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Harnessing the Therapeutic Potential of Extracellular Vesicles for Biomedical Applications Using Multifunctional Magnetic Nanomaterials

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Extracellular vesicles (e.g., exosomes) carrying various biomolecules (e.g., proteins, lipids, and nucleic acids) have rapidly emerged as promising platforms for many biomedical applications. Despite their enormous potential, their heterogeneity in surfaces and sizes, the high complexity of cargo biomolecules, and the inefficient uptake by recipient cells remain critical barriers for their theranostic applications. To address these critical issues, multifunctional nanomaterials, such as magnetic nanomaterials, with their tunable physical, chemical, and biological properties, may play crucial roles in next-generation extracellular vesicles (EV)-based disease diagnosis, drug delivery, tissue engineering, and regenerative medicine. As such, one aims to provide cutting-edge knowledge pertaining to magnetic nanomaterials-facilitated isolation, detection, and delivery of extracellular vesicles and their associated biomolecules. By engaging the fields of extracellular vesicles and magnetic nanomaterials, it is envisioned that their properties can be effectively combined for optimal outcomes in biomedical applications.

1. Introduction

Cell-to-cell communication plays a pivotal role in any multicellular organism and is often controlled by either direct cell–cell interactions or the secretion of soluble factors to extracellular spaces.[1,2] Notably, in most mammalian cells and some of the lower eukaryotes and prokaryotes, cell-secreted biomolecules, including RNAs, DNAs, proteins, and lipids, can be enveloped in membrane-derived nanoscale vesicles to regulate activities of both neighboring and distal cells.[3,4] Such membrane-coated extracellular vesicles, or EVs, were initially discovered 38 years ago and have been purified from nearly all mammalian cell types, including cancer cells, immune cells, neural cells, muscle cells, and stem cells.[5–12] Although initially considered as membrane debris with no biological significance, the crucial roles of EVs in immune surveillance, viral infection, blood coagulation, tissue repair, and stem cell maintenance, have been sequentially identified since 1996.[6,13–19] Additionally, biomolecular compositions inside EVs have also been closely associated with the pathology of various kinds of diseases such as cancers, musculoskeletal diseases, as well as degenerative neurological disorders.[20–22]

As a result of the fundamental role of EVs in mediating cell-to-cell communications and regulating various tissue functions, there has been a critical need to develop novel therapeutics and diagnostics (or theranostics) using EVs and their associated biomolecules.[23] For example, cell-free therapy using EVs derived from mesenchymal stem cells (MSCs) has been exploited and is currently under clinical trials to enhance tissue regeneration after cardiac infarction.[6,7,17,18] Similarly, EVs capable of crossing the blood-brain barrier (BBB) have been utilized for intranasal delivery of therapeutics for treating central nervous system (CNS) injuries.[24–27] Moreover, minimally invasive, highly sensitive/accurate diagnosis of cancer metastasis, viral infection, Prion diseases, Alzheimer's disease (AD), and Parkinson's disease (PD), has also been realized by analyzing the biomolecular formulation of EVs extracted from human body fluids.[28,29]

Although the potential of using EVs for theranostic applications is enormous, their current clinical translation is still impeded by several critical barriers, which can be attributed mainly to the high heterogeneity of EVs. In general, there are four levels of heterogeneity of EVs, including size, composition, function, and source heterogeneities.[2,30] Specifically, most clinical applications often require a well-defined source of therapeutics. Sizes, compositions, functions, and sources have all been closely associated with the outcome of clinical treatment and diagnostic diseases.[30–36] Having the ability to isolate a specific population of EVs with well-defined sizes, biomolecular contents, disease-specific biological functions, and biodistributions would be crucial for the clinical application of EVs. Failing to achieve this can lead to compromised therapeutic effects and sometimes even adverse outcomes. For example, the multifaceted roles of EVs have been identified for regulating

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viral infections.[37,38] EVs derived from specific cell types (e.g., trophoblasts, sperms, and leukocytes) can limit viral infection via stimulation of immune surveillance or transcriptional suppression of viral replication.[39,40] However, EVs derived from other cell types have been reported to promote viral infection by acting as immune-escaping delivery vehicles during blood circulation.[38,41–43] Therefore, novel approaches that can precisely isolate, analyze, and deliver EVs and their associated biomolecules would be crucial for realizing the full clinical potential of EV-based theranostics.

In the past two decades, engineered magnetic nanomaterials (MNMs), including 0D nanoparticles, 1D nanorods, 2D nanosheets, and hybrid 3D nanomaterials, have attracted intense interest and showed clear advantages for the isolation and target-specific delivery of various types of biomolecules, lipid vesicles, as well as living organisms. Previous reviews and progress reports have extensively covered these topics on the synthesis, engineering, functionalization, and application of magnetic nanoparticles for biomedical applications.[44–49] To facilitate the isolation, capturing, analysis, delivery, monitoring, and imaging of extracellular vesicles for biomedical applications, it is not surprising that magnetic nanomaterials have already played significant roles, with over 300 published articles on this topic. Despite a continuous surge of research activity in this field, a comprehensive review covering magnetic nanomaterial-based detection, delivery, and engineering of extracellular vesicles for biomedical applications is lacking.[50–54] These approaches are also rapidly applied for the advanced theranostics of various types of diseases, including immune dysfunctions, neurological disorders, myocardial infarctions, and tumor metastasis. Besides, other than using their nanoscale magnetism, magnetic nanoparticles (MNPs) can be engineered to be multifunctional, capable of enhancing magnetic resonance imaging (MRI) for the in vivo tracking of EVs, incorporating magnetic hyperthermia for the spatiotemporally controlled release of biomolecules, and integrating magnetic field-based biomolecular sensing mechanisms for EV-based theranostic applications. However, despite its enormous potential, the field of magnetic nanoparticle-enabled EV research is still at an early stage, and there is plenty of room for developing novel tools for advanced theranostic applications. For example, in vivo regiospecific delivery of EVs to target tissues is of top-most interest for clinical translation of EV-based therapeutics; however, design and engineering principles to achieve this goal require establishing a structure–function relationship in MNM–EV hybrid systems. Similarly, in situ, noninvasive, and real-time biosensing of EV-derived biomarkers using MNMs have enormous potential for point-of-care disease diagnosis. However, research on this topic is still in its early stage. Thus, we aim to synergize both fields and provide insights into the opportunities and challenges of bioapplications of EVs using multifunctional MNMs. Addressing the aforementioned issues, the current paper will provide a comprehensive overview of the current state-of-art advances with our own insights and vision on challenges and opportunities in the field.

In the first part of our review, we will introduce the biological context of extracellular vesicles and their composition, function, tissue origin, and size heterogeneities (**Figure 1**). In this section, we hope to familiarize readers from fields of material science, chemistry, and bioengineering with the history and current status of extracellular vesicle-based research. Another goal of this section is to present readers with a diverse array of critical biological problems of synthetic and cell-derived vesicles that can potentially inspire novel synthesis, engineering, and applications of magnetic nanomaterials. Next, we will briefly overview current approaches to engineer magnetic nanomaterials, including doping, hybridization, functionalization, and synthesis of magnetic nanomaterial for biomedical applications. By learning from the many exceptional examples of magnetic-facilitated biomedical applications, we hope to inspire the novel design of magnetic nanomaterials for extracellular vesicle-based therapeutic and diagnostic development.

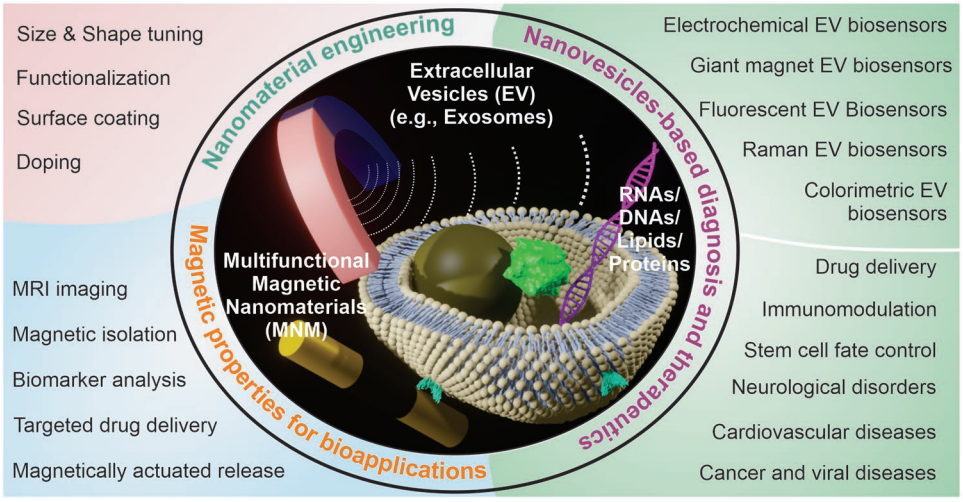

Figure 1. A scheme showing the overall structure of our proposed review on magnetic nanoparticle-facilitated biological applications of EVs. In this review, the biological context, especially different types of EVs shown in the center of the schematic diagram, will be first discussed. As a basic tool, state-of-arts on precise engineering of MNMs (pink background) for varying biomedical applications (blue background) will be briefly introduced, shown in the left panel. Then the focus of the review will be on how to harness the therapeutic potential of EVs using engineered MNMs, which mainly include diagnostic applications and therapeutic applications, shown in the right panel (in the green background).

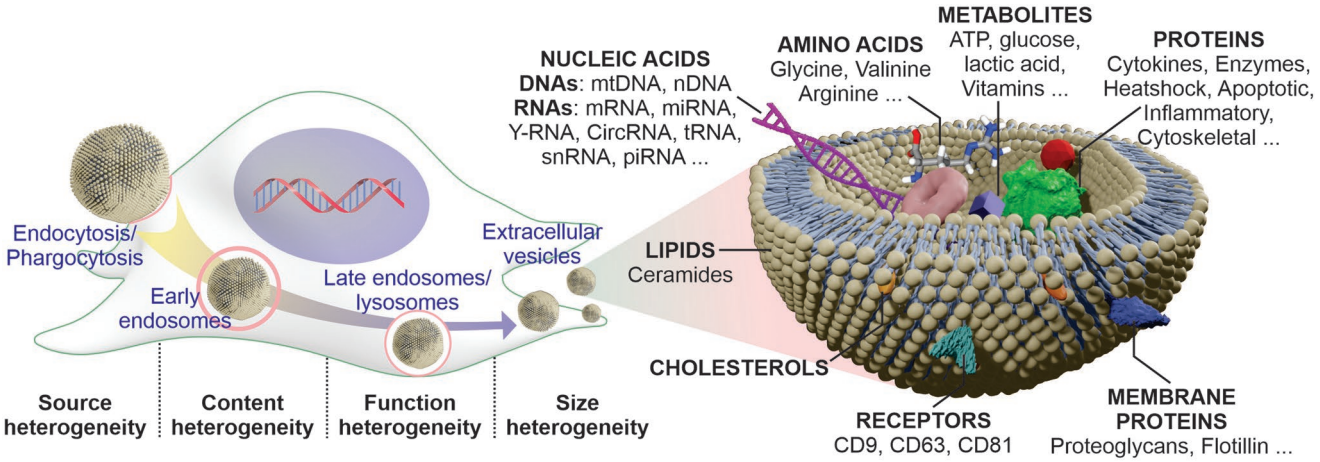

Figure 2. A scheme illustrating the biogenesis and different types of biomolecules in extracellular vesicles (EV). Biomolecules including lipids, proteins, metabolites, and others can enter cells through endocytosis, phagocytosis, or diffusion, which plasma membrane enveloping could occur. The budding of the plasma membrane will further lead to intracellular vesicle formation, vesicle fusion, and late endosome formation. Depending on the biomolecule distribution and sizes of the intracellular vesicles, different kinds of EVs can be formed with varying levels of heterogeneities, including source heterogeneity, content heterogeneity, function heterogeneity, and size heterogeneity. After exocytosis, EVs will be released. The schematic diagram on the right panel illustrates different types of biomolecules that can exist in EVs, including nucleic acids, amino acids, metabolites, proteins, lipids, and other biomolecules. These biomolecules can be used for both diagnostic applications as biomarkers, or therapeutic applications. The three major types of biomolecules that will be discussed in this review will be nucleic acids (e.g., miRNAs and mRNAs), lipids, and proteins.

Most importantly, in the third and fourth sections, we will overview the current state-of-arts that integrate engineered magnetic nanomaterials into the extracellular vesicle-based diagnostic and therapeutic applications. All key literature in the field will be comprehensively summarized and systematically. And then, we will further explain the general design principles of magnetic nanomaterial facilitated theranostic applications of the extracellular vesicle by focusing on the most exciting articles as case studies. In this way, readers with varying backgrounds could obtain both in-depth and in-breath knowledge of the field and have the opportunity to learn how to engineer magnetic materials to address the most critical challenges of extracellular vesicle-based biomedical applications.

Please note that there have been a few excellent reviews on the synthesis, functionalization, and biological applications of magnetic nanomaterials; the research topic of nanoscale vesicles has also been comprehensively discussed.[2,55–63] We would encourage readers interested in the general backgrounds of magnetic nanomaterials and EVs to read those review papers to have a complete picture of these two fields. However, to the best of our knowledge, few of the current literature has focused on magnetic nanomaterials-facilitated theragnostic applications of extracellular vesicles, which is a rapidly emerging field according to the trend of publications. The current review paper fills this critical gap by providing cutting-edge knowledge about magnetic nanomaterials-facilitated isolation, detection, and delivery of extracellular vesicles. In this way, the two important fields pertinent to material science and engineering could be engaged in dialogue to better develop theranostic systems in the future.

2. EV and Their Biological Functions

In molecular biology, EVs can be broadly categorized into two major categories, ectosomes and exosomes.[64] The fundamental difference between these two categories is the origin of the vesicles. The former is derived from the outward budding of the plasma membrane, and the latter is derived from the inward budding of the plasma membrane as part of the endosome pathway.[64] While initially thought to mainly be involved in the excretion of unneeded proteins, lipids, and other waste from the cell, further investigation has shown that EVs have a diverse role in many aspects of cell–cell communication, including soluble signaling, antigen presentation, anchorage-independent growth, and more (**Figure 2**).[1,30,65–67] In this section of the review, we will only give a brief introduction to the main types of EVs, their cargo, and some biological applications of cell-derived EV research, since there are several much more comprehensive review papers available on the subject.[1,3,64,68]

2.1. Types of Mammalian Cell-Derived Vesicles and Their Functions

2.1.1. Ectosomes or Microvesicles

While similar in several areas to exosomes, ectosomes, or microvesicles differ in their biogenesis. While exosomes are typically formed by an endosomal pathway, ectosomes or microvesicles can be formed by the outward budding of the plasma membrane, which results in fission from the cell body.[64] Microvesicle biogenesis is also highly complex, resulting in various machinery and pathways involved in microvesicle formation. One such pathway includes calcium-dependent machinery such as flippases and floppases, scramblases, and calpain, which further can induce asymmetry and bending of the plasma membrane.[11,69] This, in turn, leads to a restructuring of the actin cytoskeleton through RHO and ROCK pathways, resulting in the formation of microvesicles.[70] Like exosomes, microvesicles can also contain a diverse range of biomolecules that can be shuttled to other cells and used for cellular signaling.

2.1.2. Exosomes

The first class of cell-derived vesicles is exosomes. Exosomes are derived from the inward invagination of the plasma membrane. While the molecular mechanisms of exosome biogenesis are still being uncovered, many studies have to clarify various mechanisms that play a role in exosome biogenesis.[68] The endosomal sorting complex required for the transport (ESCRT) pathway plays a role in membrane cargo clustering, leading to the first level of invagination of the plasma membrane.[71] However, this has also been demonstrated to occur in ESCRT-independent mechanisms, such as ceramide biogenesis by sphingomyelinase.[72,73] This process creates intraluminal vesicles (ILVs). Exosomes are further developed by the endosomal sorting system, which creates multivesicular endosomes (MVEs) by a secondary invagination of the ILVs. MVEs can either be shuttled for degradation by fusing with lysosomes or autophagosomes or fuse with the plasma membrane and release the exosomes.[74] Once released, exosomes can bind with other cells through receptor-mediated or independent mechanisms to transfer the contents of the exosome to neighboring cells.

In addition to exosomes and ectosomes, recent studies have also highlighted that most current methods used to isolate exosomes also have coisolated combinatorial EVs from different biogenic origins. The term "small EVs (sEVs)" has also been suggested to describe EVs with a size range of 100–1000 nm.[29] As sEVs do not exclusively belong to exosomes or microvesicles, they will be separately described as a specific term when mentioned in the current review.

