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REVIEW ARTICLE

Engineering of Cell Derived-Nanovesicle as an Alternative to Exosome Therapy

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Abstract

BACKGROUND Exosomes, nano-sized vesicles ranging between 30 and 150 nm secreted by human cells, play a pivotal role in long-range intercellular communication and have attracted significant attention in the field of regenerative medicine. Nevertheless, their limited productivity and cost-effectiveness pose challenges for clinical applications. These issues have recently been addressed by cell-derived nanovesicles (CDNs), which are physically synthesized exosome-mimetic nanovesicles from parent cells, as a promising alternative to exosomes. CDNs exhibit structural, physical, and biological properties similar to exosomes, containing intracellular protein and genetic components encapsulated by the cell plasma membrane. These characteristics allow CDNs to be used as regenerative medicine and therapeutics on their own, or as a drug delivery system.

METHODS The paper reviews diverse methods for CDN synthesis, current analysis techniques, and presents engineering strategies to improve lesion targeting efficiency and/or therapeutic efficacy.

RESULTS CDNs, with their properties similar to those of exosomes, offer a cost-effective and highly productive alternative due to their non-living biomaterial nature, nano-size, and readiness for use, allowing them to overcome several limitations of conventional cell therapy methods.

CONCLUSION Ongoing research and enhancement of CDNs engineering, along with comprehensive safety assessments and stability analysis, exhibit vast potential to advance regenerative medicine by enabling the development of efficient therapeutic interventions.

Keywords Cell-derived nanovesicles · Drug delivery · Exosomes · Regenerative medicine

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1 Introduction

Extracellular vesicles (EVs) encompass a range of plasma membrane-derived vesicles released by living cells, including both mammalian cells and microorganisms. These vesicles consist of membranous and intracellular components [1]. These EVs can be categorized by size, with the term 'exosomes' specifically referring to those originating from mammalian cells, typically falling within the size range of 30–150 nm in diameter. Given their role in facilitating long-distance intercellular communication, exosomes have garnered substantial attention in the field of biomedical engineering. They are viewed as promising biomarkers for diagnosis or potential alternatives to cell therapy [2].

While cell therapy, particularly involving stem cells, faces challenges such as (i) unforeseen cell fate post-transplantation, leading to reduced therapeutic effectiveness or potential tumorigenesis due to the living nature of the biomaterial [3], (ii) pulmonary capillary entrapment when administered systemically [4], and (iii) time and efficiency issues during preparation and storage, exosomes offer a solution. Exosomes, despite being non-living biomaterials, carry the biological information of cells at the time of release. They are small enough to evade microvascular entrapment and can be cryopreserved in a ready-to-use form [5, 6].

Exosomes show superior potential in the field of regenerative medicine and drug delivery owing to their structure, which inherits the plasma membrane and intracellular components, including proteins and genetic molecules, from the parent cell [7–10]. Therefore, the selection of the parent cell determines the function of the released exosome. For example, exosomes released by mesenchymal stem cells (MSCs), one of the most frequently used cell types in cell therapy, inherit properties such as angiogenic, anti-apoptotic, anti-inflammatory, immune-privileged, and long-lasting circulation characteristics. Consequently, they are applied in numerous studies as a substitute for MSC therapy [4, 11–14]. The specific therapeutically active molecules in MSC-derived exosomes have not been fully elucidated due to batch-to-batch heterogeneity, though recent studies have focused on their transmembrane proteins, lipids, intracellular proteins (e.g., transcription factors, enzymes, growth factors, etc.), and nucleic acids (DNA, mRNA, and miRNA)[15–21]. Computational analyses are now being used to correlate therapeutic efficacy with the intravesicular molecules [22, 23].

Despite its numerous advantages and increasing global interest, exosome therapy faces two major limitations: low productivity and cost-effectiveness, which present significant obstacles to clinical translation. For example, only 1.7–3.8 μg of EVs are harvested daily from one million MSCs cultured *in vitro* [24], while recent clinical trials using MSC-derived exosomes require more substantial quantities (typically between 40 and 50 mg) per injection. This discrepancy leads to significant time and cost inefficiencies in exosome preparation. Consequently, various techniques and devices for increasing exosome secretion and improving harvesting efficiency are under development, though this research is still in its early stages.

Cell-derived nanovesicles (CDNs) have emerged as an alternative to exosomes. These nanovesicles are not naturally secreted by cells or harvested from conditioned media but instead are artificially synthesized using physical methods with living cells. This new approach to producing exosome mimetics has ushered in a new era of exosome therapy by increasing productivity, with yields 100 to 250 times higher than naturally secreted exosomes [25, 26]. The therapeutic efficacy of CDNs in comparison to naturally secreted exosomes is still under investigation, but their biochemical, structural, and functional properties have been consistently reported to be similar to those of exosomes [25, 26]. Therapeutic CDNs also pose a superior advantage over other artificial lipid nanoparticles such as liposomal drug delivery, since CDNs contain no chemical component, but contain a cocktail of therapeutic biomolecules that can trigger multiple therapeutic mechanisms. In the forthcoming sections, we will introduce a CDN engineering technique utilizing liposomes that leverages their higher controllability and homogeneity to create hybrids, thereby mitigating the limitations inherent in CDNs.

This review focuses on the progress made in the development of CDNs as a potential alternative to naturally secreted exosomes. Various methods for CDN synthesis and current techniques for their analysis are comprehensively presented. Additionally, recent advances in various CDN engineering approaches aimed at enhancing lesion-targeting efficiency and/or therapeutic efficacy are reviewed, depending on their therapeutic applications, such as cancer therapy, tissue regeneration, and diagnosis. Finally, we describe the issues to be addressed and the prospective directions for future research, particularly concerning the differences with naturally secreted exosomes.

2 Exosomes and cell-derived nanovesicles

EVs are categorized into three forms: exosomes, microvesicles, and apoptotic bodies, based on their morphological characteristics and content [27]. During the formation of early endosomes, numerous vesicle components come together, allowing multivesicular bodies to encapsulate various cytoplasmic substances. They are

produced by internal budding of the restricted multivesicular body membrane from late endosomes [28]. Exosomes, in particular, have been extensively studied for drug delivery, disease research, and clinical applications [29–33]. They exhibit a nearly-spherical structure with a diameter ranging from 30 to 150 nm and possess a lipid bilayer similar to that of the cell membrane [7–10]. Exosomes are generated by a wide range of normal cells, including human umbilical vein endothelial cells, mesenchymal stem cells (MSCs), T cells, B cells, macrophages, dendritic cells (DCs), and natural killer (NK) cells [34].

Various methods have been developed for exosome isolation, each grounded in distinct principles. These methods include ultracentrifugation, ultrafiltration, immune affinity capture, microfluidic techniques, and commercially available kits [35, 36]. Exosomes, which are typically spherically-shaped, can be categorized into nine types based on their shape, each serving specific functions. These functions are attributed to surface proteins specific to the endosomal pathway, such as CD63, CD81, heat shock proteins (Hsc70), lysosomal proteins (Lamp2b), tumor-sensitive gene 101 (Tsg101), and fusion proteins, which are used to identify exosomes (CD9, flotillin, and annexin) [37–39]. Exosomes contain complexes of proteins both inside and on their surface, including receptors, transcription factors, enzymes, extracellular matrix components, lipids, and nucleic acids (DNA, mRNA, and miRNA). These components contribute to diverse cellular functions, including intercellular communication, antigen presentation, and the transfer of mRNA and miRNA proteins [15–18].

