibit the capacity to target specific tissue or cell types and transform the behavior of recipient cells, contributing to both homeostasis and disease progression. In response to inflammatory signals, MSCs can migrate to injury sites and release EVs containing growth factors and regulatory miRNAs that can promote angiogenesis, tissue repair and regeneration, and anti-inflammatory responses, reviewed in (*13*). Cancer-derived EVs have been shown to promote organotropism and facilitate the transfer of pro-tumorigenic properties to stromal cells, preparing the microenvironment for metastatic cancer cell colonization (*65*, *66*). Peinado *et al.* (*66*) demonstrated that exosomes derived from highly metastatic melanomas could "educate" bone marrow progenitor cells through the receptor tyrosine kinase MET, promoting the metastatic potential of primary tumors. Hoshino *et al.* (*65*) revealed that distinct exosomal integrin expression patterns of cancer cells are associated with distinct organotropism. Specifically, it was identified that exosomal integrins α6β4 and α6β1 are linked to lung metastasis, while exosomal integrin αVβ5 is linked to liver metastasis. Furthermore, cellular uptake of exosomal integrin induces pro-inflammatory reactions in the recipient cells. Another mechanism by which EVs influence recipient cells is through horizontal gene transfer. For example, Douanne *et al.* (*67*) provided evidence that drug-resistant Leishmania parasite EVs transferred drug-resistance genes, enhancing the survival and fitness of naïve parasites.

Together, the function of EVs is multifaceted and highly dependent on the types and conditions of their source cells, underscoring their versatility in biomedical applications.

CARGO DELIVERY

Effective EV-mediated intracellular communication relies on the successful cytosolic delivery of EV cargo. This process is mediated by specific molecular interactions and pathways, generally involving the recognition and binding of EVs to recipient cells, followed by internalization and subsequent escape from endosomal pathways to avoid degradation (Fig. 2).

EV-cell interaction

EVs can bind to the surface of the recipient cells, be internalized via various pathways, or fuse with the membrane of the recipient cells, reviewed in (*68*). This interaction is mediated by specific molecules, such as ligand and receptor proteins, ECM components, lipids, and proteoglycans, present on the surface of the EVs and target cells (*69*). Proteins present on the surface of EVs, such as integrins and tetraspanins, interact with their specific receptors expressed on the plasma membrane of recipient cells, promoting EV internalization (*68*, *70*). In contrast, certain proteins, such as CD47, can play negative regulatory functions, suppressing phagocytic uptake (*71*).

EVs interact dynamically with components of the ECM, such as fibronectin, collagen, and glycosaminoglycans (*68*, *70*). EVs can bind directly to ECM molecules, or cells synthesize ECM molecules that bind to secreted EVs (*72*). Cells can also endocytose ECM components and resecrete them on EV surfaces, aided by proteins like integrins (*70*, *73*). Fibronectin bound to integrins on EVs was found to support cellular motility (*73*). Fibronectin can also bind to heparan sulfate proteoglycans on the membrane of EVs and cells, forming a bridge and thus facilitating internalization by target cells (*74*). Human MSCs can synthesize and secrete hyaluronan (HA)–coated EVs, where HA could enhance the regenerative properties of the EVs by facilitating interactions with target cells through HA receptors such as CD44, promoting ECM remodeling, and supporting immune regulation (*72*). These findings highlighted the important role of ECM components in influencing the function of EVs and their interactions with target cells.

In addition, glycoproteins and glycolipids on the membrane of EVs can bind to cell surface receptors, such as lectins, promoting EV adhesion and capture (*68*). PS, on the outer membrane of EVs, serves as a universal target by PS receptors or PS-binding proteins on target cells for attachment and internalization (*68*, *70*). Moreover, electrostatic interactions between EVs and receptor cells can modulate their initial interaction by attractive or repulsive electrostatic forces (*69*).

Internalization of EVs

The internalization of EVs occurs through various endocytic pathways, categorized into phagocytosis, macropinocytosis, and endocytosis mediated by clathrin, caveolin, or lipid rafts, reviewed in (*75*). Phagocytosis involves the internalization of large particles, such as apoptotic bodies, by specialized cells like macrophages (*75*). Macropinocytosis of EVs is a nonselective process that internalizes extracellular fluid containing EVs by forming large membrane protrusions that pinch off into the cytosol (*76*). Clathrin-dependent endocytosis of EVs begins with clathrin-coated pits forming on the plasma membrane to selectively internalize specific EVs. This process involves the recruitment of adaptor proteins, membrane invagination, vesicle closure by dynamin, and subsequent uncoating, allowing the internalized vesicles to fuse with early endosomes for further processing (*77*). Caveolin-dependent endocytosis of EVs involves the formation of caveolae, small invaginations of the plasma membrane enriched with caveolin proteins, which facilitate the uptake of specific EVs in a ligand-triggered manner (*76*). Lipid raft–mediated endocytosis of EVs relies on cholesterol- and sphingolipid-rich microdomains in the plasma membrane. This pathway is sensitive to cholesterol depletion and can involve various mechanisms, including both caveolin-dependent and caveolin-independent routes,

providing a means for cells to selectively internalize specific cargo and regulate cellular signaling (*75*).

The uptake processes of EVs can be influenced by physicochemical properties, such as size, charge, fluidity, surface moieties, and the cellular environment (*22*, *69*, *70*). The precise characterizations of these complex factors and mechanisms remain an active area of research.

Delivery

While EVs can be uptaken by cells through various pathways, the effective delivery of cargo to the site of action is not ensured. It is presumed that direct fusion of EVs with the plasma membrane of the recipient cell would likely be the most efficient method to ensure the transmission of EV cargo. In contrast, EVs uptaken by endocytosis undergo different fates, including being directly emitted by the intact cells, recycled for EV biogenesis, or retained in the endosomes followed by lysosomal degradation (*78*).

The ability of native EVs to achieve endosomal escape remains controversial, as studies report a wide range of delivery efficiencies. Wang *et al.* (*79*) demonstrated that plant EV-delivered mRNAs were translated in recipient cells and effectively reduced fungal infection. In another study, Joshi *et al.* (*80*) examined EV-mediated cargo transfer mechanisms by using EVs engineered with green fluorescent protein (GFP) fused to the cytosolic tail of CD63 and recipient cells expressing anti-GFP nanobodies. They demonstrated that ~25% of internalized EVs released their cargo after 12 hours of incubation, suggesting that the majority of EVs undergo endosomal degradation without releasing their contents. EVs were found to be internalized via endocytosis, releasing their cargo by fusing with endosomal membranes in an acidification-dependent manner, supporting the hypothesis that EVs use mechanisms similar to certain viruses to achieve endosomal release via fusion with endosomal membranes in response to low pH (*81*).

Current RNA therapeutics exhibit very low endosomal escape efficiency (<1%) (*82*), in contrast to viral systems (e.g., up to 57% predicted for adeno-associated virus) (*83*). Consequently, further research into the molecular mechanisms governing endosomal escape in both EVs and viruses could inspire innovative engineering strategies to enhance the efficacy of nanomedicines.

VEHICLE DESIGN FOR DELIVERY

Insights gained from studying natural delivery vehicles, such as EVs, have informed the design of engineered vesicles to enhance therapeutic outcomes. In this section, we will explore a range of vesicle designs for delivery, including engineered EVs and diverse types of biomimetic SVs (CDNs, PFLs, and various hybrid vesicles) (Fig. 3). These biomimetic SVs have lipid bilayer membranes and are incorporated with proteins, which closely resemble the structure of EVs. The design of these vesicles can be directly shaped by insights learned from EV biology. In turn, they can serve as synthetic EV models to study EV-associated characteristics and functions, in addition to their potential as delivery vehicles.

Extracellular vesicles

EVs are regarded as highly promising nanocarriers due to several key attributes. In general, compared to synthetic materials, their natural origin reduces immunogenicity and toxicity, their nanoscale size allows for tissue penetration and retention, and their lipid bilayer structure allows for encapsulation of both hydrophobic payload in the membrane and hydrophilic payload in the lumen. Depending on the source of EVs, they have further specialized capabilities, such as the potential to resist clearance by the mononuclear phagocytic system and regulate immune responses, surface interaction with cells for targeted cell uptake and delivery, and tissue regeneration (Fig. 3). These characteristics can be tuned and optimized by precise selection of the parental cell type, modifying cell conditions, or modifying the isolated EVs through engineering methods.

Therapeutic cargo loading

Various therapeutic cargo components have been loaded into EVs, such as proteins, RNAs [e.g., small interfering RNAs (siRNAs) and miRNAs], metals, and synthetic drugs, through endogenous or exogenous methods, reviewed in (*10*, *26*). Endogenous agents can be expressed through the pretransfection of the producer cells. Genetically engineered EVs benefit from the intrinsic cellular machinery that precisely synthesizes and packages biomolecules. In this perspective, EVs may outperform synthetic NPs in loading high-quality functional cargo. Exogenous agents can be loaded post-EV production using methods including incubation, sonication, electroporation, extrusion, and repeated freeze-thaw cycles.

For instance, exosomes derived from normal fibroblast-like mesenchymal cells have been engineered to deliver siRNA or shRNA targeting the $Kras^{G12D}$ mutation, an oncogenic mutation commonly found in pancreatic cancer (*84*). These engineered exosomes, termed "iExosomes," demonstrate superior efficacy in targeting oncogenic KRAS compared to liposomes. This enhanced efficacy is dependent on CD47 and facilitated by macropinocytosis. Treatment with iExosomes suppressed cancer progression and substantially extended overall survival in multiple mouse models of pancreatic cancer (*84*). Moreover, EVs have been explored for delivering CRISPR-Cas9 gene editing systems. EVs loaded with CRISPR-Cas9 plasmids have been investigated for inhibiting specific targets like poly (ADP-ribose) polymerase-1 (PARP-1) in ovarian adenocarcinoma cells, showing synergistic cytotoxicity effects with traditional DNA-damaging treatments and demonstrating anticancer efficacy in mouse models (*85*). Another study successfully generated EVs containing Cas9 protein and single-guide RNA from transfected cells, enabling efficient gene modification in recipient cells such as lung adenocarcinoma A549 cells (*86*). Despite these advancements, the efficiency of EV-mediated CRISPR-Cas9 delivery remains debated due to several technical challenges. Loading efficiency, particularly for large CRISPR components, is often low, and exogenous loading methods can compromise EV membrane integrity. Achieving effective cell uptake by target cells, avoiding immune clearance, and enhancing endosomal escape of CRISPR components remain hurdles that limit the success of gene editing in target cells.

Direct comparison of different therapeutic cargo loading methods is limited in the literature. Zhang *et al.* (*87*) compared several methodologies for loading therapeutic agents into EV-liposome hybrid vesicles, each with distinct strengths and limitations. Electroporation results in high loading efficiency by creating temporary membrane pores but may compromise membrane integrity at higher voltages. Incubation preserves membrane structure with gentle mixing but often has lower loading efficiency. Sonication enhances loading by temporarily disrupting membranes, though it risks structural damage. Freeze-thaw cycles enable cargo entry but may cause irreversible damage to EV membranes. Among these methods, a combination of

