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REVIEW

Exosomes—Nature's Lipid Nanoparticles, a Rising Star in Drug Delivery and Diagnostics

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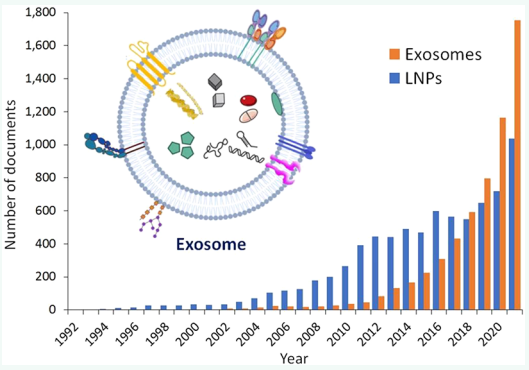
ABSTRACT: **Exosomes are a subgroup of nanosized extracellular vesicles enclosed by a lipid bilayer membrane and secreted by most eukaryotic cells. They represent a route of intercellular communication and participate in a wide variety of physiological and pathological processes. The biological roles of exosomes rely on their bioactive cargos, including proteins, nucleic acids, and lipids, which are delivered to target cells. Their distinctive properties—innate stability, low immunogenicity, biocompatibility, and good biomembrane penetration capacity—allow them to function as superior natural nanocarriers for efficient drug delivery. Another notably favorable clinical application of exosomes is in diagnostics. They hold various biomolecules from host cells, which are indicative of pathophysiological conditions; therefore, they are considered vital for biomarker discovery in clinical diagnostics. Here, we use data from the CAS Content Collection and provide a landscape overview of the current state and delineate trends in research advancement on exosome applications in therapeutics and diagnostics across time, geography, composition, cargo loading, and development pipelines. We discuss exosome composition and pathway, from their biogenesis and secretion from host cells to recipient cell uptake. We assess methods for exosome isolation and purification, their clinical applications in therapy and diagnostics, their development pipelines, the exploration goals of the companies, the assortment of diseases they aim to treat, development stages of their research, and publication trends. We hope this review will be useful for understanding the current knowledge in the field of medical applications of exosomes, in an effort to further solve the remaining challenges in fulfilling their potential.**



KEYWORDS: *exosome, extracellular vesicle, drug delivery, diagnostics, biomarker, nanoparticle, nanocarrier, blood–brain barrier, therapeutics*

Nearly 20 years after the discovery of liposomes,[1] it was found out that similar lipid vesicles form naturally in living organisms.[2,3] These include membrane-contained nanosized extracellular vesicles (EVs), secreted from cells as part of their normal process or certain pathologies. Based on the origin and size of the EVs, as well as on the current understanding of their biogenesis, they are grouped as follows: exosomes (diameter ∼30−150 nm); microvesicles or ectosomes (100 nm−1 μm); and apoptotic bodies (50 nm−5 μm).[4,5]

Exosomes are produced in the endosomes of most eukaryotic cells and subsequently released in the extracellular space by fusion with the cellular biomembrane (Figure 1). Their functions are still largely unknown but a subject of a recent burst of interest as their important roles in physiological and pathophysiological processes are steadily revealed. They have been shown to provide means of efficient intercellular communication and signaling, including transport of bioactive molecules such as proteins, lipids, and nucleic acids, between cells and across biological barriers.[6,7] These results and the physicochemical properties of exosomes are reasons that they are viewed as the rising star in drug delivery and diagnostics.[5,8,9] However, there is still insufficient knowledge regarding exosome physiology. In order to make use of the clinical potential of exosomes, it is necessary to better understand the cellular processes that govern their biology and membrane trafficking.

For a long time, synthetic drug nanocarriers have been developed to improve the efficacy of therapeutics, to refine their pharmacokinetics and pharmacodynamics, while lessening the

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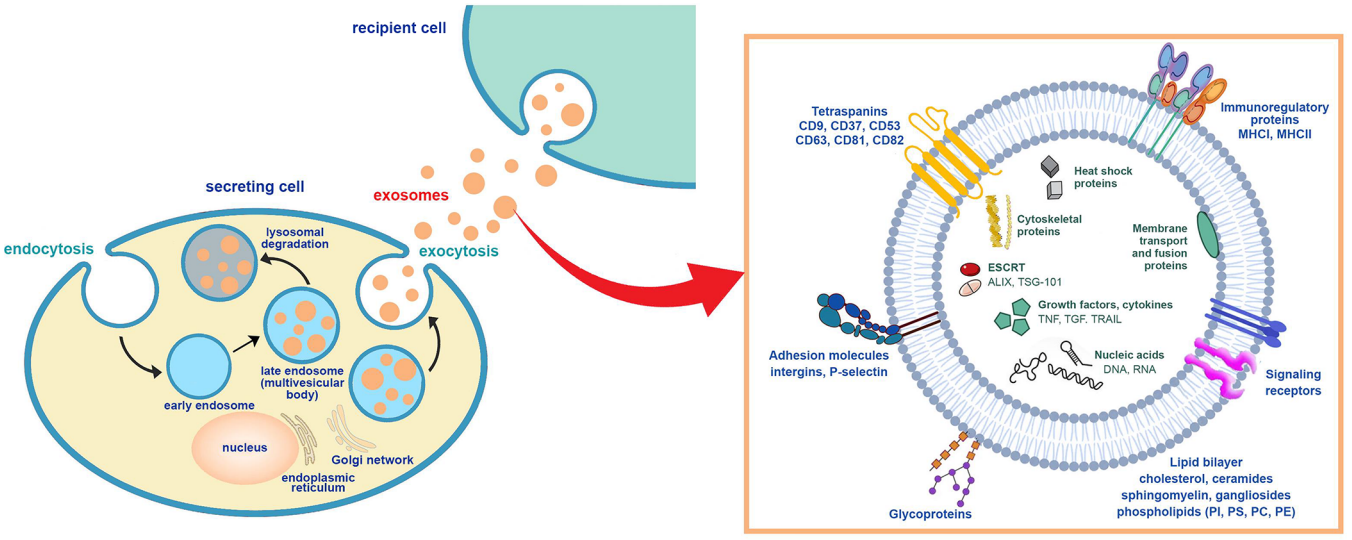

Figure 1. Scheme of exosome biogenesis and secretion. The inset exemplifies the molecular constituents of the exosomes.

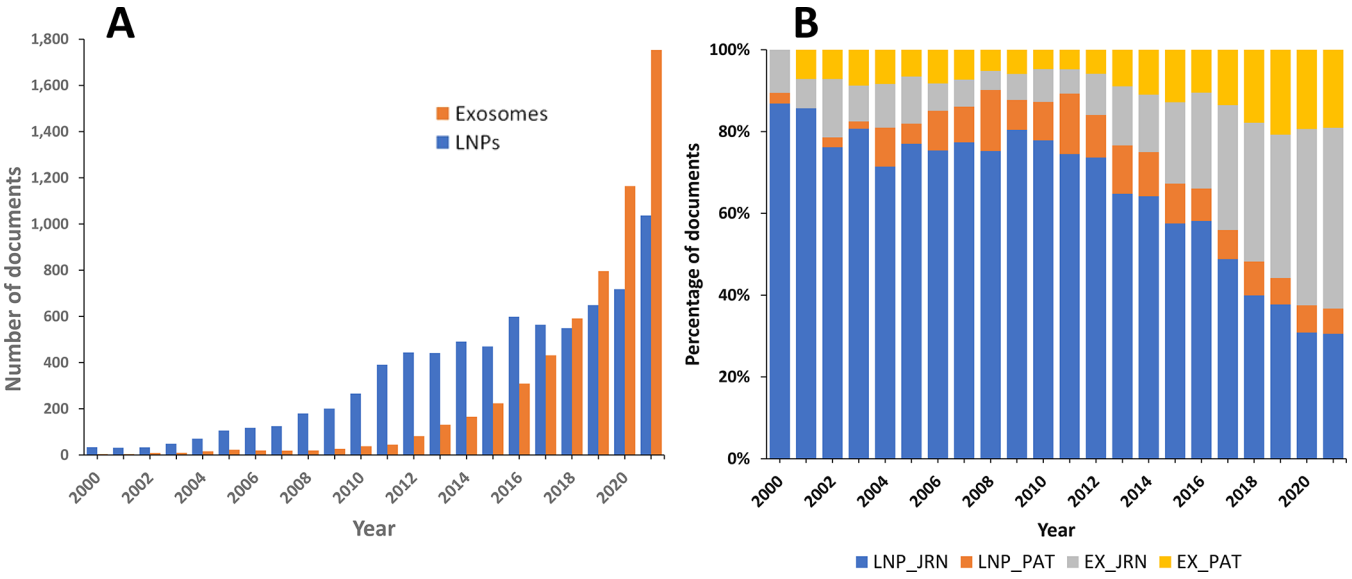

Figure 2. Publication trends of exosomes and lipid nanoparticles applied to drug delivery. (A) Comparison of the trends in the number of publications related to exosomes and lipid nanoparticles. The number of publications has been estimated by combining drug-delivery-related search terms such as "drug delivery", "pharmaceutic", and "carrier" with the terms "lipid nanoparticle" vs "exosome" or "extracellular vesicle". (B) Corresponding yearly percentages of publications related to exosomes (EX) and lipid nanoparticles (LNP) in journal articles (JRN) and patents (PAT) calculated for each specific year are compared.

toxicity and side effects.[10,11] Many smart artificial delivery systems such as various functionalized, stimuli-responsive, targeted lipidic or polymeric nanocarriers have been invented to improve key features of the delivery systems such as circulation time in the bloodstream, biodistribution, cellular interactions, and drug loading and release. However, synthetic drug delivery systems still come across many setbacks, such as non-specific drug targeting and toxicity of the carriers, immunogenicity, and unsatisfactory efficacy.[12] Specifically, lipid nanoparticles (LNPs) have been recognized as favorable vehicles to protect, transport, and deliver a wide variety of drugs and vaccines to cells.[10] Liposomes, an early kind of lipid nanoparticles, are a flexible and resourceful nanomedicine delivery system. They can significantly enhance drug pharmacokinetics. By encapsulating drugs in liposomes, they are protected against dilution and degradation or inactivation in the blood.[10,13] Lipid nanoparticle technologies together with other nanotechnological platforms for drug delivery have improved the efficiency, selectivity, residence time, and biodistribution of traditional drug carrier systems while reducing their drawbacks. However, the clinical application of the lipid nanocarriers has experienced substantial difficulties such as low bioavailability, toxicity, removal from the bloodstream, or stimulation of innate immune reactions.

After the discovery of exosomes, it was realized that they are quite similar to liposomes, in fact a more complex version of liposomes, but originating from biological systems. Despite the evident similarities, exosomes exhibit certain advantages, which make them a preferable drug delivery vehicle. Their lipid composition is rich in non-lamellar forming lipids, which may give rise to favorable curvatures in their lipid bilayer, which has been proven beneficial in drug delivery.[14] Furthermore, the

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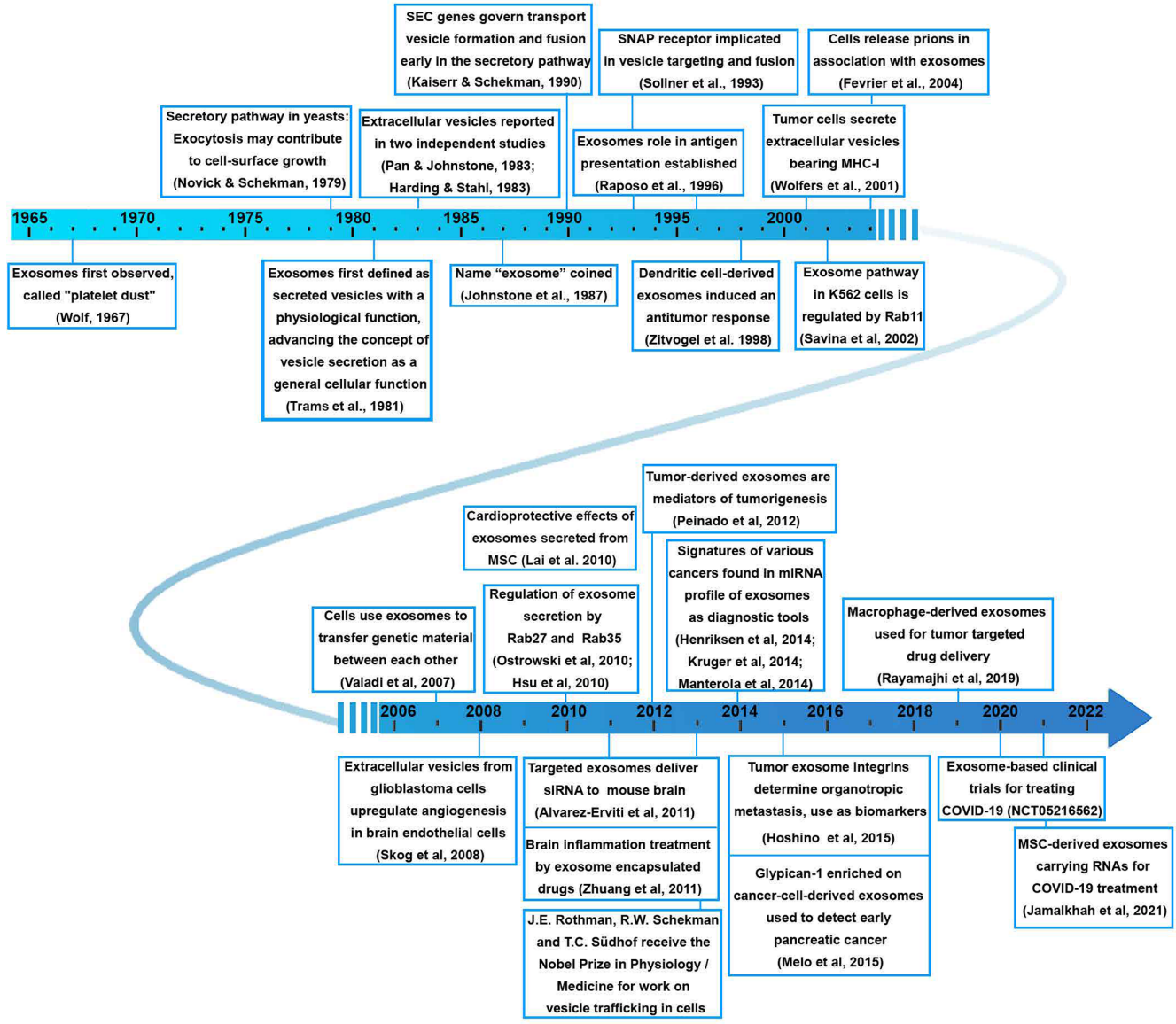

Figure 3. Timeline of major research and development milestones related to exosomes and their medical applications.[2,3,24−51]

exosome lipid bilayer is highly asymmetrical, which could be particularly advantageous for their interaction with the plasma membrane and especially with their target cells. While liposomes generally do not contain proteins, a large variety of integral and peripheral membrane proteins are found in exosomes, another favorable feature in their application in drug delivery. As a result, in the last 3−4 years, exosomes have become preferable over lipid nanoparticles as prospective drug carriers. The number of documents, both patents and journal articles, related to exosomes applied in drug delivery has significantly surpassed that of lipid nanoparticles, as revealed by a search in the CAS Content Collection[15] (Figure 2).

In enhancing exosome efficiency, valuable lessons learned from liposome development have been employed. Various techniques found useful and significantly refined in liposome/lipid nanoparticle production and drug loading, such as sonication, extrusion, freeze−thaw cycles, microfluidics, and others, have been successfully applied in exosomes. Functional modifications that have significantly improved liposome efficiency have been found useful in exosomes as well. The most noteworthy of these include targeting by surface-attached ligands for specific receptors on cells and coating with biocompatible inert polymers, typically polyethylene glycol (PEG), making the carriers invisible to phagocytes (PEGylation), considerably extending their circulatory half-life.[10]

The applications of exosomes as a natural carrier platform to deliver drugs have been regarded as a hope and promise to overcome the limitations associated with many previously studied drug delivery systems. For instance, exosomes are originated from biological systems and their components can be readily metabolized and excreted at the end of the delivery journey. In addition, exosomes produce a minimal immune response related to cell therapies, which might be rejected by the recipient.[16] Furthermore, exosomes are believed to exhibit minimum tumorigenicity,[17] as they could be readily absorbed and excreted via the blood and urine.[18] Various studies have shown the capacity of exosomes for promoting angiogenesis, providing cytoprotection, and controlling apoptosis.[17] The exciting observations on the delivery potential of exosomes such as their ability to overcome barriers for conventional colloidal

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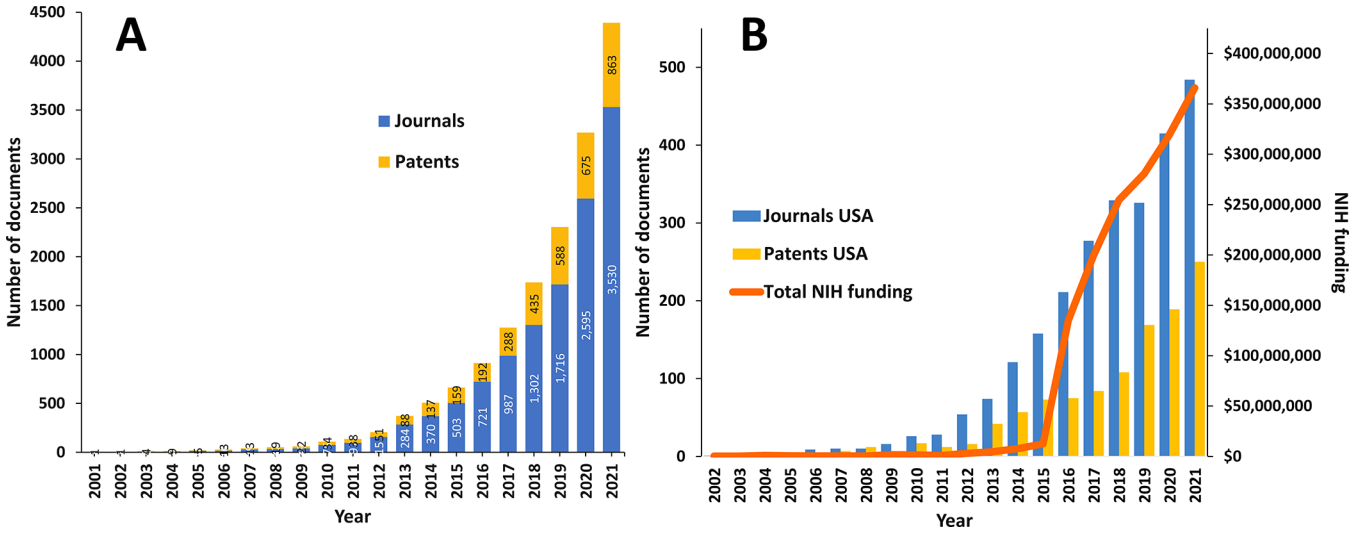

Figure 4. Journal and patent publication trends of exosome research in drug delivery and diagnostics and the association with research funding. (A) Trends in the number of publications related to exosomes in drug delivery and diagnostics, including journal articles and patents. (B) Number of documents originating from organizations in the USA as correlated with the annual NIH funding.

delivery systems, in particular the blood–brain barrier (BBB), and effectiveness for hard to deliver molecules such as proteins and RNAs have inspired intense research on their application as drug delivery vehicles.

Another especially promising clinical application of exosomes is in diagnostics. They transport biomolecules from their cells of origin, which may contain signs of pathophysiological conditions; therefore, they are widely considered to be essential for biomarker discovery in clinical diagnostics. Recent studies have shown that exosomes contain proteins and nucleic acids implicated in cancer and numerous other diseases, such as neurodegenerative, metabolic, infectious, inflammatory, and others. Moreover, exosomes can be obtained from easily achievable body fluids such as blood and urine and are thus appropriate targets for diagnostic application.[19,20]

Since the EV terminology is often confusing and has not been standardized due to the current limitations in isolating a particular type of EVs, the International Society for Extracellular Vesicles[21] on the Minimal Information for Studies of Extracellular Vesicles 2018 (MISEV 2018) guidelines suggested the use of alternative terms such as "small EVs" (<200 nm) or "large EVs" (>200 nm).[22] However, the term "exosome" is still largely used and dominates in the literature for vesicles of diameter ∼30–150 nm. The term "exosome" should also not be mixed up with "exosome complex", a multiprotein membraneless intracellular complex.[23]

Observation of small particles in plasma referred to as "platelet dust" was reported over 50 years ago.[24] The discovery of exosomes is related to two independent studies from 1983 focused on the transferrin receptor externalization.[2,3] It was subsequently realized that most viable cell types, such as B and T lymphocytes, dendritic cells, mast cells, intestinal epithelial cells, neurons, tumor cells, and various kinds of stem cells, release exosomes. It has become well-established that exosomes play an important role as messengers of intercellular communication. The interest in them was strongly enhanced after the power of antigen-loaded exosomes to eliminate tumors in mice was demonstrated[25] and phase I clinical trials in metastatic melanoma patients vaccinated with autologous dendritic-cell-derived exosomes were completed,[26] so exosomes emerged as a promising tool for autologous treatments in cancer. A timeline exemplifying some of the significant breakthroughs in the field of exosome research[2,3,24−51] is shown in Figure 3.