On the other hand, CDNs can be manufactured by physically reassembling cells by serial extrusion using porous membrane filters, a microfluidic chip, freeze–thaw, an ultra-sonic system, and ultracentrifugation [25]. Because they have a morphology and function similar to those of exosomes, they can perform functions such as creating the signal pathway and carrying genetic factors. CDNs mimic several exosome characteristics, resulting in lower clearance rates and effective cellular accumulation due to the intrinsic targeting capacity of their surface proteins. CDNs offer a promising alternative to synthetic drug delivery systems such as liposomes, carbon nanotubes, gold nanoparticles, micelles, polymer nanoparticles, and dendrimers used in the past decades. Several studies indicate that CDNs can transfer RNAs, similar to exosomes [40–42]. CDNs are generated by subjecting cells to a physical process that maintains the original protein complex of the parent cells during the generation of nanoscale vesicles. They can produce a greater quantity of nanovesicles in significantly less time than it takes to conventionally produce exosomes. Additionally, they offer specific surface functionalization and therapeutic cargo-loading possibilities comparable to other drug delivery systems currently under investigation. While CDNs and exosomes are known to share similarities in various aspects, we anticipate differences in production procedures, loading strategies, surface functionalization, and therapeutic applications. Due to the rearrangement of surface proteins and changes in lipid composition, several strategies outlined in the literature for exosomes, such as genetically modified cells, biochemical labeling, membrane modification, and hydrophobic insertion, may require alternative approaches for CDNs.

3 Synthesis of cell-derived nanovesicles

3.1 Isolation of naturally secreted exosomes

The specific characteristics of EVs, such as size, density, and surface components, are used as the basis for isolation techniques [35]. Conventionally, exosomes are produced using techniques such as ultrafiltration, chromatography, and affinity capture, and ultracentrifugation and precipitation using a polymer-based commercial kit are the most prevalent methods for purifying them [34, 43, 44]. Among them, ultracentrifugation-based isolation is one of the most commonly used methods. The differential ultracentrifugation method generally consists of a series of centrifugation cycles with different centrifugal forces and durations to isolate exosomes based on their size and density [45–47]. Size-based isolation methods are similar to traditional size- or molecular weight-based ultrafiltration techniques, which use membrane filters with specific pore sizes to isolate exosomes. This approach is one of the most commonly used size-based methods for separating exosomes from other components based on their size differences. Additionally, it is more efficient and simpler than other approaches, such as ultracentrifugation [48, 49]. Other exosome isolation methods are based on varying their solubility and dispensability using polymer-based precipitation. The most common method for precipitating exosomes from biological fluids uses polyethylene glycol (PEG) [49]. Exosomes can also be harvested by an affinity-based capture technique using their lipids, proteins, and polysaccharides, as these molecules may interact with a wide range of molecules, including antibodies, lectins, and lipid-binding proteins. A representative approach is immune affinity using a specific antibody-antigen interaction. Additionally, several other methods have been proposed, such as heparin-modified sorbents using agarose sorbent coated with heparin to capture EVs, the heat shock protein-peptide venceremin interaction, the annexin-phosphatidylserine interaction, and a method using lectin [50].

However, conventional isolation techniques have several drawbacks, as they have a limited yield and purity, are time-consuming and expensive, and are difficult to standardize [48]. To provide clinical settings with exosomes of high purity, the development of more effective strategies is necessary. The rapid advancement in microfabrication technology, microfluidic systems are a promising way to isolate exosomes based on the physical and biochemical features of exosomes [51]. In addition to traditional separation methods based on factors like size, density, and immunoaffinity, novel sorting techniques including acoustic [52, 53], electrophoretic [54–56], immunoaffinity [57–59], and magnetic [60, 61] systems can be employed. The integration of such innovative mechanisms into microfluidic devices holds the promise of achieving notable reductions in sample volume, reagent consumption, and isolation time.

Another novel technique is DNA-based hydrogel technology for exosome separation from cell culture medium and serum [62]. They designed a DNA-based hydrogel for the specific and nondestructive isolation of exosomes from complex biological media. The hydrogel was synthesized using ultralong single-stranded DNA (ssDNA) using double-rolling circle amplification (RCA). AptCD63 aptamer was incorporated to specifically recognize tetraspanin CD63 on exosomes within the RCA template, resulting in the production of positive exosome, allowing for their specific capture and efficient enrichment within the developed DNA hydrogel.

3.2 Synthesis of cell-derived nanovesicles

Whereas exosomes are typically isolated and purified from the conditioned media of densely populated cells using biochemical methods, CDNs can be directly synthesized from cells through physical disruption. The crux of the CDN synthesis process lies in developing a high-throughput method to disintegrate cells into small membrane debris, releasing their intracellular components. This enables amphiphilic molecules to self-assemble into nano-sized vesicles, reducing their thermodynamic energy [26, 42]. These vesicles comprise the intracellular components of the parent cell (such as proteins and genetic material) surrounded by membrane components (including phospholipids and proteins), forming spherical structures. This section explores the presently employed cell disruption techniques for CDN synthesis.

3.2.1 Cell extrusion through microporous membranes

Cell extrusion is the predominant technique in the field of CDN applications. The processes involved in CDN synthesis closely resemble the method used for liposome extrusion, resulting in uniform particle size. The extrusion device, often a liposome extruder, typically consists of a porous membrane firmly positioned between two Hamilton syringes. By applying high pressure, the cells are extruded through the membrane, undergoing disruption into smaller sizes due to shear stress (Fig. 1A). After multiple rounds of extrusion, the polycarbonate membranes with varying pore sizes are sequentially replaced with smaller ones to achieve higher size uniformity. The pore size of polycarbonate membranes may vary among research groups but is usually approximately 10 μm, eventually decreasing to a minimum of 0.1 μm after two replacements. For greater diameter homogeneity of CDNs, the extruded nanovesicles are filtered through syringe filters with a pore size of 0.45 μm and then centrifuged at 15,000 g or higher speeds to concentrate them. The quantity of CDNs synthesized through the cell extrusion method is 250 times greater than that of naturally secreted exosomes isolated from conditioned media cultured with a similar number of cells, and the intracellular contents of CDNs are approximately double that of exosomes [26].

Despite its high productivity, the entire process is manual, leading to variations in CDN diameter depending on the operator compared to the methods to be described later. Thus, the control of cell suspension viscosity (i.e., cell density) before extrusion and the extrusion pressure are vital to minimize batch-to-batch heterogeneity. To address these challenges, several devices have been developed to facilitate large-scale extrusion. These devices are designed to fit within a centrifuge device, and either a syringe [26] or a spin cup [63] serves as support. High centrifugal force is applied to pass the cells through microporous membranes for extrusion, a more tunable and controllable approach compared to manual pressure. Among various methods, the extrusion technique offers a significant advantage in preserving intracellular components. Notably, the extrusion method facilitates the incorporation of metallic nanoparticles, as several studies have consistently reported the presence of iron oxide nanoparticles, taken up by human cells, in CDNs after extrusion [64–66]. Metallic nanoparticles can serve as tools for imaging [67], photodynamic therapy [68], targeted therapy through magnetic forces [64], or even for enhancing the expression of therapeutic biomolecules in cells [69], demonstrating a synergistic effect alongside the therapeutic biomolecules inherited from the parent cells.

3.2.2 Microfluidic devices for cell disruption

The development of microfluidic devices for CDN synthesis has garnered attention due to their ability to create a controlled microenvironment that facilitates high-throughput and homogeneous nanovesicle production. A typical