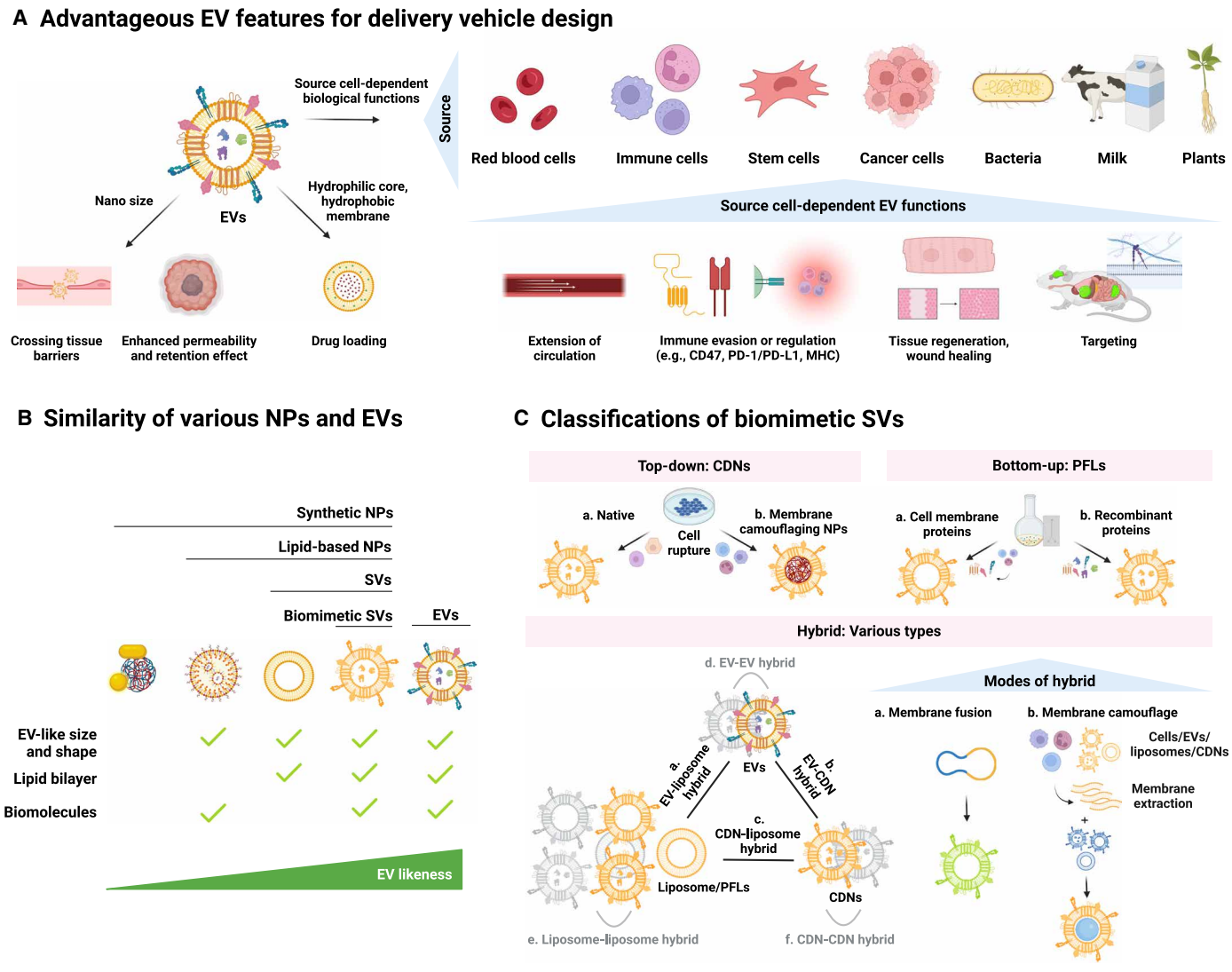

Fig. 3. Key features of extracellular vesicles (EVs) and classifications of biomimetic synthetic vesicles (SVs). (**A**) Advantageous EV features for delivery vehicle design, (**B**) similarity of various types of NPs and EVs, and the (**C**) classifications of biomimetic SVs. Created with www.biorender.com.

electroporation and EV-liposome hybridization demonstrated the highest drug loading efficiency compared to other tested approaches, as well as EVs or liposomes alone (*87*). Further research is needed to establish optimal protocols for therapeutic cargo loading.

Advances in engineering EVs

EVs can be edited to enhance their inherent properties and to introduce additional functions. These modifications primarily aim to increase the precision of delivery, reduce off-target effects, and improve scalability (*10*, *19*, *22*, *26*).

To maximize targeting capacity, EVs can be genetically engineered to express or conjugate biological and chemical molecules such as peptides, nanobodies, antibodies, polysaccharides, and nucleic acid aptamers, thereby endowing them with specific biological functions (*10*, *26*). Genetic engineering for EV modification primarily involves fusing a gene encoding a targeting motif, such as a protein or peptide, with the gene of an EV membrane protein. The LAMP2B method is a notable advancement in this area, widely used for precise targeting. In one study, Alvarez-Erviti *et al.* (*88*) engineered dendritic cell–derived exosomes to express LAMP2B fused with a rabies virus glycoprotein peptide, enabling targeted delivery of therapeutic siRNA to neurons in the brain. Kooijmans *et al.* (*89*) engineered EVs with tumor cell targeting capacity by incorporating anti-EGFR nanobodies into their membranes. This was achieved by transfecting the EV producer cells with plasmids encoding a fusion protein of anti-EGFR nanobodies and GPI anchor signal peptides, which naturally localize to the EV membrane. The engineered EVs exhibited tumor-targeting capacity. In addition, click chemistry methods, such as copper-free variants like strain-promoted azide-alkyne cycloaddition, allow postisolation modifications to attach various molecules to EV surfaces (*90*). Other targeting enhancements include conjugating RGD peptides (for cell adhesion) and aptamers (specific DNA or RNA molecules) through click chemistry or chemical conjugation methods (*26*). Moreover, membrane fusion with liposomes allows ease for modifying the lipid profile and enhances drug loading capacity (*16*).

To prolong circulation time, the expression of immune checkpoint molecules such as CD47, CD24, MHC, and PD-1/PD-L1 could be one potential approach (*10*, *26*). The conjugation of polyethylene glycol (PEG) (*91*) has been shown to protect EVs from clearance by the mononuclear phagocytic systems. Enhancing targeting specificity could reduce systematic toxicity. For example, A33 antibody-functionalized exosomes loaded with Dox showed targeted delivery against colorectal tumors, reducing cardiotoxicity and prolonging mouse survival (*92*).

To scale up EV production, several strategies in cell culture have been implemented (*10*), including shifting from two-dimensional (2D) to 3D cell culture, applying chemical and physical stimulation, and using stressed culture conditions (e.g., hypoxia, low pH, and oxidative stress). Commercial fabrication for EV-based therapeutics is also underway. For example, Codiak uses bioreactors for EV production and modifies EV using scaffold proteins at an industrial scale (*93*). Furthermore, improvements in EV isolation methods, such as transitioning from ultracentrifugation to tangential flow filtration, have substantially enhanced yields. Haraszti *et al.* (*94*) demonstrated that combining 3D culture with tangential flow filtration resulted in a 140-fold increase in EV yield. Although these approaches enhance overall yield, the biological variation in EVs isolated from different cultures and isolation techniques requires further investigation.

Strengths and limitations

The multifaceted biological properties of EVs make them a powerful therapeutic platform, specifically their low immunogenicity and toxicity, resistance to immune evasion and endosomal degradation, and targeting ability. EVs extracted from different cell types, such as immune cells, RBCs, cancer cells, and MSCs, have additional specialized functionalities, making them suitable for a wide range of downstream applications. Moreover, EVs can be tailored through engineering methods to enhance their utility, particularly as delivery systems.

Despite the numerous strengths, challenges persist in manufacturing. EV isolation involves large-scale cell culture and several purification steps, which are labor-intensive and time-consuming. The heterogeneity of EVs, coming from different cell types, culture conditions, isolation techniques, and inherent diverse subpopulations, makes the production of homogeneous vehicles difficult. Research efforts continue to unravel EV subpopulations in terms of sizes, lipid profiles, surface markers, and cargo characteristics. In the perspective of biosafety, the broad spectrum of cargo in EVs may lead to unintended effects on recipient cells. The balance between the therapeutic benefits and off-target effects remains to be thoroughly researched. Achieving high EV purity presents another challenge, with multistep isolation methods improving purity but often reducing yield (*95*).

Comprehensive ADME (absorption, distribution, metabolism, and excretion) data are essential for translating EVs and biomimetic SVs into clinical applications. Although ADME studies on EVs are emerging (*22*, *96*–*98*), notable gaps and controversies remain. EV pharmacokinetics can be affected by intrinsic factors such as size, lipid composition, and surface proteins, as well as by methodological variations like administration routes, labeling techniques, and animal models, as noted by van der Koog *et al.* (*22*). Labeling EVs with imaging dyes is a common approach to examine circulation time, biodistribution, and excretion; however, results can vary based on factors like dye type, staining procedure, concentration, and purification of free dyes (*99*, *100*). For instance, Lazaro-Ibanez *et al.* (*99*) analyzed EV biodistribution using various labeling techniques, from noncovalent and covalent fluorescent dyes to bioengineered CD63 fused with fluorescent or bioluminescent proteins. EV half-lives in tumor-bearing BALB/c mice were generally under 10 min, yet tissue distribution, serum stability, and clearance rates varied significantly by labeling method. Continued efforts, including the use of multiple labeling methods and rigorous controls, will be essential for future ADME research on EVs.

In summary, these challenges impede the reproducibility of EV quality and quantity, which is essential for precisely characterizing their therapeutic effects in disease treatment.

Biomimetic SVs

Biomimetic SVs are strategically designed to combine the advantages of both natural vesicles like EVs and fully synthetic NPs while mitigating certain limitations (*19*, *23*–*25*, *101*) (Figs. 3 and 4). For the scope of this review, we use biomimetic SVs to refer to fully or semisynthetic lipid-bilayered vesicles loaded with functional proteins for biomedical applications. In this regard, biomimetic SVs can be classified based on their production methods. CDNs are cell-derived nanovesicles generated by top-down methods that involve the breakdown of cells into smaller vesicles. PFLs are protein-functionalized liposomes synthesized by bottom-up approaches, typically involving the assembly of individual components in specific ratios. Hybrid vesicles, focused on in this review, are created by fusing two entities, i.e., "EV-liposome," "EV-CDN," and "CDN-liposome." It is acknowledged that other hybrid types exist, such as fusion within the same entities of different sources, e.g., cancer EVs fused with RBC EVs. This section summarizes the methods used for the synthesis of each category of biomimetic SVs and the strategies in their design.

CDNs (biomimetic SVs generated by top-down methods)

CDNs have most, if not all, of the biocomplexity of their biological sources, allowing them to replicate the functions of their producer cells while exhibiting substantially reduced dimensions (100 to 1000 times smaller). CDNs can be classified based on their engineering methods and applications: extracted cell membrane camouflaging large NP-based delivery vehicles (e.g., 80 to 100 nm) or CDNs with only endogenous native biomolecules. Both types can be loaded with additional therapeutic agents.

CDNs are generally produced by extrusion, sonication, hypotonic treatment, nitrogen cavitation, repeated freeze-thaw cycles, cell homogenizer, or a combination of these methods (Table 1, table S1, and Fig. 4). Exogenous payload-loading strategies include extrusion, incubation with the source cells before CDN production or directly with CDNs, sonication, electroporation, pH gradient-driven loading, extrusion, microfluidic mixer, or pretransfection of the source cells. Density gradient ultracentrifugation and dialysis are commonly used for the purification of CDNs postproduction or post-payload incorporation.

Cell membrane camouflage has been applied to improve the circulation and targeting efficacy of conventional NP-based delivery systems. Cell types applied for this purpose include RBCs (*102*, *103*), immune cells (*104*), cancer cells (*105*–*107*), MSCs (*108*, *109*), platelets (*110*, *111*), and bacteria cells (*106*) (Table 1 and table S1). One of the earliest studies, reported by Hu *et al.* in 2011 (*102*), demonstrated that camouflaging poly(lactic-co-glycolic acid) (PLGA), a type of polymer NPs, with cell membranes extracted from RBCs extended their circulation. The rationale behind this was to leverage immune checkpoints, such as CD47, present on the surface of RBCs to evade

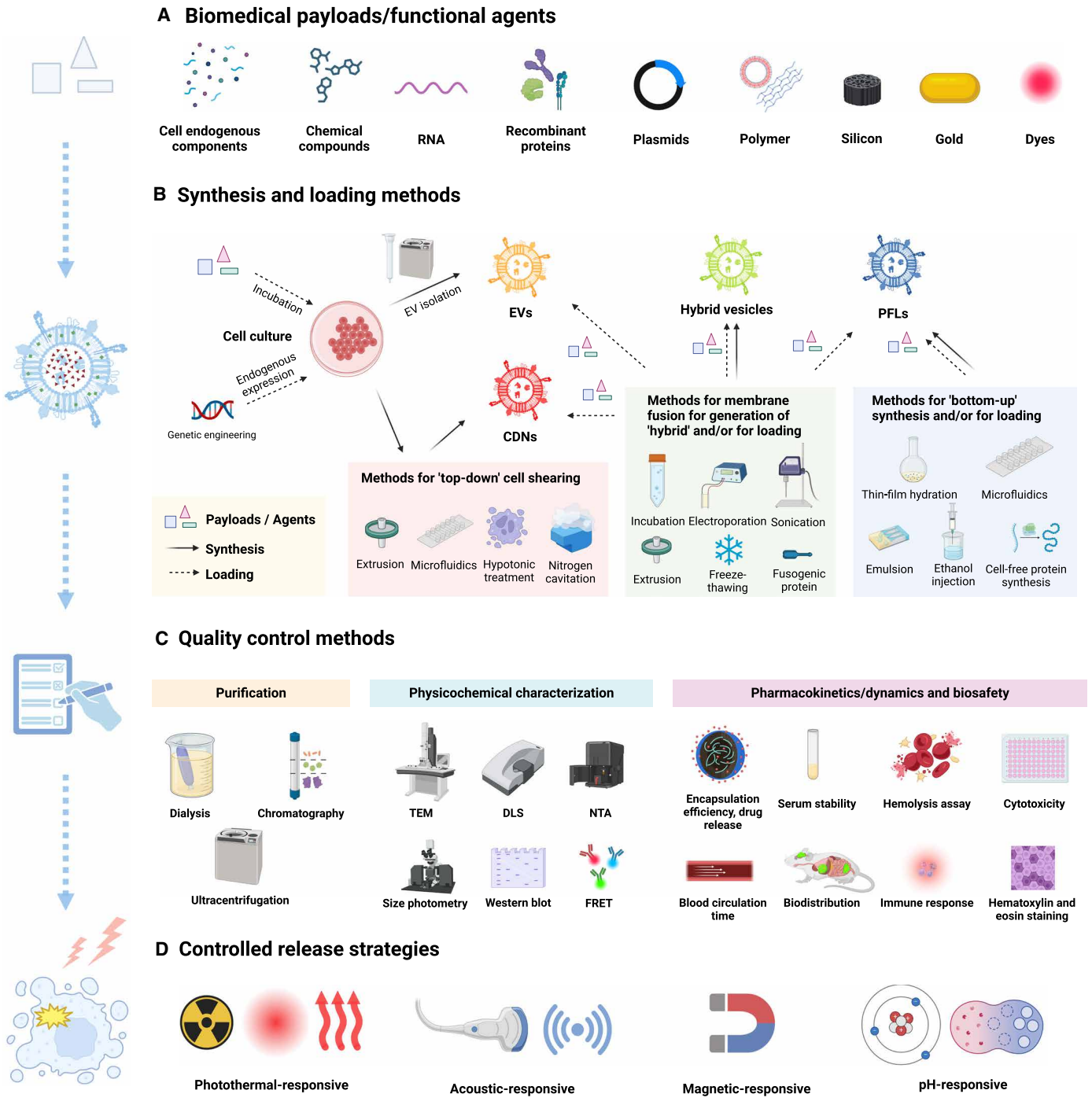

Fig. 4. Therapeutic cargo and engineering strategies. Types of (**A**) biomedical payloads or functional agents, (**B**) synthesis and loading methods for extracellular vesicle (EV) and biomimetic synthetic vesicles (SVs), (**C**) quality control methods, and (**D**) controlled payload release strategies. TEM, transmission electronic microscopy; DLS, dynamic light scattering; NTA, nanoparticle tracking analysis. Created with www.biorender.com.