In this paper, we review the advances in the exosome applications in drug delivery and diagnostics. We examine data from the CAS Content Collection,[15] the largest human-curated collection of published scientific knowledge, and analyze the publication landscape of recent research on exosome applications in therapeutics and diagnostics to provide insights into the research advances in the area. We also discuss the exosome composition and pathway, from their biogenesis and secretion from the host cells to the recipient cellular uptake. Subsequently, we assess the methods for isolation and purification of exosomes, their clinical applications in therapy and diagnostics, their development pipelines with company research focuses, disease categories, development stages, and publication trends. We hope this review can serve as a useful resource in understanding the current state of knowledge in the field of clinical applications of exosomes, in an effort to further solve the remaining challenges for fulfilling their potential.

LANDSCAPE OF EXOSOME RESEARCH—INSIGHTS FROM THE CAS CONTENT COLLECTION

The CAS Content Collection[15] is the largest human-curated collection of published scientific knowledge, representing a comprehensive resource to access and keep up to date on the world's published scientific literature across disciplines including chemistry, biomedical sciences, engineering, materials science, agricultural science, and many more, thus empowering quantitative analysis of global research publications against parameters such as time, scientific area, medical application, disease, and chemical composition. Currently, there are over 40,000 scientific publications (mainly journal articles and patents) in the CAS Content Collection related to exosomes/extracellular vesicles. Over 25,000 of them are related to the application of exosomes in drug delivery and diagnostics. There is a steady, exponential growth of these documents over time (Figure 4A). On Figure 4B, the number of documents (journal articles and patents) originating from organizations in the USA

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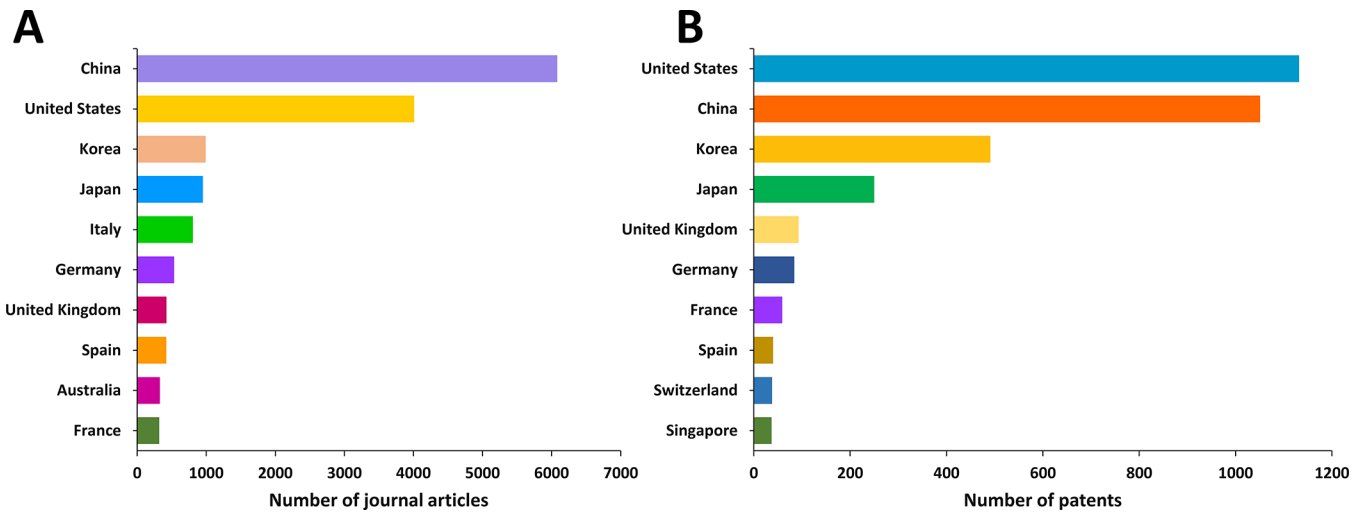

Figure 5. Top countries publishing journal articles (A) and patents (B) related to exosomes in drug delivery and diagnostics.

A

Companies	No. of patents
MD Healthcare	51
Codiak Biosciences	44
OncoTherapy Science	33
Evelo Biosciences	26
ExoCoBio	24
Evox Therapeutics	18
Figene	12
Orthogen	11
Arbor Biotechnologies	10
Samsung Life Public Welfare Foundation	10
Unicyte	9
Henry Ford Health System	8
Cavadis	7
Exosome Therapeutics	7
ExoStem Biotec	7
Reneuron Limited	7
Biorchestra	6
Flagship Pioneering Innovations VI	6
Isis Innovation Limited	6
NanoSomiX	6

B

Universities & Hospitals	No. of patents
University of California	43
University of Louisville	28
Zhejiang University	26
Xiangya Hospital Central South University	24
The University of Texas	23
Cornell University	20
National Center for Nanoscience and Technology	17
Cedars-Sinai Medical Center	16
Southeast University	15
The Catholic University of Korea	15
Korea Institute of Science and Technology	14
PLA Air Force Medical University	14
Yeditepe Universitesi	14
Massachusetts Institute of Technology	13
Mayo Foundation for Medical Education & Research	12
Morehouse School of Medicine	12
Ohio State University Innovation Foundation	12
The General Hospital Corporation	12
Jinan University	11
Soonchunhyang University	11

Figure 6. Top patent assignees from companies (A) and universities and hospitals (B) for patents related to exosome applications in drug delivery and diagnostics.

have been correlated with the funding from the National Institutes of Health (NIH),[52] increasing sharply after 2015.[53]

United States, China, Korea, and Japan are the leaders in the number of published journal articles (Figure 5A) and patents (Figure 5B) related to exosomes in therapeutics and diagnostics. Patenting activity related to exosomes is nearly equally shared between corporate and academic players (Figure 6). MD Healthcare, Codiak Biosciences, and OncoTherapy Science have the largest number of patents among the companies (Figure 6A), while University of California, University of Louisville, and Zhejiang University are the leaders among the universities and hospitals (Figure 6B).

Figure 7 presents the distribution of patents related to the application of exosomes in drug delivery and diagnostics with respect to the patent office. The World Intellectual Property Organization (WIPO) received the most patent applications, followed by the US and China patent offices, the European Patent Office (EPO), and the Korean and Japan patent offices. The percentage of Chinese patents, 27.2%, is well below the average number (63%) of chemistry-related Chinese patents in the CAS Content Collection from the last 10 years. This shows that exosome applications are emerging areas, and it may take some time to establish the technologies. At the same time, the percentage (49.8%) of patents filed through WIPO is significantly higher than the average number (18%) of chemistry-related WIPO patents in the CAS Content Collection, which indicates a strong desire of patenting exosome-related technologies internationally.

Patent protection is territorial, and thus, the same invention may be filed for patent protection in two or more jurisdictions. Therefore, we looked at all related filings on exosome applications in drug delivery and diagnostics. One patent family may be counted multiple times when it is applied in multiple patent offices. Figure 8 presents the flow of patent filings from different applicant locations to various patent offices of filing. There are diverse patent filing strategies: some patent assignees, such as those from China, file foremost in their home country patent office (CN), with a smaller proportion filing through the World International Patent Office WIPO (WO), or other jurisdictions. Others, for instance United States-based appli-

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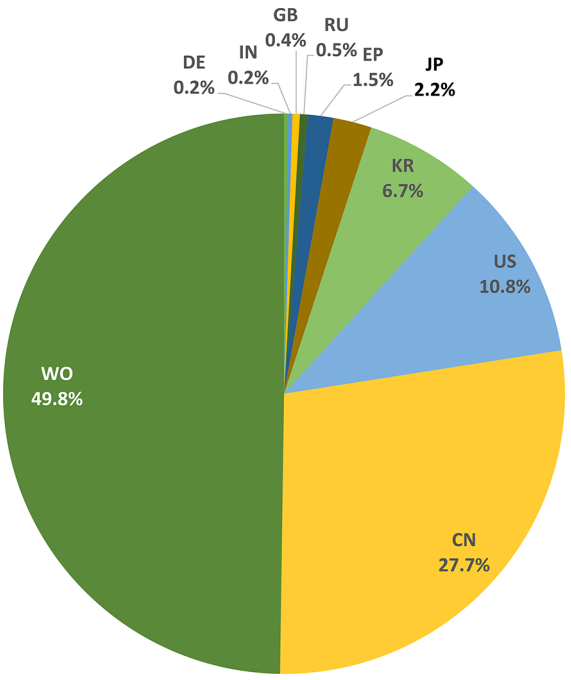

Figure 7. Top patent offices receiving patent applications for exosomes in drug delivery and diagnostics.

cants, have a nearly equal number of US and WO filings and a considerable number of filings at other patent offices such as the European Patent Office (EP).

We explored the presence and trends of selected essential concepts relevant to the exosome applications in drug delivery and diagnostics as they appear in the scientific publications (Figure 9). With respect to the cumulative number of documents, "targeting" and "biomarker" appear as top concepts in the area (Figure 9A), reflecting the rising interest in the application of exosomes in therapeutics with specificity and diagnostics. It is noteworthy that the "blood–brain barrier" concept, although with a relatively low cumulative number of publications, exhibits the greatest growth rate in the past 2 years (Figure 9B), characterizing it as the trendiest concept in the field.

The landscape of exosome research as revealed from the CAS Content Collection is further explored in the later sections of this paper with respect to the exosome components and their roles.

CHARACTERIZATION OF EXOSOMES

Exosome Pathway—Biogenesis, Secretion, Transport, Uptake. Exosomes are a population of extracellular vesicles. They are being secreted by many cell types using the endocytic pathway.[54] The formation of exosomes includes three steps: (i) the endocytic vesicles form from the plasma membrane; these early endosomes mature into late endosomes; (ii) the endosomal membrane experiences inward budding, forming multiple intraluminal vesicles (ILVs) encapsulated within multivesicular bodies (MVB); (iii) the latter either fuse with the lysosome and bring the ILVs to degradation or access the cell membrane and discharge the ILVs in the form of exosomes (Figure 1).[28,55] Thus, MVBs and late endosomes comprise ILVs, capturing certain proteins, lipids, and substances from the cytosol. The cytoskeleton and the microtubule network are the routes by which MVBs are transported to the cell membrane where they fuse with the cell membrane and undergo exocytosis.

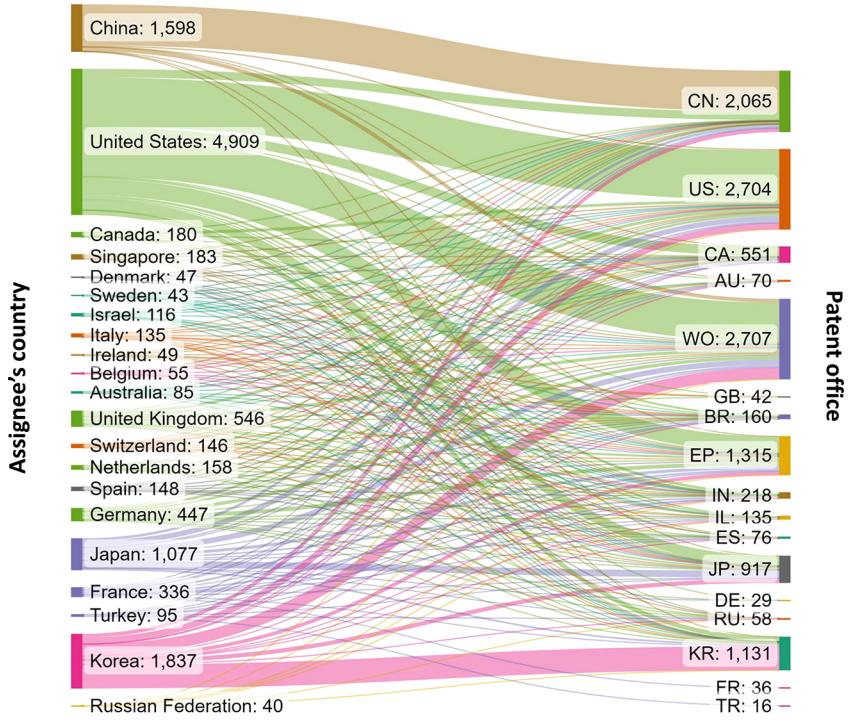

Figure 8. Flow of patent filings related to exosome applications in therapy and diagnostics from different patent assignee locations (left) to various patent offices of filing (right). The abbreviations on the right indicate the patent offices of China (CN), United States (US), Canada (CA), Australia (AU), World Intellectual Property Organization (WO), Great Britain (GB), Brazil (BR), European Patent Office (EP), India (IN), Israel (IL), Spain (ES), Japan (JP), Germany (DE), Russian Federation (RU), Korea (KR), France (FR), and Turkey (TR).

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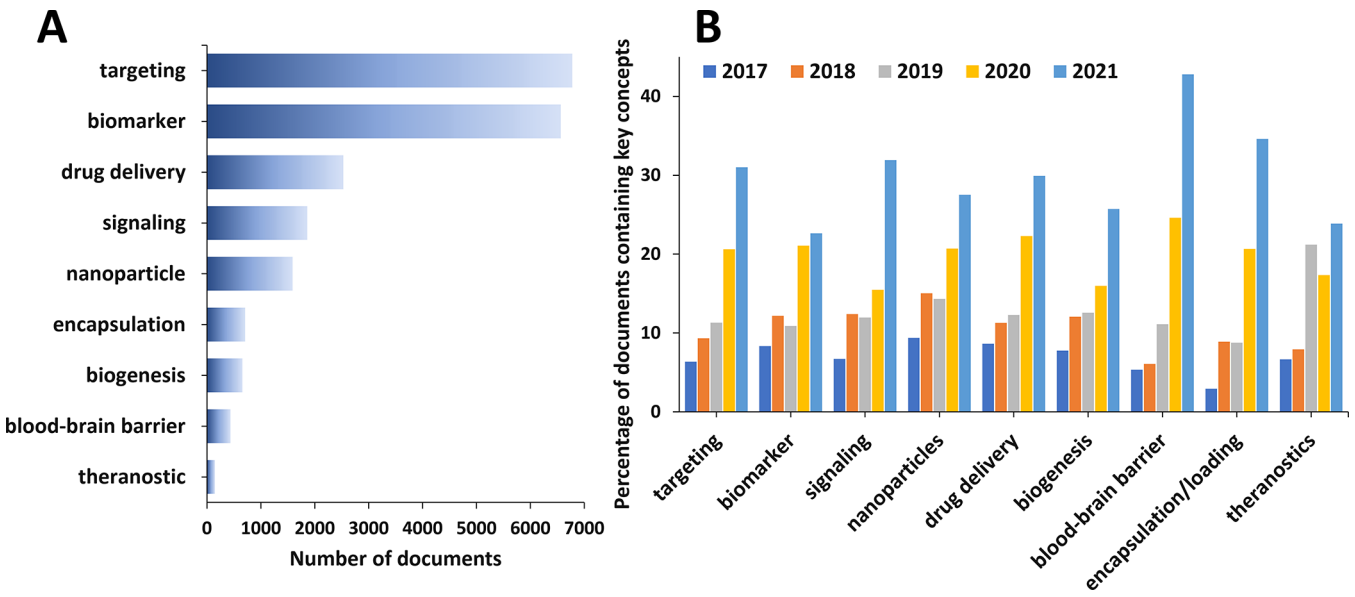

Figure 9. Key concepts in the scientific publications relevant to the exosome applications in drug delivery and diagnostics. (A) Number of publications exploring key concepts related to exosome applications in therapy and diagnostics. (B) Trends in key concepts presented in the articles related to exosome applications in therapy and diagnostics during the years 2017−2021.

Table 1. Roles of Exosomes in Health and Disease

Exosome role	Details and references
cell−cell communication	Exosomes can participate in an autocrine, paracrine, or endocrine communication reaching their target cells via the systemic or local circulation. They are important participants in cell communication including cell migration, proliferation, and senescence.[66,67]
immune response	The cells of the immune system are known to release exosomes.[29] Exosomes mediate immune modulation, both immunosuppression and immunostimulation.[68]
signal transduction	Exosomes enable intercellular communication between various types of cells, regulating gene expressions and cellular signaling pathways of recipient cells by delivering their components, such as specific lipids, proteins, and RNAs. Certain lipid components including sphingomyelin, cholesterol, and ceramides have been involved in signaling;[69,70] phosphatidylinositol-3-phosphate (PI3P) is also known to participate in regulating cell signaling.[71] The presence of multiple kinds of signaling molecules—lipids, proteins, and RNAs—results in rapid signal changes in the target cell.
material (cargo) transport	Exosomes transport their constituents involving proteins, nucleic acids, lipids, and metabolites between cells, both in the close vicinity of the parent cell and at distant sites in the body carried by biofluids. It has been reported that RNA cargo of exosomes can modify gene expression in recipient cells.[72,73]
pathogenesis	Viruses are known to make use of exosome biogenesis pathways to release a variety of pathogenic factors. Thus, a number of pathogen-derived components have been detected on exosomes after infection. These include, e.g., human immunodeficiency virus, Epstein−Barr virus, cytomegalovirus, hepatitis C virus, and herpes simplex virus.[74] Exosomes play multiple roles in the progression of cancer via various communication pathways.[75] Exosomes are more often released by tumor cells than by healthy ones and facilitate communication within the tumor microenvironment.[76]
blood−brain communication	Exosomes are able to cross the BBB in both directions—from the brain to the bloodstream and from the blood to the CNS. Moreover, exosomes can interact with the BBB, leading to changes in the barrier's properties.[77]
target cell delivery	The delivery of cargos such as bioactive RNAs, proteins, metabolites, and/or lipid makes the capture of exosomes by target cells of vital importance in a variety of key biological processes such as angiogenesis,[78] bone development,[79] and cell migration.[80]

This way, the ILVs are being secreted as exosomes.[56,57] Other MVBs exhibit degradation through lysosomes.

Indications exist that the endosomal mode of exosome formation—by endosomal budding—is not the only way of exosome biogenesis. Evidence has been accumulated indicating that exosomes may also bud from the plasma membrane directly.[4,58−60] Altogether, the exosome biogenesis is a complex process with multiple participants involved in essential cellular functions.

The extracellular circulation half-life of exosomes has been estimated to be approximately 2−30 min according to reported pharmacokinetic profiles.[61] Currently, there is certain knowledge regarding the exosome biogenesis and secretion, but there is still insufficient data regarding the uptake of exosomes by various cells and their signaling pathways. Internalization of the exosomes by the recipient cells follows the common endocytic pathways; e.g., it might be mediated by clathrin, lipid rafts, caveolins, through phagocytosis, or through micropinocytosis.[57] Likewise, after internalization, exosomes follow the usual endosomal routes.[62]

Exosomes are membrane-bound carriers. Like other EVs, they are surrounded by a lipid membrane, which encloses their cargo. The typical exosome cargo includes mainly peptides, small proteins, and nucleic acids, such as mRNA, microRNA (miRNA), and non-coding RNA (ncRNA).[63] These are used by the cell for signaling, to manage biological functions and to preserve homeostasis.[64]

Physiological Functions of Exosomes in Health and Disease. The intercellular traffic of exosomes plays a significant role in many physiological and pathological processes, including immune response, tissue homeostasis and regeneration, as well as in development of diseases such as cancer, neurodegenerative, cardiovascular, and other disorders. They are key players in cell−cell communication, signal transduction, extracellular matrix support and remodeling, and various other important physiological activities (Table 1). Furthermore, exosomes play significant roles in viral infections.[65]

Exosome Composition. Nearly 100,000 proteins and over 1,000 lipids are found related to exosomes, along with a multitude of mRNAs and miRNAs, according to various

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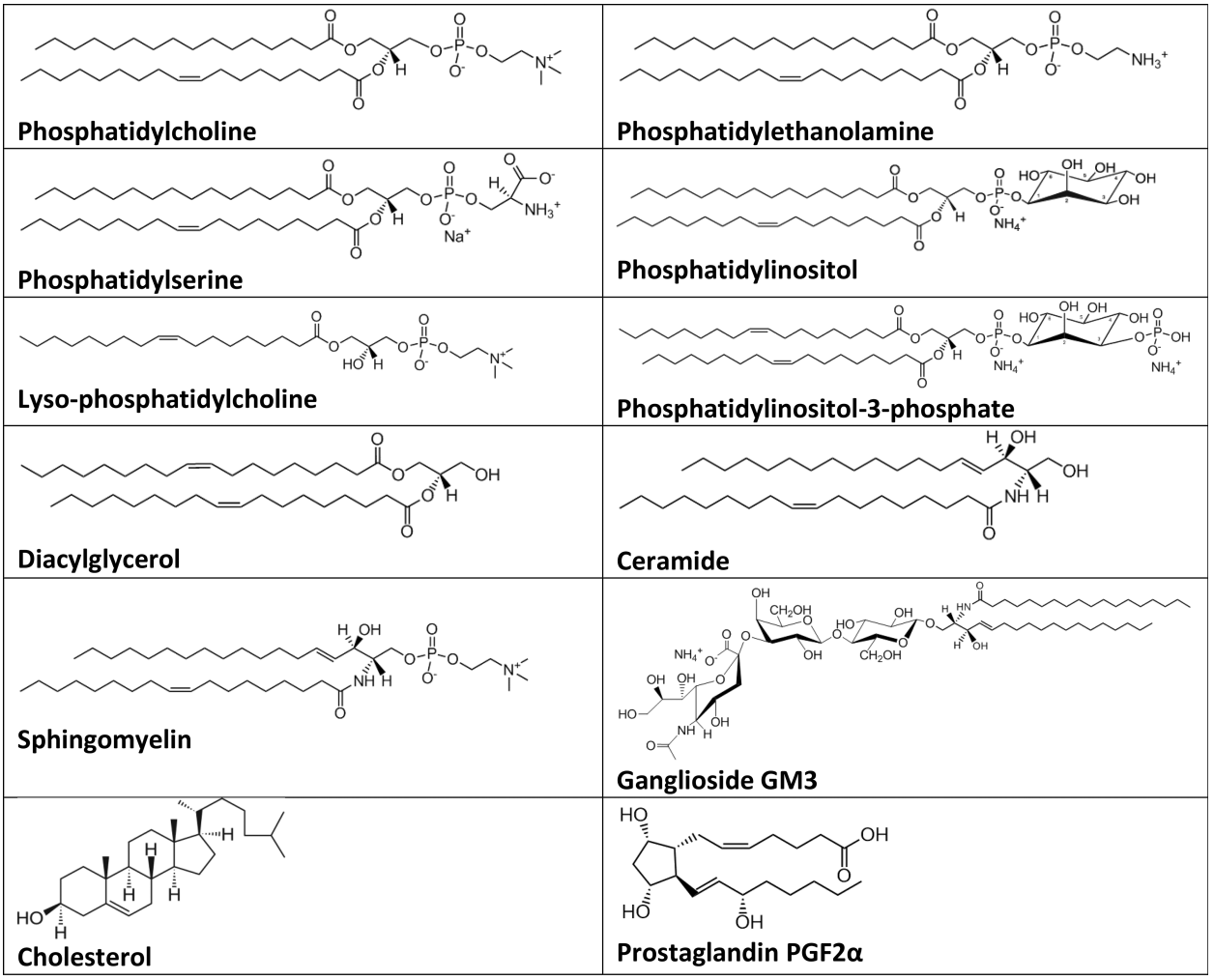