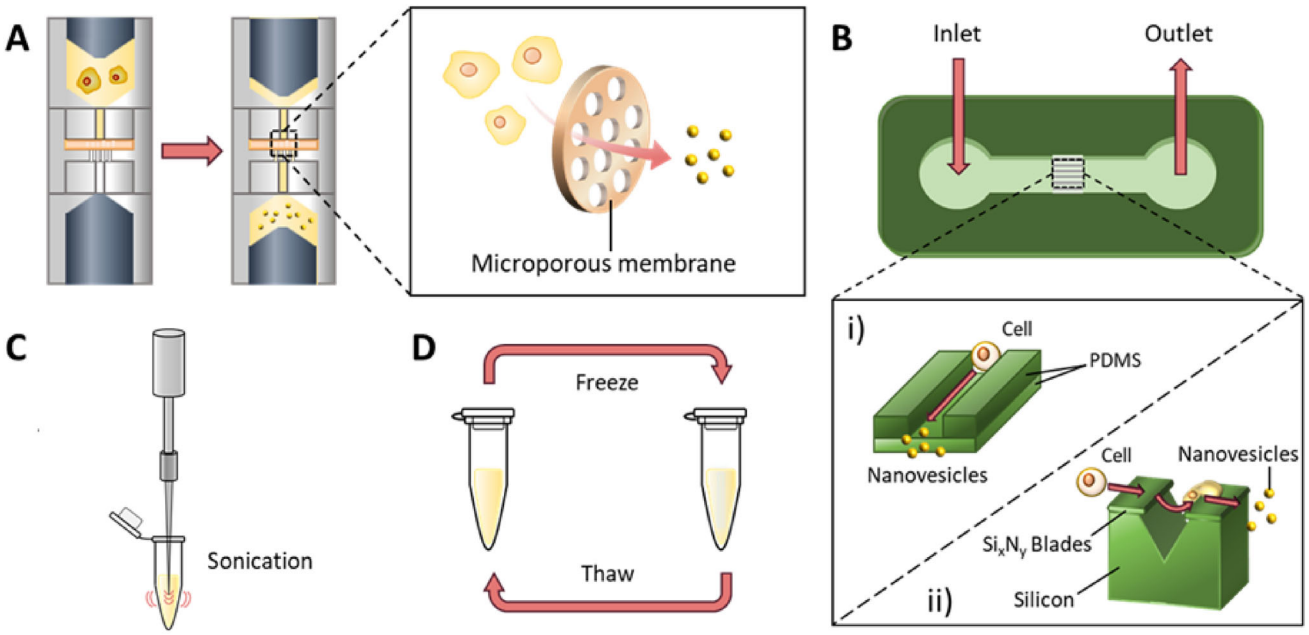

Fig. 1 Schematic of the synthesis processes of CDNs. **A** Cell extrusion method using microporous membranes equipped between Hamilton syringes, **B** microfluidic chip system with microstructures to induce physical damage to the parent cells, **C** ultra-sonication, and **D** freeze-thawing method

microfluidic device for CDN synthesis consists of multiple microchannels with inlet and outlet features, each incorporating unique structural elements (Fig. 1B). Numerous microfluidic devices have been designed for the synthesis of lipid nanoparticles, with some focusing on liposome synthesis, which can be considered a form of bottom-up exosome-mimetic synthesis [70]. However, liposomes are composed of phospholipids and defined biomolecules as cargo, making their therapeutic mechanism less natural or efficient than that of exosomes.

Conversely, a few microfluidic devices have been developed specifically for cell disruption and the subsequent reassembly of cell membranes to spontaneously form CDNs, operating on a mechanism similar to the cell extrusion method (Fig. 1B). In one such microfluidic device, high shear stress is applied to cells passing through microchannels [42]. They reported that microchannels designed with a height of 10 μm, length of 200 μm, and width of 3 μm optimized CDN synthesis from embryonic stem cells, resulting in the highest protein and RNA concentrations compared to other microchannel sizes. This configuration produced CDNs with a diameter of 100 nm. Another type of microfluidic device involves 500 nm-thick silicon nitride (Si_xN_y) cantilever blades to slice the membranes of living cells [71]. The size of reassembled CDNs depends on the width of microchannels, with channels ranging from 10 to 100 μm in width yielding CDNs with a diameter of 100 nm. This platform can also be employed for synthesizing CDN-encapsulating microbeads.

While both of these introduced devices are capable of achieving uniform CDN synthesis, the differences from the extrusion method, highlighting the advantages of using microfluidic devices, have not been extensively described. Considering the reported heterogeneity of CDNs, these microfluidic devices present a promising solution for addressing this concern in future research.

3.2.3 Synthesis of CDN without intracellular components

As aforementioned, cell membrane fragments rapidly reassemble, spontaneously forming spherical vesicles, the size of which typically ranges between 50 and 200 nm in diameter. Sonication and nitrogen cavitation are conventional methods for cell disruption. They can be used to fragmentize the cell membrane and are therefore applicable to CDN synthesis. However, these methods can only produce CDNs composed of cell membranes with minimal intracellular components. Sonication is a widely used method for drug loading in liposomes because ultrasound disrupts the membrane but does not damage its protein component. A recent study used a sonifier twice sequentially to fragmentize monocytes and load dexamethasone into the reassembled vesicles [72] (Fig. 1C). Therefore, the sonication-based method allows the users to utilize the cell membrane, which can incorporate surface molecules with a targeting moiety or camouflage effect, reject unwanted luminal cargos that might induce potential side effects after injection, and load defined molecules, such as

dexamethasone, with higher efficiency compared to the extrusion method [72]. Moreover, human mesenchymal stem cells, which show skin regenerative effects, were sonified to produce CDNs [73]. Nitrogen cavitation is another method to isolate the cell membrane; it involves placing the cell suspension in a nitrogen-cavitation chamber to disrupt the cells under high pressure (400–500 psi for 20 min) of cavitation. The synthesized CDNs have properties similar to those produced by sonication methods, as their diameters are typically below 200 nm and they do not contain DNA contents but maintain most of the protein contents [73], especially integrin [74]. This implies that the intracellular components were not included while the fragmentized membrane spontaneously formed an empty vesicle. However, drug loading through nitrogen cavitation was not confirmed in this study because they performed a pH gradient drug loading after CDN synthesis [74].

3.2.4 Synthesis of liposome-exosome hybrid nanovesicles

Liposomes can serve as a synthetic alternative to exosomes due to their similar biochemical properties, which comprise a hydrophilic interior encapsulated by a phospholipid bilayer. Liposomes offer significant advantages because of their cell membrane-like structure, which enable their delivery into target cells through a fusion process akin to that of exosomes. Moreover, liposomes can be engineered to provide specific organ-targeting capabilities or drug loading. However, liposomes have been repeatedly reported to have potential side effects related to the foreign body response, short circulation time, and residual chemicals resulting from synthesis or bioconjugation [75, 76].

On the other hand, exosomes, being nanovesicles from natural sources, possess tremendous potential for clinical use with extremely high biocompatibility. However, isolated exosomes tend to be heterogeneous, varying from batch to batch, and individual vesicles within a single batch exhibit distinct properties [77]. Thus, the fusion of liposomes and exosomes to create a liposome-exosome hybrid nanovesicle could complement each other's shortcomings by enhancing biocompatibility and homogeneity. The fusion of these vesicles employs methods similar to those used in CDN synthesis, as introduced earlier. Extrusion is the most commonly employed method, involving the dispersion of liposomes and exosomes in the same syringe, followed by multiple extrusions through serial microporous membranes with decreasing pore sizes [78]. Optional vortexing and sonication can be performed for proper mixing [79]. Additionally, the freeze-thawing method can be considered a means of membrane disruption and self-assembly [80] (Fig. 1D). Furthermore, simply incubating non-PEGylated liposomes and exosomes in the presence of PEG induces vesicle fusion [81]. To the best of our knowledge, no study to date has reported quantification to compare their fusion efficiencies. Consequently, it is imperative to identify the most stable method that minimizes damage to biomolecules during the fusion process, ensuring the preservation of the biological functionality of hybrid CDNs.

4 Characterization of exosome-mimetic nanovesicles

Understanding the molecular composition, functionality, and cargo transport of CDNs relies on the analysis of their properties. The characteristics of these exosome-like nanovesicles are typically investigated using a range of analytical techniques (Fig. 2). Since no single method can comprehensively assess CDNs, various approaches for characterization and quantification have been employed in CDN analysis [35]. Different techniques are employed to assess CDN size, structure, and quantity, as well as their molecular features, including surface properties, protein content, and RNA content [82–84]. The choice of method is guided by the specific requirements of the study and the available resources. Each technique has its own set of advantages and disadvantages, and different techniques can be used in combination to provide a detailed analysis of the intravesicular components in CDNs.

4.1 Physical characterization of CDNs

CDNs are small membrane-bound vesicles that play a critical role in various biological processes, including cell-to-cell communication and the intercellular transport of bioactive molecules. Understanding the physical characteristics of CDNs, such as their size, shape, and structure, is vital for determining their biological function. Several techniques, including electron microscopy (EM), nanoparticle tracking analysis (NTA), dynamic light scattering (DLS), and atomic force microscopy (AFM), can be employed to measure the size of CDNs (Fig. 3).