macrophage engulfment. The circulation elimination half-life of RBC membrane–coated NPs (39.6 hours) outperformed the PEG–coated NPs (15.8 hours). This strategy was subsequently applied in numerous other studies. For instance, Parodi *et al.* (*104*) used cell membranes from white blood cells to prevent the clearance of silicon NPs by phagocytic cells. Fang *et al.* (*105*) reported cancer cell membrane–coated NPs with enhanced tumor-targeting capacity, attributed to the inherent homotypic binding properties of cancer cell membranes.

CDNs themselves, without conventional NPs, are capable of delivering various payloads, such as drugs [e.g., Dox (*112*–*115*), camptothecin (*116*), piceatannol (*117*), dexamethasone (*118*), paclitaxel

Table 1. Literature review of CDNs generated by top-down synthesis methods. Source cells, payloads, methods for synthesis, loading, and purification, and proposed applications of the study were summarized.

Authors and year	Source cell	CDN synthesis method	Payload	Loading method	Purification of CDNs	Proposed applications
Wang *et al.* 2024 (*125*)	Macrophages (RAW 264.7)	Cell disruption by a homogenizer, and CDN preparation by a microfluidic mixer	ICG	Microfluidic mixer	Dialysis (3.5 kDa)	Anticancer therapy
Jiang *et al.* 2024 (*183*)	Macrophages, bacteria (*Klebsiella pneumoniae*)	Cell lysis and sonication	Endogenous biomolecules	Not applicable	Not reported	Vaccine against pneumonia
Yuan *et al.* 2024 (*106*)	Glioma cells (C6), *Porphyromonas gingivalis*	Repeated freeze-thaw cycles and extrusion	MnO_2 NPs, Dox	Sonication and extrusion	Centrifugation	Anticancer therapy
Cao *et al.* 2023 (*109*)	MSCs	Induction of apoptosis by Staurosporine	Polycarbonate, bortezomib	Incubation with cells in the process of apoptosis induction	Sequential centrifugation	Anticancer therapy, pH-responsive release
Prasad *et al.* 2023 (*127*)	4T1	Repeated freeze-thaw cycles, hypotonic treatment, and sonication	AuNRs, Dox, ICG	Sonication	Dialysis (12 kDa)	Anticancer therapy, x-ray radiocontrast, and NIR fluorescence imaging
Zhang *et al.* 2023 (*126*)	Macrophages (RAW 264.7), endothelial cells (iECs)	Cell disruption by hypotonic treatment and cell homogenizer, and CDN preparation by extrusion	4-octyl itaconate	Incubation	Centrifugation	Diabetic wound repair
Zhang *et al.* 2023 (*107*)	Breast cancer cells (MCF-7/ADR)	Hypotonic treatment and homogenization	Magnetic NPs	Sonication	Not reported	Drug screening
Liu *et al.* 2023 (*176*)	Bacteria (*Akkermansia muciniphila*, *Bifidobacterium longum*, and *Bifidobacterium breve*)	Sonication and extrusion	Endogenous biomolecules	Not applicable	Centrifugation	Anticancer therapy
Jin *et al.* 2022 (*111*)	Platelets	Repeated freeze-thaw cycles and extrusion	Curcumin-resveratrol NPs	Extrusion	Not reported	Inflammatory lung injury therapy, inhalation administration
Qin *et al.* 2022 (*108*)	Mouse bone marrow–derived MSCs	Cell membranes were extracted by the Membrane and Cytosol Protein Extraction Kit, and cell membranes were freeze-thawed repeatedly, sonicated, and extruded	PLGA/DOTAP NPs	Sonication and extrusion	Not reported	Anti-inflammation therapy
Wu *et al.* 2021 (*115*)	Endothelial cells (bEnd.3)	Extrusion	Dox	Sonication	Ultracentrifugation	EV-mimicking, cancer therapy
Guo *et al.* 2021 (*114*)	Breast cancer cells (MDA-MB-231), fibroblasts (3T3)	Cell disruption by hypotonic treatment and a homogenizer. NP-encapsulating endosomes were isolated and extruded	Dox	Incubation	Dialysis (20 kDa)	EV-mimicking, cancer therapy
Zhang *et al.* 2021 (*184*)	Bacteria (*Mycoplasma hyopneumoniae*), macrophage (RAW 264.7)	Hypotonic treatment, repeated freeze-thaw cycles and extrusion	Endogenous biomolecules	Not applicable	Centrifugation	Vaccine against pneumonia

(*Continued*)

(*Continued*)

Authors and year	Source cell	CDN synthesis method	Payload	Loading method	Purification of CDNs	Proposed applications
Güliz *et al.* 2020 (*103*)	Erythrocytes	Hypotonic treatment and extrusion	Dox-loaded NPs	Extrusion	Centrifugation	Anticancer therapy
Nasiri Kenari *et al.* 2019 (*137*)	Neuroblastoma cells (SH-SY5Y)	Extrusion	Endogenous biomolecules	Not applicable	Density gradient ultracentrifugation	Assessment of EV-mimicking
Go *et al.* 2019 (*118*)	Monocytes (U937)	Alkaline treatment and sonication	Dexamethasone	pH neutralization and sonication	Density gradient ultracentrifugation, size exclusion chromatography	Anti-inflammation
Wu *et al.* 2018 (*131*)	Hepatocyte	Extrusion	Endogenous biomolecules (especially Sphk2)	Not applicable	Density gradient ultracentrifugation	Regenerative medicine for liver, EV-mimicking
Zhang *et al.* 2018 (*124*)	Embryonic kidney cells (HEK293T)	Cell lysis and sonication	ICG, Dox, hEGF, HER2-targeting affibody	Sonication for loading ICG and Dox, genetic engineering for expressing targeting proteins	Not reported	Cancer therapy, tumor imaging
Zhu *et al.* 2018 (*190*)	Natural killer cells (NK92-MI)	Extrusion	Endogenous biomolecules	Not applicable	Density gradient ultracentrifugation	Anticancer therapy, EV-mimicking
Tao *et al.* 2018 (*123*)	Embryonic kidney cells (HEK293)	Extrusion	LncRNA-H19	Genetic engineering	Ultrafiltration and density gradient ultracentrifugation	Regenerative medicine for diabetic wound
Kalimuthu *et al.* 2018 (*119*)	Human bone marrow–derived MSCs	Extrusion	Paclitaxel	Extrusion	Ultracentrifugation	Exosome-mimicking, cancer therapy
Choo *et al.* 2018 (*132*)	Macrophages (RAW 264.7)	Extrusion	Endogenous biomolecules	Not applicable	Density gradient ultracentrifugation	Anticancer therapy
Wu *et al.* 2018 (*113*)	Dendritic cells (DC2.4)	Extrusion	Dox	Extrusion	Centrifugation	Anticancer therapy
Kim *et al.* 2017 (*130*)	Adipose stem cell	Extrusion	Endogenous biomolecules, especially FGF2	Not applicable	Ultracentrifugation	Regenerative medicine for lungs
Gao *et al.* 2017 (*117*)	Neutrophil-like cells (HL-60)	Nitrogen cavitation and sonication	Piceatannol	pH gradient-driven loading	Ultracentrifugation	Anti–lung inflammation therapy
Dehaini *et al.* 2017 (*110*)	Erythrocytes and platelets	Hypotonic treatment or repeated freeze-thaw cycles	PLGA	Sonication	Centrifugation	Anticancer therapy
Lunavat *et al.* 2016 (*122*)	Monocytes (U937)	Extrusion	GFP siRNA, c-Myc shRNA	Electroporation for exogenous loading and genetic engineering for endogenous loading	Density gradient ultracentrifugation	EV-mimicking, cancer therapy, gene delivery
Gao *et al.* 2016 (*120*)	Neutrophil-like cells (HL-60)	Nitrogen cavitation and extrusion	TPCA-1	Incubation	Ultracentrifugation	Anti–lung inflammation therapy
Yang *et al.* 2016 (*121*)	Breast epithelial cells (MCF-10A)	Extrusion	CDK4 siRNA	Electroporation	Density gradient ultracentrifugation and RNase treatment	Gene delivery, cancer therapy
Gao *et al.* 2016 (*201*)	Macrophage-like cells (J774)	Hypotonic treatment, sonication, and extrusion	Endogenous biomolecules	Not applicable	Differential centrifugation	Anticancer therapy
Oh *et al.* 2015 (*134*)	Pancreatic β-cell line (MIN6) and fibroblast (NIH3T3)	Extrusion	Endogenous biomolecules	Not applicable	Density gradient ultracentrifugation	Regenerative medicine for diabetes
Hsieh *et al.* 2015 (*116*)	RBCs	Hypotonic treatment and sonication	Camptothecin, perfluoro-*n*-pentane	Sonication	Centrifugation	Remotely triggered drug delivery, ultrasound imaging enhancement

(*Continued*)

(*Continued*)

Authors and year	Source cell	CDN synthesis method	Payload	Loading method	Purification of CDNs	Proposed applications
Jo *et al.* 2014 (*133*)	Embryonic stem cells (ES-D3)	Cell disruption by passing through microfluidic channels	Endogenous biomolecules	Not applicable	Density gradient ultracentrifugation	Gene delivery
Jeong *et al.* 2014 (*129*)	Embryonic stem cells (ES-D3)	Cell disruption by passing through microfluidic channels	Endogenous biomolecules	Not applicable	Density gradient ultracentrifugation	Exosome-mimicking, regenerative medicine
Fang *et al.* 2014 (*105*)	Melanoma cells (B16-F10), breast cancer cells (MDA-MB-435)	Hypotonic treatment	PLGA	Extrusion	Centrifugation	Anticancer vaccine
Parodi *et al.* 2013 (*104*)	Leukocytes (Jurkat cells)	Isolation of cellular membrane through a discontinuous sucrose gradient	Nanoporous silicon particles, Dox, FITC-BSA	Incubation	Centrifugation	Anticancer therapy
Jang *et al.* 2013 (*112*)	Monocytes (U937), macrophages (RAW 264.7)	Extrusion	Dox	Extrusion	Density gradient ultracentrifugation	Anticancer therapy
Hu *et al.* 2011 (*102*)	RBCs	Hypotonic treatment, sonication, and extrusion	PLGA	Extrusion	Dialysis (30-nm porous membranes)	Anticancer therapy

(*119*), and TPCA-1 (*120*)], RNAs [e.g., siRNA (*121*, *122*), shRNA (*122*), and lncRNA (*123*)], and fluorescent dyes (*124*, *125*). Common source cell types for CDNs include RBCs (*116*), immune cells (*112, 113, 117, 120, 122, 125, 126*), cancer cells (*114*), MSCs (*119*), and normal tissue cells (*121, 123, 124*). In 2013, Jang *et al.* (*112*) created CDNs from monocytes and macrophages and loaded them with Dox. These CDNs have a natural targeting ability and a 100-fold higher production yield than exosomes. CDNs have also been used for gene delivery. For example, Yang *et al.* (*121*) encapsulated siRNA in CDNs derived from epithelial cells, demonstrating tumor targeting and antitumor effects.

Multiple types of payloads can be coloaded to CDNs (*124*, *127*). For instance, Prasad *et al.* (*127*) encapsulated Dox, gold nanorods (AuNRs), and an imaging dye, indocyanine green (ICG), into breast cancer cell–derived CDNs. These CDNs showed potential for synergistic chemo-phototherapeutics for solid tumors. CDNs from various cell types may be applied for the same therapeutic purpose, with each offering unique advantages. For example, in brain-targeted drug delivery, CDNs have been developed from a range of cell types, including immune cells, MSCs, neural stem cells, and tumor cells (*128*). In addition, hybrid membranes from multiple cell types have been developed to merge specialized cell-like functions (*106*, *114*, *126*), offering customization options that are hard to achieve by natural EVs. These examples highlight that the functional properties of source cells can be translated into biomimetic solutions for enhanced therapeutic targeting.