Figure 10. Representative molecular structures of the major lipid classes in exosomes.

available database collections such as ExoCarta,[81] a web-based compendium of exosome proteins, RNA, and lipid database information;[82] Vesiclepedia,[83] a community compendium for extracellular vesicles; and EVpedia,[84] a web-based resource providing bioinformatics tools for extracellular vesicles research.[85] The contents enclosed into exosomes vary depending on the cell types and cellular conditions. Exosomes include proteins originating from the intracellular endosomal component. They include heat shock proteins, membrane transport proteins and fusion proteins, as well as a multitude of tetraspanins, a transmembrane protein family.[86,87] With respect to lipid constituents, the exosomal content of cholesterol, sphingomyelin, saturated phosphatidylcholines, and phosphatidylethanolamines is higher than that of the plasma membrane.[88]

With respect to substance classes represented in the publications related to the exosome applications in drug delivery and diagnostics in the CAS Content Collection, nucleic acids have the highest share (Supporting Information Figure S1). Indeed, the capability of exosomes to carry nucleic acids from cell to cell is one of their major features attracting attention nowadays. As natural intercellular shuttles of RNAs, they affect many physiological and pathological processes and are the appropriate nanocarriers for targeted delivery of nucleic acids.[89] Moreover, they have been identified as biomarkers for diagnosing of diseases, particularly various cancers. The RNAs, which have been examined include ncRNAs: microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs) carried by exosomes.[90]

Lipids. Lipids are essential constituents of biological membranes. As such, they are also abundant in exosomes. The major membrane lipid classes include phosphoglycerolipids, sphingolipids, and sterols. Certain lipid kinds are enriched in exosomes compared to their parent cells, suggesting that some membrane reorganization occurs upon exosome biogenesis.[91] Lipid species enriched in exosomes include ceramides (Cer), sphingomyelins (SM), and gangliosides GM3 from the class of sphingolipids; phosphatidylserines (PS), phosphatidylethanolamines (PE), phosphatidylcholines (PC), lyso-phosphatidylcholines, and phosphatidylinositols (PI) from the class of phospholipids; diacylglycerols (DAG); as well as cholesterol (Figure 10).[92,93]

The parental cell types and their physiological status are determinants of the proportion of the lipid content in exosomes.[94−96] Lipids are critical players in exosome biogenesis. As a result of their various molecular shapes—cone or inverse cone—they tend to generate negative or positive membrane curvatures. Lipids with large headgroups such as PIs and gangliosides or single-chain lipids such as lyso-PCs induce positive membrane curvature, while smaller headgroup lipids such as PEs or lipids lacking a hydrophilic headgroup such as ceramides and DAGs induce negative membrane curvature.[97,98] Since these lipid classes are enriched in exosomes, they can

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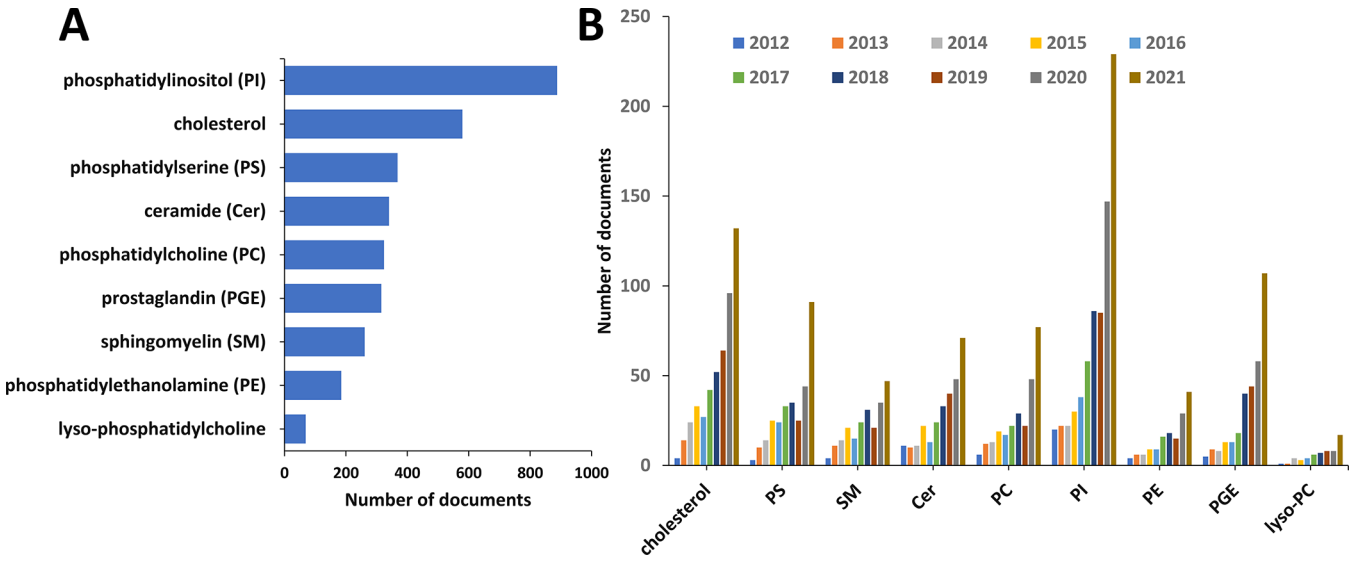

Figure 11. Number of documents mentioning specific types of lipids related to exosome applications in therapeutics and diagnostics. (A) A top list of classes of lipids with the numbers of associated documents. (B) Annual growth of document numbers.

significantly modify their membrane curvature. As a rule, membrane curvature is very important for cellular functions and trafficking.[99]

It has been reported that exosomes adjust their lipid composition to adjust to their biological function. Such enrichment of specific lipid classes with respect to the parental cells has been commonly observed.[94,100,101] Thus, exosomes are typically enriched in cholesterol[102] which supposedly accumulates in MVBs. It is important for the generation of intraluminal vesicles, the precursors of exosomes.[100] Plasma membrane lipid rafts—ordered and tightly packed membrane microdomains organizing the assembly of signaling molecules for promoting signal transduction—are supposedly the origin of the high sphingomyelin content in exosomal membranes.[100,103]

The distribution of lipids in the two layers of the exosomal membrane lipid bilayer has been reported to be asymmetrical.[93] Generally, asymmetric arrangement of lipids in the two membrane leaflets is a fundamental feature of the biological membranes. It is a consequence of various factors, including the biophysical properties of lipids, including their miscibility, the ionic composition of the media on both sides of the membrane, as well as the presence of transporter enzymes that actively support and maintain the lipid distribution across the bilayer, including flippases, floppases, and scramblases.[104−106] Membrane asymmetry is believed to be associated with important biological functions such as apoptosis, cell fusion, and signaling.[107] In the exosomal membranes, the sphingomyelin is typically found mostly in the outer leaflet and phosphatidylserines in the inner leaflet, while phosphatidylethanolamines seem to be randomly distributed across the bilayer.[108] However, phosphatidylserine is externalized in apoptotic and malignant cells, attracting macrophages from the immune system.[109] This finding is also useful from the viewpoint of possible use as exosomal lipid biomarkers for cancer diagnosis.[110] Certain lipids are predominantly allocated in certain types of exosomes.[111] The process of biogenesis of exosomes and cargo packaging appears as a well-controlled process with lipids playing an important role.[112]

Figure 11 illustrates the results from a search on various lipid classes in the CAS Content Collection in documents related to the medical application of exosomes. Phosphatidylinositol and its derivatives appear to attract significant attention in the field. Seven phosphorylated phosphatidylinositols (PIPs) have been identified in membranes. They can be transformed into each other by phosphorylation or dephosphorylation of the 3-, 4-, and 5-hydroxyl groups of the inositol headgroup (see the example of PIP3 in Figure 10). PIPs are known as precursors for certain second messengers involved in signal transduction and, noteworthy, regulate membrane dynamics and vesicular transport. They have been reported to significantly affect exosome secretion[69] and macrophage targeting,[113] which may have provoked the strong attention in the published literature (Figure 11).

Cholesterol is another lipid that attracts strong attention in the recent exosome publications (Figure 11). Indeed, cholesterol has been reported to be essential for the entire development of exosomes, for their biogenesis and release, for their membrane stability, and for entrance into the target cells.[114] Furthermore, reports show that exosomes constitute part of the cellular machinery taking care of the cholesterol balance and that they can assist in detecting and combating cholesterol-related pathologies.[114]

Exosomal enzymes are responsible for the production of bioactive lipids in exosomes. For example, exosomes contain A2 phospholipases, which hydrolyze glycerophospholipids to generate arachidonic acid and other free fatty acids.[115] Arachidonic acid can be processed to release leukotrienes such as LTB4, involved in the inflammation, and LTC4/LTD4 which promote angiogenesis. Exosomal cyclooxygenases COX1/2 and the PGE synthase promote the transformation of arachidonic acid to the pro-inflammatory prostaglandin E2 (PGE2) (structure included in Figure 10) or to the anti-inflammatory and tumor-suppressing 15-deoxy-prostaglandin J2.[63,115] The role of exosomes in mediating lipid metabolism during cancer progression is attracting much attention recently. Bioactive exosomal lipids, e.g., the prostaglandins PGE2α, PGE1, and PGE2, are known to be released from macrophages into the cancer microenvironment.[116] Such bioactive lipids are a subject of increasing interest in the recent exosome publications (Figure 11).

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Table 2. Exosomal Proteins with respect to Their Location and Role

Proteins	Examples
	Exosomal Proteins with respect to Their Location
integral transmembrane proteins[117−119]	tetraspanins (CD81, CD82, CD37, CD63)
lipid-anchored outer membrane proteins[43,120−123]	ectonucleotidases (CD39, CD73); sperm receptor Juno; complement-inhibiting proteins (CD55, CD59); glypican-1; prion proteins (PrP^{C}, PrP^{SC})
lipid-anchored inner membrane proteins[34,35,58,124−127]	prenylated GTPases (Rabs, Ras, Rho), myristoylated signaling kinases (Src), palmitoylated membrane proteins, acylated Gag proteins
peripheral outer surface proteins[128−135]	wingless (Wnt) proteins; BMPs; TGF β; tumor necrosis factor (TNF); cytokines; extracellular matrix (ECM) proteins (fibronectin, tenascin C, ECM1)
peripheral inner membrane proteins[4,102,136−142]	scaffolding factors including the ezrin-radixin-moesin (ERM) proteins and ERM ligands (EBP50, CD43, CD44, IGSF8, PTGFRN); syntenin; ALIX; heat shock proteins (HSP70, Hsp40/DnaJ proteins, Hsp90, Hsp20, Hsp27, α/β-crystallins)
enzymes[27,120,143,144]	ATPase, pyruvate kinase, fatty acid synthases, phosphatases, pyrophosphatases, calcium-binding annexins, phosphate transporters; RNA editing enzymes, lipases, proteases, glycosyl transferases, glycosidases, metabolic enzymes
cytosolic proteins[117,145]	clathrin, HSC70, HSP70, HSP60, HSP90, ALIX, YWHAE, ubiquitin, TSG101, ESCRT
	Exosomal Proteins with respect to Their Role
membrane transport and fusion-related proteins	annexin, Rab-GTPase, heat shock proteins (HSPs), e.g., Hsp60, Hsp70, Hsp90
membrane organization and trafficking	tetraspanins: CD9, CD63, CD81, CD82, CD106, Tspan8, ICAM-1
multivesicular-body (MVB)-related proteins	ALIX, TSG101
cell-adhesion-related proteins	integrins
cytoskeletal proteins	actin, myosin

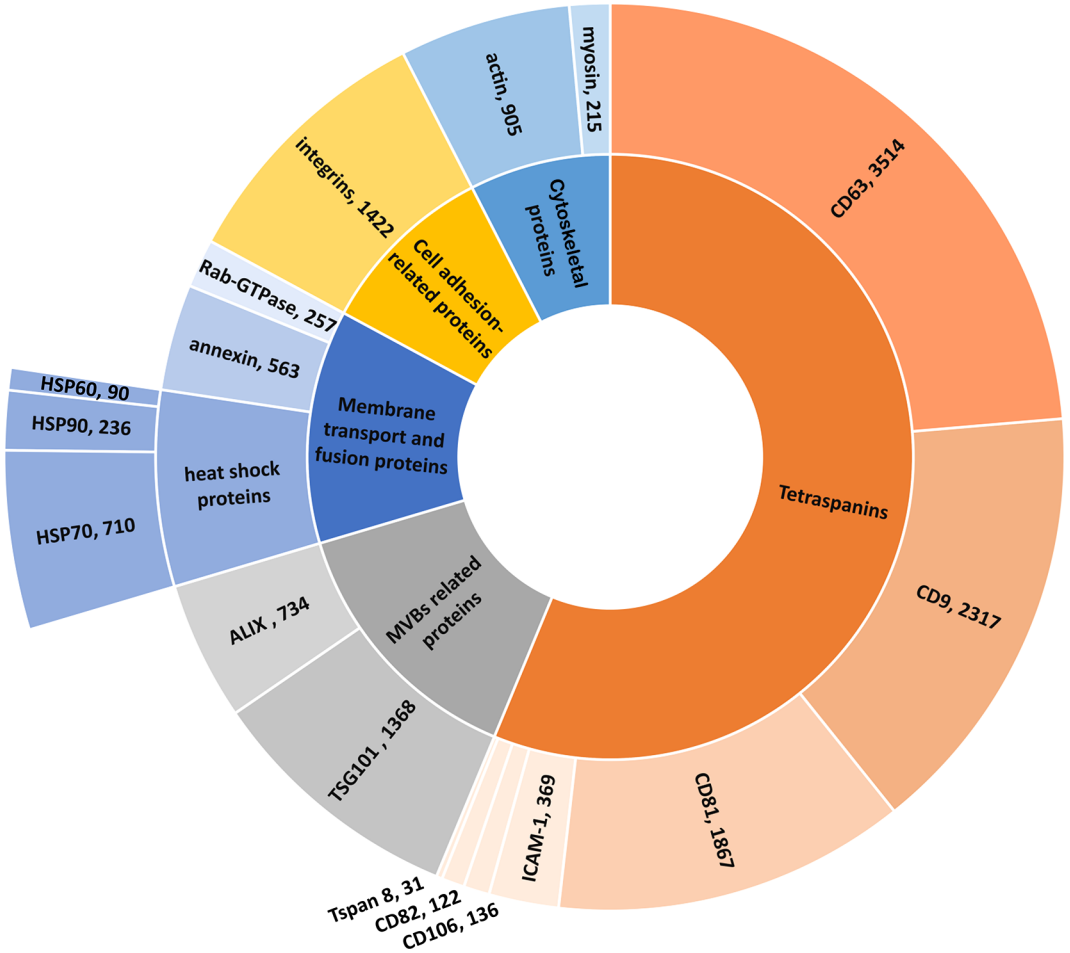

Figure 12. Number of documents concerning major exosome proteins in the documents related to exosome applications in therapeutics and diagnostics.

Proteins. Exosomes comprise a broad collection of proteins including transmembrane proteins, lipid-anchored membrane proteins, peripheral membrane proteins, as well as soluble proteins inside the exosome core (Table 2).[4]

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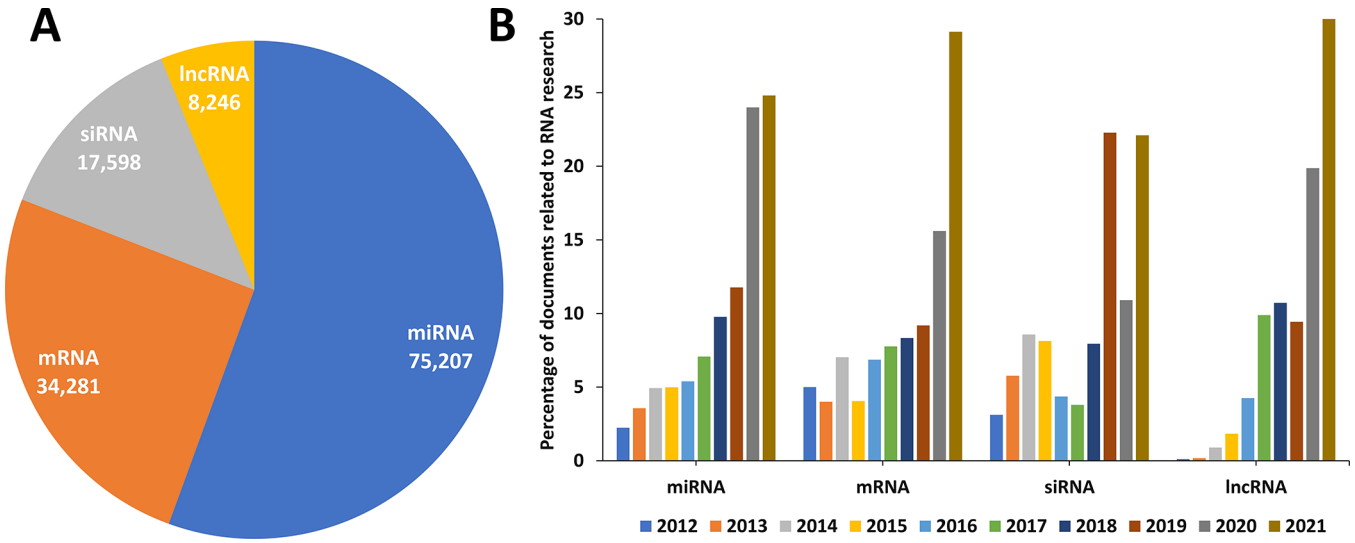

Figure 13. Types of RNA molecules in exosome applications and their document counts. (A) Distribution of the number of documents related to exosome applications in therapy and diagnostics concerning various RNAs during the years 2012−2021. (B) Annual trends in the number of the same documents. (Percentages are calculated with yearly publication numbers for each RNA, normalized by the total number of publications for the same RNA in the same time period.)

Exosomes are rich in certain tetraspanins such as CD81, CD82, CD37, and CD63, representatives of the class of the integral membrane proteins.[117] They are membrane proteins including four transmembrane α-helices. Tetraspanins are believed to have an important role as organizers of transmembrane and cytosolic proteins, as well as lipids (e.g., cholesterol) into a membrane network, the tetraspanin web. Tetraspanins do not exhibit catalytic activities; their function is to facilitate the trafficking, functioning, and stability of the other membrane proteins.[118] CD81 and CD63 are frequently used exosomal marker proteins, as well as CD9, another exosomal tetraspanin, which mediates the membrane metalloendopeptidase CD10 loading into exosomes.[118,119] Certain surface proteins alter the exosome circulation time. For example, the occurrence of CD47 or CD55/CD59 on the exosomal membrane can extend the blood circulation time by avoiding phagocytic clearance.[12] CD47 has been found on the surface of EVs secreted by fibroblasts, T-cells, and MSCs,[146] and EVs secreted by antigen-presenting cells and retinal pigmented epithelium express CD55 and CD59.[147]

Lipid-anchored proteins including some glycosyl-phosphatidylinositol-anchored proteins are present on the exosome surface. Between them are the ectonucleotidases CD39 and CD73,[120] the complement-inhibiting proteins CD55 and CD59,[121] glypican-1,[43] prion proteins,[122] and the Hedgehog morphogens attached to the outer layer by their cholesterol portion.[123]

Exosomes also involve peripheral surface proteins playing key roles in signaling. Representatives of this class are the wingless (Wnt) proteins,[128] surface-bound bone morphogenetic proteins,[129] transforming growth factor β,[130] tumor necrosis factor,[131] cytokines,[132] and a large collection of other surface signaling molecules. Extracellular matrix proteins including fibronectin, tenascin C, and ECM1 are also at the exosome surface.[133,134] Some peripheral proteins are attached to the exosomal phosphatidylserine.[135]

The exosome inner membrane leaflet carries acylated, lipid-anchored proteins, including prenylated GTPases, myristoylated signaling kinases, and palmitoylated membrane proteins.[34,35,124] A large part of the composition of exosomes released by infected cells include acylated Gag proteins.[58,125] Furthermore, some Gag proteins, such as the activity-regulated cytoskeletal (ARC) protein, play a critical role in cognition.[126,127]

The distribution of publications in the CAS Content Collection among exosomal proteins is presented in Figure 12. Because of the high content of tetraspanins in exosomes, they are most often used as specific exosome markers,[117] which explains the abundance of tetraspanin-related publications in those concerning exosome applications in therapy and diagnostics (Figure 12). The distribution of documents concerning the major tetraspanin classes as well as their roles, in particular in therapy (THU) vs diagnostics (DGN), are shown in Supporting Information Figure S2.