Transmission electron microscopy (TEM) is a well-established and conventional method for high-resolution imaging of CDNs [85, 87, 88]. It provides information about their size, shape, and ultrastructural characteristics, including membrane morphology and content distribution, with a resolution in the nanometer range. However, commonly used TEM method requires sample preparation, such as drying (including freeze drying), thin-sectioning and negative staining, which may influence the morphological structure of CDNs [89]. Linda et al. discussed three common TEM methods: drying, staining, and cryo-EM, highlighting their applications, limitations, and interpretations [90]. They offered background information on these

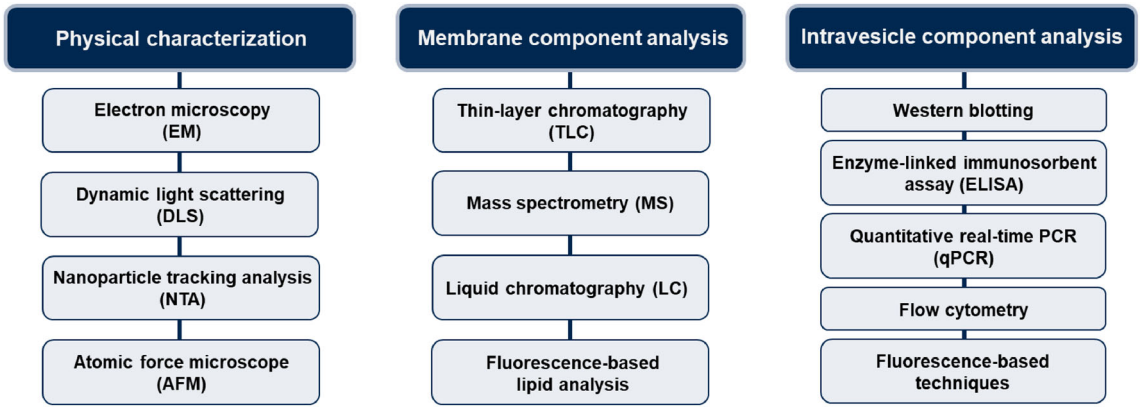

Fig. 2 Categorization of CDN characterization methodologies

three techniques, outlines what information they can and cannot provide, and helps to choose the appropriate method for soft matter imaging.

DLS is a widely used method for determining the size distribution of CDNs in solution. It relies on the measurement of scattered light proportional to the size of CDNs. It provides information on the hydrodynamic diameter and size distribution of CDNs and is a quick and straightforward approach for characterizing CDNs in solution. Nevertheless, DLS might not distinguish CDNs from other similarly sized particles in a sample and does not provide information about CDN shape. NTA utilizes video microscopy to track individual CDN motions and determine their size distribution. This method offers high-resolution data on CDN size and distribution and can differentiate CDNs from similarly sized particles. However, NTA requires well-dispersed samples and may not provide information on the shape of CDNs. Rebecco et al. quickly determined the size and characteristics of cellular vesicles using NTA [91], highlighting the higher sensitivity of NTA compared to conventional flow cytometry. They compared the NTA, DLS, electron microscopy, and flow cytometry to analyze vesicles. They showed the capacity of NTA to measure cellular vesicles, as small as approximately 50 nm, using human placental vesicles and plasma, showing its superior sensitivity compared to conventional

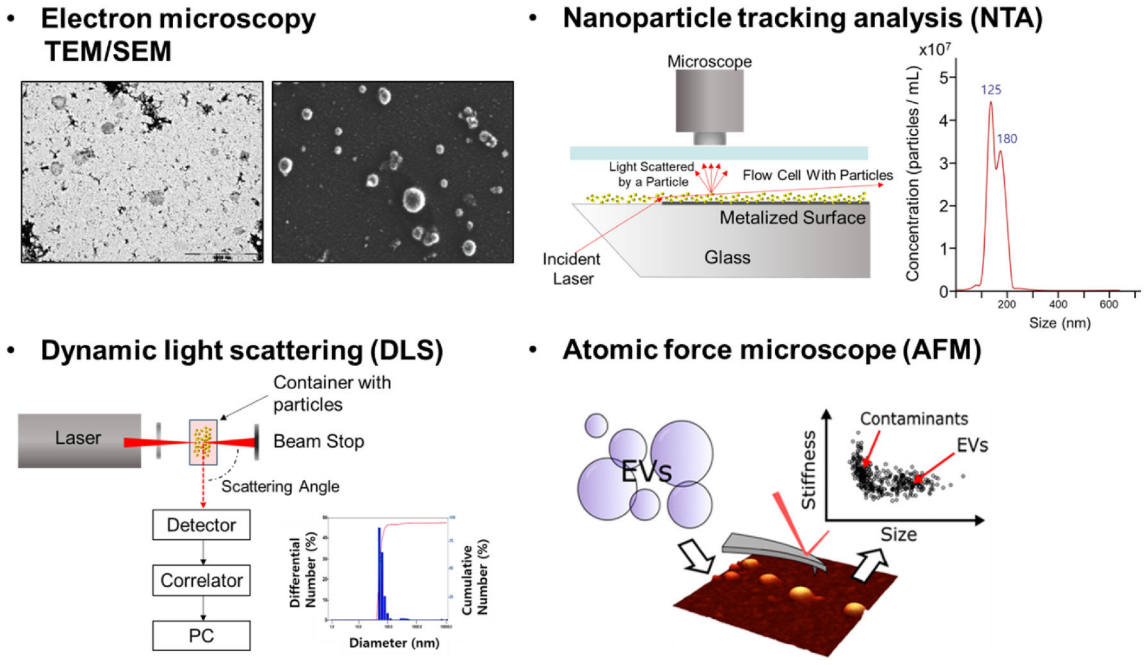

Fig. 3 Physical characterization of CDNs. Various tools used for CDNs analysis, such as electron microscope (TEM/SEM) (Reprinted with permission from Enderle et al. [85], Copyright © 2015 PLOS), NTA, DLS, and AFM (Reprinted with permission from Ridolfi et al. [86], Copyright © 2020 American Chemical Society)

flow cytometry, which has a lower detection limit of around 300 nm.

AFM is a technique that employs a probe to scan a sample's surface and generate high-resolution images of CDNs with nanometer-scale resolution [86]. AFM can provide information not only on size and shape but also on mechanical properties like membrane stiffness. Nevertheless, it necessitates complex sample preparation and does not provide size distribution information. Sera et al. studied multiple extracellular vesicles including membrane protein composition, size, and mechanical properties using a combination of fluorescence and AFM [92]They explored the correlations and heterogeneity among these parameters across different cellular sources, including human embryonic kidney 293, cord blood mesenchymal stromal, and human acute monocytic leukemia cell lines. The findings reveal both shared and cell line-specific small extracellular vesicle subpopulations with distinct distributions of common tetraspanins (CD9, CD63, and CD81) and biophysical properties. Notably, the levels of CD9 and CD63 are strongly correlated, even though individual small extracellular vesicle tetraspanin abundances do not depend on their sizes. Furthermore, in all cell lines, a small extracellular vesicle subpopulation with relatively high abundance of all three tetraspanins, average diameters of < 100 nm, and relatively low Young moduli is identified.

These techniques are essential for the physical characterization of CDNs, offering valuable insights into their size, shape, and structural properties, all of which are crucial for understanding their biological function. These methods are complementary and can be used together for a comprehensive characterization of CDNs. However, the limitations of each method and the impact of sample preparation on CDN morphological features must be considered. Moreover, diverse particle size measurements have been reported depending on the selected analysis method, highlighting the need for multiple techniques[93]. For example, NTA measures particle sizes in the range of 10 nm–2 μm by tracking particle motion in a suspension and calculating the mean square velocity. Zeta potential measurements involve applying an electric field across the solution and assessing the mobility of isolated vesicles due to electrophoresis.

4.2 Membrane component (lipidomic) analysis

Molecular markers, including surface receptors and membrane proteins on CDNs, can be identified using various biological analysis techniques, such as thin-layer chromatography (TLC), liquid chromatography/mass spectrometry (LC/MS), and fluorescence-based lipid analysis [94–98]. The selection of a method depends on the specific study requirements and the availability of technical expertise and resources.