CDNs without exogenous payload primarily rely on endogenous biomolecules from the source cells to exert therapeutic effects. Stem cell–derived CDNs particularly stand out for their ability to promote cell proliferation, highlighting their potential in regeneration medicine (*129*, *130*). Similarly, CDNs generated from primary hepatocytes can promote hepatocyte proliferation, indicating their capacity for liver regeneration (*131*). In addition, endogenous biomolecules in CDNs have been found to regulate immune response (*132*), induce horizontal gene transfer (*133*), and promote cell differentiation (*134*), opening doors for novel therapeutic perspectives.

While cell membranes are the primary source of CDNs, EV membranes could be an alternative for coating synthetic NPs to improve drug delivery and treatment efficacy. For instance, Wang *et al.* (*135*) used exosomal membranes derived from natural killer cells to coat NPs loaded with therapeutic miRNA. This method not only enhanced tumor inhibition but also offered increased biocompatibility in an allogeneic setting, potentially avoiding graft-versus-host disease. Similarly, Han *et al.* (*136*) developed cancer exosomal membrane–coated NPs modified with AS1411 aptamers. The exosomal coating extended the in vivo circulation time, while the aptamers ensured high tumor-targeting efficiency by specific binding to nucleolin on the tumor cell membrane. Despite this promising approach, scalability remains a challenge. Nevertheless, these studies suggest the unique and potentially irreplaceable properties of EV membranes compared to cell membranes.

CDNs present a manufacturing advantage with high scalability compared to other types of biomimetic NPs. Compared to their producer cells, CDNs lack nuclei and many cell machinery, rendering them safer and more stable in terms of storage. Compared to conventional liposomes, CDNs have high biocomplexity, facilitating improved circulation and targeting. However, unintended effects can result from the diverse biomolecules on the membranes or enclosed in the lumen of CDNs, which are hard to eliminate. Compared to EVs, CDNs can achieve substantially higher yield, and therefore be an alternative to EVs in many applications, especially for cell types like primary cells that have a low yield of EVs. However, it has been noted that CDNs generated from cells have distinct proteomic profiles compared to EVs derived from the same cells

(*137*). Furthermore, cells and EVs differ in lipid composition (*58*). These findings emphasize the importance of precisely characterizing their cargo when applying them to biomedical applications.

PFLs (biomimetic SVs generated by bottom-up methods)

Liposomes are clinically proven drug delivery systems for therapeutics. They have an EV-like lipid bilayer nanostructure and the capacity to encapsulate both hydrophilic and hydrophobic agents (*20*). In contrast to the heterogeneous nature of EVs, liposomes provide a unique opportunity to control homogeneous properties, such as cargo, lipid formulation, size, and charge, to suit specific targets. PFLs can exert biological functions by incorporating specific proteins, making them highly customizable and versatile for therapeutic applications (Table 2, table S2, and Fig. 4).

The synthesis of liposomes typically involves mixing lipids dissolved in an organic solvent with an aqueous buffer by methods such as thin-film hydration, ethanol injection, detergent removal, and microfluidics (*20*). Microfluidic-based liposome synthesis is a cutting-edge technique that leverages microscale fluid dynamics to produce liposomes with enhanced control over size and uniformity (*20*, *138*–*141*). Compared to thin-film hydration, one of the most widely used liposome synthesis methods, liposomes synthesized by microfluidics typically do not require extrusion, sonication, or homogenization to reduce the size and lamellarity (*20*). PFLs are made in similar ways to liposomes, with proteins added to the aqueous phase during synthesis or loaded postsynthesis (*139*, *140*, *142*–*145*). Phosphate-buffered saline (PBS) and water are commonly used as aqueous buffers for protein solutions during synthesis. Dialysis, ultracentrifugation, ultrafiltration, and size-exclusion chromatography are widely accepted methods for their purification postsynthesis.

PFLs can be loaded with a selected group of biomolecules (e.g., cell membrane proteins) or a defined ratio of individual components (e.g., specific recombinant proteins and miRNAs). Notably, in 2016, Molinaro *et al.* (*144*) synthesized liposomes incorporated with leukocyte cell membrane proteins, termed "leukosomes," by thin-film hydration using a lipid formulation composed of dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), and 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC). These leukosomes exhibited inflammation-targeting properties. Subsequently, in 2018, the same group reported the use of microfluidics for the synthesis of leukosomes (*139*). The effects of total flow rates (1, 3, 6, 9, and 12 ml/min), flow rate ratios of aqueous phase to organic phase (1:1, 2:1, and 3:1), and protein-to-lipid weight ratios (1:300, 1:100, and 1:50) on the physicochemical properties of liposomes were assessed. Rampado *et al.* (*141*) conducted a study to optimize microfluidic-based synthesis. The influence of the experimental parameters on leukosome features such as size and protein loading was analyzed using a design of experiment approach. The design of microfluidic devices has also been consistently improved. For instance, Forbes *et al.* (*138*) developed a microfluidic system for in-line production and purification of protein-encapsulated liposomes. Various lipid formulations, protein concentrations, encapsulation efficiency, and protein retention and release were systemically analyzed. Across studies, there has been no consensus on an optimal set of parameters for the encapsulation of proteins, which could be due to the variation in experimental details from study to study, such as buffers, purification methods, lipid formulations, and protein sources (*138*–*141*).

In terms of protein source, a number of studies focused on using leukocyte membrane proteins to advance therapeutic delivery (*139*–*147*), leveraging their excellent circulation and immune regulation properties. Cancer cell and RBC membrane proteins have also garnered research interest. For instance, Li *et al.* (*148*) produced liposomes incorporated with a combination of 4T1 breast cancer cell membrane proteins and neutrophil elastase, exploiting the tumor-homing property of cancer cell membranes and the ECM destruction property of elastase, resulting in enhanced tumor penetration of encapsulated drugs. Similarly, Zhang *et al.* (*149*) produced liposomes with a combination of RBCs and cancer cell membrane proteins to improve circulation and targeting properties. These studies demonstrated the strategy of combining molecules of interest to design multifunctional PFLs.

In contrast to the high biocomplexity associated with vehicles like EVs, CDNs, and PFLs loaded with bulk proteins, another branch of this field has explored the loading of selected proteins in specific identities and amounts (*138*, *150*, *151*). In this context, Vazquez-Rios *et al.* (*151*) produced liposomes loaded with commercial human integrin α6β4, alongside therapeutic agents, resulting in lung organotropism. This EV-mimetic showed great similarities to natural EVs in terms of physicochemical properties, drug loading capacity, and cancer cell targeting in vitro and in vivo while being easier to produce and having a higher yield compared to EVs. Staufer *et al.* (*150*) produced liposomes loaded with tetraspanins (CD9, CD63, and CD81) individually or in combination with miRNAs. Cellular behavior assays and RNA sequencing profiles validated the expected functionality of the incorporated proteins. These developments indicate the feasibility of producing bio-inspired liposomes with fine control of individual biomolecules.

PFLs offer advantages such as their high customizability, enabling precise control over the lipid formulation, physical characteristics, and selection of biomolecules. This level of control allows for isolated studies on individual factors (*139*–*141*). While the incorporation of recombinant proteins is feasible, the production of PFLs is largely restrained by the availability of biomolecules and the considerable costs for large-scale production. Furthermore, quantitative and qualitative characterization of the functionality of encapsulated biomolecules poses challenges, as they may degrade, aggregate, and adopt incorrect orientation or localization in the vesicles.

Despite the growing interest and advancement in the field of bio-inspired liposomes, guidelines for their assessment remain relatively scarce. In addition, various nomenclatures have been adopted in different studies, and there is no universal definition for each of the nomenclatures and categories. For example, "EV-mimetics" or "synthetic EVs" has been used for SVs that are empty (no protein incorporation) liposomes with similar physical properties to EVs (*152*), as well as for SVs that encompass a certain degree of EV-associated marker proteins (*150*). "Nanovesicles" is generally used to refer to vesicles generated by the top-down method, while it was also used for vesicles synthesized by the bottom-up method in other studies (*153*). On the basis of MISEV2023, "synthetic vesicles" is the recommended nomenclature for EV-mimetic vesicles that are synthesized de novo from molecular components or made as hybrid entities (*1*).

Hybrid vesicles (biomimetic SVs generated by fusion methods)

Hybrid vesicles represent a novel class of vesicles that combine the advantages of EVs (e.g., high biocompatibility and targeting capacity), CDNs (e.g., high biocompatibility, targeting capacity, and yield), and liposomes (e.g., high controllability, customizability, and yield) for advanced therapeutic delivery.

Table 2. Literature review of PFLs produced by bottom-up synthesis methods. Protein source and other payloads, lipid formulation, synthesis method, aqueous buffer used for synthesis, protein-to-lipid weight ratio, liposome purification methods, and proposed application of the study were summarized. CHOL, cholesterol.

Authors and year	Proteins and other payloads	Lipid formulation (molar ratio unless specified)	Synthesis method	Aqueous buffer	Protein:lipid (weight ratio)	Purification of liposomes	Proposed applications
Koo *et al.* 2024 (*153*)	Ovalbumin, monophosphoryl lipid A	DPPC, EcoCeramide ENP, and CHOL in varying ratios	Thin-film hydration followed by extrusion	PBS	Not reported	Not reported	Vaccine
Rampado *et al.* 2022 (*141*)	Leukocyte (THP) membrane proteins, Dox	DPPC:DOPC:CHOL = 4:3:3	Microfluidic mixer (Nanoassemblr chip)	Ammonium sulfate (for the purpose of Dox loading)	1:300 to 1:20	Dialysis (300 kDa), overnight, room temperature	Anticancer therapy
Zinger *et al.* 2021 (*140*)	Leukocyte cell membrane proteins	DPPC:DOPC:CHOL = 4:3:3	Microfluidic mixer	MilliQ water	1:100, 1:50, 1:20	Dialysis (1000 kDa)	Anti-inflammation therapy
Staufer *et al.* 2021 (*150*)	CD9, CD63, CD81, miRNAs	CHOL:SM:DOPC:DOPS:DOPE:DOPG:PA:DAG:DOPI 43:16:15:11:6:5:2:1:1, 1% molar ratio of DGS-NTA(Ni)	Shear stress emulsification, thin-film hydration, then incubation of vesicles with CD peptides	PBS	1:2	Ultracentrifugation	EV-mimicking
Li *et al.* 2021 (*148*)	Breast cancer cell (4T1) membrane proteins	DPPC:CHOL:DSPE-PEG 2000 = 5:4:1 (weight ratio)	Thin-film hydration followed by extrusion	PBS	1:200	Ultrafiltration (1000 kDa)	Anticancer therapy
Molinaro *et al.* 2020 (*145*)	Leukocyte cell membrane proteins	DPPC:DSPC:DOPC:CHOL = 5:1:3:1	Thin-film hydration followed by extrusion	PBS	1:300	Dialysis (1000 kDa)	Anticancer therapy
Zinger *et al.* 2020 (*147*)	Leukocyte cell membrane proteins	PC, CHOL	Thin-film hydration followed by extrusion	PBS	1:300	Dialysis (1000 kDa) and 0.2-μm filtration	Anticancer therapy
Forbes *et al.* 2019 (*138*)	Ovalbumin, insulin, BSA	PC:CHOL or DMPC:CHOL or DPPC:CHOL or DSPC:CHOL or DSPC:CHOL:PS in various ratios	Extrusion and sonication, or microfluidics (Nanoassemblr chip)	PBS	1:5 to 1:6	Tangential flow filtration fitted with a column (750 kDa)	Approach development (in line synthesis and purification)
Lu *et al.* 2019 (*191*)	Conexin 43, VEGF siRNA	DOPC:SM:CHOL:DOPS:DOPE = 21:17.5:30:14:17.5	Thin-film hydration followed by extrusion	Nuclease-free water	Not reported	Not reported	EV-mimicking
Zhang *et al.* 2019 (*149*)	RBC and cancer cell (MCF-7) membrane proteins	Not reported	Not reported	Not reported	Not reported	Not reported	Anticancer therapy
Vazquez-Rios *et al.* 2019 (*151*)	Integrin a6b4, BSA, lysozyme, drug (curcumin), siRNA, dsDNA	CHOL:PC:SM:Ceramide = 0.9:1:0.4:0.3 (weight ratio)	Ethanol injection	MilliQ water	1:100	Ultracentrifugation	Anticancer therapy
Molinaro *et al.* 2018 (*139*)	RBC and macrophage (J774) membrane proteins	DPPC:DOPC:CHOL = 4:3:3	Microfluidics (Nanoassemblr chip)	Not reported	1:300, 1:100, 1:50	Ultracentrifugation followed by dialysis	Approach development (microfluidic-based synthesis)
Martinez *et al.* 2018 (*143*)	Macrophage (J774) membrane proteins	PC, CHOL, ratio note specified	Thin-film hydration followed by extrusion	Water	Not reported	Not reported	Anticancer therapy