Nucleic Acids. Exosomes comprise RNAs and can transfer them to other cells and tissues. The exosome-mediated RNA transfer has been initially reported for mRNAs and miRNAs.[32,33,148,1494,149,150] Exosomal RNA pools are enriched in small non-coding RNAs (sncRNAs) and differ from the cellular RNA profile.[72,151,152] A wide variety of RNA species are embedded in the extracellular vesicles. The extracellular vesicles compendium Vesiclepedia lists over 10,000 entries of EV miRNAs and over 27,000 entries of EV mRNAs.[83] Upon internalization of exosomes by the recipient cells, the variety of cargo RNA species can be released. The subsequent translation of mRNA into active proteins may result in phenotypical changes.[32]

miRNA is the dominating RNA found in exosomes. It has been reported that exosomal miRNAs play an important role in intercellular communication. Multiple examples of EV-mediated transfer of miRNAs have been established for a variety of physiological and pathological events. Reports show that it is highly abundant in exosomes, with the five most common miRNAs being miR-99a-5p, miR-128, miR-124-3p, miR-22-3p, and miR-99b-5p.[153] mRNAs carried in the extracellular vesicles can serve as a source of proteins in recipient cells. Active translation of exosomal mRNAs into recipient cells was reported, such as expression of reporter proteins from mRNA

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transferred by extracellular vesicles between mast cells and from glioblastoma to endothelial cells.[32,33]

Exosomes also contain multiple kinds of DNAs, including single-stranded, double-stranded, genomic, and mitochondrial DNAs.[154−157] Certain inflammatory processes and cell aging are thought to rationalize the presence of DNA in exosomes.[64] There is more DNA in exosomes derived from cancer cells than from healthy cells. It has been suggested that DNA secretion originating form exosomes may affect the inflammation regulation. It is viewed as a potential marker of cancer, viral infection, or chemotherapeutic resistance.[4,155,158]

Analysis of the data available in the CAS Content Collection confirmed that exosomal RNAs are dominated by miRNAs (Figure 13A). Annual distribution of the RNA-related documents within the pool of publications concerning exosome applications in therapy and diagnostics shows an explosive growth in the last 2−3 years for all RNA types (Figure 13B), which is impressive, even considering the overall rapid growth in interest in all RNA medicines.[150]

Glycoconjugates. Polysaccharides and glycans are other exosomal constituents located on their outer surface.[159,160] They are predominantly enriched in

- mannose
- α-2,3- and α-2,6-sialic acids
- complex N-linked glycans
- high-mannose N-glycans
- heparan sulfate
- polylactosamine

The role of glycans in exosome biology is less well understood than that of proteins, lipids, and nucleic acids, yet there is evidence that surface glycoconjugates play important roles in exosome biogenesis, release, targeting, and uptake by cells.[161] Glycoconjugates appear to be an additional source of exosome biomarkers as well (Table 3), since variation in glycosylation is characteristic of different types of cancers.[162,163]

Table 3. Examples of Glycoconjugate Tumor Markers in Exosomes

Tumor marker[a]	Cancer	Body fluid/cells
α-fetoprotein	liver and germ cell tumors	blood
β-human chorionic gonadotropin	choriocarcinoma, germ cell tumors	urine, blood
C-kit/CD117	gastrointestinal tumor, melanoma	tumor cells
CA15-3/CA27.29 (MUC1)	breast cancer	blood
CA19-9	pancreatic, gallbladder, bile duct, stomach cancers	blood
CA-125 (MUC16)	ovary cancer	blood
carcinoembryonic antigen (CEA)	colorectal cancer	blood
estrogen receptor (ER)	breast cancer	tumor cells
HE4	ovary cancer	blood
HER2/neu	breast, stomach, gastroesophageal adenocarcinoma	tumor cells
prostate-specific antigen (PSA)	prostate cancer	blood

[a]Tumor markers selected from https://www.cancer.gov/about-cancer/diagnosis-staging/diagnosis/tumor-markers-fact-sheet.

APPLICATIONS OF EXOSOMES

Methods for Exosome Isolation/Purification. Speedy, straightforward isolation methods offering high purity and recovery are a requirement for large-scale applications of extracellular vesicles in clinics. Each of the available methods discussed below brings about certain advantages and disadvantages to exosome isolation and purification. Based on the application purpose, different methods can be applied for exosome separation and analysis.

Ultracentrifugation-Based Isolation Techniques. Ultracentrifugation is capable of generating very high centrifugal forces, up to 1,000,000*g*, and is currently one of the most frequently used methods for exosome isolation,[164] considered as a gold standard before 2015 (Figure 14). This approach does not require much expertise and is affordable over time. Furthermore, it is not rather time-consuming, without considerable sample pretreatment. Differential ultracentrifugation (DUC) and density gradient ultracentrifugation (DGUC) are the two popular kinds of preparative ultracentrifugation.

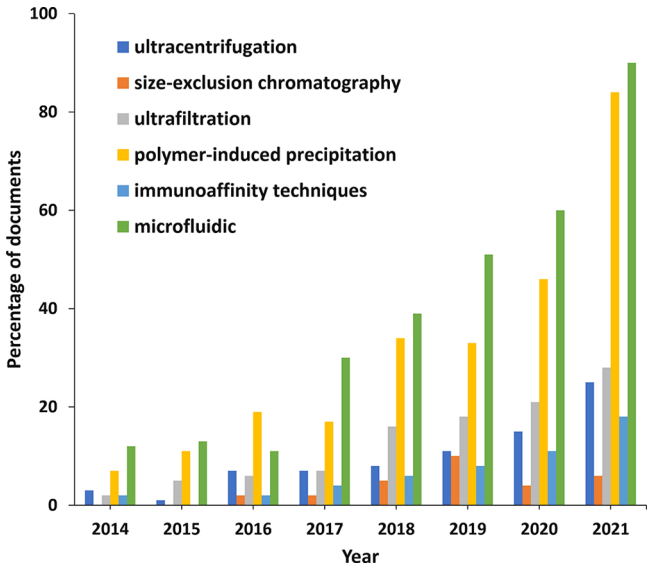

Figure 14. Trends in the number of documents related to exosome applications in therapeutics and diagnostics concerning various exosome isolation methods during the years 2014−2021. (Percentages have been calculated with yearly publication numbers for each isolation method, normalized by the total number of publications for the same isolation method in the same time period.)

DUC includes several steps with continuously increasing centrifugation forces and durations, with the purpose of sequentially isolating smaller particles from large ones, such as whole cells, cellular debris, and macromolecular proteins. Finally, exosomes are separated by ultracentrifugation at 100,000−150,000*g*. The technique is time- and effort-consuming compared with DGUC, because of the multiple steps. In addition, heterogeneity of exosomes and overlap in the size of extracellular vesicles lead to contaminations.[165−167]

The separation of particles by DGUC is based on their size, shape, and density by utilizing an inert medium of graded densities.[168] Under a given centrifugal force, components of a sample will reside in the zone corresponding to their density. This technique has a relatively higher separation efficacy and thus provides higher purity. It is noteworthy that exosomes are not likely to be crushed during the separation.[166] However, this

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Table 4. Major Methods of Exosome Isolation/Purification

Method	Principle	Advantages	Disadvantages
ultracentrifugation	density- and size-based sequential separations	• appropriate for large-volume samples	• high equipment cost
		• markers not introduced	• labor-intensive
		• cost-effective	• potential damage of exosomes
			• low yield
ultrafiltration	using a membrane filter with a defined size-exclusion limit or molecular weight cutoff	• low cost	• potential damage of exosomes
		• time efficient	• membrane clogging and blockage
		• simple	
immunoaffinity	exosome capture based on antigen−antibody-specific recognition and binding	• high specificity	• potential damage of exosome integrity
		• simple	• expensive reagents
		• scalability	• non-specific binding
polymer precipitation	hydrophilic water-excluding polymer adhering and precipitating exosomes	• broad applicability	• lack of specificity and selectivity
		• simple and rapid	• low purity
		• no exosome deformation	• contamination with polymers
microfluidics	immunoaffinity, size, density	• high efficiency	• large volumes of starting materials
		• fast processing	• low sample capacity
		• good portability	
		• easy automation and integration	
size-exclusion chromatography	exosome separation based on hydrodynamic radii	• preserve biological activity	• potential contamination
		• no preprocessing	• high equipment cost
		• high yield	

method also has the issue of unwanted aggregation of particles as well as the contamination of proteins and nucleic acids.

Ultrafiltration. Ultrafiltration is a size-based technique frequently used for exosome isolation. Exosomes are being isolated using membrane filters with a specific pore size defining their molecular weight or size-exclusion limits. A microfluidic device consisting of ciliated micropillars has been fabricated to isolate exosomes.[169] Commercial exosome isolation kits have been designed for exosome isolation from serum, cell culture medium, and other body fluid.[170] The kits apply rapid fractionation including a syringe filter with two membranes to capture the exosomes and the larger extracellular vesicles. It is noteworthy that a combination method of ultrafiltration with size-exclusion chromatography (SEC) has been successfully applied in the isolation of exosomes from adipose tissue.[171]

Immunoaffinity Techniques. As more cell-type and disease-specific protein receptors in the exosomal membrane are being identified, opportunities are created to develop highly efficient techniques for exosome isolation. Immunoaffinity techniques have been developed employing the affinity interactions between surface proteins and corresponding antibodies. Preferably, markers for exosome immunoisolation are membrane-attached without soluble parts and are only located on the surface of exosomes. Thus, the widely popular enzyme-linked immunosorbent assay (ELISA) has been established for isolating and quantifying exosomes from sources such as blood serum, plasma, and urine.[172−176] ELISA results are typically expressed as absorbance measures to enable a quick comparison with standards of known exosome counts, thus enabling absorbance measures to be calibrated to quantify the resultant exosomes. By means of the microplate immunoaffinity approach, the distinction and yield of exosomes can be assessed with respect to ultracentrifugation. This method is highly specific, resulting in extremely pure exosome populations. A corresponding kit was developed for exosome isolation based on this theory and enables fast isolation of high-purity and high-yield exosomes.[177]

Exosome Precipitation—Polymer-Based Precipitation Techniques. By modifying their solubility or ability to disperse, exosomes can be precipitated from biological bodily fluids. Polymers that exclude water such as PEG are utilized for this purpose. Such polymers bind water and components of lower solubility out of the solution. Samples are incubated with a PEG (MW ∼ 8000 Da) precipitation solution. PEG binds water molecules and thus expels less soluble components out of the solution.[178] Subsequently, the sediment containing exosomes is settled out by centrifugation or filtration. This approach is easy to conduct and scalable for large sample size, which allows easy transition to clinical applications. To date, several commercial kits utilizing PEG for the isolation of exosomes have been developed. One of the most widely used kits is ExoQuick (System Biosciences, Mountain View, CA, USA).[171] Some of these kits are developed to be compatible with body fluids, as well as culture medium. Selected kits are summarized and discussed in the following section. Samples usually require precleaning from cells and cellular debris before carrying out the precipitation. Urinary exosome precipitation by these kits has been shown to achieve the highest yield compared to other techniques. The disadvantage of the method is that the presence of the polymer may affect downstream analysis due to the positive charged molecules.[179]

Microfluidics-Based Isolation Techniques. These techniques utilize high-throughput microfluidic tools to isolate exosomes based on concepts including size, density, and immunoaffinity. The immuno-microfluidic method is developed based on the immunoaffinity capture technique. Exosomes are

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isolated by the specific binding of antibodies immobilized on the microfluidic chips and bind specifically to exosome markers (antigens).[180−182] The advantages of this technique include efficient, speedy processing and high grade of purity. Modifications of the microfluidic method such as size-based microfluidic isolation using the exosome total isolation chip (ExoTIC),[183] acoustofluidic,[184] and dielectrophoretic[185] techniques have been successfully applied. The tools are very complicated and expensive with requirement of specific fabrication skills. In summary, microfluidics is an advanced and promising technology, but it still needs certain regularization in order to be considered as a standard exosome isolation method.

Size-Exclusion Chromatography. According to the accumulating evidence, size-exclusion chromatography (SEC) has been considered as the most preferred method for isolation and purification of exosomes.[186] Exosome isolation using SEC has a low level of contaminants, resulting in a homogeneous isolation of exosomes. This circumstance has promoted the use of SEC among its competitor techniques for body fluid exosome-related biomarker identification. SEC has been used successfully for isolation, purification, and enrichment of exosomes from an assortment of biological fluids including plasma, serum, urine, cerebrospinal fluid, saliva, milk, and tears. SEC is advantageous because it does not require a large sample volume and the shearing force generated in SEC does not likely damage the original structure of the vesicles. These distinctive properties make this technique preferable compared to centrifugation.[187] Presently, SEC is a commonly used technique for isolation of exosomes from both blood and urine samples.[188,189]

A summary of the most widely used exosome isolation techniques is highlighted in Table 4, including the isolation mechanisms, advantages, and disadvantages.[168,186,190−192] Other isolation methods and method modifications have also been applied, e.g., asymmetric flow field-flow fractionation (AF4),[193] aptamer-based isolation,[194] and others.[168] Even though various exosome purification approaches have been developed, it is difficult for one method to solve all the associated challenges such as low yield, contaminations, and variations between batches. The combination of several methods to isolate and purify exosomes would be needed to characterize exosomes effectively and comprehensively. And it has been suggested as a promising strategy for improvement of the isolation outcome, in order to provide exosome subsets with high purity, in particular with respect to size, morphology, density, number, presence of exosome-enriched markers, and lack of contaminants.[168]

The annual trends in the number of documents related to exosome applications in therapeutics and diagnostics concerning various exosome isolation methods during the years 2014−2021 are shown in Figure 14. The precipitation and microfluidic methods are dominating the field, because of their broad applicability and high efficiency.

Exosomes as Drug Delivery Vehicles. Exosomes provide distinct benefits as highly efficient drug carriers. They have been recognized as a successful platform for delivery of various drugs because of their ability to mediate cellular communications.[195] Exosomes can be modified by means of their parental cells to exhibit the desired targeting capability and being loaded with therapeutic agents with anticipated biological activity. Exosomal drug formulations are applicable to many diseases including cancers and infectious, cardiovascular, and neurodegenerative disorders. Generally, exosomes exhibit a combination of advantages characteristic of both synthetic drug carriers and cell-mediated delivery methods, at the same time preventing their drawbacks.

Multiple encapsulation approaches for exosomes utilizing physical/chemical/biological techniques have been developed for stocking therapeutic agents into exosomes, to achieve diverse therapeutic effects and optimum efficiency.

Cargo Loading. Therapeutic agents can be introduced into exosomes either before or after exosome isolation.[5,195−197] Pre-isolation loading methods introduce the therapeutic molecules into the parental cells before the EV production, so that they are encapsulated before exosome biogenesis.

Cell transfection of RNA, peptides, and proteins has been used.[198,199] This is the most commonly used approach for loading therapeutic molecules into exosomes. Another way of pre-isolation cargo loading comprises simple incubation of the parental cells with the drug, allowing passive diffusion of the drugs into cells or exosomes during their biogenesis.[200]

Advantages: Appropriate for loading nucleic acids and proteins; large cargos

Disadvantages: Cytotoxicity, difficult purification

Post-isolation loading methods introduce the therapeutic agent after the exosome being collected applying techniques such as co-incubation, sonication, electroporation, freeze−thaw cycles, and extrusion.[197,201] Most of these methods have been acquired from their application in the liposome-based drug delivery.

In the **direct co-incubation** method, the therapeutic agent and the exosomes are mixed and incubated for a certain time period at room temperature. It is driven by the passive transport mechanism exploiting the concentration gradient. As a result, therapeutic small molecule drugs enter through the membrane into the exosomes or cells, with subsequent secretion of drug-loaded exosomes.[202] Loading is highly dependent on the drug hydrophobicity, with hydrophobic molecules being loaded more efficiently into exosomes.[203] An incubation time of ∼90 min has been reported to result in the most efficient loading of exosomes with synthetic oligonucleotides.[204] The size of the drug molecule is a substantial loading controlling factor.[205] Loading capacity can be strongly modulated by tuning the cells/exosomes−drug ratio.

Advantages: Simple, convenient, mild

Disadvantages: Low loading efficiency

Sonication is a technique using mechanical energy to produce temporary pores in the exosomal membranes allowing the cargo to be encapsulated, with subsequent reorganization and recovery of the lipid bilayer.[197,206] Sonication exhibits higher loading efficiency, but it could cause deformation of exosomes with subsequent compromising of their integrity. Also, sonication may lead to heating and damage of the active agents; therefore, careful temperature and process controls are critical.[197,205]

Advantages: High loading efficiency

Disadvantages: Heat generation, possible active agent damage, aggregation

Electroporation makes possible the entry of the therapeutic cargo by applying electrical pulses to modify the dielectric properties of the membrane, thereby opening recoverable pores and enhancing its permeability.[205] This way of permeabilization of exosome membranes is one of the most common techniques applied for exosome loading. The applied potential can vary significantly in different cases, from 0.1 to 1000 kV. Disruption of the membrane lipid bilayer allows hydrophilic compounds such as small DNAs,[207] miRNAs,[208,209] and siRNAs[210] to diffuse

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into exosomes. The method is simple to operate, has a high loading efficiency, and has been widely applied to encapsulate siRNAs or miRNAs. However, possible aggregation of therapeutic nucleic acids during loading caused by metal ions originating from the electrodes is a likely disadvantage.[211,212] Aggregation can be prevented by using protectors such as the trehalose, citric acid, and EDTA.[212]

Advantages: High loading efficiency, controllable

Disadvantages: Cargo aggregation

Freeze−thaw cycles are also successfully used for drug loading after exosome isolation. The exosomes are being frozen together with the drug in liquid nitrogen at −80 °C with subsequent thawing at room temperature for several cycles.[205] A minimum of three freezing−thawing cycles is needed, and 5−10 cycles are recommended.[213] It is a relatively mild method appropriate for miRNA and protein loading.[210] Drug penetrates through exosomal membrane as a result of minor disordering of the lipid bilayer during the procedure. Moderate loading efficiency is characteristic for this method.[210] It can be combined with the co-incubation and/or sonication techniques for enhancing efficiency.[214] The freeze−thaw technique has been successfully used to fuse exosomes and liposomes, thus producing exosome-mimetic particles.[213]

Advantages: Mild and simple, appropriate for RNA and protein loading

Disadvantages: Uncertain efficiency, aggregation

The **extrusion** technique includes forcing the exosomes to mechanical destruction by using an extruder device. The device has a heating block and polycarbonate filters with specific pore sizes (usually ∼100−400 nm). The exosome components are subsequently reconstructed into a population of nanovesicles incorporating the intended drug.[215] The method exhibits good loading efficiency, but the applied excessive shear stress can damage the vesicles and their protein components.[205] The extrusion method has been found to be appropriate in constructing exosome-mimetics.[5]

Advantages: Good loading efficiency, uniform size

Disadvantages: Possible damage of the exosome membrane

The distribution of documents in the CAS Content Collection related to exosome applications in therapy and diagnostics with respect to the applied exosome loading methods is illustrated in Figure 15. Dominating are physical methods—electroporation, freeze−thaw, sonication, and extrusion—while chemical and biological methods, such as transfection and incubation, are less popular. In fact, various methods turn out to be appropriate for different cargo loadings.

A selection of small molecule drugs, which have been frequently used as exosome cargo in drug delivery, as seen from the CAS Content Collection, have been exemplified in Supporting Information Table S1.

Cell Sources for Derived Exosomes. As a form of cell−cell messenger, exosomes play a crucial role in different physiological processes. Exosomes secreted by different tissues and cells exhibit specific properties. Moreover, understanding the properties of different cell-derived exosomes can also help us understand the pathogenesis mechanism of various diseases.

Exosomes Derived from Tumor Cells. Tumor-derived exosomes are able to modify tumor progression, including growth, angiogenesis, invasion, and metastasis. They promote cell development, adhesion, and cell polarity.[216,217] Exosomes derived from tumors may be involved in various immunomodulatory outcomes, since they carry both immunosuppressive and immunostimulatory mediators. Furthermore, tumor-derived exosomes may be used as immunoadjuvants and antigens in cancer vaccines.[218] They secrete cytokines and growth factors and can thus protect T-cells from cancer-cell-mediated apoptosis.[219] Also, tumor-derived exosomes exhibit a composition related to that of their cells of origin. When administered, they prefer to fuse with their parent cancer cells, indicating that exosomes might be distinctively suitable, as Trojan horses, to deliver anticancer therapeutics.[220]

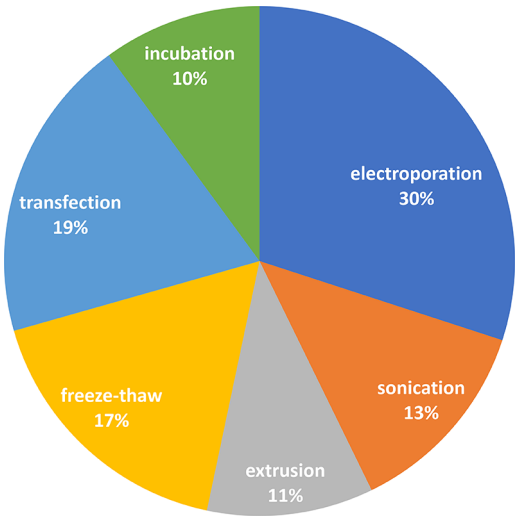

Figure 15. Percentages of documents related to exosome applications in therapy and diagnostics concerning various exosome loading methods.