LC and MS are recognized as the most powerful and quantitative techniques for the lipidomic analysis of CDNs, but they also demand a high level of technical expertise and resources [94]. LC separates lipids based on their hydrophobic properties and is frequently used for lipidomic analysis. It is often coupled with MS, allowing for highly sensitive and quantitative lipidomic investigations of CDNs. LC can be employed in various configurations, including reverse-phase LC (RP-LC), normal-phase LC (NP-LC), and ultra-performance liquid chromatography-MS/MS (UPLC-MS/MS), which have been utilized in lipidomic analysis of CDNs[97, 99, 100] (Fig. 4A). MS is a potent tool for lipidomic analysis but requires a substantial level of technical expertise and is relatively expensive.

TLC is a simple and cost-effective method for lipid separation and visualization. It separates lipids based on polarity and can be used to analyze CDN composition. While TLC is frequently used as an initial step in the lipidomic analysis of CDNs, it has limited resolution and cannot provide quantification of individual lipids. The recent instrumental addition to TLC, known as high-performance thin-layer chromatography (HPTLC), offers effective separation of samples into lipid classes, detection, and quantitative measurement by UV/FL densitometry, utilizing suitable standards, and direct integration with MS.

MS is a highly sensitive and quantitative approach for lipid analysis. It can identify and quantify lipid species in CDNs, delivering a comprehensive lipidomic profile. MS can be employed in various configurations, including electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI), both of which have been used in the lipidomic analysis of CDNs [96] (Fig. 4B).

Fluorescence-based lipid analysis employs fluorescent dyes or probes to visualize and quantify lipids in CDNs [101] (Fig. 4C). It is a simple and cost-effective method for lipidomic analysis, although it offers limited resolution and sensitivity compared to MS and LC.

4.3 Intravesicular component analysis

The intravesicular components of CDNs play a pivotal role in their biological function and intercellular communication. Analyzing these components is crucial for understanding the molecular composition, functionality, and cargo transport of CDNs. Various methods, such as western blotting [], MS, enzyme-linked immunosorbent assay (ELISA), quantitative real-time PCR (qPCR) [], and fluorescence-based techniques, are commonly employed to analyze the intravesicular components of CDNs, each having its own strengths and limitations. The choice of

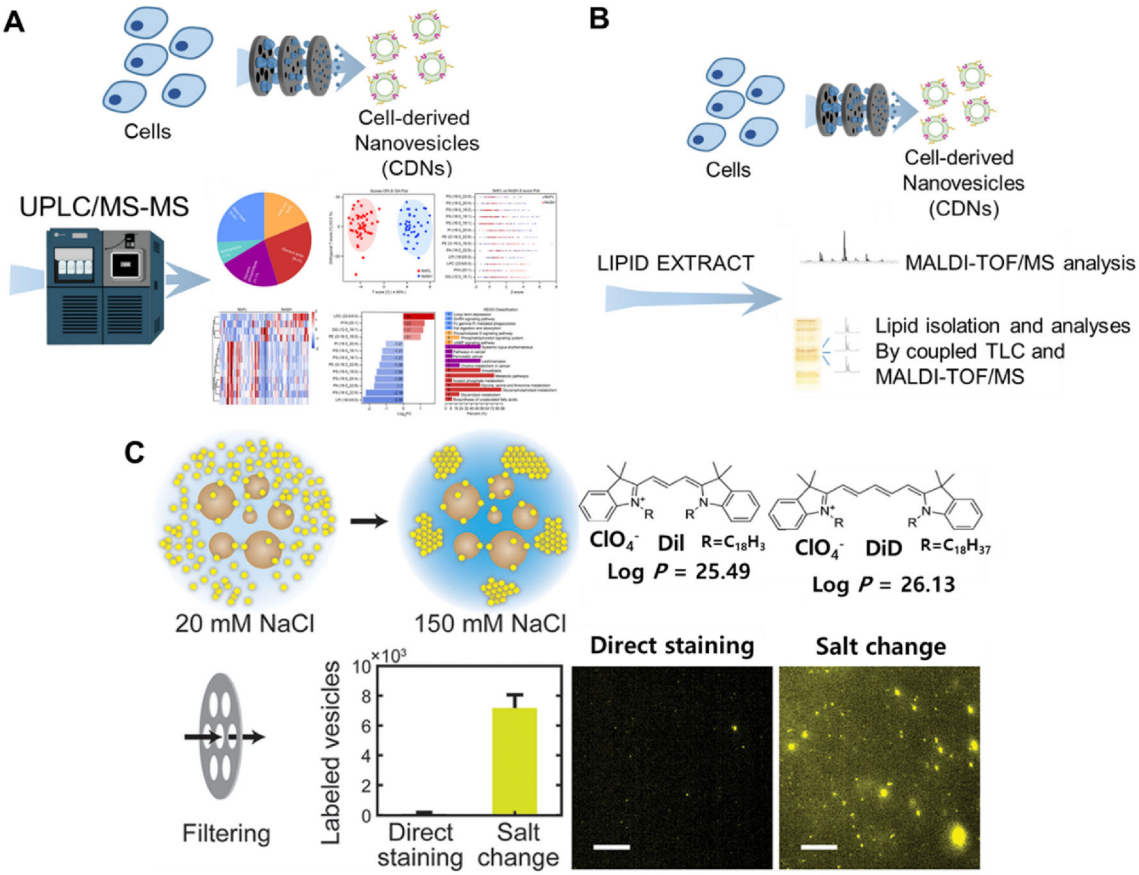

Fig. 4 Lipidomic analysis of CDNs. **A** UPLC-MS/MS analysis (Reprinted with permission from Xhu et al. [97], Copyright © 2022 Springer Nature), **B** MALDI-TOF/MS and coupled TLC and MALDI-TOF/MS (Reprinted with permission from Lobasso et al. [96], Copyright © 2021 Frontiers), and **C** fluorescence-based lipid analysis (Reprinted with permission from Cha et al. [101], Copyright © 2023 American Chemical Society)

method depends on the specific study requirements and the availability of technical expertise and resources.

The chosen characterization methods should meet several criteria: they should be rapid, high-throughput, repeatable, cost-effective, applicable for small- and large-scale detection, and sensitive. Long sample turnaround times should be minimized, and the methods should be capable of identifying both small and large vesicles.

Western blotting is a widely used method for the detection and quantification of specific proteins in a sample. It is valuable for determining the functional characteristics of these vesicles, allowing the identification and quantification of intravesicular proteins in CDNs. MS, ELISA, flow cytometry, and qPCR can be used to determine the quantity of CDNs [48, 84, 102, 103]. MS, in particular, is highly sensitive and quantitative for the analysis and quantification of intravesicular proteins and RNA in CDNs. It provides a comprehensive molecular profile of these vesicles, although it is relatively expensive and requires a high level of technical expertise.

ELISA is another well-established method for quantifying CDNs based on the detection of specific antigens or markers on the vesicle surface [104]. It is highly sensitive and reliable, with established protocols for sample preparation and analysis. Flow cytometry is a high-throughput technique suitable for single-particle analysis of CDNs. It enables the determination of particle size, surface marker expression, and particle count, offering important insights into the biological properties of CDNs. qPCR is a highly sensitive and specific technique for measuring RNA levels in a sample, providing information on the biological content of CDNs.

Fluorescence-based techniques, such as fluorescence in situ hybridization (FISH) and fluorescence microscopy, can visualize and quantify intravesicular RNA in CDNs [105–108]. This approach enables the direct visualization of DNA targets without the need for time-consuming optimization steps or specialized expertise. Furthermore, using formalin-fixed paraffin-embedded (FFPE) blocks of CDNs allows for the long-term preservation of samples, facilitating future research. Vishal et al. introduced a novel

approach that combines CDNs, microfluidics, and single-molecule fluorescence colocalization microscopy to monitor individual binding events at the cyclic nucleotide-gated TAX-4 ion channel, which is crucial for sensory transduction [108]. The findings revealed insights into the dynamics of both nucleotide binding and a conformational change that likely precedes pore opening. Kinetic modeling suggests that the second ligand's binding is either independent of the first ligand or shows approximately ten-fold positive binding cooperativity.

These methods are essential for the accurate quantification of CDNs and provide valuable information on the biology and function of these critical extracellular vesicles. They are crucial for the development of therapeutic applications and the study of cellular communication and signaling. Western blotting is ideal for protein examination and identification, whereas fluorescence-based techniques are suitable for visualizing and quantifying specific components. They are simple and cost-effective methods for the analysis of intravesicular RNA in CDNs but have limited resolution and sensitivity compared to MS and qPCR.