(*Continued*)

(*Continued*)

Authors and year	Proteins and other payloads	Lipid formulation (molar ratio unless specified)	Synthesis method	Aqueous buffer	Protein:lipid (weight ratio)	Purification of liposomes	Proposed applications
He *et al.* 2018 (*178*)	Insulin	SPC:CHOL = 0.3:0.075	Reversed-phase evaporation	Hydrochloride solution	16:254	Size-exclusion chromatography	Therapy for diabetes
Corbo *et al.* 2017 (*142*)	Leukocyte cell membrane proteins	DPPC:DSPC:CHOL:DOPG = 5:1:1:3	Thin-film hydration followed by extrusion	Bi-distilled water	Not reported	Not reported	Anti-inflammation therapy
Corbo *et al.* 2017 (*146*)	Leukocyte cell membrane proteins	DPPC:DOPG:DSPC:CHOL = 5:3:1:1	Thin-film hydration followed by extrusion	PBS	Not reported	Not reported	Analysis on corona profile of SVs in in vivo circulation
Molinaro *et al.* 2016 (*144*)	Leukocyte cell membrane proteins	DPPC:DSPC:DOPC:CHOL = 5:1:3:1	Thin-film hydration followed by extrusion	PBS	1:600, 1:300, 1:100	Dialysis (1000 kDa)	Anti-inflammation therapy
Liguori *et al.* 2016 (*193*)	VDAC	DOPC:DOPE:DMPA:CHOL = 20:20:20:40	Thin-film hydration followed by filtration	Diethyl pyrocarbonate-treated water	Not applicable	Ultracentrifugation	Approach development (cell-free production of protein directly into liposomes)
De La Peña *et al.* 2009 (*192*)	MHC/peptide complexes and Fab regions	PC:CHOL:DSPE-PEG:DSPE-PEG-MAL = 2:1:0.08:0.02	Thin-film hydration followed by extrusion	25 mM Hepes and 140 mM NaCl	Not reported	Size-exclusion chromatography	EV-mimicking

Hybrid vesicles are prepared by membrane fusion, such as through repeated freeze-thaw cycles, coextrusion, incubation, sonication, the addition of EVs or CDNs during liposome synthesis by thin-film hydration, or a combination of these strategies (Table 3, table S3, and Fig. 4). EV isolation, liposome synthesis, and payload loading methods are similar to the methods used for the aforementioned vesicles. Hybrid vesicles have been loaded with NPs (*154*, *155*), drugs [e.g., semaglutide (*156*), gemcitabine (*87*), Dox (*157*–*159*), reversine (*158*), and DL-3-*n*-butylphthalide (*160*)], curcumin (*161*)), siRNAs (*162*, *163*), plasmid DNA (*162*, *164*, *165*), and fluorescence dyes (*166*–*168*). The biological sources of EVs or CDNs include immune cells, cancer cells, MSCs, and bacteria, as mentioned in the CDNs (biomimetic SVs generated by top-down methods) section. In addition, cow's milk (*156*) and ginseng roots (*169*) have also been used. Fusion efficiency is frequently studied by the FRET (fluorescence resonance energy transfer) method, i.e., monitoring the fluorescence change resulting from the fusion of distinctly labeled vesicles (*170*). Ultracentrifugation, dialysis, ultrafiltration, and size-exclusion chromatography are the most common methods for their purification.

One of the earliest studies emerged in 2016, in which Sato *et al.* (*170*) mixed exosomes and liposomes and created EV-liposome fusions by repeated freeze-thaw cycles in liquid nitrogen and at room temperature. Modifying the lipid composition of liposomes changed the cell uptake of these hybrid vesicles, representing an innovative strategy for modifying EV design. In another study, Lin *et al.* (*164*) demonstrated enhanced transfection efficiency of EV-liposome hybrid vesicles for delivery of CRISPR-Cas9 plasmids to MSCs, surpassing the efficacy of using exosomes or liposomes (Lipofectamine 2000) alone. These hybrid vesicles were created simply by incubation at 37°C for 12 hours.

In addition to EV-liposome fusion, EV-CDN and CDN-liposome hybrid vesicles have appeared as novel types of biomimetic SVs for biomedical applications (Table 3 and table S3). Hybrid vesicles offer unique possibilities and strategies for tailoring the functions of vesicles, as highlighted in the "Applications" section. However, further efforts are required to overcome challenges associated with both EV and SV preparations, as discussed in previous sections.

APPLICATIONS

In addition to therapeutic delivery, EVs and synthetic NPs expand their influence across diverse biomedical fields for disease diagnosis and treatment. This section will present the biomedical value of EVs and explore insights and possibilities for innovative applications using biomimetic SVs (Fig. 5 and Table 4).

Biomarker discovery

EVs are enriched in multiple biofluids (liquid biopsy), such as blood, urine, milk, and saliva, and can be extracted as noninvasive diagnostic and prognostic analytes in various disease contexts (*7*–*9*, *47*). Growing evidence has shown that EV-associated biomarkers from biofluids could be a more reliable diagnostic analyte than total biofluids (*8*).

EV RNAs and proteins are the most extensively studied cargo for biomarker discovery. For instance, miRNAs in cancer-derived EVs have been shown to regulate cellular processes including proliferation, migration, invasion, cell death, and response to inflammation,

Table 3. Literature review of biomimetic hybrid SVs. Hybrid types (EV-liposome, EV-CDN, and CDN-liposome), fusion method, biological source, payload, payload loading method, SV purification method, and proposed applications in the study were summarized.

Authors and year	Hybrid type	Fusion method	Biological source for EVs or CDNs	Payload	Loading method	Purification of hybrid SVs	Proposed applications
Jiang *et al.* 2024 (*162*)	EV-liposome	Extrusion	MSCs	pTREM2 plasmid DNA and BACE1 siRNA	Thin-film hydration	Not reported	Therapy for Alzheimer's disease
Xiao *et al.* 2024 (*156*)	EV-liposome	Thin-film hydration and extrusion	Cow's milk	Semaglutide	Thin-film hydration	Ultracentrifugation	Therapy for diabetes, oral administration
Kang *et al.* 2023 (*154*)	EV-liposome	Extrusion	Melanoma cells	Fe_3O_4 NPs	Incubation	Not reported	Approach development (isolation of CTCs)
Barone *et al.* 2023 (*166*)	EV-liposome	Repeated freeze-thaw cycles	Macrophages (J774A.1)	Disodium fluorescein	Thin-film hydration	Size-exclusion chromatography	Anticancer therapy
Zhang *et al.* 2023 (*87*)	EV-liposome	Incubation	Breast cancer cells (MCF-7)	Gemcitabine, miR-21 inhibitors	Electroporation or thin-film hydration	Ultracentrifugation	Anticancer therapy
Liang *et al.* 2022 (*165*)	EV-liposome	Incubation	Dendritic cells	CRISPR-Cas9 Plasmid	Incubation	Not reported	Therapy for osteoarthritis
Zhou *et al.* 2022 (*163*)	EV-liposome	Thin-film hydration and extrusion	Hepatocellular carcinoma cells (Sk-hep1)	CDK1 siRNA	Electroporation	Size-exclusion chromatography	Anticancer therapy, gene delivery
Rayamajhi *et al.* 2019 (*157*)	EV-liposome	Sonication and extrusion	Macrophages (J774A.1)	Dox	Extrusion	Ultrafiltration (10 kDa)	Anticancer therapy
Lin *et al.* 2018 (*164*)	EV-liposome	Incubation	Embryonic kidney cells (HEK293FT)	CRISPR-Cas9 plasmid	Incubation with liposomes before hybridization	Not reported	CRISPR-Cas9 delivery
Sato *et al.* 2016 (*170*)	EV-liposome	Repeated freeze-thaw cycles	Macrophages (RAW 264.7)	Not applicable	Not applicable	Not reported	Approach development (one of the earliest studies on EV-liposome hybridization methods)
Liu *et al.* 2024 (*185*)	EV-CDN	Extrusion	EVs isolated from bacteria (*E. coli*), CDNs derived from melanoma cells (B16F10)	Endogenous biomolecules	Not applicable	Not reported	Cancer vaccine
Wang *et al.* 2024 (*169*)	EV-CDN	Extrusion	EVs isolated from ginseng roots, CDNs derived from resected tumors	Endogenous biomolecules	Not applicable	Density gradient ultracentrifugation	Cancer vaccine
Liu *et al.* 2024 (*177*)	CDN-liposome	Sonication	Macrophages (RAW 264.7)	Ginsenoside Rg3 and *Panax notoginseng* saponins	Thin-film hydration	Ultracentrifugation	Therapy for ischemic stroke, intranasal administration
Qian *et al.* 2024 (*158*)	CDN-liposome	Thin-film hydration and extrusion	Breast cancer cells (4T1)	Dox, reversine	Thin-film hydration	Centrifugal filter devices (100 kDa)	Anticancer therapy
Qiao *et al.* 2024 (*155*)	CDN-liposome	Thin-film hydration of the dry lipid layer using isolated CDNs followed by sonication and extrusion	Macrophages (RAW 264.7)	Dexamethasone sodium phosphate-loaded NPs	Thin-film hydration	Centrifugation	Therapy for acute respiratory distress syndrome, pH-responsive drug release

(*Continued*)

(*Continued*)

Authors and year	Hybrid type	Fusion method	Biological source for EVs or CDNs	Payload	Loading method	Purification of hybrid SVs	Proposed applications
Liu *et al.* 2024 (*167*)	CDN-liposome	Thin-film hydration, repeated freeze-thaw cycles and extrusion	Melanoma cells (B16F10) and glioblastoma cells (G422)	ICG	Thin-film hydration	Not reported	Tumor imaging
Dong *et al.* 2024 (*160*)	CDN-liposome	Sonication	MSCs	DL-3-*n*-butylphthalide	Thin-film hydration	Dialysis	Therapy for ischemic stroke
Liu *et al.* 2023 (*161*)	CDN-liposome	Thin-film hydration, repeated freeze-thaw cycles and extrusion	Natural killer cells	Curcumin	Extrusion	Not reported	Therapy for Parkinson's disease, neuroprotection
Xiong *et al.* 2022 (*168*)	CDN-liposome	Extrusion and repeated freeze-thaw cycles	Neutrophils	ICG	Thin-film hydration	Dialysis (7 kDa)	Imaging and PTT for echinococcosis
Wang *et al.* 2021 (*159*)	CDN-liposome	Incubation	Erythrocytes	Dox, hematoporphyrin monomethyl ether	Thin-film hydration	Not reported	Cancer therapy, ultrasound-responsive imaging

and their levels are related to tumor stages (*171*). Several promising EV-associated miRNAs, including miR-21, miR-375, and the miR-200 family, have been identified as promising biomarkers across various cancer types, reviewed in (*172*). In addition to EV miRNAs, Su *et al.* (*51*) identified plasma-derived EV long RNA signatures that could identify early-stage breast cancer with high accuracy (AUC of 0.94). Abnormal levels of oncogenic protein expression in EVs, such as Wnt family proteins, EGFR, and VEGF, have been observed in various cancer types (*9*). For neurodegenerative diseases, EVs are in association with amyloid-β (Aβ) peptide release and accumulation in the brain of patients with Alzheimer's disease, as well as α-syn aggregation in the brain of patients with Parkinson's disease (*8*). In the context of cardiovascular disease progression, endothelial cells, smooth muscle cells, platelets, and leukocytes release EVs containing apoptotic factors, cytokines in inflammatory regulation, and adhesion molecules (*9*).

EV-DNA and lipid composition are emerging biomarkers. EV-DNA carries mutation signatures that indicate disease onset and treatment response (*47*). For instance, Balaj *et al.* (*173*) demonstrated the presence of EV-associated single-stranded DNA, reflecting tumor genetic status and c-Myc oncogene amplification. Furthermore, alterations in the lipid composition of EVs have been demonstrated to affect the development of several diseases, such as ovarian and prostate cancer, reviewed in (*6*).

NVEPs are enriched with metabolic enzymes, proteins, DNA, and RNA, indicating diagnostic and prognostic potential across various diseases. In Alzheimer's disease, exomeres have been found to carry proteins associated with the disease, such as amyloid precursor protein (APP) (*42*). In cancer, supermeres carry the membrane protein GPC1, which serves as a potential biomarker for pancreatic cancer, while supermere-associated TGFBI may be a biomarker for colorectal cancer (*42*). The abundance of miR-1246 in supermeres has also been suggested as a biomarker for colorectal cancer (*42*). Extracellular RNA is enriched in supermeres and is correlated with the presence of RNA binding proteins, while DNA is detected in exosome subpopulations and exomeres (*40*). These findings highlight the value of NVEPs as potential cancer biomarkers, warranting further analysis across diverse cell types and clinical samples, reviewed in (*2*).

The unique biomarker properties of native EVs cannot be fully replicated by SVs. However, understanding disease-specific biomarkers and their functions in EVs can inform the design of biomimetic SVs, enhancing their targeting accuracy, therapeutic efficacy, and potential applications in basic research. Kang *et al.* (*154*) presented an innovative approach for capturing melanoma CTCs using EV-liposome hybrid vesicles. These biomimetic SVs are modified with click chemistry to target CTCs and camouflaged with magnetic NPs to enhance isolation efficiency. This method showed more than 80% capture efficiency in both in vitro and in vivo models and allowed for effective CTC isolation from blood samples. This example represents an innovative application of biomimetic SVs in research.