2.2. Biomolecules Found in Cell-Derived EV

2.2.1. Nucleic Acids

Nucleic acids are the building blocks of the genetic code and play a vital role in every cellular process. While mainly thought of as simply storing the code for protein synthesis, nucleic acids have several other functional tasks in the cell, including structural makeup of protein-complexes (e.g., rRNA and tRNA), translational regulation (e.g., RNAi), formation of coenzymes (e.g., NAD^+ and FAD), and acting as secondary messengers in signal transduction pathways (e.g., cAMP).[75] Because of the complex role of nucleic acids in cellular function, research regarding the direct cell-to-cell communication through the delivery of nucleic acids in EVs is of the utmost interest. It has been widely revealed that a range of nucleic acids, including DNA, mRNA, miRNA, and lncRNA, can be incorporated into EVs; however, the exact mechanism of encapsulation is unclear.[30] It has been suggested that some RNAs a preferentially loaded in EVs with sequence specificity.[76] This has been assumed to be achieved through interactions and loading with RNA-binding proteins or lncRNAs.[77,78] However, detailed mechanisms remain undiscovered, and the specific roles of active and passive loading of nucleic acids in EVs may require more comprehensive research. What has been reported is nucleic acid-containing exosomes' ability to deliver their cargos to neighboring cells and for these nucleic acids to retain their function.[79,80] This proves that the transcriptomic state of one cell can directly affect neighboring cells through sharing of nucleic acids through EV release.[33,79,81] This makes studying the nucleic acid components in EVs vital for learning about cell-cell communication and developing novel tools for disease diagnostics and new potential therapeutics.

2.2.2. Lipids

Lipids have diverse structures and functions in cell biology. They are structural components of the cell membrane that regulate several aspects of cell signaling, including cell–cell interactions and cytoskeletal signaling, and act as a barrier for soluble signals. Also, they are involved in energy storage, hormone generation, and insulation.[82] Regarding EVs, lipids have mainly been studied about their structural formations and functions. The affinity of lipids to form lipid rafts and create asymmetries in the plasma membrane is one of EV biogenesis's key regulators.[83,84] This affinity can be one potential mechanism for sorting lipids in EVs. For instance, cholesterol is one of the most highly enriched lipids in EVs, and cholesterol withdrawal has been shown to impair EV biogenesis significantly.[19] In addition, the structural makeup of the EVs' lipids also plays a role in cargo targeting. Cytoplasmic proteins and lipids can anchor onto lipid rafts during EV biogenesis and remain trapped as the vesicles are released. Palmitoylation, prenylation, and myristoylation can play a prominent role in this anchoring and potentially enrich specific EV proteins.[84] Lastly, incorporating lipid and proteins into the lipid membrane of EVs may play a role in targeting EVs to other cells. The exact mechanism of targeting is still being explored, but the end fusion of EVs to target cells is highly regulated by the lipids and surface proteins of which the EVs are composed.[85–87]

2.2.3. Proteins

Another major type of molecule found in EVs is proteins. There are two major classes of proteins that can be incorporated into EVs: 1) transmembrane proteins that can be incorporated into the outer membrane of EVs; 2) cytosolic proteins that can be trapped in the inner lumen of EVs.[33] These types of proteins can play essential roles in cell signaling by EVs.[64] Transmembrane proteins can be vital in the cellular trafficking and targeting of EVs to other cells.[88] These membrane proteins can be composed of various proteins, including membrane organizers such as tetraspanins, adhesion proteins such as integrins, intracellular trafficking proteins such as annexins, and other cell type-specific proteins such as MHC-1.[89,90] All of these play a role in the biogenesis and targeting of EVs. There can be various proteins in the lumen of EVs, including enzymes, signal transduction proteins, biogenesis factors, and chaperone proteins.[33,91] The exact mechanisms of sorting these proteins into EVs are still not fully understood; however, interactions with lipid rafts, membrane proteins, and other chaperone proteins have been revealed to contribute to cargo loading in EVs.[92] It has also been revealed that proteins in EVs transferred to other cells can be used functionally. For instance, dendritic cells can

use cargo from exosomes for antigen presentation to elicit an immune response.[13,23] Therefore, the investigation of EV-based cell signaling can have broad applications in immunology, neurodegeneration, cancer, and much more. Beyond nucleic acids, lipids, and proteins, other biomolecules (mainly metabolites) exist in specific types of EVs. The detailed updates on different biomolecules in EVs can be found in Vescilpedia, EVpedia, or Exocarta. Besides various types of biomolecules existent inside or on the surface of EVs, recent studies have shown that EVs, after entering gastrointestinal tract, blood, or even cell culture media, can readily bind proteins and form a protein corona on the surface.[93,94] Protein corona on nanoparticles can significantly affect their surface charges and overall sizes, affecting biodistribution and theranostic functions.[95] Hence, it would be essential to consider protein corona when EVs were used for in vivo drug delivery applications.

2.3. Theranostic Applications of Cell-Derived EVs

2.3.1. Diagnostics

EVs are of great interest for diagnostic purposes for two major reasons. First, they can be found in bodily fluids such as blood, urine, cerebral spinal fluid, and saliva, allowing them to be gathered noninvasively. Second, their components are often related to their cells of origin, which means they could be used to understand the body's intercellular communications.[2,96] Because of this, EVs can make an ideal candidate for biomarker detection and disease diagnostics. For instance, in diseases where surface markers reveal disease states, such as many cancers, EVs can be applied to diagnose a wide range of cancers in the body effectively. It has been revealed that the presence of epithelial cell adhesion molecule (ECAM) in EVs has diagnostic value in colorectal cancer, while other surface markers such as EGFR subtypes in EVs can predict the efficacy of various treatments for glioblastoma.[97] In cancers, various proteins in EVs and microRNAs can be detected to diagnose specific types of cancers. For instance, detecting eight miRNAs in exosomes has been used to screen ovarian cancer cells from benign cells.[98] In patients with lung cancer, the protein CD151 is upregulated in EVs. In contrast, microRNA-1246 and -21 can be upregulated in breast cancer EVs.[99] Recently, researchers discovered a series of proteins and nucleic acids isolated from EVs that can provide a 90% accuracy and 95% selectivity for cancer diagnosis, albeit nonspecifically.[100] EV-based screening can be applied prognostically where downregulation of exosomal miR-638 predicts a poor prognosis in colon cancer.[101] In addition to cancers, it has also been reported that the detection of several microRNAs is improved in patients suffering from acute myocardial infarction, demonstrating their ability to use as a diagnostic marker for myocardial infarction.[102] EVs characterized by $CD31^+$/annexin $A5^+$, or containing miR199a and miR126, can be interesting biomarkers for stable coronary artery disease. At the same time, it has also been proven that the ICAM1 measurements from EVs may provide more reliable and effective diagnostic predictions of adverse events than measurements of soluble ICAM1 in the plasma.[103–106]

Because of the delicate and complex nature of neurodegenerative disorders, it is challenging to diagnose and treat before significant neurological deficits. Therefore, using EVs to diagnose neurodegenerative diseases would be an attractive strategy owing to the non-invasive nature of EV harvesting. In Parkinson's disease, the upregulation of alpha-synuclein and LRRK2 have been discovered in EVs.[107,108] In addition, decreased miR-19b, miR-1 levels, and increased miR-195 miR-153, miR-409-3p, miR-10a-5p, let-7g-3pand miR-24 levels have been found to be associated with PD.[109,110] In Alzheimer's disease, miR-16-5p, miR-125b-5p, miR-451a, and miR-605-5p isolated from exosomes of patients differed from controls showing their potential use as a diagnostic tool.[111] Similarly, after injuries to the CNS, changes in exosome profiles can be observed. For instance, after traumatic brain injury, expression levels of miR-21, miR-146, miR-7a, and miR-7b have been shown to increase.[112] In spinal cord injury, exosomal miR-125b-5p, miR-152-3p, and miR-130a-3p expression levels are all increased.[113] Such changes in protein and miRNA expression can be invaluable tools for diagnosis, prognosis, and therapeutic discovery for various devastating diseases and disorders.

2.3.2. Therapeutics

In addition to the diagnostic relevance of EVs, several researchers have also proved the unique therapeutic effects of EVs. It has been widely shown that cells, including stem cells, can provide beneficial effects by releasing various factors, including growth factors, cytokines, chemokines, and immunomodulatory molecules. However, the lack of quality control, uncontrolled cell behaviors, immunogenicity (from allogenic sources), and abundant cell death after transplantation severely limit cell therapies from clinical success. To this end, research has turned toward EVs as a novel therapeutic candidate for treating several diseases and disorders.[29] For cancers specifically, EVs derived from immune cells have been a promising avenue for cancer therapy. EVs derived from B cells, macrophages, T cells, and NK cells showed beneficial effects in various tumor models.[13,23,114–116] EVs can also be engineered to target specific cell types or load-specific cargo (**Figure 3**). Engineering exosomes from mesenchymal stem cells to carry short interfering RNA or short hairpin RNA specific to oncogenic $Kras^{G12D}$ led to a reduction in tumor volume across several mouse models of pancreatic cancer.[117] More recently, the delivery of miR-21i and 5-FU in engineered exosomes could reverse drug resistance in colon cancer cells.[118]

Exosomes can be ideal candidates for advanced therapy in neurodegenerative diseases because of their ability to cross the BBB.[119–121] For Parkinson's disease, exosomes loaded with catalase were shown to have a neuroprotective effect in a mouse model.[122,123] In Alzheimer's disease, extraction and delivery of exosomes from mesenchymal stem cells that were preconditioned in hypoxic conditions led to improved memory and reduced amyloid-beta plaques.[124] It has been revealed that engineering dendritic cells to express a Lamp2b-RVG fusion protein allowed targeted delivery of GAPDH siRNA carrying exosomes to the neurons, glia, and oligodendrocytes in the brain after intravenous injection.[25] In spinal cord injury, exosomes from

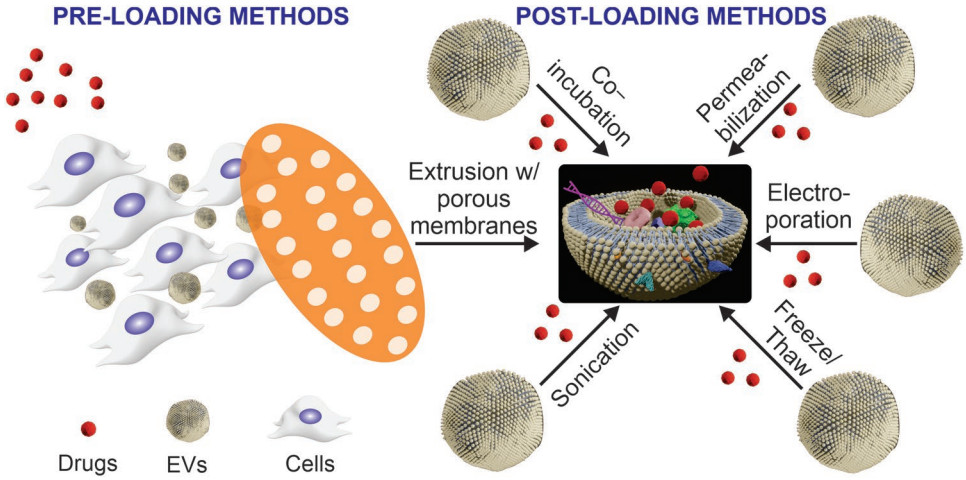

Figure 3. Strategies for drug loading and delivery by EVs. In general, the loading strategies can be divided into preloading and postloading methods. Preloading of drugs is achieved by incubation of cells with drugs which leads to the formation of drug-encapsulated vesicles inside cells. After secretion from the cells, EVs encapsulating drugs can be harvested by extrusion with a porous membrane. Postloading of drugs can be achieved by multiple methods including direct coincubation of EVs with drugs in the presence or absence of permeabilization reagents or facilitated by electroporation of EVs that leads to pore formation on the lipid vesicles. Freeze-thaw cycles and sonication also lead to pore formation and facilitate the loading of drugs into the EVs. EVs loaded with drugs can then be harvested by conventional ultracentrifugation or size exclusion columns, or by magnetic nanomaterial-facilitated magnetic isolation for therapeutic applications.

mesenchymal stem cells have been used to attenuate inflammation and have been displayed to polarize macrophages from M1 to M2-like phenotypes.[125] Similarly, EVs have been studied for their ability to reduce or control inflammation in several other diseases, including diabetes, rheumatoid arthritis, neurodegenerative diseases, cancers, and atherosclerosis.[126]

Most recently, researchers have looked at the use of EVs to treat the global COVID-19 pandemic. While literature regarding the use of EVs for therapy for the COVID-19 pandemic is lacking, stem cells, specifically mesenchymal stem cells, have been explored to treat COVID-19. Mesenchymal stem cells have the unique ability to regulate the inflammatory response and potentially reduce the cytokine storm known to occur with severe COVID-19 cases.[127–130] However, limitations with cell injections, including potential aggregation of cells after injections, uncontrolled cell attachment and fate, and immunogenicity, make EVs of mesenchymal stem cells an attractive candidate.[131] Therefore, there is much room for the use of EVs to treat the novel coronavirus, specifically concerning severe cases where immunomodulation can be of the utmost importance.

2.4. Section Summary

Because of their unique biological properties, EVs are an ideal candidate for many types of disease diagnostics and therapies. In this section of the review, we briefly outlined some of the critical features of EVs, including their biological origin, payloads, and bio-applications. While there are many more comprehensive reviews on EVs and their applications, we highlighted some of the essential aspects of EV research. While there is much promise of EVs for biomedical applications, there are still many hurdles to overcome. Limitations regarding isolation, purification, and characterization remain to be addressed. Due to their high heterogeneity in size, structure, and components, understanding the complex signaling, packaging, and therapeutic potential of EVs is challenging. Ramirez et al. listed a table comprehensively summarizing critical challenges in the field of EVs, covering isolation, analysis, and applications of different types of EVs,[132] and following this framework, we will further discuss how MNMs helped to overcome these challenges in the following sections.

3. Synthesis and Engineering of MNM for EV-Based Biological Applications

3.1. Synthesis and Engineering Strategies of MNMs

Many metallic nanomaterials, including MNMs have been synthesized and engineered in the past few decades because of their unique physical (magnetic, optical, mechanical) properties, biocompatibility, and in vivo stability. MNM-based magnetic nanoparticles (MNPs) of different sizes, shapes, morphology, and configuration have been synthesized using different methods, such as sol-gel, coprecipitation, hydrothermal, thermal decomposition, sonolysis, pyrolysis, microemulsion, and electrochemical deposition. The engineering of MNMs has been pivotal for safe and effective use in biomedicine because of unfavorable physicochemical properties such as stability, dispersibility, and inherent hazard of chemicals which can interfere with the normal physiological process (**Figure 4**). Engineering of MNMs involved tuning the shape, size, surface functionalization, surface coating, and binding with targeting ligands and peptides on the particle's surface. The principal motive of engineering of MNMs is not only to improve their bioavailability and functionality, but also to enable their use for on-demand therapeutic and diagnostic applications. The engineering of MNMs has proven to be a potential candidate to facilitate personalized (i.e., patient-specific) medicine care by enabling targeted delivery, imaging, selective tumor targeting, and regeneration. In these regards, attempts have been made to

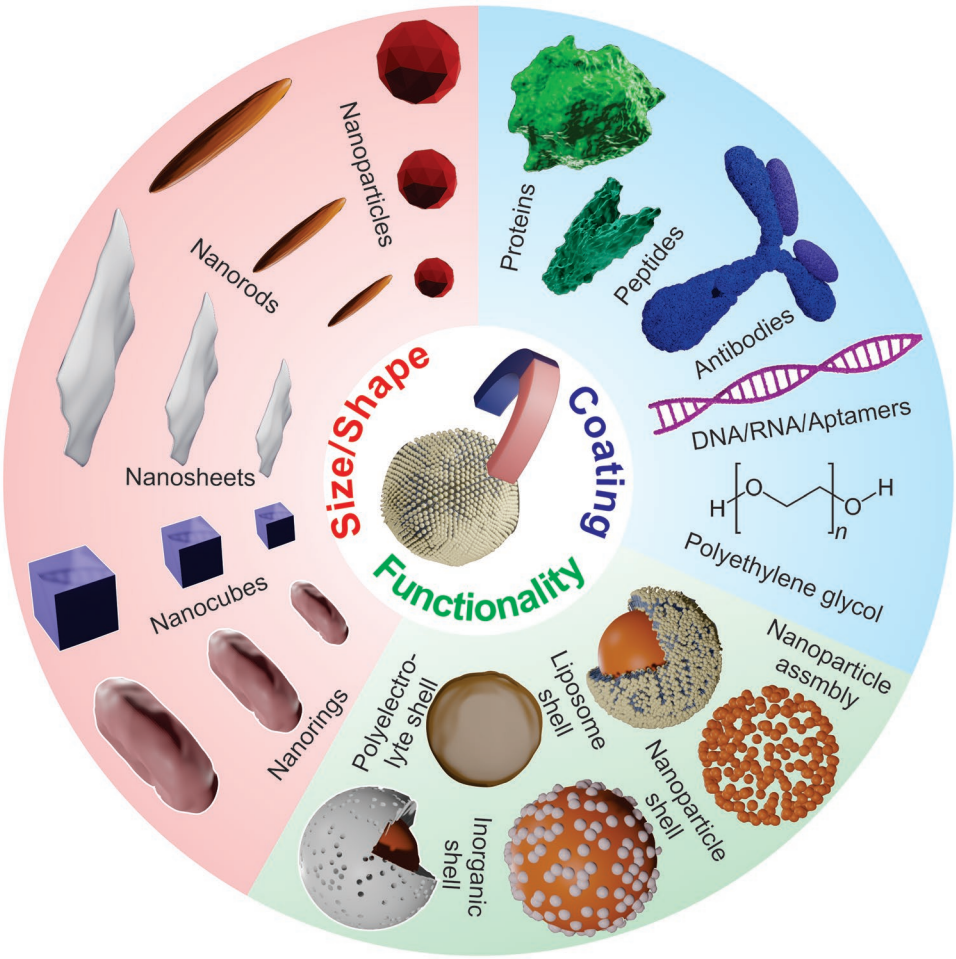

Figure 4. Illustrating the merits of engineering of magnetic nanomaterials (MNMs). Different features of engineering such as size, shape, surface coatings, and surface functionalities are presented as multifunctional tailoring to improve the biomedical applications of MNMs.

coat amorphous silica and mesoporous silica, gold, silver, thermoresponsive polymers, peptides, and specific binding proteins on the surface of MNMs. The coating and functionalization of MNMs involved advanced fabrication approaches to precisely control morphology, shape, and size. Moreover, the stability and distribution of MNMs in cells and tissues require even more attention to achieve favorable outcomes. Several factors, such as shape, size, surface functionality, and porosity, should be considered to design and synthesize MNMs for biomedical applications, particularly theranostic applications. As these parameters of MNMs can drastically influence the expected therapeutic outcome, the control of both physical and chemical properties of MNMs is critical, and various synthesis methods have been developed.[133,134] For theranostic applications of MNMs, it is vital to have suitable surface chemistry for loading and delivering biomolecules and magnetic property for imaging under the response to exogenous stimuli through the magnetic field.