5 Engineering of exosome-mimetic nanovesicles

Improving the therapeutic efficacy and targeting efficiency of CDNs is essential to mitigating side and off-target effects. Various engineering techniques have been explored to achieve this goal, including strategies to enhance the concentration of therapeutic molecules within the vesicles and to modify the membrane components for targeted delivery. Given their structural similarity to liposomes, these techniques often draw inspiration from liposome engineering. However, an alternative approach worth considering is the engineering of parent cells before nanovesicle synthesis, enabling the nanovesicles to inherit the engineered membrane and intracellular components. Although this approach carries the risk of unexpectedly altering cell viability or the original therapeutic effect, it offers the advantage of avoiding potentially toxic chemical conjugation methods. In this section, we will review the engineering techniques used for modifying the membrane or intravesicular components of exosome-mimetic nanovesicles with the goal of enhancing tissue targeting efficiency and therapeutic efficacy (Fig. 5).

5.1 Pre-synthesis engineering

Considering the synthesis process of CDNs and their physicochemical structure inherited from the parent cells, engineering the parent cells before CDN synthesis holds promise for improving the therapeutic efficacy of CDNs. One notable example of membrane engineering is the development of MSC-derived CDNs expressing a disturbed blood flow-targeting peptide (GSPREYTSYMPH; PREY) on their outer surface [4] (Fig. 5A). A custom plasmid was transfected into MSCs to express PREY on the outer surface of the cell membrane [4]. The PREY-expressing MSCs were subsequently extruded to synthesize CDNs that also express PREY on the surface, resulting in significantly enhanced atherogenic lesion-targeting efficacy under disturbed flow and superior protective capabilities.

Moreover, several studies have explored the incorporation of iron oxide nanoparticles (IONPs) into CDNs extruded from MSCs [64–66] (Fig. 5B). This approach aimed to improve the regenerative efficacy of MSCs through iron ion-mediated signaling cascades and enhance the targeting capabilities of the resulting CDNs due to their magnetic properties. By treating parent cells with IONPs, these nanoparticles were endocytosed and incorporated into the CDNs during the extrusion process. Compared to CDNs synthesized from MSCs without IONPs, these modified CDNs featured a magnetic targeting unit and significantly improved therapeutic efficacy. In practical applications, placing a magnetic bar at the desired tissue site, such as the brain [64], spinal cord [65], or heart [66], significantly increased the targeting efficiency and retention of the CDNs, promoting tissue regeneration.

In another study, high-yield engineered CDNs were produced (from human mesenchymal stem cells (hMSCs) that were modified with bone-targeting moieties [111]. The precursors of sialic acid modified with azide were formed on the surface of hMSCs via metabolic glycoengineering. Following the preparation of azide-modified exosome mimetics using an extrusion method, alendronate (ALD), a bone-targeting ligand, was implemented on the CDN surface via a click reaction with azide. These bone-targeting EMs demonstrated significant binding efficiency and affinity for hydroxyapatite (HA), implying the possibility of future immobilization on HA-coated implantable devices. In a different study, lentiviral infection was used to genetically modify TC-1 cells (a mouse lung cancer cell line) to ensure consistent programmed cell death-1 (PD-1) protein expression on the cell membrane [112]. They created PD-1-displaying CDNs using an extrusion technique with pre-modified cells, followed by loading doxorubicin (DOX) and 2-deoxy-D-glucose (2-DG) into these CDNs. According to their findings, integrating CDNs with PD-1 improved their tumor-targeting ability, and the loading of 2-DG and DOX considerably improved the therapeutic efficacy for non-small cell lung cancer (NSCLC) in a synergistic manner. These findings suggest that PD-1-displaying and drug-co-loaded CDNs are effective therapeutic options for the treatment of NSCLC.

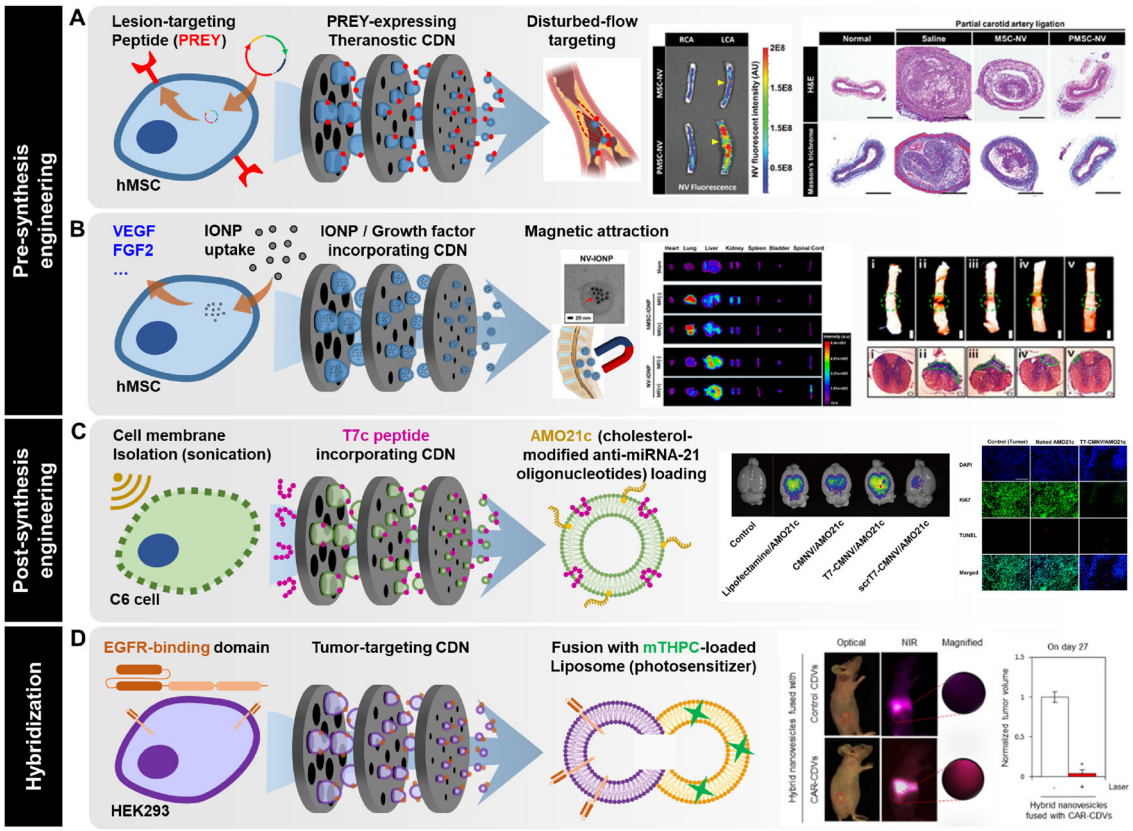

Fig. 5 Various engineering techniques for CDN synthesis, categorized as pre-synthesis engineering, post-synthesis engineering, and hybridization. **A** Theranostic CDNs were synthesized for atherosclerosis. PREY, a disturbed-flow targeting peptide, was expressed on hMSCs through plasmid transfection, followed by CDN synthesis. The results demonstrate successful lesion-targeting effect with lesion formation prevention. Reprinted with permission from Yoon et al. [4], Copyright © 2020 John Wiley & Sons, Inc. **B** CDNs were synthesized from IONP-treated hMSCs resulting in enhanced growth factor release. Magnetic attraction was employed to recruit CDNs to the spinal cord injury site. Reprinted with permission from Kim et al. [65], Copyright © 2018 American Chemical Society. **C** C6 cell membrane fragments were extruded with tumor targeting (T7c) peptides to synthesize T7c-incorporating CDNs. Anti-miRNA-21 oligonucleotide, modified with cholesterol, was sequentially loaded through hydrophobic interaction. Reprinted with permission from Lee et al. [109], Copyright © 2022 John Wiley & Sons, Inc. **D** CDNs were extruded from HEK293 cells engineered to express an EGFR-binding domain for cancer targeting. The CDNs were fused with photosensitizer-loaded liposomes for cancer therapy. Reprinted with permission from Shin et al. [110], Copyright © 2023 The Korean Society of Industrial and Engineering Chemistry