Theranostics and controlled-release strategies

Theranostics combines diagnostic and therapeutic functions within a single platform, traditionally using metal-based NPs, carbon nanomaterials, and polymers (*174*). However, these synthetic materials pose biocompatibility and biosafety challenges. Emerging evidence supports EVs as biocompatible theranostic agents, with examples such as melanoma-derived exosomes loaded with superparamagnetic iron oxide NPs for magnetic resonance imaging (*175*). In recent years, biomimetic SVs gained recognition for their enhanced scalability and versatility. Their highly customizable membrane structures can effectively encapsulate therapeutic and imaging agents for controlled-release applications.

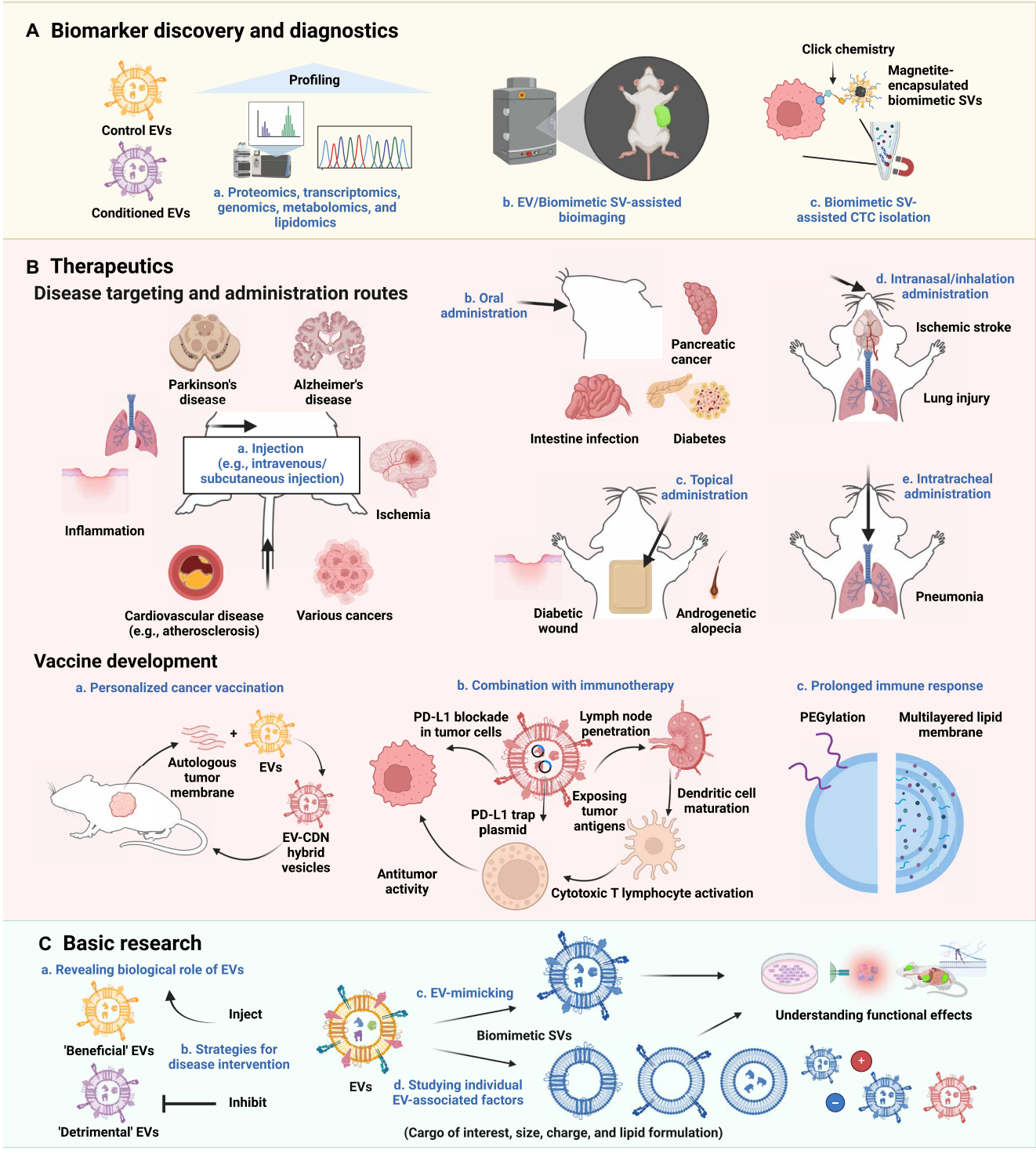

Fig. 5. Biomedical applications of extracellular vesicles (EVs) and biomimetic synthetic vesicles (SVs) in diagnostics, therapeutics, and basic research. (**A**) For diagnostics, EVs are profiled for biomarker discovery. Both EVs and biomimetic SVs deliver imaging agents for bioimaging. Biomimetic SVs enhance circulating tumor cell CTC isolation. (**B**) For therapeutics, EVs have been used in mouse models to target diverse diseases via various administration routes. They are also designed to innovate vaccine development. (**C**) In basic research, EV functions are continuously revealed and applied to therapeutic interventions, while biomimetic SVs serve as synthetic models to mimic EVs or study EV-associated factors. Created with www.biorender.com.

Biomimetic SVs have been explored for enhancing the effectiveness of photothermal therapies (PTTs). Prasad *et al.* (*127*) developed cancer cell–derived CDNs integrated with AuNRs, Dox, and ICG, creating a multifunctional system for imaging and therapy. The vesicles' cancer cell–mimetic surface facilitates immune evasion and tumor targeting. ICG is unique in its near-infrared (NIR) fluorescence for imaging and photothermal conversion properties. AuNRs provide a powerful photothermal effect by intensifying the heat generation upon NIR light exposure, which synergizes with ICG, making the system highly effective for precise tumor ablation with minimal off-target effects. In another example, Liu *et al.* (*167*) created ICG-loaded CDN-liposome hybrid vesicles with a combination of glioblastoma and melanoma cell membranes to enhance BBB penetration and glioblastoma margin delineation during surgery. Similarly, Xiong *et al.* (*168*) used neutrophil membrane–camouflaged ICG liposomes to target echinococcosis, a zoonotic infectious disease. Beyond ICG, DIR (another NIR dye) (*113*) and thermoresponsive lipids (*166*) are also applied for photo- or thermo-controlled drug release.

Ultrasound-responsive materials offer another theranostic approach. For instance, Hsieh *et al.* (*116*) developed erythrocyte-derived CDNs encapsulated with perfluoro-*n*-pentane, which vaporizes upon exposure to high-intensity focused ultrasound, enhancing ultrasound imaging contrast. Similarly, Wang *et al.* (*159*) designed ultrasound-responsive erythrocyte membrane–derived CDNs hybrid with liposomes, loaded with Dox and a sonosensitizer, enabling controlled drug release upon ultrasound exposure.

Magnetic-responsive systems use external magnetic fields to localize and release therapeutic agents at target sites. Güliz *et al.* (*103*) developed a folate-linked erythrocyte membrane–derived CDNs for ovarian cancer treatment. These vesicles encapsulate Dox-loaded magnetic NPs. The erythrocyte membrane enhances targeting to tumor cells through folate receptors while evading immune detection. Magnetic fields allow for the controlled release of Dox, reducing the IC_{50} (median inhibitory concentration) value against ovarian cancer cells.

pH-responsive systems leverage acidic microenvironments to enable controlled drug release and improve therapeutic efficacy. Qiao *et al.* (*155*) developed pH-responsive mineralized CDN-liposome hybrid vesicles with dexamethasone sodium phosphate and calcium, coated with M2 macrophage membranes, achieving targeted and controlled release in acidic inflammatory sites. Similarly, Yuan *et al.* (*106*) developed hybrid vesicles combining glioma cell membranes and bacterial outer membranes with MnO_2 NPs and Dox for targeted glioma therapy. The glioma membrane component aids tumor targeting, while bacterial outer membranes enhance BBB penetration. MnO_2 NPs generate reactive oxygen species under the tumor's acidic microenvironment, enhancing anticancer efficacy.

These advanced controlled-release systems reduce systemic toxicity and provide localized treatment. Compared to conventional theranostic materials, the adaptable membrane structures of biomimetic SVs offer diverse functionalities, further improving therapeutic precision.

Disease targeting

The efficacy of disease targeting with biomimetic SVs has been evaluated in preclinical models across a broad range of diseases. While cancer remains the primary area of research, applications of biomimetic SVs have expanded to include diseases of the central nervous system, diabetes, cardiovascular conditions, and respiratory diseases (Tables 1 to 3 and tables S1 to S3). Derived from various cell types, these biomimetic SVs exert therapeutic effects through mechanisms such as disease homing, immune modulation, and tissue regeneration. In addition, different administration routes have been explored to optimize therapeutic outcomes based on lesion location and disease type.

Biomimetic SVs have shown promise in enhancing cancer treatment through targeted delivery and immune activation. Wang *et al.* (*125*) developed small macrophage-derived CDNs (51 nm) for glioma targeting via intravenous injection, achieving a 78% increase in targeting efficiency compared to larger CDNs (>100 nm). For colorectal cancer, Qian *et al.* (*158*) used CDN-liposome hybrid vesicles incorporated with cancer cell membranes and loaded with Dox and reversine. Upon subcutaneous injection, these vesicles induced immune activation and prolonged survival in tumor-bearing mice. For pancreatic cancer, Liu *et al.* (*176*) developed hybrid CDNs derived from multiple strains of bacteria. Administered orally, these vesicles enhanced αPD-1 checkpoint therapy by reprogramming the tumor microenvironment, inhibiting oxidative phosphorylation, and promoting innate immunity activation. This approach achieved tumor regression comparable to live bacteria transfer but with fewer side effects like inflammation and diarrhea.

In central nervous diseases, biomimetic SVs have been used for targeted delivery and neuroprotection. For neurodegenerative diseases, Liu *et al.* (*161*) administered natural killer cell membrane–coated NPs containing curcumin through meningeal lymphatic vessels to treat Parkinson's disease. This route enhances curcumin's brain delivery by 20-fold compared to intravenous injection, targeting damaged neurons to clear reactive oxygen species and inhibit alpha-synuclein aggregation, which improved motor function and reduced neuron death in Parkinson's mouse models. In Alzheimer's disease treatment, Jiang *et al.* (*162*) used intravenously injected MSC EV-liposome vesicles with *BACE1* siRNA and *TREM2* plasmid to cross the BBB, reducing Aβ accumulation and reprogramming microglia, thus mitigating neuroinflammation. For ischemic stroke treatment, Dong *et al.* (*160*) developed MSC-derived CDNs with DL-3-*n*-butylphthalide for intravenous injection. These vesicles selectively targeted infarcted brain regions, promoting neuroprotection and motor recovery. In another study, Liu *et al.* (*177*) used intranasal macrophage membrane–coated CDN-liposome vesicles with neuroprotective agents for ischemic stroke, achieving high brain targeting and reduced inflammation.

For diabetes and wound healing, biomimetic SVs have shown potential in drug delivery and tissue repair. He *et al.* (*178*) developed vitamin-decorated PFLs for oral insulin delivery, achieving prolonged hypoglycemic effects through vitamin receptor targeting. For diabetic wound healing, Zhang *et al.* (*126*) created hybrid CDNs combining endothelial and macrophage membranes delivered via hydrogel. These vesicles promoted inflammation reduction, protected endothelial cells, and supported wound repair.

Overall, diverse biomimetic SVs, including CDNs, PFLs, and various hybrid vesicles, have demonstrated substantial therapeutic potential in disease targeting and have been extensively tested in preclinical models via different administration routes. Fine-tuning the selection and hybridization of source cell membranes or EVs, or by loading with recombinant proteins, enables multifunctional capabilities that enhance treatment outcomes.

Table 4. Summary of emerged biomedical applications, advantages, and limitations of biomimetic SVs.