Exosomes secreted by cancer stem cells mediate cell−cell communication and substance exchange, thus regulating processes of tumor growth metastasis, epithelial−mesenchymal transition, and angiogenesis by transporting tumor-related mRNA, non-coding RNA, surface proteins, and encapsulated proteins.[221] In colorectal cancer, exosomes derived from fibroblasts activate the Wnt signaling pathway, rendering cancer cells to exhibit stem cell properties, including spherocytosis and tumorigenicity, and increase the number of cancer stem cells in colorectal cancer cells.[222] Also, exosomes derived from mesenchymal stem cells can boost breast cancer cell proliferation by activating the Wnt signaling pathway.[223] Growing evidence indicates that targeting signal pathways regulated by exosomes could act on CSCs to inhibit the incidence and development of tumors, which has become a trending topic in recent years.

Mesenchymal-Stem-Cell-Derived Exosomes. Mesenchymal stem cells (MCS) are pluripotent stem cells and can be derived from certain adult tissues and organs. MSCs are an ideal exosome source. They inhibit the proliferation of immune cells. MSC-derived exosomes inherit the immunomodulatory properties.[224] In addition, MSCs have exhibited the highest amount of CD81 expressed exosomes.[225,226] It has been shown that upregulated miRNAs, especially miR-320C, from MSC-derived exosomes promote osteoarthritis chondrocyte proliferation. In a myocardial I/R injury study, MSC-derived exosomes carrying miR-182-5p showed a cardioprotective effect with improving cardiac function and reducing myocardial infarction, accompanied with reduced inflammation *in vivo*.[227] Exosomes from mesenchymal stem cells play an important role in many diseases and can be used as an adjuvant in supporting and complementing other therapeutic modalities. Bone marrow MSC-derived exosomes are being utilized by Direct Biologics, a

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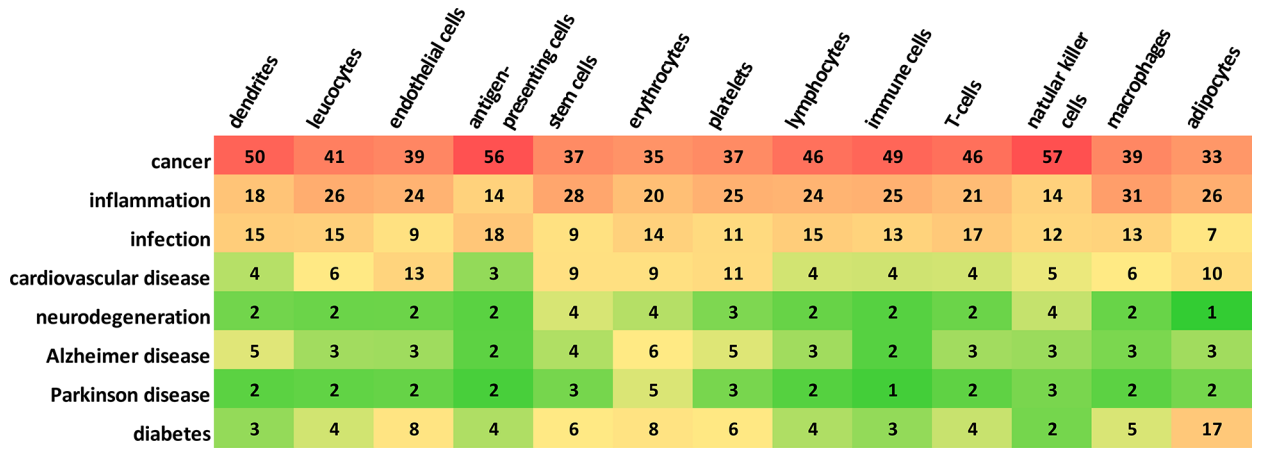

Figure 16. Correlation between exosome donor cells and the diseases to which the exosomes have been applied to in the studies related to exosomes in therapy and diagnostics, as represented by the number of documents in the CAS Content Collection.

regenerative biologic company, in many different clinical trials.[228] Their therapeutic product ExoFlo is currently available under FDA expanded use protocol for the treatment of COVID-19 acute respiratory distress syndrome (ARDS) (NCT04657458).[229,230] It is also under clinical trial for ulcerative colitis (NCT05176366),[231] Crohn's disease and irritable bowel disease (NCT05130983),[232] solid organ transplant rejection (NCT05215288),[233] and mild/moderate COVID-19 (NCT05125562).[234]

Macrophage-Derived Exosomes. Macrophages are known to exhibit phagocytic ability in the immune system.[235] They are able to identify and eliminate pathogenic microbial products and tumor cells and are thus important for the prevention of diseases.[236] Studies have reported that they are an essential regulator in injury and repair. After chemotherapy, macrophage-derived exosomes stimulate breast cancer proliferation and metastasis. Thus, inhibition of exosome secretion is identified as beneficial for breast tumor prevention.[237] M2-macrophage-derived exosomes could promote cardiac repair in a mouse model of acute myocardial infarction. miR-1271-5p-enriched macrophage-derived exosomes suppressed cell apoptosis and enhanced the viability of hypoxia-induced cardiomyocytes. By downregulating SOX6, miR-1271-5p decreased cardiomyocyte apoptosis induced by hypoxia and alleviated cardiac injury.[238]

Exosomes Derived from T-Cells. Exosomes derived from T-cells are a subject of growing interest because of their potential role in controlling innate immune responses. Similarly to the exosomes from other sources,[32,239] these exosomes carry bioactive miRNA.[240] Exosomal carriers can transport miRNA from T-cells to antigen-presenting cells.[240]

In addition to modulating the immune response, T-cell-derived exosomes participate in tumor inhibition. T-cell secreted exosomes containing Fas ligand promote tumor infiltration in lungs by enhancing the expression of matrix metalloproteinase 9,[241] and exosomes released from $CD8^+CD45^+$ regulatory T-cells inhibit the response of the $CD8^+$ cytotoxic T-lymphocyte and the antitumor activity.[242] Exosomes contribute to the tolerance to transplantation as well.[243] Through a clinical trial (NCT04389385), TC Erciyes University in Turkey is researching the use of COVID-19-specific T-cell-derived exosomes.[244] This clinical trial is testing the safety and efficacy of the agent following a metered inhalation for targeted delivery (Turk-Patent Application Number: PCT/TR2020/050302).[244]

Exosomes Derived from Other Cells. A lot of studies have been dedicated to identifying the roles of other living-cell-derived exosomes. Exosomes obtained from fibroblasts rich in miR-21-3p could induce cardiomyocyte hypertrophy by targeting SORBS2 and PDLIM5. Inhibition of miR-21-3p diminished cardiac hypertrophy in animals treated with Ang II. Exosomes extracted from endothelial cells expressing KLF2 can attenuate the formation of atherosclerosis. Exosomes derived from neural stem cells are being researched by Aruna Biomedical for the treatment of stroke along with other neurological and neurodegenerative diseases. Their candidate AB126 shows the ability to cross the BBB and demonstrates central nervous system specificity.[245] Their preclinical data supports that neural stem-cell-derived exosomes were more effective than MSC-derived exosomes in improving cellular, tissue, and functional outcomes in the tested mouse thromboembolic stroke model.[245]

The frequency of using various kinds of exosome donor cells in the studies related to exosome applications in therapy and diagnostics, as presented by the number of documents in the CAS Content Collection, is illustrated in Supporting Information Figure S3. Tumor cells and stem cells (specifically, mesenchymal stem cells, MSC) are the most frequently used exosome sources. Figure 16 illustrates the correlation between the exosome donor cells and the diseases to which the exosomes have been applied to in studies related to exosome application in therapeutics and diagnostics, as represented by the number of documents in the CAS Content Collection. Cancer studies clearly dominate, followed by inflammation and infection studies. Furthermore, in cancer studies, antigen-presenting cells and natural killer cells have been frequently used. Macrophages and stem cells are the most frequently used in inflammation, while antigen-presenting cells and T-cells are frequently used in infection.

Delivery of Small Molecules. Exosomes have been recognized as prospective vehicles for therapeutic small molecules. Generally, exosomal delivery vehicles exhibit higher biocompatibility due to their endogenous origin, tissue-specific targeted delivery, drug deposition in target cells, and favorable drug stability and blood circulation time, thus improving the effectiveness and pharmacokinetics of the small molecule drugs, such as curcumin, paclitaxel, doxorubicin, and withaferin. Exosome-encapsulated curcumin has been reported as able to reduce inflammation.[246] Exosomes derived from macrophages and packed with the antitumor drug paclitaxel produced a strong

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Table 5. Exemplary Exosome-Based Drug Delivery Systems

Exosome source	Disease	Drug/therapeutic agent	Study type/disease model or cell line
non-small-cell lung cancer H1299 cells and MRC9 lung fibroblasts[256]	lung cancer	doxorubicin/gold nanoparticles	*in vitro*/human cell
Raw264.7 macrophages[257]	pulmonary metastases	paclitaxel	*in vivo*/mouse model
pancreatic adenocarcinoma PANC-1 or MIA PaCa-2 cells[258]	pancreatic cancer	curcumin	*in vitro*/human cell
mesenchymal stromal cells (SR4987)[200]	pancreatic adenocarcinoma	paclitaxel	*in vitro*/mouse cell
human brain glioblastoma−astrocytoma U-87 cells and endothelial bEND.3 cells[259]	brain cancer	doxorubicin and paclitaxel	*in vivo*/zebrafish model
mouse macrophages Raw264.7[260]	glioma	curcumin/SPIONs[a]	*in vitro*/mouse and human cell
human brain glioblastoma−astrocytoma U-87 cells[261]	glioblastoma	paclitaxel	*in vitro*/human cell
human endometrial stem cells (hEnSCs)[262]	glioblastoma	atorvastatin	*in vitro*/human cell
immature mouse dendritic cell transfected by vector expressing iRGD-Lamp2b fusion protein[263]	breast cancer	doxorubicin	*in vivo*/mouse model
bone marrow mesenchymal stem cells[264]	neuroinflammation	miR-193b-3p	*in vivo*/mouse model
mesenchymal stem cells[265]	traumatic brain injury	MSC generated exosomes	*in vivo*/rat model
macrophages[266]	Alzheimer's disease	curcumin	*in vivo*/mouse model
blood plasma[267]	Alzheimer's disease	quercetin	*in vivo*/mouse model
adipose-derived stem cells[268]	Alzheimer's disease	neprilysin	*in vivo*/mouse model
blood plasma[269]	Parkinson disease	dopamine	*in vivo*/mouse model
human mesenchymal stem cells[270]	Parkinson disease	catalase mRNA	*in vivo*/mouse model
murine dendritic cells[271]	Parkinson disease	shRNA	*in vivo*/mouse model
Raw264.7 macrophages[248]	Parkinson disease	catalase	*in vivo*/mouse model
HEK 293 cells[272]	Huntington disease	miR-124	*in vivo*/mouse model
Schwann cells[273]	Huntington disease	siRNA	*in vivo*/mouse model
mesenchymal stem cells[274]	bacterial infection	antimicrobial peptides: cathelicidin LL-37, β-defensin-2, hepcidin, lipocalin-2	*in vitro*/mouse and human cells and *in vivo*/mouse model
human amniotic fluid[275]	COVID-19	zofin	clinical trial identifier NCT04657406 for expanded access use/*in vivo*/human

[a]SPIONs, superparamagnetic iron oxide nanoparticles.

antitumor effect.[247] Paclitaxel, doxorubicin, and withaferin were encapsulated in exosomes isolated from bovine milk and exhibited better antiproliferative activities against A549 lung cancer cells than the free drugs.

Delivery of Proteins. Exosomes have been examined and found particularly promising as delivery vehicles for macromolecular proteins. The routes of inserting proteins into exosomes include either genetic engineering—by transfecting the donor with a plasmid carrying the gene of interest—or direct loading into the exosomes. A delivery construct for the potent antioxidant catalase has been developed for treating inflammatory and neurodegenerative disorders, in particular Parkinson's disease.[248] SIRPα has been loaded into exosomes for antitumor therapy by blocking the CD47 receptor on tumor cells.[249] Hyaluronan degradation has been applied to stimulate tumor penetration by using exosomes holding PH20 hyaluronidase.[250] Moreover, it was reported that the exosome codelivery of PH20 hyaluronidase and doxorubicin inhibit tumors.[250]

Delivery of Nucleic Acids. Because of their ability to protect nucleic acids from degradation, exosomes have been identified also as superior carriers for nucleic acids for gene therapy. Thus, B-cell-derived exosomes has been employed for delivery of a miRNA-155 inhibitor in order to decrease the lipopolysaccharide-stimulated TNFα production in macrophages.[251] The tumor-suppressing agent miR-199a-3p encapsulated into exosomes from fibroblasts of ovarian cancer successfully suppressed c-Met production and inhibited cancer cell proliferation and invasion.[208] Substantial inhibition of postoperative breast cancer metastases was attained by an exosome-based siRNA delivery system comprising biomimetic nanoparticles including albumin and siS100A4 with an exosome membrane coating.[208,251−253] CRISPR/Cas9 genome editing technology has recently become a preferred tool due to the high precision and efficiency modifying, deleting, or replacing specific genes.[254] Exosomal nanocarriers were reported to have achieved efficient delivery of CRISPR/Cas9 plasmids with cancer cell tropism and produced advanced antitumor effects.[255]

Table 5 exemplifies some exosome drug delivery systems with relation to diseases and exosome sources.

Exosomes as Therapeutics. Exosomes are considered a promising drug delivery system due to their specific structure and composition allowing them to be used as efficient natural nanocarriers, as well as their impressive preclinical success. Yet another rapidly expanding and noteworthy application of exosomes is their use as therapeutic agents.[276−281]

Exosomes can modify tumor growth because of some proteins and RNAs which they deliver to the tumor cells.[282] Reports show that tumorigenesis is being controlled, specifically downregulated, by the transport of miR-139-5p encapsulated into exosomes in bladder cancer cells.[283] A similar effect has been observed when miR-381 packed in exosomes is transfected into triple negative breast cancer cells. miR-140-3p in exosomes isolated from human colorectal cancer blood samples inhibits cancer cell proliferation. miR-5100 in exosomes derived from mouse breast cancer xenograft model-associated macrophages hinders the CXCL12/CXCR4 spreading tumor cells to regional nodes of the primary tumor.[284,285] Other miRNAs secreted by

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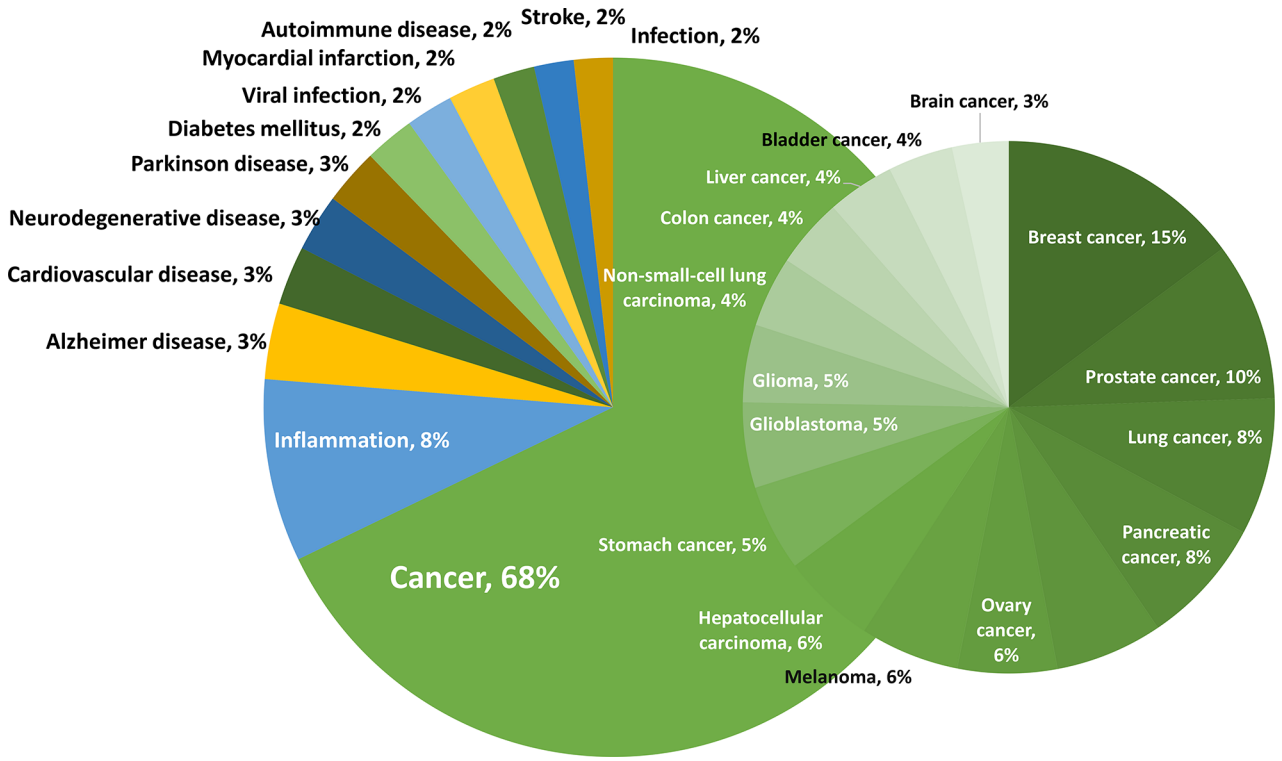

Figure 17. Distribution of the publications in the CAS Content Collection related to exosome applications in therapy and diagnostics with respect to the target diseases.

colorectal cancer exosomes hinder angiogenesis in colorectal cancer.[286]

With respect to neurodegenerative diseases, the therapeutic power of exosomes is augmented thanks to the capability of exosomes to cross the BBB. For example, the enzymes neprilysin and insulin degrading enzyme (IDE), which degrade amyloid β peptide, can be found in exosomes. Uptake of these enzymes results in reduction of amyloid β levels.[287] Exosomal miRNAs have been found helpful in neurological diseases including Alzheimer's disease. For example, analysis of exosomal miRNAs isolated from mesenchymal stem cells has been shown to improve various brain disorder pathologies, including Alzheimer's disease, Parkinson's disease, subarachnoid hemorrhage, and traumatic brain injury.[288,289]

Exosomes are reported to be helpful for treatment of cardiovascular diseases as well. An example includes cellular conditioning after acute myocardial infarction. Stem cell exosomes have been reported to promote angiogenesis, impart cytoprotection, and control apoptosis.[17,279] Progenitor cell exosomes supplemented with certain cardioprotective miRNAs reduce infarct size in an animal model of ischemia-reperfusion injury.[290] Such exosomes also protect from ischemia-reperfusion injury, advancing cardiac performance.[291]

In infectious diseases, exosomes have been shown to incorporate pathogen-originated molecules or immunomodulators favoring eradication of the microorganism and immune balance.[280,281] Hence, exosomes are considered as appropriate carriers of substances to prevent or manage infection, e.g., to control bacterial infections, sepsis, and COVID-19.[281] MSC-derived exosomes have been shown to be able to treat infections by expression of microbicidal peptides cathelicidin LL-37, human β-defensin-2, hepcidin, and lipocalin-2 and/or by immunomodulation.[274,292] Compared to antibiotics, antimicrobial peptides exhibit certain advantages, such as lower toxicity and immunomodulatory activities, and are thus preferable.[292] Exosomes have also become a valuable tool for treatment of sepsis.[293] Thus, miR-27b carried by MSC-derived exosomes induces decline of the production of pro-inflammatory cytokines.[294] miR-21 carried by exosomes gives rise to substantial renoprotection imparted by remote ischemic preconditioning, proposed as an efficient therapeutic approach for renal damage caused by sepsis.[295] Patients with severe COVID-19 disease have been reported to develop a "cytokine storm syndrome", leading to acute lung injury, acute respiratory distress syndrome, organ failure, and ultimately death.[296] Using the model developed for sepsis, exosomes might perform as a therapeutic strategy for the immunomodulatory cure of COVID-19.[297] The safety and therapeutic efficacy of exosomes overexpressing CD24 have been assessed, as they are able to directly suppress a cytokine storm.[294,298,299] CD24 is an important factor in many human cancers. It is also a significant participant in controlling the T-cell proliferation and as such may suppress inflammation. T-cell-derived exosomes have also been suggested as a useful medication for pneumonia in patients with early stage COVID-19 infection. A clinical study assessing the safety and efficacy of such exosomes has been delivered for inhalation by aerosol.[244] Research on treating severe COVID-19 pneumonia is carried out based on exosome inhalation as well.[300,301]

The variety of diseases to which exosome systems have been applied as therapeutic or diagnostic tools, as demonstrated by our exploration of the publications in the CAS Content Collection, is shown in Figure 17. The largest part (68%) of the publications are associated with cancer, and neurodegenerative, inflammatory, and cardiovascular diseases are also highly represented (Figure 17).