3.1.1. Size and Shape Control

The size and shape of MNMs have great importance in tuning the magnetic properties for a specific application. In theranostic applications, paramagnetic ultrasmall nanoparticles of <4 nm have been developed for positive contrast agents for MRI. Superparamagnetic nanoparticles of 5 to 25 nm have been exploited for drug delivery, bioimaging, and diagnostic applications. In addition, MNPs of smaller size have longer blood circulation time in vivo compared to the large (60–80 nm) spherical-shaped nanoparticles and cause less toxicity with improved efficiency.[135] A successful attempt has been made to synthesize varying size, monodispersed, defect-free, and high crystallinity superparamagnetic nanoparticles in organic solvent using the thermal decomposition method.[136] The key factors affecting the final size of nanoparticles are the ratio of the solvents, surfactants used, reaction temperature, and time. This method has produced magnetic nanoparticles from 5 to 25 nm with excellent reproducibility of physical size. The thermal decomposition method has produced surface-functionalized water-soluble iron oxide nanoparticles with high stability,[137] and protein-encapsulated gold fluorescent nanoclusters functionalization on IONPs [138] through minor modification in the chemical reaction. A hydrothermal method has also been employed to synthesize the size control over 4–16 nm of MNPs functionalized with glucose and gluconic acid using $FeCl_3$ and sucrose decomposition.[139] Mizutani et al. synthesized

the size-controlled from 9.5 to 38.6 nm of magnetite nanoparticles through the coexistence of lactate and sulfate ions using the organic solvent-free hydrothermal method.[140] Moreover, variation of particle size depends on the concentration of lactate and sulfate ions, and lactase ions can affect decreasing the size, while sulfate ions promote particle growth.

The shape is another important aspect in the engineering of MNMs, which requires different surfactant solvents and a tedious optimization process. Much effort has been devoted to synthesizing the MNMs of different shapes such as spherical magnetic nanoclusters,[141] nanoflowers,[142] nanoplates,[143] nanocubes,[144] nanowhiskers,[145] nanowires,[146] nanorods,[147] nanocoils,[148] and nanoworms.[149] Various leading research groups have thoroughly investigated the roles of different shapes and structures on effectiveness in theranostics. For example, 1D magnetic nanoworms have been utilized as enhanced contrast agents, showing long blood circulation and targeting the tumor site with enhanced retention due to the increased binding sites.[150] 2D magnetic nanocrystals have shown magnetic anisotropy, which could stabilize the long-range magnetic ordering by opening the excitation gap to resist thermal agitation.[151] 3D magnetic nanostructures can also play an essential role in tuning the optical and magnetic properties, significantly influencing theranostic applications' outcomes. Moreover, particles with shape anisotropy can also transfer a mechanical force, significantly influencing cell fate. Gupta and Sharma have demonstrated the bimodal application of manganese-doped nanoclusters for hyperthermia and photothermal glioblastoma therapy.[152] Thus, engineering the shape of nanoparticles has emerged as an excellent platform for theranostic applications.

3.1.2. Surface Coating, Doping, and Functionalization

Surface modification via coating of functional bioactive materials, stimuli-responsive polymers, and biomolecules are regarded as practical strategies for engineering MNMs. Amorphous silica coating of MNPs is an old and reliable strategy to engineer the MNMs, improving the aqueous dispersibility and biocompatibility for biomedical applications. A sol-gel approach for the amorphous silica coating of superparamagnetic nanoparticles with varying shell thickness from 2 to 100 nm has been developed.[153] Moreover, fluorescent dyes, namely 7-(dimethylamino)-4-methylcoumarin-3-isothiocyanate (DACITC), and tetramethylrhodamine-50isothiocyanate (5-TRITC), were conjugated in a silica shell through covalent bonding during the reaction. Lately, mesoporous silica coating of MNMs has gained notice because of high porosity, large surface area, and high aqueous dispersibility. The porous silica shell allows the efficient loading of small molecules for theranostic applications. Attempts have been made to engineer the different types of core–shell (silica-MNPs) structures such as sandwich structure core–shell,[154] hollow core–shell,[155] magnetic yolk–shell mesoporous silica microsphere supported Au nanoparticles,[156] silica-coated magnetic nanocluster,[157] and multiple MNPs coated uniform mesoporous silica nanoparticles.[158] These engineered MNMs have been developed for simultaneous use of enhancing magnetic resonance (MRI), fluorescence imaging, and therapeutic delivery applications.

In addition to silica coatings, gold, silver, and other inorganic nanoparticles have also been used as coatings to create a second shell structure with specific functionality. The gold coating is another strategy to engineer the MNPs because gold-coated magnetic nanoparticles (Au@MNPs) can improve the optical, magnetic, and plasmonic properties and endow their use in potential use in analytical chemistry and nanomedicine. These properties can be easily tuned through gold shell thickness and the size of the core magnetic nanoparticles. Gold shells on MNPs can be formed directly on the core MNPs through an aqueous-organic phase, in which particles dispersed in an aqueous solution bind with Au^{3+} and are reduced on the surface using sodium citrate and sodium borohydride. The use of gold chloride and sodium citrate reagents is a simple and commonly used method for synthesizing Au@MNPs. In this method, MNPs are mixed in boiling gold chloride aqueous solution and mixed with reduced-sodium citrate under rigorous magnetic stirring.[159,160] It is important to note that sodium citrate acts as a reducing agent and provides citrate surface capping on Au@MNPs surface, subsequently improving dispersibility and preventing aggregation. Gold shell thickness can be easily tuned by controlling the ratio of MNPs/gold precursor and concentration of reducing agent sodium citrate. However, if these ratios and experiment parameters are not well optimized, then free gold nanoparticles rather than Au@MNPs may form.

MNPs synthesized by the thermal decomposition method often possess oleic acid and oleylamine on their surfaces. Therefore, gold shells can be directly formed on top of the capping agent, or the capping agent can be washed, and then shell Au can be formed on the core MNPs. MNPs dispersed in a mixture of chloroform and oleylamine can form Au@MNPs by reduction of gold chloride. Oleylamine plays a crucial role as a capping agent to enable high dispersibility in chloroform and a reducing agent to form the gold shell. Silva et al. have summarized the various approaches for synthesizing Au@MNPs core–shell NPs synthesis and their functionalization for analytical and biomedical applications.[161]

3.1.3. Hybridization with Other Functionalities

A hybridization is a good approach for engineering MNMs, improving physicochemical and biological functionalities, and enabling precise targeting and detection. The hybridization of MNMs with plasmonic nanoparticles such as silver, platinum, and gold can positively influence the optical properties and eventually provide extra leverage in diagnosing and detecting exosomes and imaging tumors. The presence of a plasmonic nanoparticle shell on Fe_3O_4 NPs surface protects the oxidation of the magnetic core and produces surface-enhanced plasmonic resonance (SEPR) and magnetic properties. Moreover, magnetoplasmonic nanoparticles can be functionalized with antibodies, siRNA, DNA, proteins, and other biomolecules through gold-amide (Au-NH_2) or gold-thiol (Au-SH) surface chemistry. These specific biomolecule-functionalized plasmonic nanoparticles can be utilized for magnetic isolation, biolabeling, optical, and biosensing. Nowadays, ligand-bearing MNPs have been developed using Au–NH_2 and Au–SH chemistry to construct remotely and temporally controlled stimuli-responsive platforms

for stem cell adhesion, differentiation, and immune modulation. For example, Thangam et al. have developed a unique ligand-bearing gold nanoparticle on the surface of silica-coated MNPs through Au–NH_2 reaction for macrophage recruitments and polarization toward tissue regeneration.[162] They used the thermally decomposed method synthesized MNPs and followed the sol-gel method for the synthesis of amine-functionalized silica-coated MNPs (SiO_2@MNPs-NH_2) by use of tetraethyl orthosilicate (TEOS) and (3-aminopropyl) triethoxysilane (APTES). A solution of separately prepared sodium citrate capped gold nanoparticles was mixed with SiO_2@MNPs-NH_2 for decoration of gold nanoparticles. Lee et al. have developed the magnetoplasmonic nanorods using the electrodeposition method and functionalized with the CD63 antibody, and 5(6)-carboxyfluorescein (FAM)-tagged molecular beacon (MB) against miR-124 for exosomal miRNA detection and characterization of stem cell neurogenesis.[163] The electrodeposited magnetoplasmonic nanorod-based detection platform possessed several components, such as immunomagnetic, plasmonic-enhanced fluorescence for exosome isolation, and selective exosomal siRNA detection. Min et al. have developed CoFe nanocoils through electrochemical deposition and then engineered with cell adhesive peptide RGD to create ligand presenting remote control magnetic nanosystem for cyclic adhesion and differentiation of stem cell.[164] They found that the RGD ligand-presenting magnetic coils experienced mechanical stretching and eventually changed the surface area of ligand-presenting nanocoils, which significantly influenced stem cell adhesion and differentiation. Choi et al. have fabricated slidable nanoligand coating on the surface of silica-coated MNPs through PEGylation for macrophage adhesion and regenerative polarization.[165]

Electrochemical hybridization assay-based biomolecules detection, DNA-sequence analysis, and disease diagnosis have become very popular. Compared to the conventional optical method, electrochemical assay-based methods are more efficient, low-cost, and effective in detecting small amounts of biomolecules. Efforts have been made to detect the hybridized DNA using an electrochemical assay to combine MNPs-modified electrodes.[166] Jeong et al. have developed an integrated magneto-electrochemical assay-based exosome detection kit, in which immunomagnetically captured exosomes from patient samples were analyzed via an electrochemical reaction.[167] They used MNPs to capture the exosome, then hybridized the MNPs–EVs with CD63 antibody, and oxidizing enzyme horseradish peroxidase to enable sensitive, cell-specific exosomes detection and scale-up for scale-up high-throughput measurements.

3.2. Synthesis of MNM-Tagged Nanovesicles

Engineered MNMs have many properties which endow their broad-range application in regenerative medicine. Tagging MNMs with exosomes can give us more opportunities with multimodal, safe, and precise use of a combined system for theranostics. Therefore, it is vital to develop the engineering MNMs-tagged nanovesicles (i.e., magnetosomes) in either natural or synthetic approaches. There are three different approaches to synthesize the MNMs-tagged exosomes, which are, first, an in situ synthesis in vivo, second, a chemical process through internalization of MNPs, and third, a fusion of MNMs in isolated exosomes. In this section, we will describe the process involved in these approaches and summarize the limitations of each approach.

3.2.1. Nanovesicles-Derived from Cells with In Situ Synthesized NMPs

Biosynthesis of the MNPs is a unique natural process in aquatic microorganisms that allows magnetotactic bacteria to navigate along the Earth's magnetic field[168] (**Figure 5**). The organelles in the magnetotactic bacteria, produce and accumulate in situ natural intrinsic MNPs, called magnetosomes, and the process is known as magnetogenetics.[169] The MNPs have been of great importance in nanomedicine. They have gained extensive attention since the successful use of magnetically labeled antibodies for the isolation of cells, therapeutic delivery, and imaging in both in vitro and in vivo. The magnet-sponsored cell separation technique has been established for cell separation, purification, and detection from the complex mixture. Therefore, the possibility for the biosynthesis of MNPs and subsequent isolation and detection of magnetosomes/nanovesicles can be an advance platform for theranostic application.

In magnetogenetics, the biosynthesis of MNPs and accumulation in noncell/microorganisms occur through a redox-controlled synthesis mechanism. Moreover, the shape, size, and their assembly in the form of a regular chain were formed to achieve the highest magnetization.[170] Synthesis of magnetosomes from microaerophilic alphaproteobacterium *Magnetospirillum gryphiswaldense* synthesize has been achieved in intracytoplasmic vesicles of magnetosome membrane, which comprise crystal of magnetite (Fe_3O_4). Further, magnetosome biomineralization can be achieved in vesicles provided by magnetosome membrane by controlling the cumulation of Fe and deposition of MNPs with a specific morphology.[171] The magnetosome membranes are composed of unique biochemicals and subsets of magnetosome membrane proteins, which endows spatial and physicochemical control over the magnetite biomineralization process.[172] Scheffel et al. have demonstrated the magnetosomes alignment along with the filamentous structure and coupled to the presence of the *mamJ* gene product using gene deletion in *Magnetospirillum gryphiswaldense*.[168] Their findings suggest that MamJ, an acidic protein interacts with the magnetosome surface and other structures like the cytoskeleton. However, the mechanism governing the formations of the magnetosome chain and any additional structure to preserve the structure have not been fully understood. In consideration of in situ MNPs formation in the cell, the unpaired electron is required for the magnetic attraction, and the electronic configuration of Fe^{2+} and Fe^{3+} suggests 4, and 5 unpaired electrons, respectively, which indicate the possibility of formation of natural intrinsic magnetic cells through the intracellular accumulation of ions. It has also been proposed that the magnetite crystal nucleation proceeds via coprecipitation of Fe^{2+} and Fe^{3+} ions.[173] However, ions in the cytoplasm interact with the proteins and form complex and exhibit cytotoxicity. Therefore, biosynthesis of MNPs in cells is possible through precipitation and crystallization of iron oxide or iron sulfide (greigite).