Biomimetic SV type	Emerged biomedical applications	Advantages	Limitations
CDNs	Camouflage—Coating synthetic NPs with cell membrane to enhance biocompatibility in blood circulation.	Conveniently translating cell functions into nanovesicles. e.g., Homotypic cell-specific surface markers enables precise targeting to diseased cells.	Biocomplexity nature poses concerns over off-target cellular signaling, especially with tumor-derived CDNs.
	Therapy delivery—Disease targeting or immune regulation for various diseases like cancer, infection, inflammation, diabetes, neurodegeneration. Crossing tissue barriers like BBB.	Versatile by leveraging features of multiple cell types, including immune, stem, cancer, and bacterial cells, improving efficacy across diverse conditions.	Uncontrolled protein orientation can affect protein activity and targeting precision.
	Regenerative medicine—Stem cell–derived CDNs carry biomolecules that facilitate tissue repair and regeneration.	Multifunctional CDNs can be created by merging membranes of multiple cell types.	Lack of standardization. Technique-to-technique comparison is needed for standardized production for high-purity CDNs.
	Vaccine development—Immunogenic particle delivery for infectious diseases and cancer.	Relatively high scalability comparing to other types.	
	Imaging and controlled-release therapies—Loading CDNs with NIR dyes or ultrasound/magnetic/pH-responsive materials for imaging or therapy.		
	EV-mimicking—CDNs developed as EV-mimetic models.		
	Drug screening—Testing drug binding to the cellular targets.		
PFLs	Camouflage—Coating synthetic NPs with liposome-loaded cell membrane proteins to enhance biocompatibility in blood circulation.	Precise control of composition, allowing for studying individual factors such as size, surface charge, target proteins, and experimental parameters.	Requiring identification of specific protein targets to achieve recapitulation of intended biological functions.
	Therapy delivery—Disease targeting or immune regulation for various diseases like cancer, infection, inflammation, diabetes, neurodegeneration. Crossing tissue barriers like BBB.	Simpler composition and potentially enhanced control over pharmacokinetic and biosafety profiles.	Limited availability of target proteins can make them costly and difficult to scale.
	Vaccine development—Immunogenic particle delivery for infectious diseases and cancer.		Technically challenging, especially for ensuring efficient encapsulation of internal proteins and incorporation of membrane proteins.
	EV-mimicking—PFLs developed as EV-mimetic models.		Uncontrolled protein orientation can affect protein activity and targeting precision.
EV-liposome hybrid	Camouflage—Coating synthetic NPs with EV-liposome membrane to enhance biocompatibility in blood circulation.	High biocompatibility conferred by EV membrane.	Limited scalability due to EV isolation challenges.
	Therapy delivery—Disease targeting or immune regulation for various diseases like cancer, inflammation, diabetes, androgenetic alopecia, neurodegeneration. Crossing tissue barriers like BBB.	Conveniently leveraging the combined engineering advantages of EVs (e.g., genetical engineering and targeting capacity) and liposomes (e.g., high loading capacity).	Combined challenges associated with each entity.
	Research tool for biomarker discovery—e.g., EV-liposome vesicles incorporated with CTC-binding click chemistry and magnetic NPs were developed to assist CTC isolation.		Biocomplexity nature of EVs poses concerns over off-target cellular signaling.
	Tissue engineering—EV-liposome vesicles have been embedded in hydrogel to create bioinks that can delivery regulatory cargo.		
	CRISPR-Cas9 delivery—EV-liposome vesicles enhanced the delivery plasmid of CRISPR-Cas9 to the cells that are hard to transfect.		

(*Continued*)

(*Continued*)

Biomimetic SV type	Emerged biomedical applications	Advantages	Limitations
EV-CDN hybrid	Vaccine development—Immunogenic particle delivery for cancer.	High biocompatibility conferred by EV and cell membrane.	Limited scalability due to limited EV yield.
	Therapy delivery—Disease targeting or immune regulation for various diseases like cancer and atherosclerosis.		Combined challenges associated with each entity.
			Biocomplexity nature of EVs and CDNs poses concerns over off-target cellular signaling.
CDN-liposome hybrid	Therapy delivery—Disease targeting or immune regulation for various diseases like cancer, infection, ischemic stroke, respiratory disease, diabetes, neurodegeneration. Crossing tissue barriers like BBB.	Relatively high scalability compared to other types.	Combined challenges associated with each entity.
	Imaging and PTT—Loading CDN-liposome vesicles with NIR dyes.	High biocompatibility conferred by cell membrane.	Biocomplexity nature of CDNs poses concerns over off-target cellular signaling.
	Regenerative medicine—Stem cell–derived CDNs carry biomolecules that facilitate tissue repair and regeneration.		Combined challenges associated with each entity.
	Imaging and controlled-release therapies—Loading CDN-liposome vesicles with NIR dyes or pH/magnetic-responsive materials for imaging or therapies.		

Vaccines

Since the 1980s, numerous studies have observed differential expression of surface proteins in immune cell and cancer cell–derived EVs, suggesting that EVs carry molecular signatures of their parent cells and can modulate immune responses (*59*, *60*, *179*). These discoveries led to the idea of using EVs from different cell sources, including dendritic cells, tumor cells, and MSCs, as vaccine platforms for the treatment of cancer and various infectious diseases, reviewed in (*179*).

EV-based vaccines

In particular, EVs derived from dendritic cells, termed "dexosomes," are rich in antigen presentation machinery, making them attractive candidates for cancer immunotherapy and vaccine development (*15*). Dexosome-based cancer vaccines typically involve activating the patient's dendritic cells ex vivo with tumor-associated antigens or neoantigens. The dexosomes are then derived from these activated dendritic cells, displaying the antigens on their surface. These antigen-presenting dexosomes are injected back into the patient to stimulate a robust immune response against the cancer cells. The first clinical trials using dexosomes were conducted in the mid-2000s in patients with non–small cell lung cancer and melanoma (*180*, *181*). A phase 2 clinical trial was conducted using a "second generation" of dexosomes (IFN-γ-Dex) derived from dendritic cells matured with interferon-γ (IFN-γ) in patients with advanced non–small cell lung cancer, demonstrating the enhanced natural killer cell arm of antitumor immunity in patients compared to the previous "first-generation" dexosomes (*182*). These trials established the feasibility of using personalized dexosome-based vaccines. Moreover, MSC-derived exosomes have been used in several clinical trials to treat chronic diseases including type 1 diabetes and interstitial nephritis, macular holes, and SARS-CoV-2 pneumonia, reviewed in (*179*). While exosome vaccines hold promise, efficient antigen loading and cargo delivery, as well as large-scale production of exosomes, remains a challenge.

Biomimetic SV–based vaccines

Biomimetic SVs are a rising platform for both prophylactic and therapeutic vaccines. These engineered vesicles can be tailored to extend circulation, promote immune responses, and target specific diseases, offering advantages over traditional vaccines.

For infectious diseases, biomimetic SVs have been explored in promoting localized immunity and long-lasting protection. Jiang *et al.* (*183*) developed a CDN-based vaccine against pneumonia using *Klebsiella pneumoniae* and alveolar macrophage membranes. These vesicles successfully stimulated local mucosal immunity in a mouse pneumonia model. Similarly, Zhang *et al.* created CDNs targeting mycoplasma-related respiratory infections by fusing *Mycoplasma hyopneumoniae* and IFN-γ–primed macrophage membranes and adding PEGylation to extend lymph node residence. These vesicles prolonged immune memory through $CD8^+$ T cell activation (*184*). Koo *et al.* (*153*) took a different approach to prolonging immune responses, developing stable multilamellar PFLs functionalized with an ovalbumin antigen and monophosphoryl lipid A adjuvant. The multilamellar structure allowed for enhanced stability and induced strong antibody and cytokine responses, providing a strategy for prolonged durable antigen delivery.

Personalized cancer vaccines represent a promising frontier, as they can be tailored to incorporate autologous antigens, inducing highly specific immune responses. Liu *et al.* (*185*) developed a multi-antigenic nanovaccine by combining bacterial EVs with melanoma

cell membranes (EV-CDN vesicles). This approach promoted M1 macrophage polarization and robust T cell activation, resulting in the inhibition of melanoma growth and metastasis. Combined with anti–PD-1 therapy, the autologous vaccine further improved the survival of melanoma-bearing mice, demonstrating the potential of personalized vesicles in cancer therapy. Similarly, Tong *et al.* (*186*) combined *Akkermansia muciniphila* outer MVs, tumor EVs, and PD-L1 trap plasmid-loaded liposomes to create a cancer vaccine. This hybrid system activated dendritic cells and cytotoxic T cells in combination with PD-L1 blockade, effectively inhibiting tumor growth in mice. Wang *et al.* (*169*) developed a personalized vaccine combining ginseng-derived EVs with tumor-derived membranes (EV-CDN vesicles) exposing autologous tumor antigens. These vesicles promoted dendritic cell uptake, activated cytotoxic T cells, and provided long-term immune protection against metastasis.

In conclusion, these studies demonstrate the broad applicability of biomimetic SV–based vaccines across bacterial infections and cancer. As research progresses, the focus on scalability, efficiency, personalization, and biosafety will be essential to transition these promising technologies from bench to bedside.

Basic research

Delineating the heterogeneity and diverse roles of EVs remains an active area of research in basic science. EVs act as a "double-edged" sword, with "beneficial" EVs contributing to physiological homeostasis, and "detrimental" EVs are involved in pathogenesis. Modulating the secretion of beneficial EVs or inhibiting detrimental EVs offers potential avenues for therapeutic intervention. For instance, in the brain, EVs have been demonstrated to transport Aβ to microglia via the endocytic pathway for degradation in microglial lysosomes (*187*). Continuous intracerebral administration of neuroblastoma-derived exosomes has been shown to reduce Aβ deposition in murine models, with glycosphingolipids in these exosomes playing an essential role in Aβ binding (*187*). Conversely, in cases where EVs promote disease progression, such as cancer-derived EVs, inhibitors like Pantethine, GW4869, and Calpeptin can target EV biogenesis or release (*188*). Cancer-derived EVs can express immune checkpoint proteins such as PD-L1, which can impede the efficacy of immune checkpoint blockade therapy (*188*). Combining EV inhibitors with anti–PD-L1 therapy has been shown to increase antitumor immunity (*189*). These examples demonstrate the diverse intervention strategies that harness the therapeutic potential of EVs (*9*).

Biomimetic SVs, in addition to their therapeutic delivery potential, can serve as valuable research tools for biological discovery by modeling EVs. Numerous groups have attempted to develop EV-mimetics or assess how closely biomimetic SVs resemble natural EVs (*114*, *115*, *119*, *122*, *129*, *131*, *137*, *150*, *190*–*192*). CDNs are frequently used as EV-mimetic models. For example, Wu *et al.* (*115*) produced CDNs derived from endothelial cells, which exhibit similar Dox loading and tumor-suppressive capacities to exosomes but with a 500-fold higher yield. Nasiri Kenari *et al.* (*137*) reported that EV-mimetic CDNs display a protein profile similar to the parental cell proteome, distinct from the exosomal proteome that reflects an endosomal origin. In addition, natural killer cell–derived CDNs were shown to exert stronger anticancer effects than their exosomal counterparts (*190*). These studies indicate that while CDN-based EV-mimetics have valuable applications, further research is needed to fully elucidate their biocomplexity and specific functions. In contrast, EV-mimetics generated from bottom-up approaches allow for precise control over lipid formulation, proteins, and other biomolecular cargo, enabling the study of individual targets of interest (*150*, *192*). This model avoids the need for genetic engineering of the EV-producing cells and the challenge of EV heterogeneity. Large-scale synthesis of PFLs presents a critical technical challenge due to the high cost associated with the requirement for large amounts of pure proteins. To address this, cell-free protein synthesis has emerged as a strategy for scaling up PFL production (*191*, *193*). Future advances in engineering techniques could enable these models to drive EV research forward, shedding light on solving the molecular mechanisms of immune regulation, tissue and cell tropism, endosomal escape, and facilitating efficient drug screening.

TOXICITY AND BIOSAFETY

Toxicity and biosafety is a critical factor for clinical applications. EV-based therapies generally demonstrate low toxicity profiles. Mendt *et al.* (*96*) evaluated the toxicity and immune response associated with long-term administration of human BJ fibroblast exosomes (every 48 hours for 4 months). No significant hematologic abnormalities, and minimal to mild inflammation were found. For a shorter-term administration (every 48 hours for 3 weeks) of BJ exosomes, MSC exosomes, and MSC iExosomes (loaded with siRNA), no significant immune response was observed, supporting a safe profile of exosome-based therapy. The role of EV dosage and the condition of source cells is evident. For instance, lower doses of exosomes have shown neuroprotective effects in treating neurodegenerative diseases, while higher doses (from later passages) may be harmful to neurons (*97*). In addition, various factors are critical for evaluating the efficacy and safety of EV-based therapies, such as the source of EVs, therapeutic cargo, and administration routes. Currently, EV-based therapies have been conducted in several clinical trials for their safety and efficacy in targeting diseases such as cancers, SARS-CoV-2, Alzheimer's disease, and type 1 diabetes mellitus, reviewed in (*98*).

While synthetic NPs offer greater design flexibility compared to EVs, their toxicity and safety profiles require increased attention. Synthetic materials like metals and polymers may exhibit immunogenicity and limited biodegradability. Studies have reported that synthetic NPs can cause cellular damage, including mitochondria, dysfunction, reactive oxygen species generation, and DNA damage, reviewed in (*194*). The toxicity of these NPs varies based on their design parameters, such as size, charge, shape, composition, and administration routes, which affect tissue-specific penetration, cell type–specific uptake, subcellular localization, retention, and clearance.