Advantages and Disadvantages of Exosomes in Drug Delivery versus Lipid Nanoparticles. Exosomes are small

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and flexible and exhibit adhesive proteins on their surface, so they can cross the BBB. At the same time, they are endogenous; their membrane is composed of cellular lipids, which imparts them with negligible immunogenicity and toxicity. Exosomes are rich in proteins and genetic material and are thus useful for early and accurate diagnosis. More studies on exosome *in vivo* biodistribution are required to establish biodistribution mechanisms and their important features, such as the route of administration, disease progression, their cells of origin, and the recipient cell types available to uptake circulating exosomes.[302]

The endogenous origin of exosomes may also have disadvantages in the clinical practice. The yield of exosomes is considerably lower than that of liposomes. The yield of exosomes is strictly limited by the secretion abilities of cells, the complexity and expenses for large-scale cell culturing, and the time- and effort-consuming, low-efficiency procedures for exosome production, making industrial scale-up manufacture of exosomes a hard to ignore obstacle.[303]

Additionally, the cargo carrying efficiency of exosomes is restricted. They intrinsically carry a large load of natural components, which significantly complicates and restrains the anticipated cargo loading.[304] Although approaches to engineer exosomes with enhanced loading capacity are being elaborated, they are still less efficient than the synthetic liposomes.

As an additional drawback, the quality control of exosomes is harder than that of liposomes. Exosomes are highly heterogenic, even when generated by a single cell type. Due to the lack of sensitive high-throughput analysis methods, it is hard to separate the heterogeneous exosome population into homogeneous ones.[4] Furthermore, since one of the essential functions of exosomes is to remove the harmful substances from cells, they may be left with undesired and unsafe macromolecules from their parent cells.[305] Strategies to precisely control the contents of exosomes are currently still insufficient.

Exosomes in Diagnostics. To be practicable in clinical use, a blood biomarker needs to be easy to assess, cost-effective, specific for the targeted disease, highly sensitive, and easily and reliably measured. Exosomes are favorable with respect to conventional biomarkers especially in their higher diagnostic sensitivity and accuracy. Thus, exosomes are an appropriate tool for clinical diagnostics for the following reasons:

- The disease progression strongly modulates the content of exosomes; exosomal bioactive substances have been shown to be altered and are thus highly informative regarding the pathological status.[306,307]
- Exosomes can be obtained non-invasively from easily available biological fluids including urine, blood, saliva, and even tears for early and fast diagnosis of diseases such as cancers, cardiovascular diseases, and neurodegenerative diseases such as Alzheimer's disease.[308,309]
- Exosomes are highly stable due to their lipid bilayer membrane. They can thus circulate even in a harsh tumor microenvironment. Moreover, the biomembrane protects the exosomal content from degradation by extracellular proteases.[309,310]
- Exosomes express surface markers characteristic to their cells of origin, so their source can be identified.[311]
- Exosomes can be stored by freezing, freeze-drying, or spray-drying and are highly stable, which is of significant importance for their clinical application.[312]
- Exosomes can permeate through the BBB in both directions; they thus afford collecting information about brain cells non-invasively.[310,313]
- Exosomes exhibit advantages compared to conventional biomarkers in their higher diagnostic sensitivity and accuracy.[314−316]

The significant potential of exosomes in diagnostics is already widely appreciated, and exosomes are drawing intense attention as evidence is being accumulated that exosomes contain biological molecules characteristic of cancer, neurodegenerative, infectious, and metabolic diseases and can be possibly used as diagnostic biomarkers.[19,41,276,317−320]

Exosomal Proteins as Diagnostic Biomarkers. Tetraspanins, a group of membrane scaffolding proteins, are abundant in exosomes. One of the members of this family is the exosomal marker CD63. It has been reported that there is a much higher amount of plasma exosomes comprising the CD63 marker in patients with melanoma as compared to healthy ones.[321] Furthermore, CD63 has been found to be elevated in exosomes from various types of human cancer cells. Thus, exosomal CD63 is suggested as an appropriate protein marker for cancer.[322] Another tetraspanin, CD81, is found essential in hepatitis C pathology, seemingly associated with inflammation and fibrosis. It has thus been identified as a marker for hepatitis C diagnosis.[323] A higher expression of CD151, CD171, and tetraspanin 8 (TSPAN8) is reported in blood serum exosomes collected from lung cancer patients.[324] These findings suggest exosomal proteins are appropriate biomarkers for cancer diagnosis. Other members of the tetraspanin family such as CD91, CD82, CD147, CD9, and TSPAN8 have also been explored as cancer biomarkers.[319]

Numerous exosomal protein biomarkers have been identified that can be used to diagnose diseases of the central nervous system. Glioblastoma-specific receptor EGFRvIII has been detected in glioblastoma-patient-derived exosomes, suggesting that exosomal EGFRvIII is an appropriate source of glioblastoma diagnostic information.[33] Exosomes from brain tumor patients were found to comprise EGFR, EGFRvIII, and TGF-β.[325] Exosomal amyloid peptides are found in brain plaques indicative for Alzheimer's disease.[326] Tau protein phosphorylated at Thr-181, which is an established biomarker for Alzheimer's disease, was detected at elevated levels in exosomes isolated from cerebrospinal fluid of Alzheimer's disease patients.[307] Thus, exosomes are possibly valuable for early diagnosis of Alzheimer's disease. Exosome Sciences[327] partnered with Boston University researched the use of their TauSome biomarker (exosomal tau) for diagnosis and monitoring of chronic traumatic encephalopathy (CTE) in living individuals.[328,329] With Boston University's DIAGNOSE CTE study (clinical trial NCT02798185),[330] they have enrolled 120 former National Football League Players, 60 former college football players, and 60 healthy controls to develop methods to diagnosis CTE and to examine potential risk factors.[330] Another biomarker, α-synuclein, the aggregation of which is considered to play a key role in Parkinson's disease pathology, has been reported to be released from exosomes in a Parkinson's disease model system.[331] The study showed that lysosomal dysfunction typical for Parkinson's disease increases exosomal α-synuclein release.

The easily and non-invasively attainable proteins in urinary exosomes have also been examined as diagnostic biomarkers, especially for urinary tract diseases. Urinary exosomal fetuin-A

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has been found to be elevated in acute kidney injury occurrences.[332] Exosomal proteins in urine have also been examined as potential biomarkers for bladder cancer and prostate cancer. Eight urinary exosomal proteins have been identified as possible biomarkers for bladder cancer.[333] Two identified prostate cancer biomarker proteins were found in urine exosomes from prostate cancer patients.[334] Twenty-four urinary exosomal proteins notably differ between bladder cancer and control patients.[335]

Table 6 exemplifies some candidate exosomal protein biomarkers reported to date for diagnostic applications.

Table 6. Examples of Exosomal Proteins for Clinical Diagnostic Applications

Protein(s)	Disease	Body fluid
CD81[323]	chronic hepatitis C	blood plasma
CD63, caveolin-1, TYRP2, VLA-4, HSP70, HSP90[37,321]	melanoma	blood plasma
epidermal growth factor receptor VIII[33]	glioblastoma	blood plasma
survivin[336]	prostate cancer	blood plasma
c-src[337]	plasma cell dyscrasias	blood plasma
NY-ESO-1[338]	lung cancer	blood plasma
PKG1, RALGAPA2, NFX1, TJP2[339]	breast cancer	blood plasma
Her2[340]	breast cancer	blood plasma
glypican-1[43]	breast cancer	blood serum
glypican-1[43]	pancreatic cancer	blood serum
glypican-1[341]	colorectal cancer	blood plasma
CEA[342]	colorectal cancer	blood serum
AMPN VNN1, PIGR[343]	cholangiocarcinoma	blood serum
PSA[344]	prostate cancer	blood plasma
GGT1[345]	prostate cancer	blood serum
CD24, EpCAM, CA-125[346]	ovarian cancer	blood plasma
CD91[347]	lung cancer	blood serum
TSPAN8, CD151[348]	lung cancer	blood plasma
CD82[349]	breast cancer	blood serum
CD9, CD147[350]	colorectal cancer	blood serum
TSPAN8[351]	pancreatic cancer	blood serum
fetuin-A, ATF 3[332,352]	acute kidney injury	urine
CD26, CD81, S1c3A1, CD10[353]	liver injury	urine
NKCC2[354]	Bartter syndrome type 1	urine
EGF, α subunit of Gs, resisitin, retinoic acid-induced protein 3[333]	bladder cancer	urine
PSA, PCA3, ERG, SPDEF[334,355]	prostate cancer	urine
L1CAM, CD24, ADAM10, EMMPRIN, claudin[356,357]	ovarian cancer	blood plasma, cell culture medium, ascites
A2M, HPA, MUC5B, LGALS3BP, IGHA1, PIP, PKM1/M2, GAPDH[358]	squamous cell carcinoma	saliva
Annexin Al, A2, A3, A5, A6, All, NPRL2, CEACAM1, HIST1H4A, MUC1, PROM1, TNFAIP3[359]	lung cancer	saliva
LMP1, galectin-9, BARF-1[360]	nasopharyngeal cancer	blood, saliva
CALML5, KRT6A, and S100P[361]	dry eye disease	tears

Exosomal Nucleic Acids as Diagnostic Biomarkers. Exploration of exosomal RNAs as diagnostic biomarkers has been triggered by the finding that exosomes contain RNAs.[32,362] Indeed, exosomal RNAs are protected from RNase degradation by the lipid bilayer membrane and thus can be steadily detected in blood, making them perfect diagnostic biomarkers. Among all exosomal cargo substances, miRNA has drawn attention because of its complex roles in regulating the cancer microenvironment involving angiogenesis, cell proliferation, and metastasis. Its roles in regulating cellular behaviors *in situ* or in the remote recipient cells are under intensive investigation.[363−365]

Exosomal miRNAs are being most commonly utilized as cancer biomarkers. Thus, eight miRNAs were identified in serum exosomes from ovarian cancer patients which are missing in healthy controls, suggesting that easily attainable exosomal miRNAs are appropriate diagnostic markers.[363] Exosomal miRNAs from lung adenocarcinoma were significantly different from the control patients. Thus, exosomal miRNAs are a possible tool for screening for lung adenocarcinoma.[366] Similarly, the miR-141 level is supposedly a forceful diagnostic marker for prostate cancer.[364] Researchers from Hackensack University Medical Center are also currently recruiting for a clinical trial (NCT03694483) that will purify prostate-cancer-derived exosomes and characterize their miRNA for the potential development of a prostate cancer liquid biopsy assay.[367]

A simple, urine-based liquid biopsy test has been developed by Exosome Diagnostics called ExoDx[368] and is commercially available to provide risk probabilities of aggressive prostate cancer in patients. The ExoDx test was granted FDA Breakthrough Device Designation in 2019[369] and uses RNA copy numbers of ERG, PCA3, and SPDEF to develop a predictive count to correlate the probability that a patient may develop prostate cancer.[355]

Exosomal miRNAs are reported as hopeful biomarkers for esophageal squamous cell cancer. Exosomal miR-21 has been found to be high in esophageal squamous cell cancer patients' serum.[370] Serum miRNA-1246 exhibits a sensitivity of 71.3% and a specificity of 73.9% for esophageal squamous cell cancer diagnosis. Serum miRNA-1246 has been found to also be significantly correlated with the tumor, lymph node, and metastasis stage and is a strong risk factor for poor survival.[371]

Exosomal miRNAs have been identified as possible biomarkers for diagnosing cardiovascular diseases and renal fibrosis as well.[372−375] Recently, a study of tear exosomes concluded that miR-145-5p, miR-214-3p, miR-218-5p, and miR-9-5p are dysregulated during diabetic retinopathy development.[361] Furthermore, tears were established as another easily accessible body fluid expected to improve molecular diagnostics to diagnose ocular, neurodegenerative, and systemic diseases, as well as cancer. Thus, the study of miRNAs in tear exosomes has shown that miR-145-5p, miR-214-3p, miR-218-5p, and miR-9-5p are dysregulated during diabetic retinopathy development[361]

Another possible diagnostic biomarker besides miRNAs are the exosomal mRNAs.[375,376] For example, specific features for diagnosing prostate cancer have been identified in circulating exosomal mRNA.[377] Urinary exosome mRNA has been suggested as a tool for non-invasive detection of kidney disease.[375]

Searching for biomarkers among RNAs to be used in non-invasive diagnostics has been booming in recent years. Representative examples of exosomal miRNAs reported as cancer therapeutic and diagnostic agents are shown in Table 7.

A search in the CAS Content Collection[15] found an extensive increase of the number of documents related to exosome applications in diagnostics (Figure 18A). A comparison with the therapy-related exosome documents demonstrates that, although at present they outnumber the diagnostic-related documents (Figure 18), the annual growth of the diagnostic exosome documents has begun to dominate (Figure 18A, inset).

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Table 7. Exosomal miRNAs as Cancer Therapeutic and Diagnostic Agents

miRNAs	Cancer types	Applications
miR-378[378]	non-small-cell lung cancer	prognostic
miR-323-3p, miR-1468-3p, miR-5189-5p, and miR-651359[379]	non-small-cell lung cancer	prognostic; osimertinib therapy management
miR-486-5p and miR-146a-5p[380]	non-small-cell lung cancer	early diagnosis
miR-375-3p[381]	non-small-cell lung cancer	therapeutic
miR-433[382]	non-small-cell lung cancer	therapeutic
miR-148a[383]	breast cancer	prognostic
miR-423, miR-424, let7-i, and miR-660[384]	breast cancer	diagnostic
miR-567[385]	breast cancer	therapeutic; reversing trastuzumab resistance
miR-9 and miR-181a[386]	breast cancer	therapeutic; expanding early myeloid-derived suppressor cells (MDSCs)
miR-423-3p[387]	prostate cancer	prognostic; castration resistance
miR-16-5p, miR-451a, miR-142-3p, miR-21-5p, and miR-636[388]	prostate cancer	prognostic; metastasis
miR-125a-5p and miR-141-5p[389]	prostate cancer	diagnostic
miR-375 and miR-451a[390]	prostate cancer	diagnostic
miR-143 (from cancer tissue)[391]	prostate cancer	therapeutic
miRNA-92a-1-5p[392]	prostate cancer	therapeutic
miR-24-3p[393]	oral squamous cell carcinoma	diagnostic
miR-130a[394]	oral squamous cell carcinoma	diagnostic and prognostic
miR-30a[395]	oral squamous cell carcinoma	therapeutic; cisplatin sensitivity
miR-130b-3p[396]	oral squamous cell carcinoma	therapeutic
miR-139-3p[383]	colorectal cancer	diagnostic
miR-126, miR-1290, miR-23a, and miR940[397]	colorectal cancer	diagnostic
miR-106b-3p[398]	colorectal cancer	therapeutic
miR-221/222[399]	colorectal cancer	therapeutic

Exosomes as Therapeutic Targets. Exosomes are known to be related to the pathogenesis of various illnesses such as cancer, neurodegenerative, cardiovascular, and others. Provided that exosome amounts are frequently enhanced and related to the severity of the diseases, in particular for cancers, a successful therapeutic strategy may involve reducing exosome production and circulation to normal levels to prevent disease progression.[400−403] With this perception, numerous studies are intended to modify the exosome pathway at its various steps, including production, release, and uptake.[404]

A number of approaches have been explored for **inhibiting exosome formation**. The endosomal sorting complexes required for transport (ESCRT) are known to be involved in multivesicular body biogenesis[112] Several reports have correlated the exosome secretion to the ESCRT-0 protein hepatocyte growth factor-regulated tyrosine kinase substrate (HGS, HRS), by reporting decreased exosome release in HRS depleted dendritic cells and tumor cells.[405,406] Mechanisms of exosome formation which do not depend on ESCRT are known too. They include ceramide or the tetraspanins. The small-molecule inhibitors of sphingomyelinase, the enzyme generating ceramide from sphingomyelin, are able to reduce endosomal sorting and production, causing a reduction in tumor growth.[407,408] Otherwise, the formation of exosomes may be controlled by certain signaling pathways triggered by Ras homologue family member A or ADP-ribosylation factor 6 (ARF6).[409,410] Targeting these pathways may produce a distinct therapeutic effect on tumor progression.

Other strategies that block exosome secretion have been developed as well. The sphingomyelinase inhibitor drug GW4869 causes inhibition of intraluminal vesicle formation and release of exosomes.[239,411] Inhibition of exosome production has been accomplished by attenuation of sphingomyelinase 2, which manipulates the synthesis of ceramide and restrains angiogenesis and metastasis in breast cancer.[412] Numerous modulators of exosome fabrication from prostate cancer cells have also been reported recently.[413]

Another way of modulating extracellular exosome levels is by **inhibiting exosome release**. Certain proteins, such as small GTPases of the Rab family, are associated with the discharge of exosomes. Thus, Rab27a and Rab27b are significant regulators of exosome release and the same is true for their effector proteins.[34,414] Silencing Rab27a by RNA interference can reduce tumor growth.[415] Lipids are also shown to be involved in the exosome secretion regulation. Diacylglycerol kinase down-regulation results in the suppression of the secretion of exosomes containing the Fas ligand.[416] Exosome discharge includes fusion of MVBs with the cell membrane as a final step. This process is mediated by the SNARE complex machinery, with the SNARE protein Ykt6 involved.[417] Lastly, cellular pH also modulates exosome secretion, via modulation of proton pump inhibitors.[418] Furthermore, studying the exosome roles has revealed that suppression of melanoma progression is correlated with exosomes released by natural killer cells.[419]

Exosome uptake inhibition is another way to modulate exosome activity. Cells uptake exosomes using various endocytic pathways, such as clathrin-dependent endocytosis as well as clathrin-independent routes, e.g., macropinocytosis and phagocytosis.[420−422] Exosome treatment with proteinase K has been reported to significantly reduce uptake by ovarian cancer cells, which is an indication that proteins located on the exosomal surface may operate as uptake receptors.[423] The uptake of tumor exosomes is supposedly mediated by the membrane phosphatidylserine that is possibly inhibited by diannexin.[424] Heparan sulfate proteoglycans allegedly operate as internalization receptors of cancer cell exosomes. Such an uptake route appears to be significant, since heparin treatment considerably inhibits the cancer cell migration stimulation mediated by exosomes.[425] Besides, exosome uptake is inhibited by dynamin2 knockdown, required for clathrin and caveolin endocytosis pathways.[421]

Another successful strategy of treating cancer has been exploited by **physical elimination of exosomes** secreted by cancer cells. Communication among cells in tumors is mostly via chemokines, cytokines, or growth factors.[426,427] Exosomes from tumor cells are noted to facilitate these kinds of communications, thus playing a role in tumor progression.[428] Therefore, the removal of exosomes secreted by cancer cells is one of the exosome-targeting therapeutic approaches. A hemofiltration system capable of targeting cancer cell exosomes by specifically targeting at human epidermal growth factor receptor 2 (HER2) on the exosome surface was utilized.[429] That caused selective elimination of cancer-derived exosomes, which proved to be very valuable for cancer treatment.[219]

Collectively, these data reinforce the hypothesis that elimination of exosomes or inhibition of their secretion, release,

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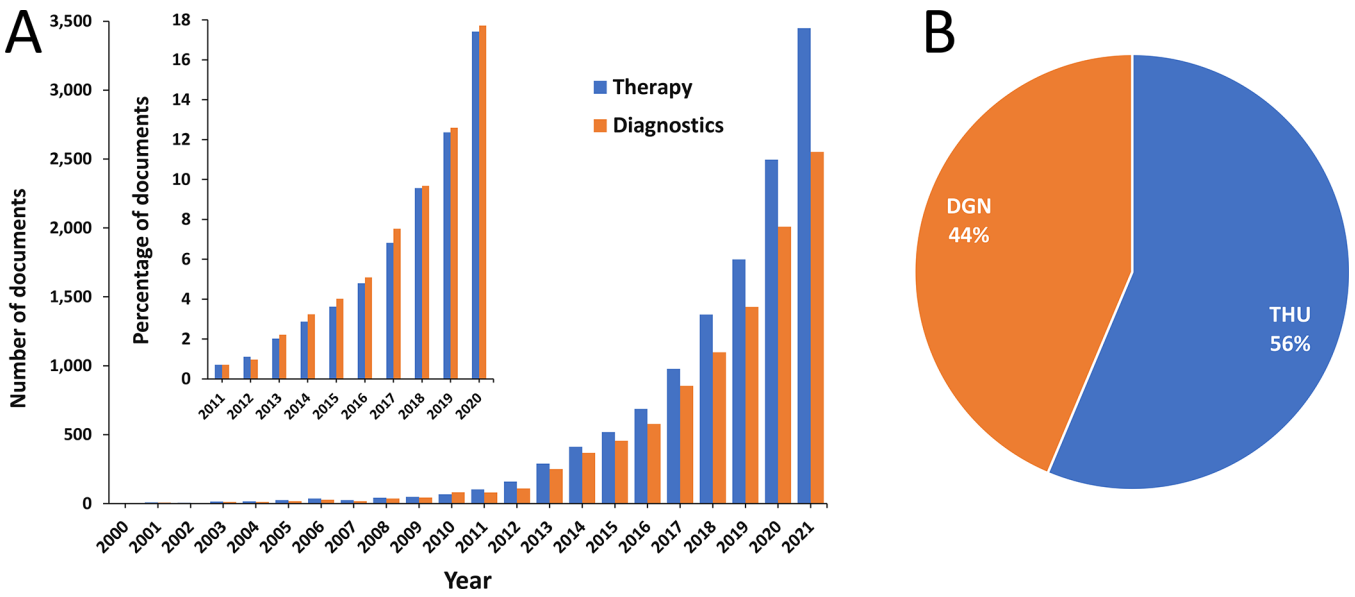

Figure 18. Diagnostic vs therapeutic applications of exosomes. (A) Comparison of the number of documents related to exosome applications in therapy vs diagnostics. Inset: Annual growth of the number of documents related to exosome applications in therapy vs diagnostics. (B) Comparison of the number of documents related to exosome applications in therapy vs diagnostics with respect to their role indicators (THU, therapeutic; DGN, diagnostic).

or internalization mechanisms may have favorable effects in cancer therapy. Thus, a good understanding of the disease-specific mechanism of exosome pathways is needed in finding specific therapies intervened by targeting exosomes.[427]

Other Applications. *Exosomes in Food and Cosmetics.* Prospective applications of exosomes are also in cosmetics and food.[430] It has been reported that stem-cell-derived exosomes are able to perform significantly in skin cosmetology, specifically in promoting wound healing, alleviating skin aging, and preventing scar formation.[431,432] For example, exosomes derived from induced pluripotent stem cells are able to modulate the expression of MMP-1/3 and enhance the expression of type I collagen in senescence skin fibroblasts.[433] Exosomes from adipose stem cells were reported as able to promote wound healing through the PI3K/Akt signaling route and to increase the amount of collagen type I and type III in fibroblasts.[434] A search in the CAS Content Collection revealed a sharp growth in the number of documents related to applications of exosomes in cosmetics in the last 3 years (Supporting Information Figure S4A).