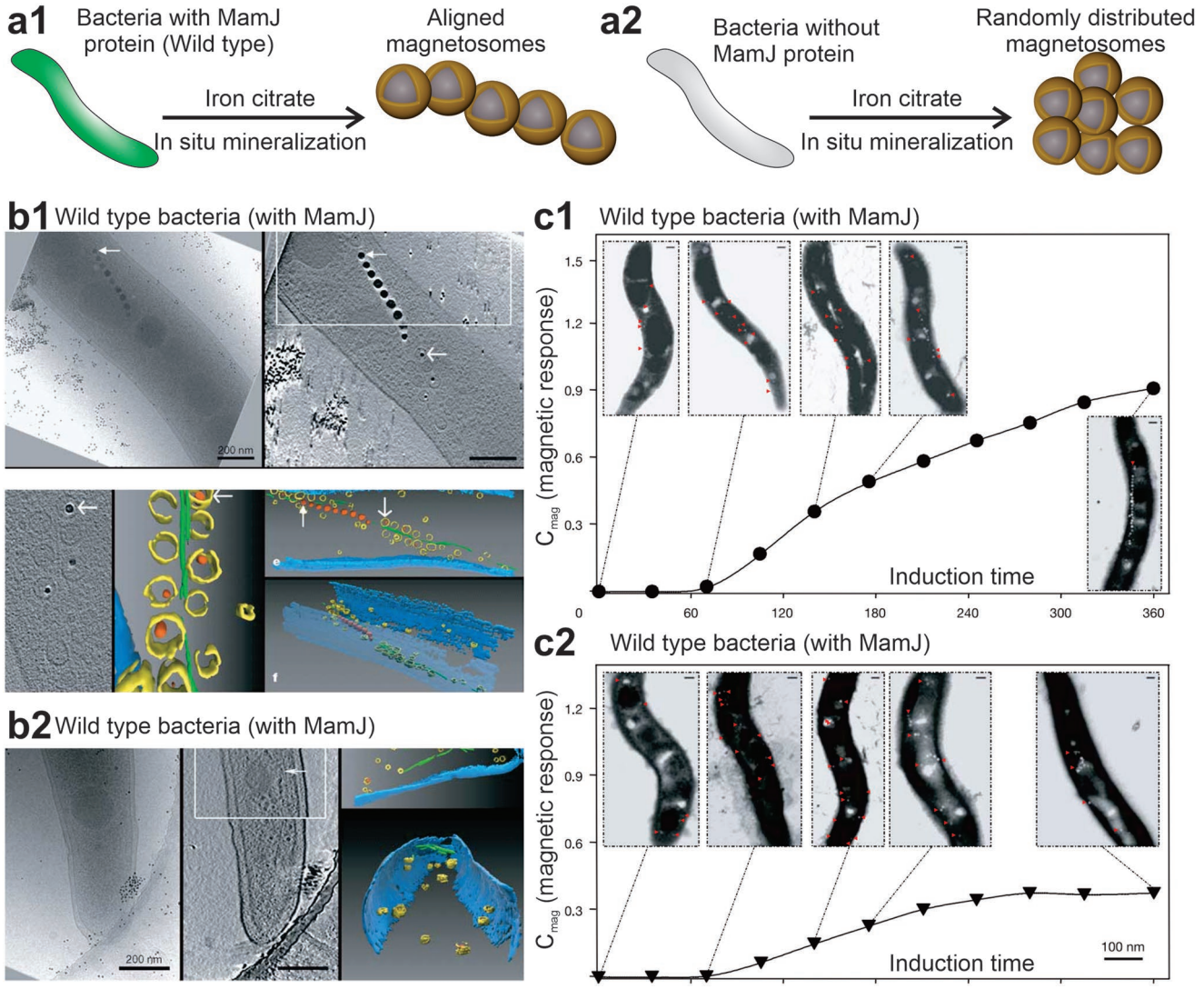

Figure 5. In situ synthesis of cellular vesicle enveloped magnetic nanomaterials (magnetosomes) by *Magnetospirillum gryphiswaldense*. a) MamJ is a protein in the bacteria species *Magnetospirillum gryphiswaldense*. Wild-type bacteria that contain MamJ protein can assemble magnetosomes mineralized from iron citrate mineralized at discrete sites into aligned fibrous shape. In contrast, bacteria with deletion of *MamJ* gene assemble the mineralized magnetosome in a different, aggregated form. b) Cryo-TEM photographs of the formation and assembly of magnetosomes synthesized in situ in *Magnetospirillum gryphiswaldense*. Colored images indicate the simulation on vesicles (yellow) and magnetic nanoparticles (red) along with a filament (green). c) Time-dependent study on magnetite formation in wild type and MamJ deleted *Magnetospirillum gryphiswaldense*. Magnetite formation is induced by ferrite citrate addition, and the magnetic response measurements were performed in energy-filtered TEM. Red arrows indicate growing magnetite crystals. Images were adapted with permission.[168] Copyright 2006, Nature Publishing Group.

3.2.2. Nanovesicles-Derived from Magnetic Nanoparticles-Transfected Cells

Since nanoparticles-based detections (biosensing, bioimaging, and biotracing) have significantly influenced the diagnosis and therapy of various diseases, it is utmost time to develop a comprehensive platform for the chemical synthesis of MNPs-tagged nanovesicles. Usually, magnetic nanostructures prepared by various methods and functionalized with appropriate molecules can be used for EV-tagging for specific applications. Two approaches, MNPs-transfection, and coupling with antibodies/aptamers, have been developed to achieve the MNPs-tagged EVs. In the first approach, synthesized MNPs were incubated with the cells for some time for internalization via endocytosis, subsequently captured by endocytic organelles, and finally transported through endosomal/lysosomal vesicles into the culture medial. The second approach involved functionalizing MNPs with antibodies or aptamers and dispersed in the isolated exosomes for selective coupling with the EVs. The most common antibodies to detect, target, and coupled with the EVs are protein markers such as tetraspanins (CD9, CD63, CD81), TSG101, and ALIX.[174]

It is imperative to understand the complexity of the extraction and isolation of MNPs-tagged/associated EVs from the cellular microenvironments (cytosol). The minimum disturbance is required during the extraction and isolation process to achieve

EVs intact with the MNPs. Apart from mechanical disturbance and damage to MNPs-EVs tagging, cell lysis or extraction steps also significantly impact the extraction process. The cell lysis process enables the release of various biomolecules such as EVs, organelles, cell membranes, debris from the various cell compartments, which can interact, absorb, and bind nonspecifically with MNPs or MNPs-EVs. Therefore, to minimize or avoid the nonspecific binding of biomolecules to microvesicles with MNPs, steps in the extraction process should involve appropriate cell lysis technique, magnetic isolation, and careful washing and managing postextraction magnetic-tagged EVs. For example, Nemati et al. have developed the Fe/Au-based MNWs to isolate tumor-derived MNWs-tagged exosomes.[175] In this study, 3×10^5 canine osteosarcoma OSCA-8 cells were cultured in six-well plates for 18 h, and then 30 µg of Fe/Au MNWs were added to the culture wells. After 48 h of incubation, the cell-cultured medium was transferred to the glass vial placed on the magnetic stand, and exosomes-containing magnetic nanostructures were collected at the wall near the magnetic bar. It was hypothesized that Fe/Au MNWs were internalized into the cells and then captured by exosomes, and further released into culture media. Song et al. have developed the magnetic-photonic self-assembled pH-responsive bilayer vesicles for theranostic applications.[176] In this approach, Fe_3O_4–Au NPs were modified with poly(ethylene glycol) on a gold surface and then functionalized with a reactive oxygen species (ROS) generative poly(lipid hydroperoxide) (PLHP) on iron oxide surface, and further assembled into vesicles containing Fe_3O_4–Au NPs layers. Lee et al. have demonstrated the use of iron oxide nanoparticles for tagging nanovesicles derived from iron oxide nanoparticles-incorporated mesenchymal stem cells for cardiac repair.[177] They have developed the IONPs-tagged exosome mimetic EVs using the extrusion method. For extrusion, cells were processed five to six times through 10–15 µm and 400 nm pore size filter papers made of polycarbonate and separated from free protein and debris by centrifugation, ultracentrifugation, and mixed with IONPs to achieve IONPs-EVs.

The use of antibody functionalized MNPs and coupling with isolated exosomes is the second approach to achieve the specific MNPs-tagged EVs. This is a combination of a two-step process, in which the first step involves functionalization of MNPs with a specific antibody, and the second step involves isolation of exosomes. Qi et al. have prepared the drug-loaded superparamagnetic nanoparticles-tagged exosomes via transferrin conjugation for cancer therapy.[178] Carboxylic functionalized superparamagnetic nanocluster crystals (SMNC) were conjugated with Tf and then incubated in serum extracted from fresh blood of mice to achieve SMNCs-exosomes via Tf–Tf receptor interactions. Boriachek et al. have used CD9 and CD63 antibodies to functionalize Au–Fe_3O_4NC for tagging exosomes for sensitive detection.[179] Antibody functionalized Au–Fe_3O_4 NCs surfaces were dispersed in the culture media to bound with a bulk population of exosomes and collected through a magnet. The isolated Au-Fe_3O_4 NCs-tagged exosomes were purified and further transferred to the tissue-specific, antibody-modified screen-printed electrode and utilized for detection of exosomes secreted from placental cells via specific placental markers.

A microfluidic electroporation-based approach has been utilized to synthesize the cell membrane-coated MNPs for enhanced MRI-guided cancer therapy. Yu et al. have demonstrated that microfluidic electroporation can endow MNPs to infuse into red blood cells-vesicles (RBC-vesicles) and effectively promote the entry of MNPs into RBC-vesicles through an applied electric field to form biomimetic cell membrane coated MNPs (CM-MNPs).[180] The collected CM-MNPs were injected into the mice to investigate in vivo MRI-guided cancer photothermal therapy (PTT) performance. Moreover, they found that the microfluidic electroporation-based synthesis of CM-MNPs gives better colloidal stability and enhanced in vivo imaging and PTT performance compared to the conventional extrusion method.

3.3. Biological Applications of MNMs

3.3.1. Magnetic-Field Responsive Drug Delivery

Many kinds of stimuli-responsive nanocarriers, such as thermo-, photo-, electro-, pH-, and magnetoresponsive drug/gene delivery carriers, have been developed for cancer and other disease treatments. The idea behind the development of these stimuli-responsive nanocarriers is to reduce the nonspecific biodistribution and subsequent cytotoxicity and rapid clearance to improve on-demand targeted drug delivery with high efficacy and high dose release at the tumor site. MNMs are well known for their immense application in drug/gene delivery, therapeutics, and cancer diagnostics.[181] Magnetically triggered drug/gene delivery nanosystems have already proven high demand in personalized medicine, which can be featured as an innovative on-demand delivery method using an external magnetic field and endow controlled and effective drug/gene release (**Figure 6**). The magnetically triggered drug/gene delivery nanosystems work on the principle of energy conservation; when the external alternating magnetic field (i.e., magnetic energy) is applied to the delivery nanosystems, magnetic cores generate thermal energy, which subsequently triggers the drug release. However, the possibility of local tissue damage due to the heat generated at the nanosystem cannot be ignored, and it can be controlled through an external magnetic field. Guisasola et al. have demonstrated the magnetic-responsive controlled drug release from thermosensitive polymers such as poly-N-isopropylacrylamide (NIPAM) and N-hydroxymethyl acrylamide (NHMA) coated mesoporous magnetic silica nanosystem by a hot spot effect.[110] They engineered the MNPs with mesoporous silica and thermosensitive polymers that can respond to altering magnetic fields and activate drug release when the local temperature reaches 43 °C. In this study, magnetic cores act as hot spots, which generate enough heat to endow payload release under the external altering magnetic field (AMF). Moon et al. have developed titania nanotube arrays filled with dopamine-functionalized IONPs and drug-loaded polymers micelles for magnetic-responsive drug delivery.[182]

Magnetic nanoparticles encapsulated in exosomes nanocarriers (MNPs-EVs) are called magnetosomes. Magnetosome-based (MNPs-EVs) nanosystems have drawn significant attention in magnetically triggered drug delivery approaches due to their biocompatibility, biological and chemical functionality, and magnetic properties. MNPs-EVs nanosystems have

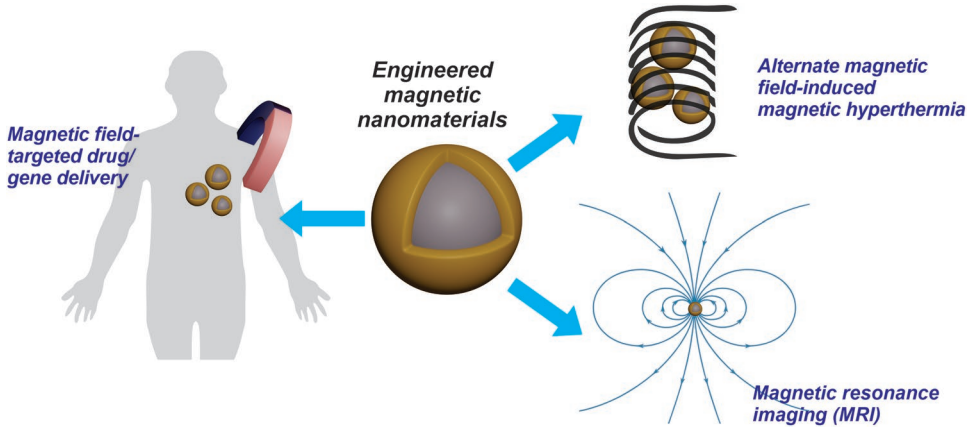

Figure 6. Illustrating MNM-facilitated therapeutic functions. In general, engineered MNMs (scheme in the center) that target specific locations under the guidance of magnetic field can be used for regiospecific delivery of drugs and genes (left panel). MNMs that can convert the magnetic field into thermal energy can be used for magnetic hyperthermia-based applications, shown in the top right panel. MNMs can also be used as imaging contrast reagents for MRI, either in T1 or T2 modes, shown in the bottom right panel.

been used as drug/gene delivery nanocarriers that can target and accumulate at the tumor site using magnetic gradient and eventually endow the drug release under external AMF and MRI applications.[183–185] In this process, a high-frequency AMF of 50–400 kHz is applied to generate local heat of MNPs to kill the cancerous cells by thermal ablation without damaging the surrounded healthy tissues.[186–188] In addition, safe and effective low-frequency AMF ranging from 0.01 to 10 kHz have been applied for local drug release from MNMs.[189] Nappini et al. have described the low-frequency AMF responsive drug release behavior in two different biofluids, namely, aqueous physiological buffer and bovine serum from magnetoliposomes nanosystem.[190] They have used liposomes encapsulated with two different types of MNPs (citrate-coated Fe_3O_4, and oleic acid-coated γ-Fe_3O_4 NPs) and entrapped dyes capable of self-quenching of carboxyfluorescein for stimuli-responsive release in biofluids. Nobuto et al. have developed the magnetic liposome drug delivery system and investigated the systemic release of anticancer drug doxorubicin under the external AMF in osteosarcoma-bearing hamsters.[184] They found that systemic chemotherapy using a magnetic field can effectively reduce the primary tumor progression and restrain lung metastasis due to the effective release of doxorubicin. These exemplary research reports confirmed the potentials of magnetic responsive-drug/gene delivery from MNP-EVs nanosystems in theranostics.

3.3.2. In Vivo Biosensing and Imaging

MNMs are an effective, versatile, and powerful candidate for contrast agents in diagnostics imaging in nanobiomedicine. The primary purpose of MNP-based biosensors is to achieve high sensitivity with specificity, noninvasive minimal sample penetration, simultaneous measurement of different molecules, and high throughput screening format using a single instrument. The MNPs-based biosensor has received extensive attention because of easy surface engineering, chemical stability, biocompatibility, and excellent magnetic property. It has been used in pharmaceutical drug development, medicine, genomic and proteomic research, clinical diagnostics, and in vivo biosensing.[191]

Recently, real-time in vivo detection and monitoring of qualitative and quantitative changes in physiological state is a major concern for the precise identification of cancer and other neurological diseases. Much effort has been made to develop MNPs-based biosensors for the detection of EVs. Boriachek et al. have developed a sophisticated gold-loaded ferric oxide nanocubes ($AuFe_3O_4$ NCs) system for isolation and electrochemical detection of exosomes via functionalization of CD63 antibody.[179] The isolated exosomes were transferred to screen-printed electrodes, which were functionalized with anti-placental exosome antibodies. Further, the oxidation of TMB reagent in the presence of H_2O_2 exhibited peroxidase-like activity of $AuFe_3O_4$ NCs, confirming PLAP-specific exosomes' presence in the complex solution of $AuFe_3O_4$ NCs/CD9/exosomes/PLAP immunocomplex. This electrochemical method-based biosensor is disposable, cost-effective, and has been successfully employed for multiplexing. Fan et al. have constructed the novel exosome-specific tumor diagnosis approach through integrating the magnetic exosome-enrichment platform and $Ru(bpy)_3^{2+}$-polymer amplified electrochemiluminescence method.[192] This method was high-speed and effective in capturing the tumor-derived exosomes via the biological-affinity identification platform of EpCAM antibody. They have also investigated the performance index from clinical blood samples from tumor patients, and their findings suggested that the exosome-specific tumor diagnosis strategy is readily and consistent with exosome-containing microRNAs (exomiRs). This is a very simplified strategy for detecting and analyzing tumor-derived exosomes in the non-destructive detection mode of fluid biopsy for tumors.

Distance-dependent magnetic resonance tuning (MRET) is a magnetism-based distance-dependent sensing and imaging technique, which works on the principle of positive T_1 magnetic resonance imaging (MRI) signals. There are two operational components in MRET, a superparamagnetic "quencher" (Q) and paramagnetic "enhancer (E)," and nanometer distance (d) between them determines the degree of the MRET phenomena.[193] This technique can be used for molecular interactions such as binding,

cleavage, folding, unfolding, and biological targets in in vitro and in vivo systems. Wang et al. have employed the MRET technique for two-way magnetic resonance tuning (TMRET) and dual-contrast enhanced subtraction imaging for noninvasive and quantitative biological imaging.[193] They first synthesized the pheophorbide a—paramagnetic Mn^{2+} chelate (P-Mn), and then conjugated the ligand to a superparamagnetic iron oxide (SPIO) nanoparticle. Next, the SPIO nanoparticle was further encapsulated in disulfide cross-linked micelle (DCM). Strikingly, the P-Mn SPIO nanoparticle encapsulated DCM exhibited varying payload release profile in response to different glutathione (GSH) concentrations. By correlating the payload release with the T_1 and T_2 magnetic resonance signals, they successfully established a model to monitor drug release based on MRI signals. Moreover, the integrated approach of TMRET and dually activable T_1 and T_2 magnetic resonance signal can significantly improve contrast enhancement with low background signal and quantitatively image the molecular features in tumors, as well as detect the small intracranial brain tumors in the patient-derived xenograft models. TMRET in combination with tailoring the dual-contrast enhanced subtraction imaging (DESI) endowed more sensitive and selective MRI than conventional imaging tools used in cancer diagnosis.