In another study, bone marrow mesenchymal stem cells were transfected with netrin-1 modified messenger RNA (modRNA) to produce netrin-1-abundant CDNs [113]. Consequently, PC12 (rat pheochromocytoma) cells and oligodendrocytes' axonal and dendritic development was accelerated by netrin1-abundant CDNs, which also reduced inflammation triggered by LPS. This *in vivo* study suggested that netrin-1-abundant CDNs may aid recovery from spinal cord damage in rats. Another study synthesized apoptotic body-mimetic CDNs coupled with two targeting agents for the treatment of cardiac ischemia–reperfusion damage [114]. Prior to inducing apoptosis and CDN synthesis, dextran and cardiac-homing peptides were introduced into NIH3T3 cells (a mouse fibroblast cell line) to improve the targeting moiety of CDNs for macrophages residing inside the ischemic myocardium. After intravenous injection, the modified CDNs prevented non-specific nanodrug distribution. Additionally, the therapeutic efficacy of the CDN platform was significantly enhanced by the ischemic myocardium-macrophage targeting method, indicating that this approach may be promising for treating cardiovascular diseases. In addition, this study used CDNs prepared from M1 macrophages to evaluate whether macrophage repolarization enhanced anticancer activity [115]. Lipopolysaccharide (LPS) treatment on RAW264.7 cells (mouse macrophage cells) induced M1-polarization, which subsequently underwent CDN synthesis [115]. When M1 macrophage-derived CDNs and aPD-L1 (PD-L1 inhibitors) were injected together into a tumor-bearing mouse model, they resulted in the repolarization of M2 tumor-associated macrophages to M1 macrophages and considerable inhibition of tumor development [115]. According to this study, the ability of

M1-derived CDNs to regulate the immune system may be useful for treating cancer [115].

Another study utilizing CDNs for cancer therapy used cancer cell-derived CDNs as cancer vaccines [116]. B16F10 (mouse melanoma) cells were treated with DOX for senescence induction prior to CDN synthesis, which enriched IFN-γ and TNF-α inside the CDN compared to healthy B16F10-derived CDNs [116]. These engineered CDNs induced dendritic cell maturation to form tumor-specific T cells [116]. Additionally, CDNs extruded from α-galactosylceramide (a vaccine adjuvant)-treated C1498 (murine myeloid leukemia) cells exhibited an effect similar to the immune system for cancer therapy. It simultaneously induced synergistic innate and adaptive immune responses [117]. Moreover, activated CD8 + T cell-derived CDNs have been reported to induce cancer cell apoptosis and prevent T cell exhaustion by receptor binding, similar to live CD8 + T cells in other cancer immunotherapies [118]. In another study, CDNs were formed by eliminating undesired luminal cargo inside cells using an alkaline solution [119]. The membrane sheets were isolated by sonication and ultracentrifugation [119]. Membrane sheets were used to create CDNs, which were loaded with dexamethasone for *in vitro* delivery [119]. These dexamethasone-loaded CDNs inhibited IL-8 release in endothelial cells [119].

Another study generated engineered CDNs by extruding HEK293 cells (human kidney cancer cells) infected with H19-overexpressing lentiviral vectors [120]. When modified CDNs were administered to diabetic wound sites, they promoted the wound-healing effect owing to greater production of long non-coding RNA (LncRNA)-H19 [120].

5.2 Post-synthesis engineering

Similar to liposome or exosome functionalization, engineering of CDNs is mostly conducted after synthesis. In contrast to previously introduced pre-synthesis engineering methods, post-synthesis engineering processes may affect the biochemical stability of molecules such as lipids, proteins, or genetic molecules. However, the development of nontoxic bioconjugation methods (e.g., click chemistry) and drug-loading methods has enabled various applications of post-synthesis engineering, opening up new possibilities for improving their targeting ability and personalizing them for specific uses.

One study employed two methods to engineer extracellular CDNs as effective siRNA delivery platforms for *in vitro* stem-cell targeting and systemic delivery: surface-engineered exosomes and plasma membrane-derived CDNs [121]. They modified the exosomal membrane to contain multiple cell-adhesion peptides (Arg-Gly-Asp; RGD) or cell-penetrating peptides to increase target delivery and cellular uptake [121]. CDNs were generated by chemically triggering membrane vesicles [121]. Compared to conventional transfection reagents, modified exosomes and CDNs demonstrated greater siRNA transfection efficiency and lower cytotoxicity [121]. Furthermore, by facilitating the effective targeted delivery of siRNAs, they demonstrated the osteogenic and neuronal differentiation of human stem cells [121]. In another study, CDNs were prepared using a combination of T7 peptides and C6 cell membranes to ensure the distribution of anti-miRNA-21 oligonucleotide (AMO21) in the brain [109] (Fig. 5C). AMO21 was modified with cholesterol (AMO21c) and then loaded onto the surface of CDNs via hydrophobic contact between cholesterol and the cell membrane, following extrusion of the cell membrane and T7 peptide to create CDNs [109]. These findings demonstrate that in C6 glioblastoma cells, the modified CDNs exhibited greater cellular uptake efficiency of AMO21c [109]. Collectively, these findings suggest that the modified CDNs may function as reliable vehicles for glioblastoma treatment [109].

In another study, CDNs produced from isolated platelets were fused to the surfaces of cardiac stem cells [122]. The ability of engineered CDNs to target myocardial infarction injury sites was demonstrated, indicating the potential of this technique for use in ischemic heart diseases [122]. Post-synthetic engineering of CDNs is often useful in cancer immunotherapy and involves the binding of tumor-specific antigens to dendritic cell-derived CDNs. For example, LPS-activated dendritic cells undergo serial extrusion to synthesize CDNs expressing CD80, CD86, and MHC class I [123]. Consequently, the binding of tumor antigens to these CDNs allowed mature T cells to form tumor-specific cytotoxic T lymphocytes for cancer therapy [123].

5.3 Hybridization

The hybridization of various types of vesicle-based nanoparticles has recently garnered interest in the field of drug delivery. This approach is seen as one of the simplest methods for providing multivalency with synergistic features. For instance, the hybridization of exosomes and liposomes can address the limitations associated with both vesicle types, particularly batch-to-batch heterogeneity and low biocompatibility, respectively. Typically, liposomes that display targeting molecules or encapsulate defined biomolecules have been shown to enhance the functionality of natural exosomes. Simultaneously, the membranous components of the exosomes contribute to prolonged circulation time and improved biosafety.

In one example, drug-loaded liposomes were combined with CDNs derived from HEK293 cells genetically

engineered with anti-EGFR-CAR plasmid to create hybrid CDNs for targeted cancer therapy [110] (Fig. 5D). CDNs were fused with photosensitizer-loaded liposomes in a fusion solution. The effectiveness of these fused hybrid CDNs for cancer cell targeting and inhibition of tumor growth was evaluated using a mouse tumor model. The results showed that under laser irradiation, hybrid CDNs with photosensitizer encapsulation efficiently targeted and suppressed tumor development. This fusion approach holds promise for potential use in cancer treatment.

In a different study, an extrusion method was employed to create tumor cell and macrophage-derived CDNs, which were then fused using sonication [124]. The results demonstrated that treatment with hybrid CDNs led to greater activation of humoral immunity in the tumor tissues. Given that tumor-derived CDNs can successfully target tumor sites and lymph nodes simultaneously, this hybrid CDN approach may be applicable for treating various types of cancer.

Another study used the PEG-mediated liposome fusion technique to create cell-derived hybrid CDNs while maintaining their biological characteristics and fundamental composition, resulting in improved drug delivery and loading efficiency [81]. A different bio-hybrid approach combined temperature-sensitive liposomes with CD47-expressed CDNs generated from genetically modified fibroblasts. These hybrid CDNs demonstrated increased drug delivery rates to tumor sites, indicating their potential as therapeutic agent cargo [125]. In another study, hybrid CDNs were created by freezing and thawing the membranes of CDNs and liposomes. Greater cellular uptake by hybrid CDNs indicates their potential as biotransporters for both hydrophilic and hydrophobic agents [126].