Bioactive compounds—polyphenols, vitamins, polyunsaturated fatty acids, and others—are common food supplements aiming to elevate nutritional value. However, their effect can be compromised by their poor bioavailability, limited water solubility, and metabolic alterations; thus, they require carriers. While extracellular vesicles and specifically their exosome subclass have emerged demonstrating an impressive potential to realize efficient delivery of bioactive compounds, they can successfully serve as carriers of such food-related bioactive compounds, as well. Indeed, the interest in applications of exosomes in food has rapidly grown in the recent years (Supporting Information Figure S4B).

Recent studies verified isolation of exosomes from food stuff such as lemon, ginger, and milk.[435] Such food-derived exosomes can be uptaken in the intestine to act locally and can allegedly play roles in alleviating diseases and especially in modulating gut microbiota, yet the underlying mechanism is still unclear.

Exosomes from Plant Cells. The existence of EVs in plants has been long debated because of the existence of the cell wall. Growing evidence implies however that plants also secrete EVs performing various functions such as unconventional protein secretion, RNA transport, and pathogen defense.[436]

It has been hypothesized that edible structures within cells of plants such as ginger, aloe, and others might have clinically valuable anti-inflammatory effects on the intestinal lining of patients with inflammatory bowel disease (clinical trial NCT04879810).[437] Exosomes from ginger or aloe are being tested for the treatment of polycystic ovary syndrome (NCT03493984).[438] Grape exosomes are in a clinical trial as an anti-inflammatory agent to decrease the frequency of oral mucositis following radiation and chemotherapy treatment of head and neck tumors (NCT01668849).[439]

EXOSOMAL DRUG/BIOMARKER IN THE DEVELOPMENT PIPELINE

Companies are working to progress exosome research from conception to commercialization. To start, many companies are offering services and products for exosomal research. Many other companies, medical centers, universities, and research organizations are looking to utilize exosomes for therapy and diagnostics to target diseases with high unmet needs. Promising preclinical therapeutic and diagnostic exosome research is explored in this section. Selected clinical trials utilizing exosomes as therapeutics and diagnostics are also highlighted. Lastly, clinical trials that research exosomes as the disease target are examined.

Companies Offering Services and Products for Exosome Isolation, Purification, Characterization, and Engineering. As exosome research has grown dramatically within the past decade (Figure 4), so have the number of companies offering services and products for exosome isolation, purification, characterization, and engineering for both therapy and diagnosis. A selection of these companies is discussed along with their services and products within Table 8.

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Table 8. Highlighted Companies Offering Services and/or Products for Exosome Isolation, Purification, Characterization, and Engineering for Research and/or Commercialization

Company (location)	Summary
Ciloa (France)	Ciloa is an exosome spin-off company from the French National Center for Scientific Research and the University of Montepellier. Ciloa is dedicated to *in vivo* development of recombinant exosomes for therapeutic and preventative applications. Their recombinant exosomes allow for loading of two types of protein cargo: the first one, at the surface for disease targeting; the second one, as the cargo inside the exosome to deliver a signal for modification, multiplication, or death.[440]
Clara Biotech (USA)	Clara provides exosome isolation using their developed ExoRelease Isolation Platform and characterization as a service for researchers. They have developed a starter kit version of their ExoRelease Isolation Platform for researchers to perform isolation of exosomes in their own lab, as well. They also offer the services of nanoparticle tracking analysis for exosome characterization, exosome proteomic analysis, exosome nucleic acid analysis, and exosome imaging.[441]
Creative Biolabs (USA)	Creative Biolabs offers a wide range of exosome-related research services. These services include exosome isolation, purification, characterization, quantification, profiling, proteomics, lipidomic and metabolomics assays, RNA sequencing, exosome engineering and manufacturing, and exosome antibody development and display and have *in vitro* and *in vivo* model platforms.[442]
EverZom (France)	EverZom is an exosome service company who provides a large panel of services for exosome development including exosome production, characterization, isolation/purification, and engineering services.[443]
Exosome Plus (Republic of Korea)	Exosome Plus manufactures MSC-derived exosomes, plant-derived exosomes, human-derived exosomes, and animal-derived exosomes. Their therapy platform is called ExoThera, and they are hoping to develop their liquid biopsy platform to diagnose 11 major cancers using body fluid exosomes. They also sell an exosome isolation kit called Exo2D and an EV characterization system called ExoCope which is a single exosome multicolor fluorescence colocalization and particle tracking analysis system.[444]
Exosomics (Italy)	Exosomics offers the services of exosome isolation and characterization, nucleic acid extraction, protein separation, nucleic acid analytical assays, and protein analytical assays.[445] They also offer kits for researchers to use in their lab including exosome purification kits and exosome-based reference standards.[446]
FUJIFILM Wako Chemicals USA Corporation (USA)	FUJIFILM offers many different exosome kits and products including exosome isolation kits, ELISA kits, and flow cytometry kits. They also offer exosome marker antibodies, blocking reagents, purified exosomes, exosome cell cultures, and labware for researchers to utilize in their own laboratories.[447]
HansaBioMed Life Sciences (Estonia)	HansaBioMed is entirely dedicated to research and development in the exosome sciences field. Their services include purification of exosomes from condition media, biofluids, or plant extracts, exosome characterization, biomarker assessment by mass spectrometry, and RNA sequencing. They also sell a broad range of purified exosomes, tools for purification, enrichment, and characterization.[448]
Lonza (USA)	Lonza acquired HansaBioMed Life Sciences in 2017. More recently in 2021, Lonza acquired Codiak's (Therapeutic exosome company) manufacturing facility and is the strategic manufacturing partner for Codiak's pipeline. Additionally, in 2021, they announced they acquired Exosomics.[449]
NanoFCM (UK)	NanoFCM has a commercial product available called the flow nanoanalyzer which is a high-sensitivity flow cytometry for exosome analysis. They also offer exosome sample analysis service.[450]
NanoView Biosciences (USA)	Nanoview Biosciences creates exosome products to help with exosome characterization. Their ExoView R200 product allows for automated exosome measurement. Their ExoView kits allow for standard or customizable assays for purification of free exosomes. The ExoView chip washer offers reliable hands-free sample preparation, along with their ExoView software suite that offers reporting of exosome size, counts, and biomarker colocalization.[451]
ReNeuron (UK)	Exosomes produced by the ReNeuron's stem cell lines or via its induced pluripotent stem cell platform have the possibility to be manufactured through a scalable process and loaded with a broad range of payloads, such as nucleic acids, proteins, as well as small molecules. They are in collaboration with universities, global pharma, and biotech companies in various stages from discovery to *in vivo* late-stage studies.[452]
RoosterBio (USA)	RoosterBio is dedicated to accelerating exosome product and process development for exosome therapeutics. They have developed an extensive panel of exosome analytical methods to support this including exosome NTA for characterization, purification, protein analysis, surface marker expression, cytosolic marker expression, miRNA quantitation and analysis, lipid content, albumin contamination, CD63 quantitation, and scratch assays for wound healing.[453] They also produce exosome production media for both research and manufacturing.[454]
Systems Biosciences (USA)	Exosome research products and services are offered by Systems Biosciences to help advance exosome research studies. They offer exosome isolation, detection, quantification, labeling, biomarker discovery, engineering, and design kits and products.[455]
ThermoFisher Scientific (USA)	ThermoFisher Scientific is a world leader in serving science, staying one step ahead for advancing science, and their products for exosomes are no different. They offer a wide range of exosome products for isolation, analysis, and cargo isolation. They also offer exosome depleted fetal bovine serum along with reagents for automated preparation products for exosome analysis.[456]

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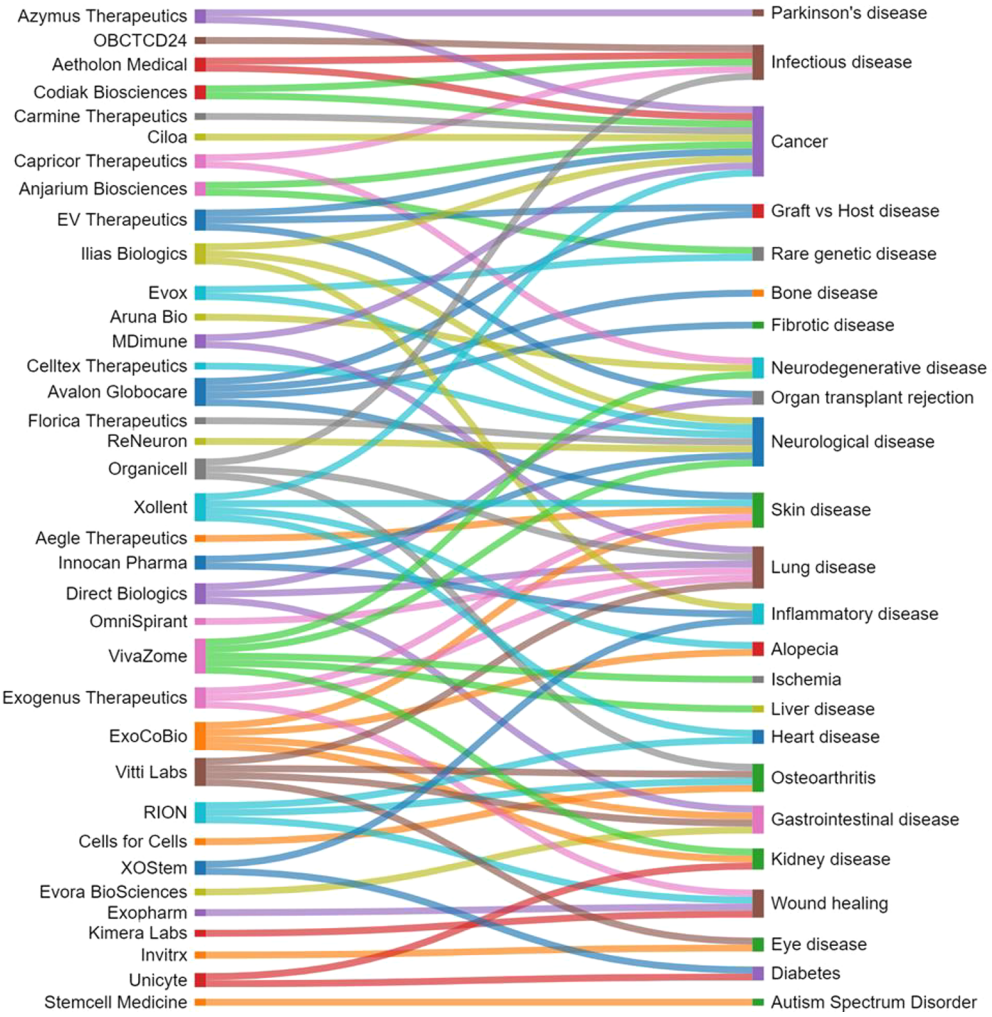

Figure 19. Promising exosome therapeutic companies and targeted diseases.

Therapeutic Exosome Companies. The number of companies that are utilizing exosomes for therapy is also expanding. Both preclinical and clinical works are progressing exosome therapeutics through companies' pipelines. A thorough review of exosome therapeutic companies reveals that the most highly represented diseases are cancer, neurological and neurodegenerative diseases, lung diseases, and wound healing (Figure 19 and Supporting Information Table S2).

Preclinical Therapeutic Exosomes. A growing number of companies are researching exosomes in hopes of advancing their therapeutic discoveries to the clinic. While historically MSC-derived exosomes were researched for therapy, a shift is taking place and companies are starting to focus research effort on organ-specific exosomes such as cardiac-derived exosomes or neural-derived exosomes for more targeted specificity in treating diseases. Table 9 displays selected preclinical companies focusing their research efforts to the highly represented targeted diseases from Figure 19.

Preclinical Diagnostic Exosomes. Companies, medical centers, and universities are also focusing their research efforts on discovering exosome biomarkers and representative tests for diagnosis of hard-to-treat diseases earlier, to help aid in the treatment success and patient survival. While cancer is the most highly represented disease for diagnosis through exosomes (Supporting Information Table S2), many other diseases can be diagnosed with exosome detection and organizations are working to develop these appropriate assays (Table 10). Many universities are also hard at work researching exosome disease diagnosis. Table 10 explores promising preclinical companies, medical centers, and universities researching exosome disease diagnosis. The current field of exosome diagnostics and developed assays is still relatively small with room to grow as more promising early disease biomarkers are researched and discovered.

CONCLUSIONS AND PERSPECTIVES

As demonstrated by data analysis of the CAS Content Collection, the interest in exosome exploration has grown significantly in the recent years. A growing number of studies provide valuable knowledge regarding this notable subtype of EVs. Indeed, exosomes exhibit distinctive functions as intercellular messengers, potential to modulate cellular bioactivities, as well as substantial therapeutic capacity, in disease diagnostics and targeted drug delivery. Their advantages over traditional pharmaceutical nanocarriers distinguish them as a rising star in both therapeutics and diagnostics. In this review, we provide a landscape of the global research effort for exosome development for medical applications, along with the challenges and growth opportunities for fulfilling their potential.

Exosomes are released from most cell types into the extracellular space following fusion of multivesicular bodies

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Table 9. Highlighted Companies Working on Preclinical Therapeutic and Cosmetic Exosomes along with Their Summaries

Company (location)	Summary
Anjarium Biosciences (Switzerland)	Anjarium is researching and developing precision exosome therapeutics. Their Hybridosome platform utilized nanotechnology and biochemistry to increase the efficiency of exosome loading with therapeutic cargo.[457] Anjarium is looking to use its exosome-based therapy platform to treat cancers and rare genetic diseases.
Aruna Bio (USA)	Aruna Bio is transforming treatment for neurological and neurodegenerative diseases. They utilize neural exosomes derived from neural stem cells that have CNS specificity and the ability to cross the BBB. Their candidate AB126 shows high uptake in the cerebellum and basal ganglia showing treatment potential for diseases such as stroke and neurodegenerative diseases.[245] Their pipeline shows that AB126 can be loaded with different cargos including siRNA, ASO, progranulin, and tripeptidyl-peptidase 1.[458]
Capricor (USA)	Capricor is developing multiple exosome platforms including cardiosphere-derived cell exosomes (CDC exosomes), engineered exosomes, and an exosome-based vaccine. They are currently researching the use of CDC exosomes for the treatment of Duchenne muscular dystrophy and engineered exosomes for RNA and protein delivery in trauma-related injuries and conditions in collaboration with the U.S. Army Institute of Surgical Research. They are also in preclinical trials for an exosome-based multivalent vaccine for COVID-19 and other infectious diseases.[459]
Carmine Therapeutics (USA)	Carmine Therapeutics utilizes red blood cell exosomes.[460] Their Red Cell EV Gene Therapy (REGENT) platform will be used to generate a pipeline of therapies for treatment of a wide range of diseases.[461]
EV Therapeutics (USA)	EV Therapeutics is developing modified exosomes (mEVs) (miR-424i and miR-424 KO) in combination with an immune checkpoint inhibitor for treatment of metastatic colorectal cancer and other GI cancers.[462] mTEV is a CD-28−CD80/86 costimulatory pathway technology platform that functions in combination with checkpoint inhibitors to enhance T-cell immunomodulation to prevent solid tumor cancer recurrence.[463]
Evora Bio-Sciences (France)	The EVOGEX therapeutic platform was developed by Evora. Their lead product EVOGEX-Digest aims to treat digestive fistula and improve patient outcomes.[464]
Evox (UK)	Evox is an exosomal therapeutic company using its DeliverEX platform to deliver proteins and nucleic acids to treat a variety of rare diseases. Their internal program is researching rare metabolic disorders. They have partnered with Takeda to treat lysosomal storage disease and other undisclosed rare diseases. Evox has also recently partnered with Lilly to research neurological treatment.[465]
Exocel Bio (USA)	Exocel utilizes placental MSC-derived exosomes for both skincare and hair care. Their products include the Evovex line called Evovex Restore, Evovex Revive, Evovex Renew, and Evovex Reveal.[466] These products are used in conjunction with facial and scalp microneedling and energy-based aesthetic device treatments to enhance results and improve recovery time.[467]
ExoCoBio (Republic of Korea)	ExoCoBio is focusing its research on stem-cell-derived exosomes to create both therapeutic and cosmetic products. They have developed ExoSCRT Exosome for the treatment of atopic dermatitis,[468] irritable bowel syndrome, acute kidney injury, and alopecia.[469] An immune-oncology drug based on exosomes derived from immune cells is also in their pipeline.
Exogenus Therapeutics (Portugal)	Exogenus's lead candidate Exo-101 is produced from umbilical cord blood mononuclear cells. It has been shown to have regenerative, anti-inflammatory, and immunomodulatory properties. Exo-101 is being investigated for treatment in inflammatory skin diseases such as psoriasis, inflammatory lung disorders such as COVID-19 ARDS,[470] and chronic wound healing.[471]
Florica Therapeutics (USA)	Florica Therapeutics aims to use hypothalamus stem-cell-derived exosome therapeutics to increase lifespan and deter neurological diseases of aging.[472]
Ilias Biologics (South Korea)	Ilias developed the platform EXPLOR that allows the loading of proteins into exosomes in a more controlled manner than conventional passive loading.[473] Ilias' lead compound ILB-202 consists of an exosome loaded with an anti-inflammatory protein super-repressor IkB targeting both acute and chronic inflammatory diseases. This lowers the risk of an off-target effect by targeting core inflammation signals.[474]
Innocan Pharma (Israel)	Innocan is a pharmaceutical company researching cannabidiol (CBD) drugs and enhancing their targeting due to its low bioavailability. Innocan is researching with Tel Aviv University the development of CBD-loaded exosomes to target inflammatory diseases and central nervous system diseases.[475]
Kimera Laboratories (USA)	Kimera specializes in the use of perinatal MSC-derived exosome products for both cosmetics and scientific research.[476] Their cosmetic products are XoGlo, XOGloPro, and Vive. They also produce a veterinarian wound healing agent called Equisome HC.[477]
MDimune (Republic of Korea)	MDimmune developed a platform technology called BioDrone that uses cell-derived vesicles for targeted drug delivery.[478] Their internal pipeline includes treatment for chronic obstructive pulmonary disease (COPD) and an undisclosed rare disease with therapeutics BDR-231 and BDR-331, respectively. They have partnered with Ildong, Kainos Medicine, and NeoCura for the treatment of cancer using various mRNAs and small molecules for cargo for therapeutic products BDR-165, BDR-166, and BDR-167. They are also partnered with Reyon for a vaccine with therapeutic BDR-761 and treatment of an undisclosed rare disease with therapeutic BDR-762 using mRNA as cargo.[479]
OmniSpirant (Ireland)	OmniSpirant's platform technology is based on inhalation and is very efficient at delivering cargos to treat respiratory diseases. The mucus penetrating exosomes will be used to develop a regenerative gene therapy for cystic fibrosis and other respiratory diseases.[480]
Regen Suppliers (USA)	Regen Suppliers developed an exosome product called ReBellaXO, derived from umbilical stem cell tissue and Wharton's jelly used for regenerative cosmetic procedures involving facial, hair, and sexual rejuvenation.[481]
Xollent (USA)	Xollent is advancing a diversified pipeline of therapeutics including exosome therapeutics treating myocardial infarction through an intravenous patch, alopecia through a spray, and skin aging through a needle-free injection.[482]

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Table 10. Highlighted Companies and Universities on Preclinical Research of Exosomes as Biomarkers for Diagnosis of Various Diseases and Their Summaries

Companies/medical centers/universities (location)	Summary
Aarhus University Hospital (Denmark)	Researchers discovered that the biomarkers CD151, CD171, and tetraspanin 8 were the main dividing factors for patients with non-small-cell lung cancer of all types versus patients without cancer.[348]
Craif (Japan)	Craif developed a medical device consisting of a zinc oxide nanowire embedded in a microfluidic channel that collects urinary miRNA for exosome-based liquid biopsy. They are using machine learning technology to analyze miRNA profiles with their original miRNA database to identify biomarkers for early cancer detection.[483]
Frankfurt University Hospital (Germany)	Researchers studied how CD81 is increased in the exosomal serum of patients with chronic hepatitis C and appears to be associated with inflammatory activity and severity of liver fibrosis.[323]
Harvard Medical School (USA)/Wenzhou Medical University (China)	Researchers have developed an incorporated tear-exosome analysis via rapid-isolation system (iTEARS) via nanotechnology to discover if exosomes from tears can diagnose ocular disorders and systemic diseases. Data show that iTEARS might be used to improve the molecular diagnostics of dry eye disease, along with diabetic retinopathy.[361] There is also a possibility that iTEARS could be used to detect other neurodegenerative diseases and cancer.
Mercy Bioanalytics (USA)	Mercy developed the Halo test for early cancer detection test with initial focus on hard-to-treat cancers such as ovarian and lung cancers.[484] Preliminary results from studies researching Halo detection of both early stage ovarian and lung cancers were positive.[485,486]
Osako University (Japan)	Researchers discovered that three p53-responsive microRNAs, miR-194, miR-34a, and miR-192 are elevated in exosomes of patients with acute myocardial infarction, suggesting that these microRNAs function as circulating regulators of heart failure. They feel that these three microRNAs are worth further exploration as biomarkers for ischemic heart failure after acute myocardial infarction.[487]
UCSF Medical Center (USA)	Researchers discovered that levels of P-S396-tau, P-T181-tau, and Aβ1−42 from neural-derived blood exosomes can predict the development of Alzheimer's disease up to 10 years before clinical onset of symptoms.[306]
University of Texas MD Anderson Cancer Center (USA)	Researchers identified a cell surface proteoglycan, glypican-1 (GPC1), specifically enriched on cancer-cell-derived exosomes. GPC1(+) circulating exosomes may serve as a potential diagnostic and screening biomarker for assays to detect early stages of pancreatic cancer.[43]

with the cellular membrane.[4,7,54,488] During the process of exosome secretion, parent cell information is stored in the exosomes, in their constituent lipids, proteins, and nucleic acids, which are then able to manipulate the functions of recipient cells on arrival. The content of the exosomes is therefore characteristic to the cell of origin, permitting parent cell signals to be communicated to neighboring cells without direct cell-to-cell contact. A foremost advantage over signaling molecule secretions is that exosomes are able to deliver signals at large distances without any dilution or degradation, because the biomolecules are being safely transported within their lipid bilayer capsule.