To achieve magnetic-based in vivo biosensors and imaging probes, it is crucial to design and develop a sophisticated system that can target tumor sites in the body in an accurate, sensitive, and selective manner. In this regard, chemical versatility, cytotoxicity, water or colloidal dispersibility, and high uptake efficacy of NPs are the essential requirements for realizing versatile biosensing, imaging, and therapeutic functions. Therefore, MNM-based multifunctional nanosystems can be an ideal platform for developing combined biosensing, imaging, and therapeutic approaches for theranostics.

4. MNM-Based Exosomal Biomarker Isolation and Detection

4.1. Biomolecules in EV as Disease Biomarkers

As mentioned in the previous section, MNMs have been applied for the separation, isolation, concentration, purification, and identification of cells and biomolecules for decades. Combining MNMs with cutting-edge detection techniques has enabled many ultrasensitive, selective, reproducible, and point-of-care in vitro and in vivo biosensing platforms. However, current EV applications such as exosomes are limited by their high heterogeneities in sizes, compositions, functions, and origins.[2] Precise isolation of specific types of exosomes from complex body fluids, and accurate analysis of the associated biomolecules are critical to the successful clinical translation of biosensing platforms built upon EVs.[54] Besides the isolation and purification of EVs, changes in the magnetic properties of nanoparticles in response to the binding of specific biomolecules have also been leveraged to develop magnetic biosensors such as giant magnetoresistance biosensors.[194] As such, magnetic nanoparticles represent a versatile tool for enabling EV-based biosensing platforms. In this section, we will first introduce the milestones of magnetic nanoparticle-enabled biosensing platforms, then introduce the progress made in MNM-facilitated isolation and detection of different biomarkers associated with EVs, including RNAs, DNAs, proteins, lipids, and the vesicles themselves. By learning from the past developments of magnetic nanoparticle-based biosensors, we could identify new opportunities for advancing magnetic nanoparticle-facilitated, EV-based biosensing platforms, and health surveillance strategies.

4.2. Design Principles of MNM-Enabled Isolation and Detection of Biomolecules in EV

MNMs have exhibited great promise for capturing, concentrating, and isolating cells and cell-derived biomolecules, including DNAs, RNAs, proteins, enzymes, and pathogens from human or animal body fluids.[45,48,195,196] There are a few clear advantages of using MNMs compared to conventional separation methods (e.g., chromatography). For instance, the large surface-to-volume ratio, excellent solvent dispersibility, ease of retrieval from solution, and high versatility of MNMs allow the rapid, sensitive, selective, and recyclable isolation of target biomarkers. To design an MNM-based isolation platform for biomarkers, MNMs are conjugated with polymers, ligands, and surfactants that bind to and capture specific biomarkers of interest.[197,198] The binding forces between the functionalization moieties and target biomolecules typically include but are not limited to antibody–antigen interactions, direct affinity absorptions, antibody–antigen interactions, and aptamer-protein conjugations.[199] After selective binding to target biomolecules in the complex biological fluids (e.g., human blood and urine), these biomolecules or cells can be separated for further analysis. Notably, there are a few crucial factors that can affect the efficiency of biomolecule or cell isolation. The first factor to be considered are densities of surface functional groups on the MNMs. The higher functional group density will cause more selective and efficient isolation of the target biomolecules and cells. The second factor to be considered is the magnetis of the nanomaterials. For instance, denser surface functionalization and higher saturation magnetization of nanomaterials can facilitate the selective and efficient isolation of cells and cell-derived biomolecules.[200] Engineering MNMs with a higher surface area through the formation of porous surfaces or embedding in porous polymers can be helpful for the isolation of EVs and associated biomolecules. Similarly, synthesizing zinc-doped, superparamagnetic iron oxide nanomaterials with high magnetization saturation would also be desired to enhance EVs' isolation.[201] We have summarized these basic design principles for engineering MNM-based isolation of EVs in **Figure 7**.

The advantages of MNM-based isolation and purification of biomolecules can be further integrated into advanced biosensing platforms. They could purify and concentrate the biomarkers to ensure both higher selectivity and sensitivity during biosensing. There are additional benefits of using MNM-facilitated and EV-based biosensors as well. First, the sizes of many biomolecules, such as DNAs, RNAs, proteins, and cellular organelles, such as mitochondria, and EVs, are at the nanoscale. Therefore, integrating MNMs as probes into biomolecules can have minimal steric hindrance.[202] This integration process can also be easily engineered by tuning the shapes and sizes of MNMs and the sensor fabrication steps. For instance, 0D magnetic nanoparticles, 1D magnetic nanowires,

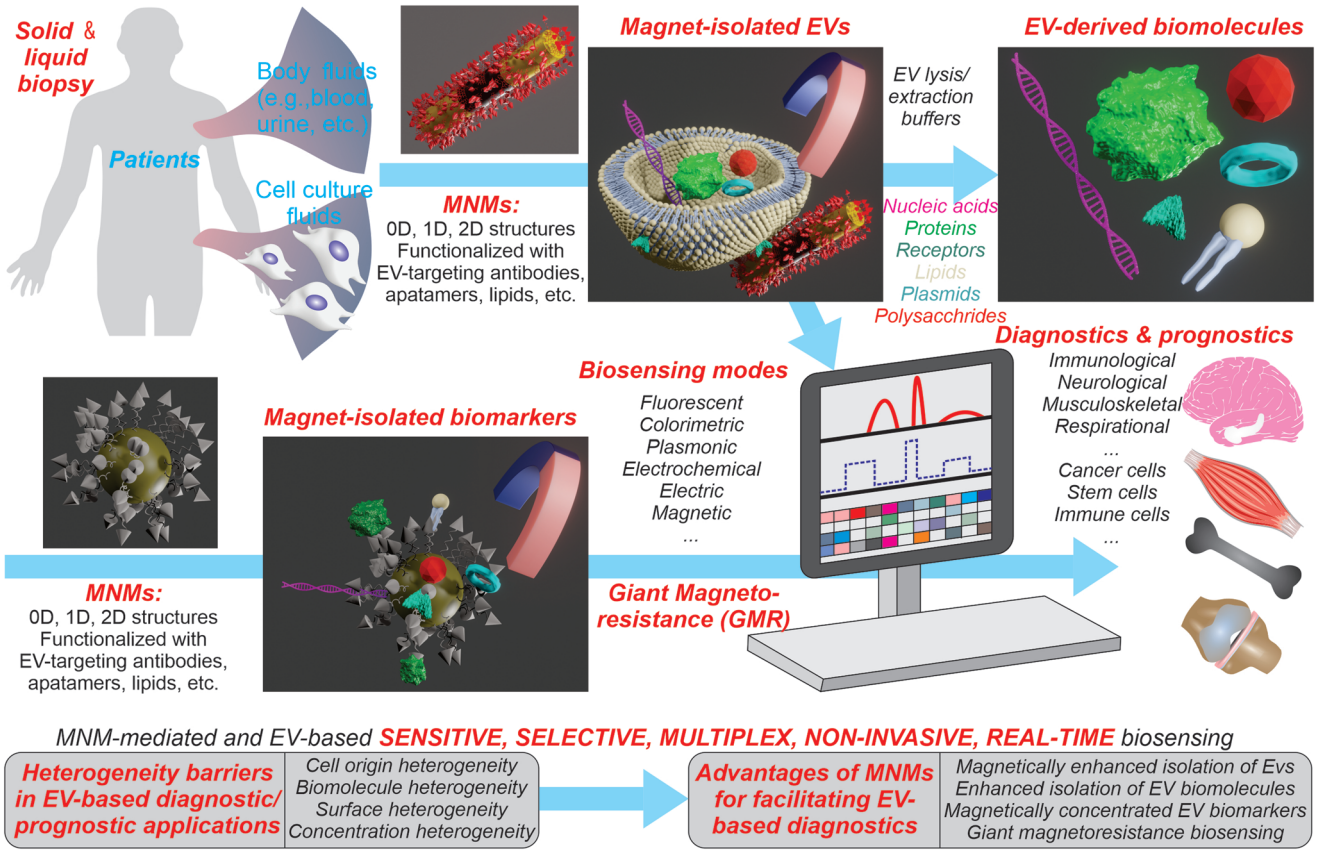

Figure 7. Design principles and workflow of MNM-enabled isolation and detection of biomolecules in EVs. Solid (e.g., tissues) and liquid (e.g., blood) biopsies harvested from individuals and containing extracellular vesicles can be extracted and isolated by MNMs followed by further purification of different types of biomolecules including nucleic acids, proteins, lipids, and other biomarkers. These biomolecules can be further concentrated to different type of biosensors for analysis of the tissue origin, disease types, and pathogen existent in EVs. In this way, MNMs can overcome heterogeneity barriers of EV-based diagnostic and prognostic applications by providing magnetically enhanced isolation of EVs, enhanced isolation of EV biomolecules, magnetically concentrated EV biomarkers, as well as unique giant magnetoresistance biosensing.

2D magnetic nanosheets, and 3D hybrid magnetic composite nanomaterials have been leveraged to tune biosensors' specific responses toward specific biomolecules.[203] Second, nanomagnetism of nanomaterials, and their alterations in response to the presence of MNM-labeled biomolecules, have been applied to develop noninvasive biosensors.[204] Based on these apparent advantages, MNMs have been integrated with different detection techniques, including fluorescent, plasmonic, electrochemical, electric, magnetic, mechanical sensors, to select DNAs, RNAs, and detect DNAs, lipids, proteins in EVs, and other cell-derived biological components.[205,206] Information provided by these MNM-facilitated and EVs has also been applied for the in vitro and in vivo diagnosis of various diseases, such as neurological disorders, viral diseases, cancer, and monitoring of stem cell differentiation.[207]

There are usually three crucial steps in terms of the design principles of MNM-based biosensors: the binding to target biomolecules, electrocatalytic amplification of signals, and signal generation. The binding step or immobilization step shares the same strategy as occurred in the isolation applications. Based on the binding forces, the conjugation of binding molecules to MNMs can be categorized into surface absorption, electrostatic interaction, antibody–antigen conjugation, and covalent modification (e.g., click reactions, EDC/NHS catalyzed amine carboxylic couplings).[205] The signal amplification step is incorporated to amplify the target biomarkers or the signals generated from the target biomarkers. For example, mass spectrophotometry, polymerase chain reaction (PCR), secondary labels based on electroactive molecules or plasmonic probes (e.g., gold nanoparticle-based Raman signal enhancement), and the combination with enzymatic amplification strategies (e.g., the conjugation of peroxidase to MNMs for electrochemical biosensing applications).[179,202,208,209]

4.3. Conventional Methods for Isolating and Detecting EV-Derived Biomarkers

EVs, especially nanoscale EVs such as exosomes, are often challenging to isolate as a result of their small sizes (50–200 nm) and low densities. This can be further complicated by the heterogeneous biological fluids where the EVs exist.[210] For example, the gold standard of EV isolation has been the ultracentrifugation method. However, this method relies on specialized and cumbersome processes. It has been reported to lead to protein and lipoprotein aggregations.[211,212] To this end, several new

isolation strategies have been developed to address the issues associated with the ultracentrifuge method. In this section, we will briefly overview these isolation methods to be compared with MNM-based isolation methods. However, there are other review articles on conventional methods for isolation of EVs that can be referred to readers.

4.3.1. Ultracentrifuge-Based Isolation of EVs

The most common approach for separating exosomes has been the differential ultracentrifugation method (Figure 7).[213] Briefly, using different centrifugal forces, components in the extracellular biofluids can be isolated based on their differences in sizes, densities, and shapes. First reported by Johnstone in 1987, and then optimized by Théry with incorporating serial centrifugal events, the differential ultracentrifugation method has been established into a highly efficient approach for extensive volume purification of extracellular biofluids without requiring highly specialized technicians.[214,215] However, the limitations of the differential centrifugation method remain in the low purity of exosomes.[216] Under a specific centrifugal force, all biological components inside the fluids, including exosomes, microvesicles, and nonvesicles (e.g., protein aggregates) with different densities, sizes, and surfaces, can be precipitated as long as they reach the centrifuge force threshold.[217] For instance, a study on detecting the differential levels of NF-κB nuclear translocation in exosomes from endothelial cells confirmed that exosomes purified by differential ultracentrifugation method resulted in inconsistent biological outcomes. In contrast, more advanced purification methods allowed identifying significant differences of the NF-κB levels in different cell types, which has implications for vascular formation and cancer metastasis.[218] Although isopycnic ultracentrifugation and moving-zone ultracentrifugation methods have also been developed to address these challenges, they have disadvantages.[219] For example, the purity of the exosomes isolated using current methods is still far from satisfactory.[220] As such, size-based and immunoaffinity capture-based isolation methods have been developed to improve the purity of the isolated EVs.

4.3.2. Size-Exclusion-Based Separation of EV

Sizes of EVs play a critical role in conveying cell–cell communications. However, the sizes of EVs are intrinsically heterogeneous, typically ranging from 50 to 200 nm.[221] Having the ability to precisely separate EVs with varying sizes is critical for understanding EVs' biological origins and their separation for specific therapeutic applications. In this regard, it has been highly challenging for the ultracentrifugation method to achieve a narrow size distribution.[222] For this purpose, nanoporous membrane-based, sequential filtration-based, size exclusion chromatography (SEC)-based, flow field-flow fractionation-based, as well as hydrostatic filtration dialysis (HFD)-based methods have been developed.

Nanomembrane has been a highly effective approach for the purification of EVs, especially those existents in cell-free biofluids such as urine, serum, blood, cell culture medium, and cerebrospinal fluid. For example, exosomes have been successfully isolated from urine volume as low as 0.5 mL (**Figure 8**).[223] One of the commercialized kits for exosome isolation has been based on nanomembrane-based approaches.[211] Briefly, a syringe filter installed with two nanoporous membranes can be used to extrude the biofluids containing EVs. The EVs and the biomolecules inside the vesicles, such as RNAs, DNAs, and proteins, can be released for analysis during the extrusion. As most biofluids rarely contain DNAs or RNAs, the nanoporous membrane-based extrusion method has been considered optimal for analyzing exosomal RNAs and DNAs.[224] However, this method is also destructive to EVs and is mainly limited to the isolation of exosomes from cell-free biofluids.

To improve the nanoporous membrane-based isolation approach, a sequential filtration method compatible with cell-containing biofluids and to allow for better size selection has been developed.[225] Compared to the single-step nanoporous membrane extrusion-based approach, this method allows for more versatile applications in more heterogeneous biofluids such as cell-containing samples.[226] Similarly, hydrostatic filtration dialysis (HFD) that does not require extrusion forces has also been applied to improve the enrichment of EVs with minimal disruption of the vesicle structures.[227,228] Still, they do not directly enable the size-dependent isolation of EVs.

Another separation technique that is widely used in the size-based isolation of EVs is size exclusion chromatography (SEC). SEC sorts macromolecules and particles in a size-dependent manner through a porous stationary phase. When biofluids flow through the stationary phase, macromolecules, or particles with large hydrodynamic radii, including EVs, will be excluded from penetrating the pores on the column.[229] By using different pore sizes in the stationary phase, exosomes with different sizes could be effectively isolated. For instance, several mesenchymal stem cell (MSC)-derived exosomes have been isolated and purified using this approach.[230–232] As exosomes purified from the SEC are typically at the nanoscale, dynamic light scattering techniques can further characterize them.[233] SEC can also be combined with the ultracentrifuge method to obtain the EVs for analysis and therapies. A more advanced technique, flow field-flow fractionation (F4) that also uses porous stationary phase but combines with a parabolic flow during the flow of biofluids, is also newly developed for more precise isolation of EVs.[31,234,235] The unique parabolic flow effectively guides the distribution of small-sized particles in biofluids along the channel wall, thereby offering an additional step of purification to be combined with the size-exclusion-based isolation technique. Applying this unique method, exosomes in the neural stem cells have been effectively isolated. These isolation methods provide a means to purify EVs based on their sizes.[236,237] However, they cannot exclude other particles of similar sizes but have different biological origins, such as fragments of cells and extracellular protein matrix found in cell and tissue extractions.