Among various types of NPs, liposomes are generally regarded as pharmacologically inactive with minimal toxicity, although several factors must be considered for safety considerations. Notably, cationic liposomes used for gene delivery have been found to be particularly toxic to macrophages and can reduce the secretion of important immunomodulators like nitric oxide and TNF-α (*195*). While PEGylation has been widely used to improve circulation, repeated administration of PEGylated liposomes can induce anti-PEG antibody production, accelerating blood clearance (*196*). Several strategies can be used to improve the safety and stability of liposomal drug delivery systems. For example, the incorporation of cholesterol has been shown to decrease macrophage uptake and immunoglobulin response in mice and decrease liver accumulation (*197*). Enhancing the targeting specificity can be another strategy to reduce the nonspecific interactions and toxicity of the cationic liposomes. For

instance, Li *et al.* (*198*) conjugated the cationic liposomes with DEC205 antibody as a specific ligand to increase the uptake specifically by dendritic cells via receptor-mediated endocytosis, while reducing cytotoxicity.

Drawn to the homotypic targeting effects of cancer cell membranes, researchers have explored the use of cancer cell–derived vesicles for tumor-targeted therapeutic delivery (*14, 104–106, 114, 163*). However, their tumor origin raises concerns about potential tumorigenic effects associated with the complex biomolecules they contain. This limitation may be addressed by using biomimetic SVs using simplified composition. Vazquez-Rios *et al.* (*151*) demonstrated that EV-mimetic liposomes functionalized with integrin α6β4 can transport therapeutic RNAs to cancer cells with similar efficiency to tumor-derived exosomes. Molinaro *et al.* (*144*) showed that leukosomes did not trigger significant antibody production against leukosome membrane antigens. Martinez *et al.* (*143*) further corroborated that leukosomes did not cause significant changes in serum cytokine levels (IL-6, TNF-α, and IL-1β), histological assessments, and organ function compared to mice treated with PBS. These biomimetic SVs potentially offer safer alternatives compared to tumor-derived exosomes and conventional LNPs containing cationic lipids.

In summary, further efforts are warranted for quality control and evaluating the biosafety of chronic exposure to biomimetic SVs and their biocompatibility. Establishing standardized protocols for characterizations, and pharmacokinetic/dynamic evaluations will also be essential for clinical translation, facilitating cross-study comparisons and ensuring safety and efficacy consistency. With careful selection of their compositions and technical optimization of their engineering processes, biomimetic SVs hold the promise of surpassing EVs or synthetic NP-based systems with their superior scalability, targeting specificity, and biosafety.

DISCUSSION AND OUTLOOK

In recent decades, research into EVs has remarkably advanced our understanding of disease mechanisms and revolutionized therapeutic delivery. Facilitated by their nanoscale dimensions and lipid bilayer structure, EVs are capable of transporting diverse cargo, targeting specific sites, and navigating tissue barriers. Compared to fully synthetic NP-based delivery systems, EVs offer high biocompatibility, which has propelled their exploration in diagnostics, targeted therapies, and regenerative medicine. However, unresolved challenges, including high heterogeneity, low yield, and scalability issues, have limited their clinical translation.

To overcome these limitations, researchers have increasingly turned to biomimetic SVs, which incorporate EV-like properties into scalable and customizable fully synthetic or semisynthetic systems. This review categorizes biomimetic SVs into three main types based on their synthesis methods: "top-down" methods referring to cell breakdown into smaller vesicles; "bottom-up" methods by de novo synthesis; and hybrid approaches. Depending on the synthesis methods and their biological incorporation, biomimetic SVs exhibit varying levels of biocomplexity, biocompatibility, controllability, and scalability. Their versatility has rapidly expanded their applications across diagnostics, targeted therapeutic delivery, regenerative medicine, vaccines, and basic research. Impressively, among the 186 studies identified through a comprehensive literature search spanning 1980 to 2024 on lipid bilayer-based and protein-functionalized biomimetic SVs, more than half (95 papers) were published within the past 3 years, which indicates the rapid advancement of this field, reflecting its novelty and the growing recognition of its transformative potential in biomedicine.

Biomimetic SVs offer innovative therapeutic opportunities that are hard to achieve by EVs or SVs alone. For instance, hybrid vesicles, such as EV-liposome constructs, combine the inherent genetic engineering potential of EVs with the remarkable drug loading efficiency of liposomes, enhancing therapeutic efficacy while mitigating individual limitations. The synthesis procedure of biomimetic SVs enables the customization of membrane and cargo profiles. For example, integrating immune cell and cancer cell membranes into CDNs results in multifunctional vesicles with enhanced immune navigation and tumor-targeting specificity (*149*). Similarly, the integration of multiple payloads, such as RNA, small molecules, and imaging agents, into a single vesicle could facilitate combination therapies and theranostics. This level of customization exemplifies the flexibility and strategic merging of strengths from multiple specialized cell or EV-derived components to meet specific biomedical needs.

Despite these advancements, major challenges remain (Fig. 6). EV biology is characterized by heterogeneity in subpopulations, complex biogenesis pathways, and variable targeting mechanisms, which are not yet fully understood. Molecular mechanisms underlying EV-cell interactions, such as receptor-ligand binding, endosomal escape, and cytosolic cargo release, require further elucidation to improve targeting specificity. On the other hand, the field of biomedical SVs faces technical limitations, such as a lack of control over membrane protein orientation during engineering, limited encapsulation efficiency or stability of therapeutic agents, and lack of standardization in synthesis.

The identification of current bottleneck challenges illuminates the future directions. First, the development of standardized protocols for the isolation, characterization, and quality control of EVs and SVs is essential. Establishing guidelines similar to the MISEV on the report of minimal experimental information will enhance reproducibility and comparability across studies (*1*, *27*). For example, while purification of vesicles by methods such as ultracentrifugation and dialysis is essential for accurate analysis of encapsulation efficiency of payloads and their downstream effects, a noticeable number of studies did not report purification postsynthesis. The techniques for the characterization of NPs are continuously evolving. For example, size photometry, an innovative label-free method that emerged recently, offers improved sensitivity and accuracy in the detection and sizing of EVs compared to conventional methods like NanoSight and flow cytometry (*199*). Second, improving scalability through innovations like 3D cell culture in bioreactors enables large-scale manufacturing of EVs, CDNs, and their hybrid vesicles. The development of the cell-free protein synthesis method could potentially reduce the cost of PFL synthesis. Third, delineating the heterogeneity and source cell–dependent functions of EVs and revealing the molecules of actions could guide the design of biomimetic SVs with targeted functions, minimizing unintended effects. Fourth, exploring the endosomal escape mechanisms of viruses and natural EVs sheds light on identifying molecular targets for engineering EVs and biomimetic SVs for efficient cytosolic delivery of therapeutic cargo. Fifth, comprehensive pharmacokinetic and pharmacodynamic studies are urgently needed. Current data on EV biodistribution, clearance, and long-term safety are limited and often inconsistent due to methodological variations. Techniques such as multimodal imaging and advanced labeling methods

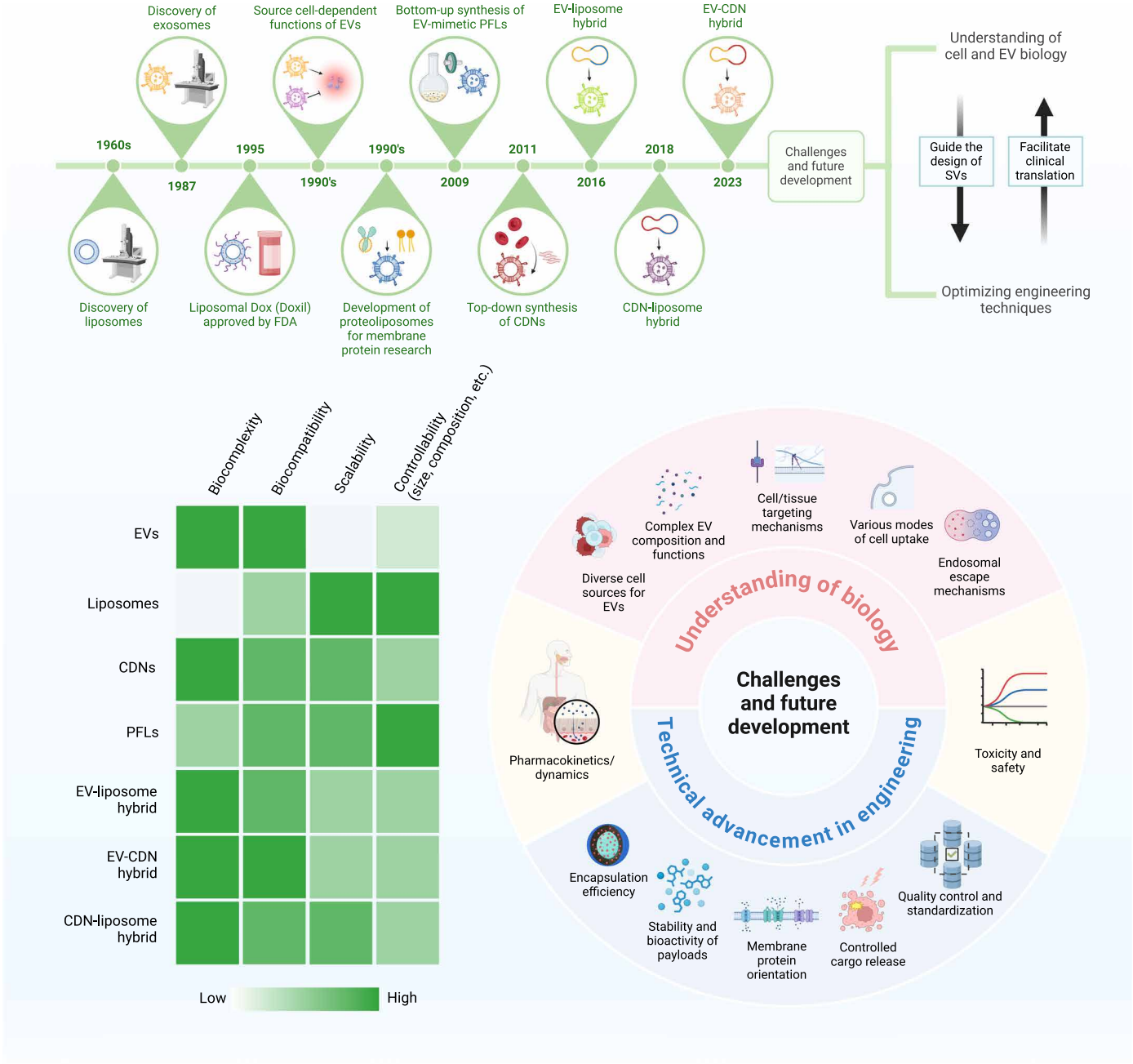

Fig. 6. Emergence of extracellular vesicles (EVs), liposomes, proteoliposomes, and biomimetic SVs. Comparisons of features of different vesicles. Challenges with understanding of cell and EV biology and engineering techniques, and future development directions. Created with www.biorender.com.

could provide real-time insights into vesicle behavior in vivo. In addition, understanding the chronic safety profiles of biomimetic SVs, particularly vesicles with complex biological and synthetic components, will be critical for clinical applications.

A shared critical technical challenge in the engineering of different biomimetic SVs is ensuring the correct orientation of surface proteins to achieve optimal functions. To verify protein orientation, techniques such as immunofluorescence, immunogold labeling, and chemical assays detecting extracellular and intracellular domains of membrane proteins are often used (*140*, *144*, *200*). To achieve targeted orientation, Staufer *et al.* (*150*) used a lipid [18:1 DGS-NTA(Ni)] to bind recombinant histidine-tagged membrane proteins to the surface of liposomes. Another method, used by Gao *et al.* (*201*), involved a layer-by-layer coating of leukocyte membranes onto polymeric capsules. The multilayer membrane and the capsulated-cushioned structure enhanced structural stability and prolonged the circulation time in mice. This multilayer membrane is likely to incorporate more right-side-out proteins compared to a single-bilayer membrane. In some applications, a flipped, "inside-out" orientation is intentionally used as a research tool. For example, Zhang *et al.*

(*107*) achieved an inside-out membrane protein orientation on CDNs designed to screen for P-glycoprotein inhibitors, where the cytoplasmic side must be exposed for effective inhibitor binding. Continued technical development is essential to enhance the quality and functionality of biomimetic SVs.

While our focus has been on the bioinspired development of SVs for biomedical applications, it is important to recognize the potential of other approaches for SV design. These include liposome-polymer hybrids, nonlipid bilayer structures such as micelles and LNPs, as well as liposomes engineered with biofunctional surfaces without relying on biological proteins, but through conjugation with synthetic peptides, aptamers, RGD motifs, and click chemistry. Although these promising methods are beyond the scope of this review, they are extensively covered in other comprehensive studies (*16*, *26*). Furthermore, "proteoliposomes," which involve the bottom-up reconstitution of membrane proteins into liposomes, have been applied as a key tool for studying protein-lipid interactions, contributing to structural biology research (*200*).

In conclusion, biomimetic SVs represent a strategic bridge between natural and synthetic systems. The interplay between advancing EV biology and improving SV engineering will be crucial for overcoming existing challenges. By unraveling the molecular mechanisms of EV function and refining SV production techniques, researchers can create next-generation delivery platforms tailored for specific therapeutic applications, with the potential to address unmet clinical needs in cancer, neurodegenerative diseases, regenerative medicine, and beyond.

Supplementary Materials

This PDF file includes:

Tables S1 to S3

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