With significant research being devoted to exosome medical applications—in drug delivery, in diagnostics, as therapeutic targets, or as therapeutics themselves—it is vital to review and recapitulate the progress made, along with the persisting challenges.[9,19,20] Although exosome analyses have intensely evolved in the recent years, their exact mechanisms of biogenesis and uptake are still largely unknown. Furthermore, the challenges in efficient and successful exosome isolation are still persisting, primarily due to the complexity of bodily fluids, the extensive overlap of the physicochemical and biochemical characteristics among the exosomes, lipoproteins, viruses, and other extracellular vesicles, as well as the heterogeneity of exosomes. Thus, developing efficient and reliable isolation and characterization techniques is critical to further advance in this area, in order to examine the cargo contents and functions, which would shed light on the biogenesis and uptake in return. Furthermore, fundamental questions in the field such as the secretory regulation mechanism of exosomes, the exosomal content sorting mechanism, and their intercellular transduction pathway are still to be answered too. To fully utilize the exosome potential, basic research and emerging advanced technologies need to be combined, which will set forth their therapeutic applications.

Clinical applications of exosomes, although highly promising, are hindered by the lack of standardization in exosome isolation and analysis, which has become a major challenge in the field.[489] The use of inconsistent protocols for sample handling, analysis, and data control leads to discrepancy that significantly affects analysis, makes interstudy comparisons difficult, and overall complicates the knowledge development. Thus, standardization in exosome preparation such as specimen handling, isolation, and quantification still has to be established.[205,206]

Thus, some appealing challenges in exosome knowledge include the following:

- Potent **isolation methods** that do not compromise on the purity of the isolated specimens are required in order to exploit exosomes in biomedical research and therapeutics—such methods are the primary prerequisite for exosomal large-scale application in medical practice. Additionally, recent studies have shown that an appropriate combination of several methods to extract and purify exosomes can effectively contribute to solving this problem.[168,171]
- The exact **mechanisms** involved in the biogenesis, secretion, and fusion of exosomes have not yet been fully elucidated and require further research. It is also mostly unknown whether incorporating cargo into exosomes is a selective or a random process, although data is accumulating that suggests a certain degree of cellular control.

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Table 11. Highlighted Exosome Therapeutic Clinical Trials[a]

Companies/ medical centers/universities (location)	Exosome	Disease treated	Clinical trial number	Clinical trial stage or status (date initiated)	Summary
M.D. Anderson Cancer Center (USA)	MSC-derived exosomes with KrasG12D siRNA (iExosomes)	metastatic pancreatic cancer with KrasG12D mutation	NCT03608631	phase I (2018)	This study researches the optimal dose and the drug toxicity of using iExosomes in treating metastatic pancreatic cancer patients.[491]
Neurological Associates of West Los Angeles (USA)	exosomes	cranial facial neuralgia	NCT04202783	suspended (due to COVID-19 pandemic) (2019)	This study will evaluate the safety and efficacy of exosome treatment in patients with craniofacial neuralgia.[492]
Organicell Regenerative Medicine (USA)	amniotic-fluid-derived exosomes/Zofin (organicell flow)	mild/moderate COVID-19	NCT04657406	expanded access status available (2020)	The therapeutic Zofin is currently undergoing clinical trials for COVID-19, COPD, and osteoarthritis.[493]
Direct Biologics (USA)	bone marrow MSC-derived exosomes/ DB-001/ExoFlo	COVID-19 ARDS	NCT04657458	expanded access status available (2020)	Their therapeutic ExoFlo is currently undergoing clinical trials for COVID-19-associated moderate to severe ARDS, ulcerative colitis, Crohn's disease/irritable bowel syndrome, and organ transplant rejection.[494]
Rion (USA)	purified exosome product (PEP)	skin graft	NCT04664738	phase I (2020)	Rion is researching with this clinical trial the application of their PEP therapeutic (a leukocyte depleted blood preparation derived from apheresed platelets) in patients with a skin graft for wounds to determine if it offers improvement in healing properties over the standard wound dressing treatment.[495]
Ruijin Hospital (China)	adipose mesenchymal stem-cell-derived exosomes (MSCs-Exos)	Alzheimer's-disease-induced dementia	NCT04388982	phase I/II (2020)	The purpose of this study is to explore the safety and efficacy of MSCs-Exos in the treatment of mild to moderate dementia due to Alzheimer's disease.[496]
Rion (USA)	purified exosome product (PEP)	acute myocardial infarction	NCT04327635	phase II (2020)	Rion's PEP exosome therapeutic is currently in preclinical and clinical studies for multiple indications[497] including acute myocardial infarction, wound healing,[498] pelvic floor disorders,[499] hair loss treatment, and degenerative joint disease[500,501] with many encouraging results.
University of Louisville (USA)	ginger exosomes with/without curcumin	irritable bowel disease	NCT04879810	recruiting (2021)	The purpose of this clinical trial is to test if edible ginger exosomes will have clinically relevant anti-inflammatory action on the gut lining of patients with inflammable bowel disease.[437] The University of Louisville also has an active clinical trial exploring edible plant exosomes conjugated to curcumin for the treatment of colon cancer.[502] They also conducted a completed clinical trial researching grape exosomes dosed as grape powder to reduce the incidence of oral mucositis during radiation and chemotherapy cancer treatments.[439]
OBCTCD24 (Israel)	exosomes overexpressing CD24/ CovenD24/ EXO-CD24	moderate or severe COVID-19	NCT04902183	phase I (2021)	OBCTCD24 has their product EXO-CD24/CovenD24 in one other clinical trial. CovenD24 is an exosome overexpressing CD24 administered through inhalation dosing for the treatment of moderate or severe COVID-19.[298]
Aegle Therapeutics (USA)	bone marrow MSC-derived exosomes/ AGLE-102	burns	NCT05078385	phase I (2021)	Preclinical data reveals that exosomes isolated by Aegle accelerated healing, minimized scars, and promoted blood vessel and nerve regeneration, as well as hair follicle growth.[503] In addition to burns, AGLE-102 is also in a clinical trial for the treatment of dystrophic epidermolysis bullosa, a group of rare genetic disorders that presents with blistering or erosion of the skin in response to little or no trauma.[504]
Maimónides Biomedical Research Institute of Córdoba (Spain)	MSC-derived exosomes	wound healing/skin ulcers in diabetic patients	NCT05243368	not yet recruiting (2022)	The focus of this clinical trial is to develop a therapeutic process to accelerate the healing of diabetic chronic skin ulcers, based on nutritional intervention and the application of MSC-derived exosomes to the wound, to improve skin regeneration.[505]
Codiak BioSciences (USA)	exosomes loaded with a synthetic lipid-tagged oligonucleotide/ CDK-004/ exoASO-STAT6	advanced hepatocellular carcinoma (HCC)/liver metastasis	NCT05375604	phase I (2022)	In addition to HCC,[506] Codiak also has clinical trials for treatment of cutaneous T-cell lymphoma, solid tumors, and non-small-cell lung cancer. They have also developed an exosome-based vaccine on their engEx platform for the treatment of beta coronavirus, Epstein−Barr virus, and HIV.[507] Recent preclinical data resulted in positive results for their pan-beta coronavirus vaccine, ecoVACC showing the probable protection from additional beta-coronaviruses and emerging variants.[508]
Vitti Laboratories (USA)	umbilical-cord-derived exosomes/EV-Pure in combination with	moderate to severe ARDS associated with COVID-19	NCT05387278	phase I (2022)	While Vitti Laboratories has two current clinical trials,[509] they have many more disease indications in their pipeline for their EV-Pure exosomal product along with exosomal-based topical and serum applications for wound healing and age-related macular

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Table 11. continued

Companies/medical centers/universities (location)	Exosome	Disease treated	Clinical trial number	Clinical trial stage or status (date initiated)	Summary
	Wharton's jelly MSCs (WJ-Pure)				degeneration, respectively. Their EV-Pure product is currently researched preclinically for treatment of COPD, osteoarthritis, traumatic brain injury, Crohn's disease, polycystic ovarian syndrome, and Alzheimer's disease.[510]

[a]Details obtained from https://clinicaltrials.gov/.

- The underlying mechanism of how exosomes communicate with the target cells and how selectivity is achieved is not yet well understood. Advanced knowledge on these processes is a prerequisite to develop effective therapeutics that target exosome communication and for the development of engineered exosome-derived therapeutic vehicles.
- Exosome **loading** capacity and methods for enhancing their **targeting** need to be optimized and improved for their large-scale application in clinic.
- Studies have demonstrated that exosomes are able to **permeate the BBB** from the brain to the bloodstream as well as from the blood to the CNS; however, only limited knowledge exists about the mechanisms exosomes use to cross the BBB.[77,245] Understanding of the surface markers required to cross the barriers protecting the brain and the ones needed to target the cells or tissues responsible for a pathology is needed in order to make use of this notable ability of exosomes.[77]
- **Standardization** of exosome preparation, including source selection, isolation, characterization, drug loading, stability, targeting, and quality control, in compliance with good manufacturing practice, is an important aspect in the clinical application of exosomes and needs to be advanced. There is thus an urgent need to develop guidelines for manufacturing, storage, and administration of therapeutically relevant exosomes, with respect to safety and quality GMP standards to be followed.
- The prospective use of exosomes as a delivery vector needs further deep assessment. The tractability of the exosomes needs to be improved, and the possibility to package multiple drugs for combination (immuno)-therapy needs to be explored. With personalized medicine models emerging and being advanced, it is important to assess the potential for developing personalized approaches for delivering therapeutically relevant exosomes.
- Advanced knowledge on the **pharmacokinetic** profile and **biodistribution** of exosomes is still particularly insufficient and is a required step toward their practical utility in clinics.
- The nature of the cargo in exosomes soundly depends on the origin of the cells where the exosomes are released. It is thus important to know how the cargo is packed in exosomes, since cancer cells are known for their heterogeneity and the nature of cargo from each cancer cell will be distinctive. Such knowledge would advance designing strategies for early diagnosis and monitoring treatment response by using exosomes.[490]
- Cells modulate the composition of exosomes in response to exogenous stress. Understanding the mechanisms involved might result in the development of therapeutics that take advantage of this property.
- An emerging area of exosome research currently gaining considerable attention is their potential application in cancer immunotherapy, in particular developing anticancer vaccines. Various cells such as B-cells, dendritic cells, macrophages, cancer cells, and normal cells have been employed for isolating exosomes as possible agents in cancer immunotherapy. These cells all exhibit characteristic composition profiles directly involved in anticancer immunotherapy.

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Table 12. Highlighted Exosome Diagnostic Clinical Trials[a]

Companies/ medical centers/universities (location)	Exosome (disease target)	Disease diagnosed	Clinical trial number	Clinical trial status (date initiated)	Summary
University of Alabama at Birmingham (USA)	blood- or urine-derived exosomes (LRRK2)	Parkinson's disease	NCT04350177	completed (2013)	Researchers used this study to determine exosome biomarkers for Parkinson's disease and to determine if LRKK2 expression within exosomes from LRRK2 kinase inhibitor sunitinub treated patients decreased after treatment. They hope to use this information to build an assay for on-target effects for future LRRK2 inhibitor clinical trials.[511]
Boston University (USA)	plasma-derived exosome (tau)	chronic traumatic encephalopathy	NCT02798185	active (2016)	Boston University researchers collaborated with Exosome Sciences and Aethlon Medical for the DETECT CTE research project, which aims to validate exosomal tau as a non-invasive CTE biomarker. Preliminary findings look promising that plasma exosomal tau may be an accurate, non-invasive biomarker for CTE.[328] Researchers are using this clinical trial as an advancement to the previous study with the goal of diagnosing CTE during life for the prevention and treatment of the disease.
Exosome Diagnostics (USA)	urine-derived exosome (ERG, PCA3, and SPDEF)	prostate cancer	NCT02702856	completed (2016)	Exosome Diagnostics developed the ExoDx test that utilizes circulating cancer exosomes from urine-derived exosomes and is commercially available. The ExoDx test was granted FDA Breakthrough Device Designation in 2019.[512] They also have current clinical trials for the use of exosomes in diagnosis of non-small-cell lung cancer[513] and kidney transplant rejection.[514] Preliminary data from their breast cancer trial reveals specific gene signatures could be isolated from plasma-derived exosomes,[515] and their kidney transplant trial showed the discovery of two separate gene signatures for the monitoring of kidney transplant rejections.[516] They have also had success preclinically with identifying plasma biomarkers for glioblastoma[517] and a saliva exosomal RNA signature for Sjogren's syndrome.[518]
miR Scientific (USA)	urine-derived exosome (442 sncRNA)	bladder cancer	NCT04155359	recruiting (2019)	miR Scientific developed the miR Sentinel test currently commercially available for prostate cancer detection with extracted sncRNA in urine-derived exosomes.[519] They are investigating through this clinical trial if there is evidence that they can also diagnose bladder cancer with the miR Sentinel test. The miR Sentinel test received FDA Breakthrough Device Designation in 2020.[520]
University of Utah Center for Clinical and Translational Science (USA)	urine-derived exosomes (sodium transporters)	heart failure with preserved ejection fraction (HFpEF)	NCT03837470	completed (2019)	This trial examines sodium transporters in the exosomes from patients with HFpEF for characterization to aid in diagnosis and treatment of these patients.[521]
Aarhus University Hospital (Denmark)	plasma-derived exosomes	acute ischemic stroke	NCT04266639	completed (2020)	This trial was performed to determine if exosome isolation with characterization of the nucleic acid (DNA and RNA, including miRNA) content will show any decrease in stroke complications and any advantage of remote ischemic conditioning.[522]
Lithuanian University of Health Sciences (Lithuania)	eosinophil-derived exosome	asthma	NCT04542902	recruiting (2020)	This clinical trial's investigation of ncRNA in eosinophil-derived exosomes will provide insights on eosinophils subtypes in airway remodeling. ncRNAs are key regulators for gene transcription, and researchers predict that altered blood levels of ncRNAs could be a diagnostic biomarker in asthma.[523]
Peking Union Medical College Hospital (China)	blood and bronchoalveolar lavage fluid (BALF)-derived exosomes	ARDS	NCT05451342	recruiting (2022)	This clinical trial will characterize exosomes from blood and BALF with transcriptome and metabolomic analysis to aid in the diagnosis of ARDS.[524]

[a]Details obtained from https://clinicaltrials.gov/.

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Table 13. Highlighted Clinical Trials That Target Exosomes (Physical Elimination) for Disease Treatment[a]

Company (location)	Exosome	Disease treated	Clinical trial number	Clinical trial status (date initiated)	Summary
Aethlon Medical (PA, USA)	circulating exosomes	squamous cell carcinoma of the head and neck	NCT04453046	recruiting (2020)	Aethon is conducting an early feasibility study for the treatment of head and neck cancer with their Hemopurifier in combination with the antibody drug pembrolizumab.[526]
Aethlon Medical (PA, USA)	circulating exosomes	COVID-19	NCT04595903	recruiting (2021)	Preclinical results had promising results showing Galanthus nivalis agglutinin affinity resin of Aethlon's Hemopurifier captures seven clinically relevant variants of SARS-CoV-2.[527] They are currently in an early feasibility study for the treatment of COVID-19, where their first patient successfully completed treatment.[528]

[a]Details obtained from https://clinicaltrials.gov/.

Since exosomes represent an advanced potential treatment strategy in a wide range of therapeutic areas, possibly as cell-free regenerative medicines, as treatments for cardiovascular, CNS, and oncological disorders, as vectors for gene therapy, as immune modulators, and as drug delivery vehicles, their innovation and range of uses means that there will be specific regulatory classification and jurisdiction issues to be clarified to enable development plans to be established. In recent years, concurrent advances have been witnessed in the exosome expertise for next-generation diagnostics, disease supervision, and individualized diagnosis and therapy. These are widely applied for early diagnosis and delivery systems with high efficacy. Further advances in the drug loading strategies and modification methods will enable clinical translation in the future, with a tangible patient benefit.

APPENDIX: EXOSOMES IN CLINICAL TRIALS

Exosome Therapeutics in Clinical Trials

Currently, the total number of clinical trials registered at https://clinicaltrials.gov for exosomal therapeutics is 59 clinical trials (Supporting Information Table S2). The most highly researched targeted diseases for exosome therapeutics include lung disease (11 clinical trials), SARS-CoV-2 infections (9 clinical trials), and cancer, heart disease, and neurological diseases (all with 4 clinical trials). Highlighted clinical trials with respect to these diseases are listed in Table 11.

Exosome Diagnostics in Clinical Trials

Currently on https://clinicaltrials.gov, there is a total of 208 clinical trials with exosomes being used for diagnosis (Supporting Information Table S2). Over half of these clinical trials (108 clinical trials) are related to cancer diagnosis utilizing exosomes. Other highly represented diseases include neurological diseases (15 clinical trials), cardiovascular diseases (13 clinical trials), and lung diseases (6 clinical trials). Early diagnosis of these diseases is crucial for better prognosis. The large number of clinical trials of exosomes in diagnosis highlighted the value and advantage of using exosomes in early disease diagnosis. Table 12 highlights the companies, medical centers, and universities related to exosome diagnosis of these diseases.

Exosomes as the Disease Target in Clinical Trials

Using exosomes as targets is another avenue that is being explored for disease treatment. Aetholon Medical is a California-based clinical company that has designed an investigational medical device called the Hemopurifier. Targeting circulating exosomes, the Hemopurifier captures viral and bacterial toxins and cancer exosomes to treat disease. To date, Aetholon has used the Hemopurifer to treat patients with ebola, hepatitis C, HIV, and COVID-19.[525] Their two current clinical trials are explored in Table 13.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsnano.2c08774.

Table S1, exemplary small molecule drugs delivered by exosomes; **Figure S1**, classes of substances represented in the documents related to exosome applications in therapy and diagnostics as found in the CAS Content Collection; **Figure S2**, percentages of documents concerning the major tetraspanin classes in the documents related to exosome applications in therapy and diagnostics, along

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with role indicators for the top three tetraspanin classes; **Figure S3**, number of documents related to exosome applications in therapy and diagnostics, in which various kinds of cells have been used as exosome donors; **Figure S4**, annual trend of the number of documents in the CAS Content Collection related to the exosome applications in cosmetics and food (PDF)

Table S2, therapeutic and diagnostic exosome clinical trials (XLSX)

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Notes

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VOCABULARY

extracellular vesicles: lipid-bilayer-surrounded particles, which are secreted by cells into the extracellular space; they represent a route of intercellular communication and contribute to a wide range of physiological and pathological processes

exosomes: a nanosized subset of extracellular vesicles (diameter ∼30−150 nm) comprising bioactive cargos, including proteins, nucleic acids, lipids, and metabolites

biomarker: indicator of normal or pathogenic biological processes, or pharmacological responses to a therapeutic intervention, which can be measured objectively, accurately, and reproducibly

drug targeting: delivering medication to a patient in a manner that results in predominant drug accumulation in a specific body area (organ, cellular, and subcellular level of specific tissue) in order to overcome the toxic effect of conventional drug delivery

blood−brain barrier: highly selective boundary of endothelial cells that prevents solutes in the circulating blood from non-selectively crossing into the extracellular fluid of the central nervous system

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