4.4. MNM-Facilitated Isolation and Detection of EV

Addressing the challenges of conventional EV isolation approaches, the immunoaffinity-based isolation strategy has

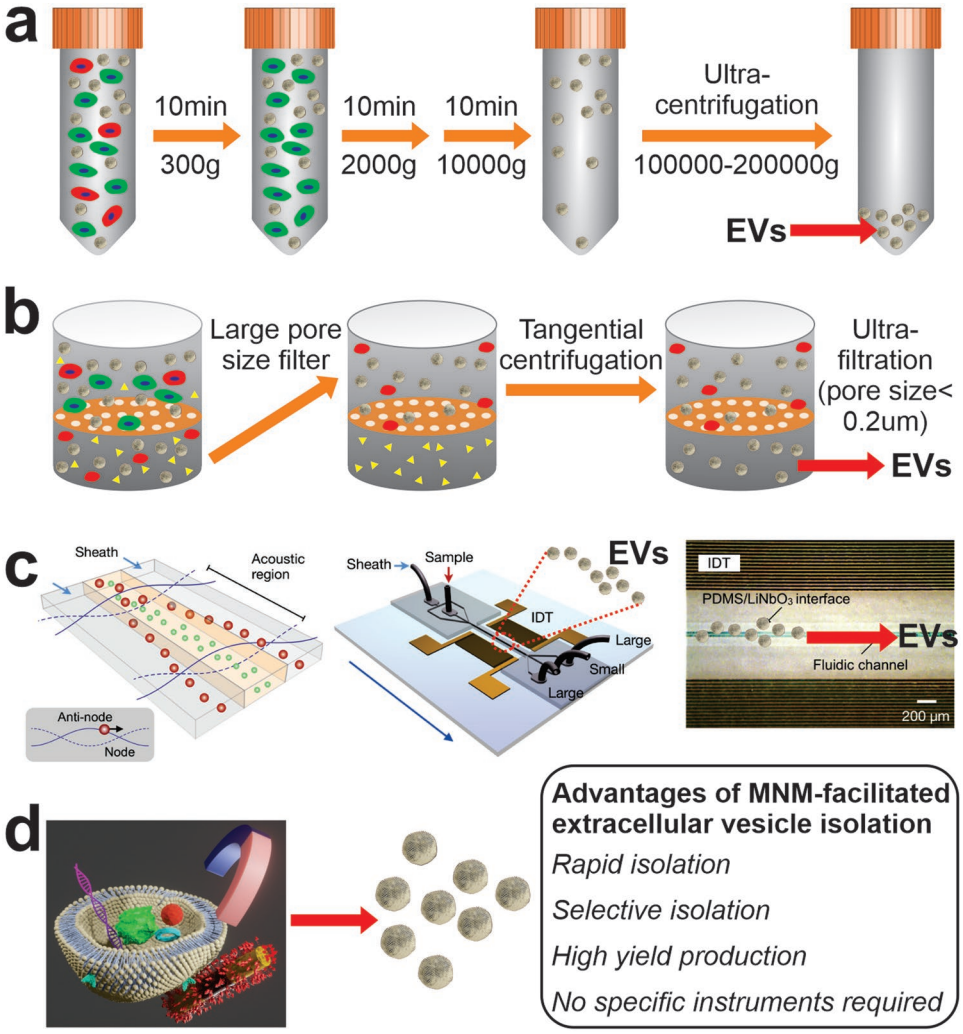

Figure 8. Comparison of MNM-based and conventional approaches for the isolation of EVs. a) Ultracentrifugal separation method. This has been conventionally used for isolation of EVs and its performance relies on the instrumentation. b) Sequential filtration-based separation method. This is a more advanced method but still has limited selectivity toward subtype EVs. c) Microfluidic-based separation of EVs. In this specific example, acoustic forces were integrated into microfluidics for the isolation of target EVs once detected by biosensors existent in microfluidics. The working principle is based on that EVs with larger sizes will move faster under acoustic forces. Therefore sheath flows integrated into the microfluidics can isolate large size EVs. The middle panel shows a schematic diagram of the device. Interdigitated transducer, or IDT electrodes are designed for generating acoustic wave vertical to the flow direction. The two side outlets are designed to collect large-size EVs and center outlets are for collecting smaller sized EVs. The right panel shows a photograph of the microfluidic device. $LiNO_3$ piezoelectric is used as the IDT electrodes. Images adapted with permission.[237] Copyright 2015, American Chemical Society. d) MNM-based immunoaffinity method for isolating EVs. MNM-facilitated EV isolation is rapid, selective, cost-effective with high yield compared to conventional EV isolation methods.

been proven effective for separating and detecting EVs with varying biological identities (Figure 8).[238,239] This strategy is mainly based on the presence of highly specific protein receptors in the EV membranes. For instance, enzyme-linked immunosorbent assays (ELISA) that target the exosome-specific receptors (e.g., CD41 and CD80) have been developed for the rapid and precise detection of diseases based on EVs.[240,241] The utilization of MNM-functionalized beads in the immunoaffinity-based isolation and detection of EVs has uniquely integrated the advantages of immunoaffinity-based isolation of biologics with the separation capability from the MNMs.[242] Therefore, the functional MNM-based immunoaffinity isolation approach has been broadly applied for the automated detection of exosomes.

There have been many surface receptors found in different types of EVs. Some of the most commonly seen receptors used for the immunoaffinity-based isolation of EVs include a cluster of differentiation 81 (CD81), CD9, CD63, and other transmembrane proteins.[243,244] Other surface receptors from specific cell origins include enhanced growth factor receptor vII (EGFRvII), vascular enhanced growth factor (VEGF) receptor, soluble N-ethylmaleimide sensitive factor attachment protein receptor (SNARE), chemokine receptor 5 (CCR5), and major histocompatibility complex (MHCs).[1] Knowing these critical receptors would be fundamentally crucial for designing the MNM-mediated isolation, detection, and delivery of EVs. Therefore, we have summarized these receptors in (**Table 1**) for readers' future references.

Table 1. A literature summary of state-of-the-art EV biosensors based on MNMs of varying compositions, structures, surface functionalities, and with varying biosensing modalities.

MNMs	Size/shape	Surface functionalities	EV types sample source	Biological targets in EV	Sensing modalities	Detection limit	Refs.
Fe_3O_4@QD	243 nm NPs	PEI	Exosome Patient samples	EpCAM	Optical (FRET)		[250]
Magnetic bead	NPs	Carboxylic group, CD63 antibody	Exosome A549/BEAS-2B	CD63	Fluorescence	100 mL^{-1}	[251]
Fe_3O_4@SiO_2	650 nm NPs	EV imprinting	Exosome CaOV3	EpCAM/CD24/CD63	Catalytic/ biochemical (HRP)	400 mL^{-1}	[209]
FeMPC	Microparticles		Exosome Gastric cancer patient urine	N-glycans	MALDI-TOF		[252]
Fe_3O_4@SiO_2	400 nm NPs	Amine groups, Ti(IV), DSPE	Exosome Prostate cancer patient urine	Phosphoprotein	Fluorescence		[253]
Fe_3O_4	1 μm	Poly-L-lysine	Exosome Blood plasma		Electrostatic		[254]
Biosilica porous diatomite@Fe_3O_4	Magnetic diatom	Concanavalin A, L-arginine, CD9/CD63/CD81 antibodies	*Salmonella typhimurium*/*Brucella ovis*/exosome from colorectal cancer cells	Lipopolysaccharides, CD9/CD63/CD81	Fluorescence	50 CFU mL^{-1},100 mL^{-1}	[255]
Fe_3O_4	NPs	Streptavidin, casein antibody	Milk-derived EVs	Casein	Fluorescence		[256]
Superparamagnetic dynabeads		CD9/CD63/CD81 antibodies	Exosome Keratinocyte-derived in murine skin	N-glycans, CD9/CD63/CD81	Fluorescence		[243]
Fe_3O_4@SiO_2	CFMZOF, 500 nm NPs	Carbonyl groups, magnetic zirconium–organic framework	Exosome Human urine	PTM peptides	MS/HRP	0.5 fmol μL^{-1}	[208]
Magnetic bead	2.8 μm dynabeads	CD63 antibody	Exosome Panc-1/SW480/C3	CD63	SERS	2.3×10^6 mL^{-1}	[257]
Magnetic bead	500 nm NPs	Carboxyl group, CD63 antibody	Exosome LNCaP/PrEC	CD63	SERS	160 mL^{-1}	[258]
Ag–Fe_3O_4	Nanoclusters		Exosome HepG2/U87	GSH–GSSG	Fluorescence		[259]
Magnetic bead	NPs	CD63-1 aptamer	Exosome MDA-MB-231/HT29	CD63	Fluorescence		[260]
Fe_3O_4	25 nm NPs	CD9 antibody	Exosome Pancreatic cancer patient blood	CD9	Biochemical (HRP)		[261]
Fe_3O_4@C	130 nm nanoflowers	Carboxyl group, CD9/CD63 antibodies	Exosome human plasma	CD9/CD63	Optical/LFIA	4×10^6 EVs μL^{-1}	[262]
SPION	4.5 μm	Polystyrene, p-toluenesulfonate group	Exosome MCF7/MDA-MB-231/SKBR3	CD81	Electrochemical/biochemical (HRP)	2.34×10^4 μL^{-1}	[263]
Fe_3O_4@Au	150 nm NPs	CD63 aptamer	Exosome HeLa	CD63	Electrochemical	6 μL^{-1}	[264]
							[265]
Magnetic bead	NPs	Biotin-labeled lectin, CD9/CD63/CD81 antibodies	Exosome PSN-1/Bxpc-3/Miya-Paca/PL45/Capan-1/Capan-2/HPAF-2/Panc-1	CD9/CD63/CD81	Optical		[266]

Table 1. Continued.

MNMs	Size/shape	Surface functionalities	EV types sample source	Biological targets in EV	Sensing modalities	Detection limit	Refs.
Iron oxide	Dynabeads	GPC-1 antibody	Exosome MDA-MB-231/HeLa/ SMMC-7721/LNCap/ H1299	GPC-1	Fluorescence	6.56×10^4 μL^{-1}	[267]
Magnetic bead		K8-/K-16 peptides	Small EVs MCF7	Phospholipid bilayers	Luminescence/HRP		[268]
Fe_3O_4	NPs	Streptavidin	MSC in vitro culture	CD81	Plasmonic	1 μg mL^{-1}	[269]
Fe_3O_4	NPs	Dopamin/succinic acid	Exosome from OVCAR3 cell culture	CD9	Chronoamperometric	6×10^5 mL^{-1}	[265]
Au–Fe_2O_3	Nanocubes		BeWo cell culture	CD63		100 mL^{-1}	[179]
Fe/Au	37 nm/2.3 μm	Polyethylene glycol–thiol	OSCA cell-derived exosomes		ExoQuick-TC kit		[175]
Ni	36 nm/ 2.2 μm nanowires	Polyethylene glycol	OSCA-8 cell-derived exosomes	CD63	Biochemical	1×10^9 mL^{-1}	[270]
Fe/Au	36 nm/ 2.2 μm nanowires	Streptavidin	Cancer patient-derived exosomes	CD9/CD63/CD81	Fluorescence		[240]
Fe_2O_3	1 μm microparticles	Polyethylene glycol	A549 lung cancer-derived and healthy human exosomes	CD63/CD9	Mass spectrometer		[271]
Magnetic beads	2.8 μm dynabeads	anti-*H. pylori* antibody	*H. pylori* From *C. albicans*		Optical, fluorescence		[272]
Fe_3O_4	2.4 μm	pCBMA, L1CAM/ CD9 antibodies	Exosome Parkinson's disease patient serum	L1CAM/CD9	Electrochemiluminescence	0.3 pg mL^{-1}α-Syn	[273]
Magnetic bead	NPs	CD63 antibody, oligonucleotide	Exosome Patient serum	CD63	Biochemical		[274]
Magnetic bead	NPs	Carboxyl group, EpCAM/CD63 antibodies	Exosome Hepatocellular carcinoma patient plasma	CD63/EpCAM	Fluorescence/HRP	576 (±15) mL^{-1}	[275]
Fe_3O_4	NPs	CD63 aptamer	Exosome MCF-7/SK-BR-3/ MDA-MB-231/BT474	CD63	Electrochemical		[276]
Magnetic bead		EpCAM antibody	Circulating exosome Esophageal squamous cell carcinoma patient serum	EpCAM	Biochemical/ELISA		[277]
Magnetic bead	NPs	CD63 aptamer	Exosome MCF-7	CD63	UV–vis/HCR	1.6×10^2	[278]
Magnetic bead	500 nm NPs	CD63 aptamer	Exosome MCF-7	CD63	Fluorescence		[279]
Magnetic bead	60–84 nm	CD63 aptamer	Exosome A549	CD63	Fluorescence	1.0×10^5 μL^{-1}	[280]
Magnetic bead		L1CAM, GLAST antibody	Exosome Human plasma	L1CAM/GLAST	Fluorescence	0.174 pg mL^{-1}	[281]
Magnetic bead	NPs	Carboxyl group, CD63 antibody	Exosome HepG2, HCC patient serum	CD63	Fluorescence	100 mL^{-1}	[282]
Fe_3O_4@chitosan	Nanorods	PNIPAM-AA microgel, Herceptin antibody	SKBR-3	HER-2	Fluorescence		[283]
Iron oxide	120 nm magnetic nanogels	Oleicacid, CHP nanogel	Exosome PC12	Lipid membrane	Fluorescence		[284]

Table 1. Continued.

MNMs	Size/shape	Surface functionalities	EV types sample source	Biological targets in EV	Sensing modalities	Detection limit	Refs.
Iron oxide	MNPs doped nanowires	CD9/CD63/CD81 antibodies	Circulating exosome Breast/lung cancer patient plasma	CD9/CD63/CD81	Biochemical, ELISA		[240]
Fe_3O_4	NPs	ssDNA complex	Exosome Prostate cancer patient urine	PSMA	Fluorescence/FRET/SERS	100 μL^{-1}	[285]
Fe_3O_4	Dynabeads	Streptavidin, CD63 aptamer	Exosome HepG2	CD63	Fluorescence	1.16×10^3 μL^{-1}	[286]
Magnetic bead		Streptavidin, EpCAM antibody	Exosome A549/MCF7/HepG2	EpCAM	Electrochemiluminescence	100 μL^{-1}	[192]
Fe_3O_4	200 nm	Thiol group	Exosome MCF-7	Maleimide moieties	Fluorescence, SERS		[287]
Magnetic bead	1 μm	CD63 antibody	Exosome Human blood plasma	CD63	ELISA	3×10^{10} mL^{-1}	[241]
Magnetic bead	NPs	CD63 antibody	Exosome Pancreatic cancer patient serum	CD63	Fluorescence		[288]
Au–NPFe_2O_3	Nanocubes	CD63 antibody	Exosome Placental cells	CD63	Electrochemical/ELISA	10^3 mL^{-1}	[179]
Magnetic bead	Array of Y-shaped micropillars	Tim4	Exosome Liver cancer patient serum	Phosphatidylserine	Electrochemical	4.39×10^3 mL^{-1}	[289]
MB@SiO_2@Au	NPs, MB@SiO_2 200 nm	CD63 aptamer	Exosome SKBR3/T84/LNCaP	CD63	SERS	73 μL^{-1}	[290]
Fe_3O_4@SiO_2	NPs	Alk5/CD105 antibodies	Exosome ARDS patient blood	Alk5, CD105	Fluorescence		[291]
SPION	9 nm		Exosome MSC-derived	Liposomes	Fluorescence		[292]
Magnetic bead		CD63/GPC-1 antibodies	Exosome MDA-MB-231	CD63/GPC-1	ELISA	10 μL^{-1}	[242]
Magnetic NP	Microbead	CD9/CD326, CD81/CD104 antibodies	Exosome PD7591/PD483 mouse pancreatic cancer cell	CD9/CD326/CD81/D104	Biochemical		[293]
Magnetic bead	200 nm	Carboxyl group, CD9/CD63/CD147/HER2/CA19–9/CEA/IgG2b antibodies	Exosome HCT116/BT474/SKBR3/A549/HEK293T, cancer patient serum	CD9/CD63/CD147/HER2/CA19-9/CEA/IgG2b	Biophysical/ELISA/FCM	0.39 μL^{-1}	[294]
γ-Fe_2O_3	4–24 nm	pAAc-b-pNIPAAm antimouse IgG	Exosome Human semen	IgG	Fluorescence/biochemical		[295]
Magnetic bead	NPs	Cholesterol	Exosome HepG2, cancer patients serum	Lipid membranes	Fluorescence, ELISA/SERS	4.8×10^4 μL^{-1}	[296]
Magnetic NP	15 nm	PLA/CD90/CD9/CD63/HLA-ABC/IgG antibodies	Exosome Human placental tissues	PLA/CD90/CD9/CD63/HLA-ABC/IgG	Biochemical		[297]
Magnetic bead	2–3 μm	Carboxyl group, PSMA aptamer	Exosome LNCaP	Prostate-specific membrane antigen	Electrochemical	70 μL^{-1}	[298]
SPION		Rhodamine B	Exosome hBM-MSCs		Fluorescence		[299]

Table 1. Continued.