In a different example, the extrusion method was used to generate a fibroblast-derived CDN and clodronate-loaded liposome hybrid system for the treatment of pulmonary fibrosis. Due to the homing ability of fibroblasts, the hybrid CDNs predominantly accumulated in the fibrotic sites and demonstrated greatly enhanced penetration of pulmonary fibrotic lesions [127]. Another study generated hybrid CDNs by utilizing a serial extrusion process to combine the surface properties of CDNs with various types of lipid-based substances. By altering the lipid type, exogenous siRNA was effectively loaded into hybrid CDNs, and the CDNs exhibited an increase in cellular uptake efficiency for lung cancer cells, indicating this might be a suitable alternative for gene delivery methods [128].

A different strategy involved the use of CDNs produced from macrophages and hybridizing them with liposomes, resulting in enhanced targeting and cytotoxicity against cancer cells [79]. Furthermore, CDN membranes can be fused into live MSCs for membrane engineering. Macrophage-derived cell membranes fused with MSCs exhibited superior spinal cord-injury theranostic ability [129] (Table 1).

6 Conclusion

CDNs, with their similar mechanical, chemical, and biological properties, offer a promising alternative to exosome therapy by significantly improving productivity and cost-effectiveness. Exosome and CDN therapies provide potential solutions to the multifaceted limitations of cell therapy, including the risks of unexpected abnormal cell fate, pulmonary microvessel entrapment, and the time-consuming preparation required to achieve an adequate cell population following diagnosis. These solutions are based on their characteristics as non-living biomaterials, nano-sized entities, and their ability to be stored in a ready-to-use state, as demonstrated in numerous preclinical studies. However, natural exosomes and CDNs require engineering to enhance their functionality, such as lesion targeting efficiency and therapeutic efficacy, as discussed earlier.

In recent times, the observed batch-to-batch variations and even particle-to-particle heterogeneity of exosomes and CDNs, along with the significance of discerning differences between CDNs and natural exosomes, have been frequently reported. This includes concerns about membrane lipid flip-flops and instabilities during or after the extrusion process, which pose a potential risk of increased inflammatory responses, such as macrophage phagocytosis, although there have been no precise reports on these phenomena yet. Additionally, the heterogeneity of CDNs, which is similar to that of exosomes, presents a natural concern that needs to be addressed. Similar to exosomes, CDN engineering is still in its early stages of research. At present, the limitations are mainly associated with identifying the heterogeneity by characterizing vesicle-to-vesicle discrepancies, which can only be identified by particle size variation. To enhance homogeneity, synthesizing hybrid CNDs through fusion with well-characterized artificial liposomes is being explored. Therefore, further investigations into proteomic, lipidomic, and genetic profiling are imperative to precisely compare their safety and stability characteristics, particularly as artificially synthesized nanodrugs. Moreover, circulation time is another method to enhance the therapeutic effect of CDNs. However, PEGylation, the most common approach for extending circulation time, may not be appropriate since polyethylene glycol molecules would potentially interfere with the function of essential transmembrane proteins. Therefore, in the case of exosome engineering, fusion with platelet-derived membranes has been employed to synthesize a hybrid nanovesicles [130].

Table 1 A summary of engineering approaches for cell-derived nanovesicles

Engineering approach	Parent cell	Method for CDNs engineering	Targeted disease	Effects & observations	References
Pre-synthesis	Mesenchymal stem cells (MSCs)	Plasmid transfection	Atherosclerosis	↑ atherogenic lesion-targeting efficacy, ↑ vascular protective effect	[4]
	MSCs	Iron oxide nanoparticle internalization	Ischemic stroke, spinal cord injury, myocardial infarction	↑ targeting efficiency, ↑ CDNs retention, ↑ tissue regeneration	[64–66]
	MSCs	Metabolic glycoengineering	Bone diseases	↑ binding efficiency and affinity to hydroxyapatite	[111]
	TC-1 cells (mouse lung cancer cell line)	Lentivirus infection	NSCLC (non-small cell lung cancer)	↑ tumor-targeting ability, ↑ therapeutic efficacy for NSCLC	[112]
	Bone marrow MSCs	RNA transfection	Spinal cord injury	attenuate inflammation and pyroptosis, ↑ axonal and dendritic development, ↑ spinal cord damage recovery	[113]
	NIH3T3 cells (fibroblast cell line)	Cell membrane conjugation	Myocardial infarction	↑ targeting ability, ↑ cardiac remodeling	[114]
	RAW264.7 cells (mouse macrophage cell line)	LPS treatment	Cancer	repolarization from M2 to M1 macrophages, inhibition of tumor growth	[115]
	B16F10 cells (mouse melanoma cell line)	Doxorubicin treatment	Melanoma	↑ IFN-γ and TNF-α inside CDNs, ↑ dendritic cell maturations to form tumor-specific T cells, ↓primary and metastatic tumor growth	[116]
	C1498 cells (murine acute myeloid leukemia cells)	α-galactosylceramide treatment	Acute myeloid leukemia	↑ innate and adaptive immune responses, ↓tumor growth	[117]
	$CD8^+$ T cells	T cell activation	Cancer	↑ cancer cell apoptosis, ↓T cell exhaustion	[118]
	U937 cells (human monocyte cell line)	Luminal cargo removal + drug loading	Sepsis	↓IL-8 release in endothelial cells, ↓symptoms of systemic inflammatory response syndrome	[119]
	HEK293 cells (human embryonic kidney cell line)	Lentiviral vector infection	Diabetic wounds	↑ wound-healing effect	[120]
Post-synthesis	HEK293 cells	Surface modification, siRNA delivery	N/A (siRNA delivery)	↑ siRNA transfection efficiency, ↓cytotoxicity, ↑ osteogenic and neuronal differentiation of human stem cells	[121]
	C6 cells (rat glioma cell line)	Surface modification	Glioblastoma	↑ cell uptake efficiency of AMO21c	[109]
	Platelets	Surface modification	Myocardial infarction	↑ targeting ability to myocardial infarction injury sites, ↑ retention in the heart, ↓infarct size	[122]
	Dendritic cells	Surface modification	Cancer	↑ tumor-specific cytotoxic T lymphocytes generation, ↓tumor growth	[123]
Hybridization	HEK293 cells	Liposome fusion	Cancer	↑ tumor cell targeting ability, suppressed tumor growth	[110]
	RAW264.7 cells, 4T1 cells (mouse mammary carcinoma cell line)	CDNs fusion	Breast Cancer	↑ humoral immunity activation in the tumor tissues, ↑ tumor sites and lymph nodes targeting ability	[124]
	Human umbilical vein endothelial cells (HUVECs)	Liposome fusion	N/A (Drug delivery)	↑ drug delivery efficiency, ↑ drug loading efficiency	[81]
	BALB/c 3T3 cells (mouse embryonic fibroblast cell line)	Liposome fusion	Metastatic peritoneal carcinoma	↑ drug delivery rates to tumor sites	[125]

Table 1 continued

Engineering approach	Parent cell	Method for CDNs engineering	Targeted disease	Effects & observations	References
	RAW264.7 cells	Liposome fusion	N/A (Drug delivery)	Modified delivery function by changing the lipid composition or CDNs property	[126]
	L-929 cells (mouse fibroblast cell line)	Liposome fusion	Pulmonary fibrosis	↑ accumulation in the fibrotic sites, ↑ penetration within pulmonary fibrotic lesions	[127]
	3T3 cells, A549 cells (lung cancer cell line)	Lipid membrane fusion	Lung cancer	↑ in cellular uptake efficiency to lung cancer cells	[128]
	J774A.1 cells (mouse macrophage cell line)	Liposome fusion	Cancer	↑ targeting and cytotoxicity against cancer cells	[79]
	MSCs	Cell membrane fusion	Spinal cord injury	↑ theranostic ability for spinal cord injury	[129]

In conclusion, the ongoing research into the engineering and refinement of CDNs, along with rigorous assessments of their safety profiles and stability characteristics, holds great promise for advancing the field of regenerative medicine and paves the way for the development of precise and efficacious therapeutic interventions.

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Data availability The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no conflict of interest to declare.

Ethical statement There are no animal experiments carried out for this article.

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