MNMs	Size/shape	Surface functionalities	EV types sample source	Biological targets in EV	Sensing modalities	Detection limit	Refs.
Fe_3O_4	200 nm	Anion-exchange-based, EpCAM/PSA aptamers	Exosome PC3/HeLa, patient plasma	Phosphatidylserine v/EpCAM	Optical	3.58×10^6 mL^{-1}	[300]
Fe_3O_4	20 nm	PEG	Exosome Human serum		Optical/biochemical		[301]
SPION	391 nm	PEI/PAA/AAB/β-CD-PEG2000-COOH, CD63 antibody	Exosome 4T1/MCF-7 Human serum/ urine/saliva	CD63	Biochemical/ fluorescence		[302]
Magnetic bead	NPs	HER2	Exosome SKBR-3/SNU-216/ MCF-7		Fluorescence		[303]
Magnetic bead	NPs	CD9/CD63 antibodies	Exosome FaDu	CD9/CD63	Fluorescence		[304]
Magnetic bead	15 nm	CD31/CD41a/CD63/ MHC antibodies	Exosome Patient blood	CD31/CD41a/CD63/ MHC	Biochemical		[305]
Magnetic bead	200 nm	b-ligand, CD81 antibody	Exosome MSCs	CD81	Optical/BLISA	0.76 μg mL^{-1}, 130×10^{-15} M	[269]
Fe_3O_4	32 nm	Carboxyl group, holo-transferrins	Exosome Kunming mice serum	Transferrin receptor	Biochemical		[306]
Fe_3O_4@SiO_2	170 nm	MPS/PMAC	Exosome HeLa	N-glycopeptide	Biochemical	10 fmol	[307]
Magnetic bead	9.1 μm	CD9/CD63/CD81 antibodies	Exosome Patient Inflamed synovial fluids	CD9/CD63/CD81	Fluorescence		[308]
FeOx	250 nm	Annexin V-biotin	Microvesicles HUVECs/MCF7		Biophysical		[309]
Magnetic bead		CD63/CD9 antibodies	Exosome BT474/SW-48, colorectal carcinoma patient serum	CD63/CD9	Electrochemical	100 μL^{-1}	[310]
Magnetic NP	15 nm	Carboxyl group, Abs	Viruses, extracellular vesicles from ACS patient blood		Fluorescence		[311]
Magnetic bead		CD9 antibody	Exosome pSS patient saliva and tears	CD9	Biochemical		[312]
Fe_3O_4@SiO_2	100 to 200 nm	CD63 antibody	Exosome SKBR3/MRC5	CD63	SERS	1200	[313]
Iron oxide	15nm	Carboxyl group, CD31/CD41a/CD63 antibodies	Exosome ACS patient blood	CD31/CD41a/CD63	Fluorescence		[314]
Magnetic bead	Microbeads	CD61/CD9 antibodies	Exosome NK cells and platelet	CD61/CD9	Fluorescence/ biochemical		[315]
Magnetic bead		CD63/CD9/ CD81antibodies	Exosome Ovarian Cancer	CD63/CD9/CD81	Biochemical (HRP)/ electrochemical	10^3 exos	[167]
Magnetic bead	Dynabeads	CD63/CD8/ CD81antibodies	Exosome Human urine	CD63/CD8/CD81	Fluorescence		[316]
Magnetic bead	Microparticles	CD63 antibody	Exosome H640	CD63	Electrochemical		[317]
Magnetic bead		CD9 antibody	Exosome PC-3	CD9	Biochemical		[318]

Table 1. Continued.

MNMs	Size/shape	Surface functionalities	EV types sample source	Biological targets in EV	Sensing modalities	Detection limit	Refs.
Iron oxide	15 nm	Carboxyl group, CD81/CD63/CD31 antibodies	Microvesicles SUPT1-CCR5 CL.30	CD81/CD63/CD31	Fluorescence/biochemical (ELISA)		[319]
Magnetic bead		CD9/PSMA antibodies	Exosome LNCaP/PC-3	CD9/PSMA	Biochemical		[320]
SPION	5 nm		Exosome B16-F10, human serum		Fluorescence/biochemical		[321]
Magnetic bead	Microbeads	CD34 antibody	Exosome Kasumi-1 AML AML patient plasma	CD34	Biochemical		[322]
VSOP	7 nm	Citrate	THP-1/THP-derived macrophages	SPIO Resovist	MRI		[323]
Magnetic bead		CD45 antibody	Exosome Human lymphoblastoid T-cell Jurkat and CEM	CD45	Biochemical		[324]
Magnetic bead	Microbeads	CD11/CD19/CD49/mPDCA-1 antibodies	Exosome Splenocytes	CD11/CD19/CD49/mPDCA-1	Biochemical		[325]
SPM polystyrene	Microbeads	Ber-EP4 Ab	Epithelial cells Human plasma	Glycopolypeptide membrane antigens	Biochemical		[326]
Iron oxide	50 nm	Streptavidin, PSMA antibody	LNCaP/DU145	PSMA	Fluorescence		[327]
$CoFe_2O_4$	8 nm	Carboxylate groups	Endosome HeLa		Fluorescence/microrheological		[328]
Magnetic bead		Poly(carboxybetaine-methacrylate), L1CAM antibody	Exosome Parkinson's disease patient serum	L1CAM	Biochemical/electrochemiluminescence		[329]
Fe_3O_4-MVs	300 nm	Fe3O4-MVs-DBCO	CTCs	Ac4ManNAz labeled	Fluorescence		[330]
Magnetic bead	100 nm microspheres	CD63 aptamers, DNA concatamers, SA-QDs	Exosome CAL27, OSCC patient saliva	CD63	Fluorescence	500 μL^{-1}	[331]
GO- $Fe_3O_4@SiO_2$	Dotted thin sheets	$Ti4^+$	Exosome HeLa, human serum	Phosphopeptides	Fluorescence		[332]
$Fe_3O_4@SiO_2$	1 µm	PEG	Exosome A549, human blood plasma		Fluorescence		[271]
MoS_2-Fe_3O_4-Au	Dotted nanocomposites	NWs-GSH	Exosome Human urine and serum	N-glycopeptides	Biochemical		[333]
Polycore magnetic NPs		PDA/PAAPBA/PAA/PPEGMA/CD63 antibodies	Exosome A498, human serum/urine	CD63/glycan moiety	Magnetoresistance (GMR) sensor	$<10^4$ EVs	[334]
Dynabeads	4.5 µm	Various tetraspanins antibodies	Exosome MCF7/MDA-MB-231/SKBR3, human serum	Tetraspanins	Electrochemical	10^5 exos μL^{-1}	[335]
$Au@Fe_3O_4$	NPs	Cationic functionalized	supported lipid bilayer TRAMP-C2		Biochemical		[336]
DeMEA system	3D printed magnetic housing	Aptasensing surface	Exosome MCF-7		Electrochemical	17 exos μL^{-1}	[337]
Magnetic bead	4.5 µm	CD63 antibody	Exosome LS174T/LSC	CD63	Biochemical		[338]

Table 1. Continued.

MNMs	Size/shape	Surface functionalities	EV types sample source	Biological targets in EV	Sensing modalities	Detection limit	Refs.
Magnetic bead		Carboxyl, CD63/PD-L1 antibodies	Exosome MDA-MB-231/L02	CD63/PD-L1	Biochemical (HRP)	334 mL^{-1}	[339]
Polystyrene magnetic bead	NPs	Carboxylic cross-linked, CD9 antibody	Exosome	CD9	Fluorescence		[340]
Magnetic bead		Anti-EpCAM	Exosome Serum	EpCAM	Fluorescence		[341]
Magnetic bead	100 nm	Polyacrylic acid, CD63 antibody	Exosome Human plasma	CD63	Biochemical/LFIA	3.4×10^6 EVs μL^{-1}	[342]
Magnetic bead		Annexin A5, lactadherin	Exosome HT29	Phosphatidylserine moieties	Fluorescence		[343]
Magnetic bead	NPs	CD63/CD81 antibodies	Exosome Ramos/SUDHL-4/SUDHL-6/Ros-50/SW480	CD63/CD81	Biochemical		[344]
Magnetic bead		A33/EpCAM antibodies	Exosome LIM1863	A33/EpCAM	Optical/biochemical		[345]
Paramagnetic bead		HLA DP/DQ/DR antibodies	Exosome B-LCL/MHC	HLA DP/DQ/DR	Biochemical		[346]

Like previous MNM-facilitated biosensing applications, MNM-facilitated exosomal isolation and detection typically involve the following crucial steps: the binding to target biomolecules, which is usually through immunoreactions with the surface receptors of EVs, the isolation of biomolecules, the electrocatalytic amplification of signals, and signal generation. While the strategies of immunoaffinity binding and the signal generation are not entirely new and could be adapted from most previous biosensing applications built upon MNMs that can be found in other reviews, the MNM-facilitated isolation and detection of EVs often require more specific design depending on the different target biomolecules and the signal generation methods. In terms of target biomolecules, RNAs, DNAs, proteins, polysaccharides (including glycoproteins), lipids, and vesicles by themselves, have been isolated and detected using MNM-based biosensing systems.[245–249] Regarding methods for signal generation, optical (e.g., UV–Vis–NIR spectroscopy, fluorescence microscopy, Raman microscopy, surface plasmon resonance), electrical (e.g., electrochemical biosensor and field-emission transistor), and magnetic biosensors have been used for the detection of biomolecules associated with EVs. A table summarizing current progress made in each category and the MNMs used can be found in (Table 1). In the following sections, we will overview how MNMs have been leveraged to empower the different sensing modalities by providing immunoaffinity-based isolation of EVs and their associated biomolecule.

4.4.1. MNM-Facilitated Fluorescence Biosensing of EVs

Among various biosensing modalities for detecting EVs, fluorescence-based biosensors have been the most commonly used with their high sensitivity and the widely used fluorescence probes such as dye-conjugated antibodies and nucleic acid probes.[347] For example, the commonly used platforms for detecting protein biomarkers inside the EVs, such as Western blotting, ELISA, or the more recently developed technologies such as small particle flow cytometry, have all relied on using fluorescent tags to identify EV-derived membrane and intravesicular proteins.[348] Similarly, commonly used platforms for detecting nucleic acids inside EVs have included quantitative real-time polymerase chain reaction (qRT-PCR), which requires DNA fluorescence probes for quantifying the nucleic acid biomarker as well.[349] However, because of autofluorescence from biological fluids and cell-derived components, the signal-to-noise ratio of the current fluorescence-based detection systems has not been entirely satisfactory.

For this purpose, multifunctional MNMs that cannot only selectively bind to target biomolecules inside the EVs but also isolate them from the biofluids for enhanced fluorescence signals would be highly desirable. For example, one of the recent studies developed a multifunctional MNM for both isolating RNAs from EVs and enhancing the fluorescence signals in molecular beacon-based nucleic acid detection (**Figure 9**). Stem cell-based therapies have been regarded as promising treatments for neurodegenerative diseases such as Alzheimer's and Parkinson's diseases.[350] The effective control and characterization of stem cell differentiation into target neural cell lineages (e.g., neurons and oligodendrocytes) are desired to maximize the therapeutic potential and clinical translation of stem cell-based treatment of neurological disorders. However, current methods for characterizing stem cell differentiation have often been destructive. Addressing this challenge, Lee et al. developed magnetoplasmonic nanorods to isolate and detect exosomal miRNA for monitoring stem cell neurogenesis. To ensure the homogeneity of the signal generation, magnetoplasmonic nanorods composed of nickel and gold were generated using anodized aluminum oxide (AAO) template-based electrochemical deposition method. The CD63 antibody was conjugated to the nickel components in the nanorod using the coordination

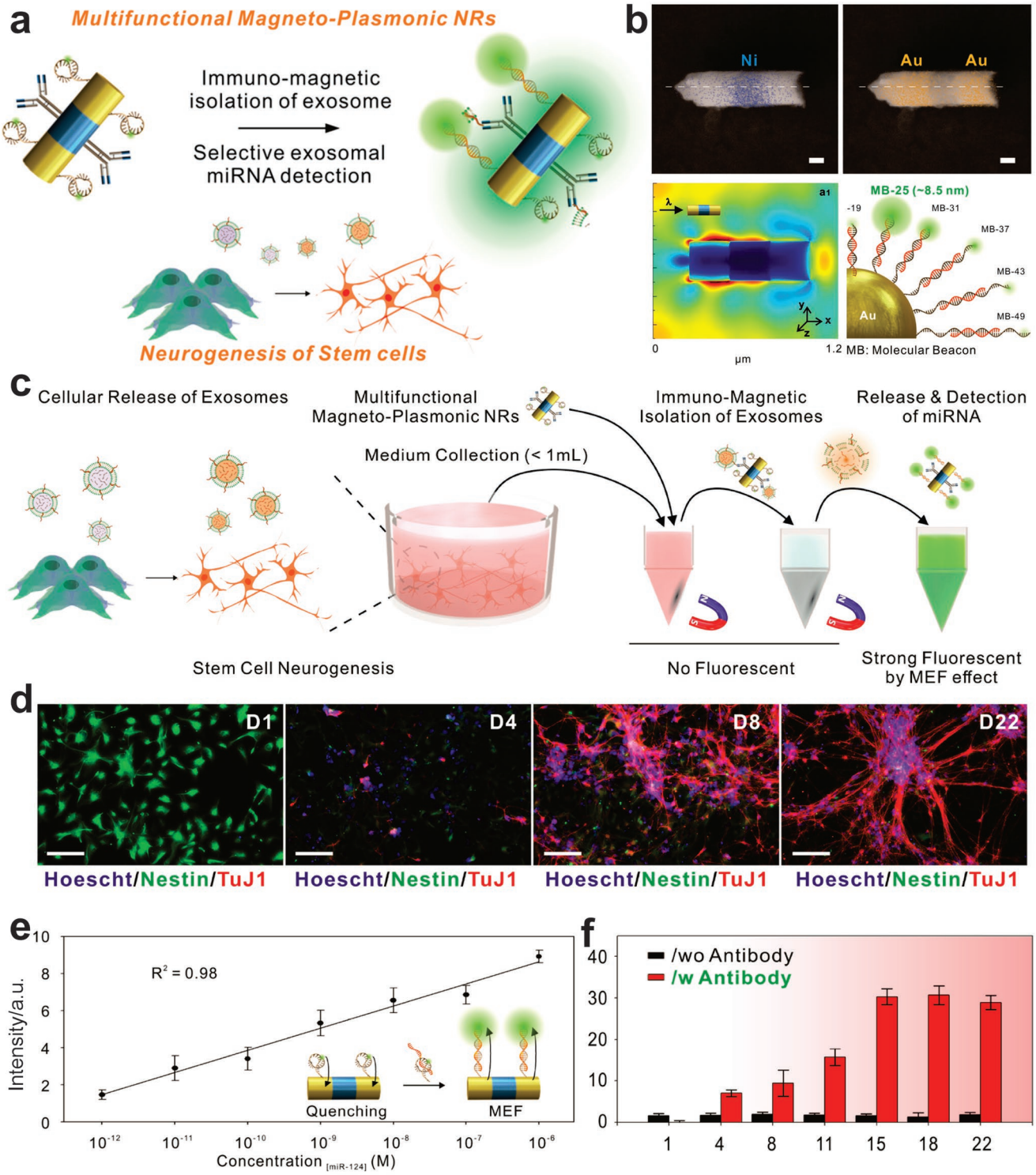

Figure 9. Design principles and workflow of MNM-enabled isolation and detection of biomolecules in EVs. a) A schematic diagram showing the gold–nickel magnetoplasmonic nanorod-enabled isolation of EVs followed by the analysis of miRNAs for monitoring stem cell neuronal differentiation. b) The characterization of the magnetoplasmonic nanorod and simulation schemes on the plasmonic-enhanced fluorescence for ultrasensitive detection of EV-derived biomolecules. c) The workflow of magneto-plasmonic nanorod-based isolation and detection of EVs from stem cells and differentiated neurons. d) Confirmation of the biosensing results from a standard, destructive immunostaining assay on the neuronal markers. e,f) Quantitative and non-destructive characterization of stem cell differentiation into neurons using MEF. *y*-axis in panel f indicates days of differentiation of stem cells. Schemes and graphs were adapted with permission.[163] Copyright 2019, American Chemical Society.

forces between the nickel and carboxylic group in the antibody to capture the exosomes derived from neural stem cells. Dye-labeled, molecular beacons targeting miRNA-124 (neuron-specific miRNA) were conjugated to the nanorod using a thiol–gold reaction to detect the exosomal RNAs. Molecular beacons are hairpin nucleic acids coupled with an internally quenched fluorescent dye. They detect RNAs through the Watson-Crick complementary binding, leading to the opening of the loop and recovery of fluorescence.

Specifically, using the magneto plasmonic nanorods, exosomes derived from the neural stem cells or differentiated neurons will be captured, and then the RNAs inside exosomes will be released through lysis. The released miRNA-124 could then be bound to the molecular beacon and generate the fluorescence signal. Notably, the plasmonic effect of gold in the nanorods was harvested for enhancing the fluorescence, the sensitivity of the biosensor, a process also known as metal enhanced fluorescence. In this way, they showed magneto plasmonic nanorods as a multifunctional platform for capturing, isolating, and enhancing the detection of exosomal RNAs. This process was further applied to monitor the neuronal differentiation of human-induced pluripotent stem cells (hiPSCs) in both 2D and 3D cell culture models.[163] The strategy using hybrid MNM to enhance the fluorescence signal and improve the selectivity of the EV-based biosensors can be applied to other platforms, such as small particle flow cytometry, Western blotting, and ELISA. Integrating the isolation and detection in a single MNM may also accelerate the biosensing of EV-derived biomolecules, which is desired for monitoring rapidly progressive diseases such as cancer.

4.4.2. MNM-Facilitated Raman Biosensing of EVs

Raman is a fingerprint analysis tool that can be developed into many ultrasensitive and multiplex biosensing systems. This is because of their narrow peak width and the low Raman background from most biological fluids, which is often desired in detecting EVs.[258] However, the critical barrier for most current Raman biosensors is their weak signals, as the Raman scattering cross-section is often too small for most analytes. To this end, surface-enhanced Raman scattering (SERS) based upon the localized surface plasmon resonance phenomenon has been developed to enhance the local electromagnetic field, leading to higher signal intensities. However, the poor signal homogeneity and reproducibility of most SERS biosensors have limited their broad applications in the quantitative analysis of EV-derived biomarkers. MNMs that can isolate target biomarkers from EVs and concentrate them to the surface of SERS biosensors can provide a promising solution toward ultrasensitive yet reproducible multiplex biosensors for disease detection.[351]

Wang et al. developed a magnetic SERS biosensing platform and applied it to multiplex detection of cancer exosomes. Specifically, they synthesized a gold shell magnetic core nanoparticle for the isolation of the exosomes, followed by the in situ analysis of biomolecules inside exosomes bound to the surface of the gold shell. To achieve the multiplex analysis of exosomes, aptamers targeting CD63 of exosomes were conjugated to the gold shell through thiol–gold interaction. Aptamers are nucleic acids that can bind to target biomarkers (e.g., membrane proteins) through stereochemistry. Compared to antibodies, aptamers have advantages such as higher affinities and smaller sizes. Additionally, the gold shell magnetic core nanoparticle can simultaneously act as a SERS substrate, having the LSPR effects on the surface of gold. When cancer exosomes were incubated with the CD63 labeled gold shell magnetic core nanoparticle, exosomes could be captured and isolated by the nanoparticle to a larger concentration without requiring ultracentrifugation. Afterward, a secondary gold nanoparticle, conjugated with another probe targeting cancer-specific biomarkers such as carcinoembryonic antigen (CEA), anti-ErbB2 (HER2), and prostate-specific membrane antigen (PSMA) were mixed for further detection of the biomolecules existent on the surfaces of captured exosomes. As the cancer exosomes and their surface proteins were sandwiched by two different plasmonic gold nanostructures, LSPR "hot spots" with exponentially enhanced electromagnetic field could occur because of the far-field coupling of two plasmonic structures. When the secondary gold nanoparticle with targeting ligands was also conjugated with Raman dye, the SERS signals could be enhanced significantly for the ultrasensitive detection of exosomal transmembrane biomarkers. Furthermore, due to the advantages of SERS and Raman for their narrow peak width, simultaneous detection of the three antigens (CEA, HER2, and PSMA) were realized in a single assay. An extremely low limit of detection (LOD) could be achieved using their method, down to 32 exosomes per microliter of biofluids.[290] Still, despite the extraordinary sensitivity of their magnetic SERS exosomal biosensors, further investigations on the repeatability would be crucial for the quantitative analysis of cancer exosomes and their derived biomolecules.

4.4.3. MNM-Facilitated Electrochemical Biosensing of EVs

Electrochemical biosensors have been among the most used devices for disease diagnostics to ensure high reproducibility in point-of-care clinical tests of biomarkers, such as glucose. Given their advantages in large-scale detection in complex biofluids, electrochemical biosensors have been developed to detect EVs.[352] However, one critical issue associated with current electrochemical exosomal biosensors has been the small volume of samples available for detection, which challenges the design of miniaturized systems and requires a highly sensitive and selective approach to ensure reliable disease diagnosis.[353]

Lee et al. developed an integrated magneto-electrochemical exosomal (iMEX) sensor for fast and streamlined exosomal analysis and cancer diagnosis. The iMEX platform integrates two orthogonal sensing modalities, namely magnetic isolation, and electrochemical biosensing, to amplify target biomarker signals. Specifically, MNMs were coated with CD63 antibodies, which bind to components that are enriched in exosomes. The exosomes captured by the magnetic nanoparticle flowed through electrochemical sensors with eight independent channels in a miniaturized form. Each channel was immobilized with a specific potentiostat that could detect broad-spectrum current signals highly sensitively. A low-pass filter was also combined to condition the input signal and suppress noises from the high-frequency signals. A digital-to-analog (DTA)

converter was employed to connect all eight potentiostats, and the whole device was packaged into a point-of-care device as a prototype. Most importantly, eight magnets were also placed below the electrode cartridge to concentrate exosomal captured magnetic nanoparticles on the target electrochemical sensor surfaces. Based on these specific designs, one would expect the iMEX platform to have a few advantages for exosomal biomarker detection: i) a high sensitivity for the exosomal detection could be achieved owing to the magnetic enrichment of the biomarkers; ii) a high cell-type-specificity could be achieved for overcoming the cell heterogeneity barrier as the specific exosomal types could be captured from complex biofluids such as patient blood without requiring tedious centrifugation or filtration processes; iii) highly portable and miniaturized device could be developed from this sensing mechanism for point-of-care detection, which is desired in clinical settings. Using this system, as low as a hundred thousand cancer cell-derived exosomes can be detected in a single assay, and ovarian cancer patient-derived EVs could be detected. Given its excellent performances in the rapid and high throughput analysis of exosomes, this magneto-electrochemical sensing platform holds excellent clinical potential in analyzing EVs for cancer diagnostic applications. However, as pointed by the authors, this magneto-electrochemical sensing assay requires further improvement on the multiplexing capability, detection sensitivity, and the ability to quantify the biological components (RNAs and DNAs) inside the exosomes.[167]

4.4.4. MNM-Facilitated GMR Biosensing of EVs

As mentioned above, MNMs can facilitate the development of ultrasensitive, selective, and multiplexed analysis of EVs in fluorescent, Raman, and electrochemical biosensors. Additionally, the change of properties of MNMs in response to target biomarkers can be leveraged for the sensitive detection of biomolecules derived from EVs. Notably, as human tissues are mainly transparent to magnetic fields, magnetic imaging and sensing have a higher potential for in vivo and clinical diagnostic applications.[354]

Wang et al. developed an innovative GMR biosensor for the selective and comprehensive analysis of EV glycans (**Figure 10**).[334] As mentioned above, EVs contain a diverse repertoire of biomolecules, including nucleic acids, proteins, lipids, and glycans. Although each of them can reflect the disease progression, most current analysis of EV biomolecules has focused on nucleic acids, proteins, and lipid components. In contrast, EV-derived glycans (e.g., lectins) that carry critical information, especially those related to sugar metabolism in host cells, have not been well characterized, primarily owing to their diverse compositions and structures. Conventional methods for mapping the diverse glycan composition have been mainly based upon mass spectroscopies (e.g., matrix-assisted laser desorption ionization-time of flight (MALDI-TOF)).[355,356] However, they typically require complicated processing of large volume samples and expensive and nonportable instruments. Addressing these challenges, Wang et al. developed an all-magnetic iMAGE platform that converts the diverse glycan signatures into the magnetic signal detectable by GMR sensors. Specifically, polycore magnetic nanoparticles (PMPs) were synthesized by assembling small Fe_3O_4 nanoparticles using reverse micelle formation followed by encapsulation in polyvinylpyrrolidone solution under heating. To enable surface functionalization, a silica shell was further coated by in situ silica deposition through the ammonium facilitated hydrolysis of organosilanes. Lectins targeting specific glycans were then grafted to the PMPs functionalized with 3-(methacryloyloxy)propyl]trimethoxysilane (MAPTS). In the presence of EVs carrying specific glycans on their surface membranes, lectin-conjugated PMPs will be bound to the EVs through lectin–glycan interactions, leading to the aggregation of multiple PMPs surrounding the same EVs. This will lead to a dramatic change of magnetic field, which can be detected by GMR sensors. As diverse lectins are discovered to bind to specific glycans, different PMPs conjugated with specific types of lectins could be mixed and used to detect multiple types of exosomal glycans in one assay, allowing for multiplex detection. To address the limitation of a small volume of blood samples available under clinical settings, a miniaturized iMAGE platform integrating all the magnetic sorting channels and the GMR sensing components was further built as a proof-of-concept. Using their miniaturized iMAGE platform, over 20 glycans were detected in a single assay, with rapid detection speeds (less than 30 minutes) and high sensitivity (LOD < 10^4 EVs). Nevertheless, the PMP aggregation-induced GMR signal alterations are not linear, resulting in high signal variations and compromising their potential in the quantitative diagnosis and prognosis of cancer.[334]

4.5. Summary and Outlook of MNM-Facilitated EV Biosensors

EVs are heterogeneous in sizes, compositions, cell origins, and functions. It is crucial and challenging to isolate and detect them in a highly sensitive, selective, and multiplex manner.[210,250] In this section, both conventional (ultracentrifugation, size-exclusion chromatography, and membrane filtration), as well as MNM-facilitated new isolation strategies, were over-viewed to address the critical heterogeneity barrier in the analysis of biomolecules associated with EVs. Integrating MNM-based isolation of EVs into fluorescent, Raman-based, electrochemical, and magnetic biosensors, has also been discussed. MNMs that can selectively isolate the surface integrins of EVs and the associated biomolecules (e.g., DNAs, RNAs, proteins, lipids, and glycans) led to enhanced sensitivity and selectivity and enabled the miniaturized EV biosensors. Therefore, MNMs hold great potential to facilitate the clinical translation of EV biosensors in the multiplex and point-of-care patient diagnosis and prognosis.

EV-based diagnostics is still at its early stage of development, and there is plenty of room for further development. For instance, MNM-integrated colorimetric, electrical, and magnetic biosensors have not been well developed yet, and their sensitivity requires further improvement for the early detection of diseases. Furthermore, most current MNM-integrated EVs have been focused on analyzing cancer patient-derived biomarkers. At the same time, there are many recent biological pieces of evidence highlighting the great potential of EVs in reflecting the disease progression of neurological and

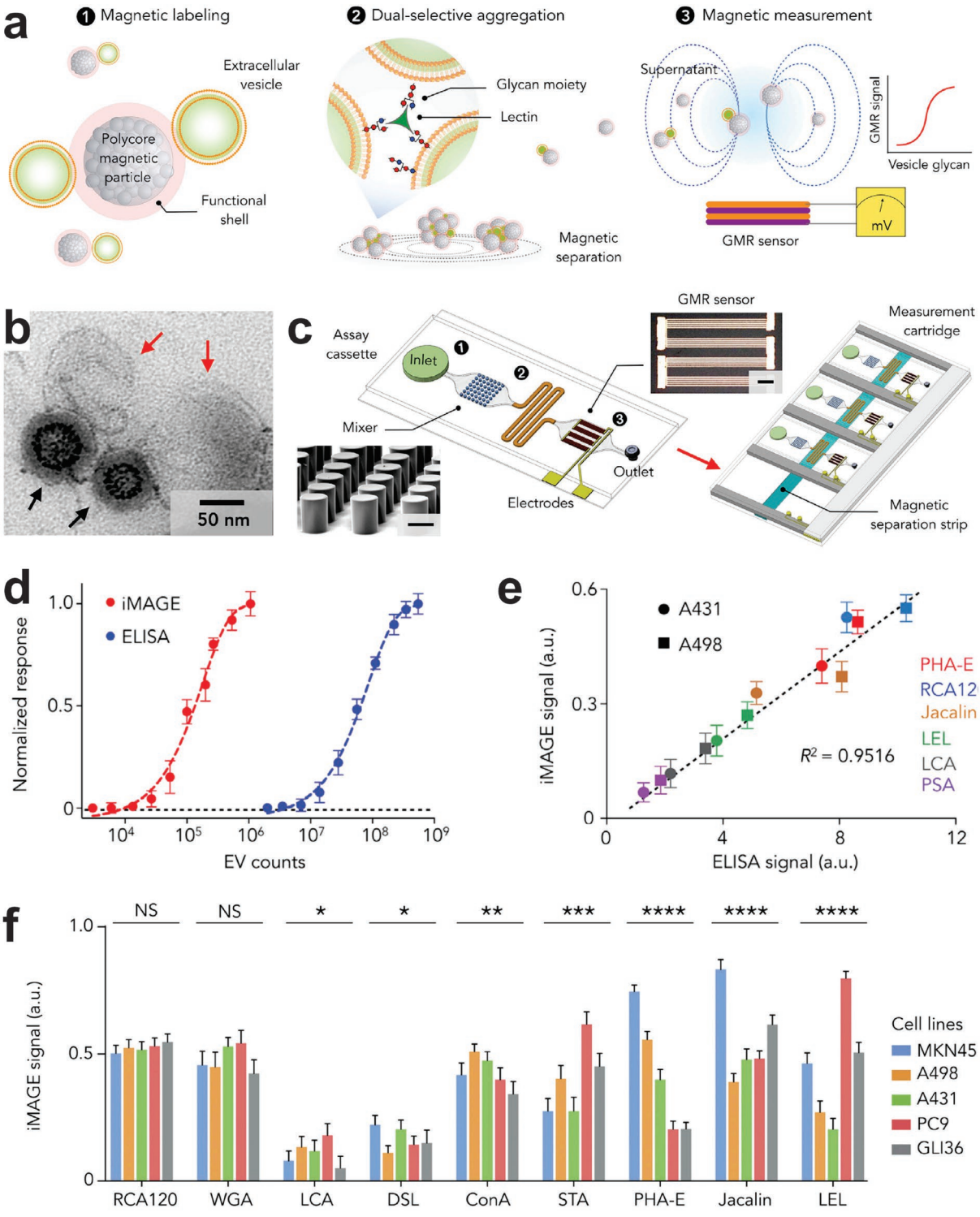

Figure 10. MNM-facilitated GMR biosensing of EVs. a) Working principles of i(integrated) M(magnetic) A(analysis) E(EVs) G(glycans), or iMAGE platform. EVs were first labeled with magnetic nanoparticles closed by a shell that binds to EVs. When the target glycan ligands exist on surfaces of EVs, the binding will occur and induce the aggregation of magnetic nanoparticles. As a result, they will induce a change of magnetoresistance (GMR) signals in the GMR sensing unit. b) TEM images of EVs that are bound to magnetic nanoparticles. A kidney cancer cell (A498) was used to study the binding process. Magnetic nanoparticles were further coated with polydopamine to enhance colloidal stability. c) An additional microfluidic-based system was used to allow multiplex detection of EVs. A micropillar array was used to improve the process of lectin binding and aggregation for amplifying GMR sensing signals. Scale bar in (c) indicates 50 mm in length. d) EV concentration-dependent GMR responses and their comparison to conventional ELISA methods showing the high sensitivity of GMR EV biosensor. e,f) Multiplex detection of EVs using iMAGE platform for monitoring different cell lines and different glycan biomarkers. Images adapted with permission.[334] Copyright 2020, Cell Press.

musculoskeletal diseases.[357–359] Harvesting EVs from these tissues may require less invasive methods. The sample volume is often less compared to cancer patient-derived EVs. Therefore, MNMs could play an even more critical role in enhancing the sensitivity, selectivity, and isolation efficiency of EV biosensors.

5. Engineered MNMs for Tracking and Delivery of Synthetic and Cell-Derived Vesicles-Based Therapeutics

5.1. Overview of the Therapeutic Potential of EV

By virtue of their diverse biomolecules, EVs also have enormous potential for tissue regeneration, pain management, cancer treatment, and various other therapeutic applications.[360] For example, stem cell-derived EVs have shown their ability to reduce apoptotic signaling, stimulate proliferation pathways, program immune cell responses, stimulate angiogenic programs, and reprogram cells of regenerative types to the sites where tissue requires repair.[361–365] In parallel, EVs derived from somatic cells have also been employed for recruiting immune cells for triggering the apoptosis of the targeted cells in cancer therapies.[366]

Stem cell-derived EVs originated from varying tissues, with bone marrow (e.g., mesenchymal stem cell (MSC)] and blood (e.g., hematopoietic stem cells (HSC)) being the most investigated.[367] Their therapeutic potential for clinical applications, especially MSCs, is strongly supported by the rapid growth of clinical trials in treating varying diseases, such as acute kidney injury, Crohn's disease, and cardiovascular diseases.[177,368–371] MSC-based stem cell therapies for tissue regeneration have been successfully established and widely tested based upon the premise that cells can home and integrate into the diseased or injured tissues, followed by differentiation into functional cells to replace diseased/injured cell types.[372] However, cells transplanted into the host tissues often encounter a hostile microenvironment, including immune responses and apoptotic signals, leading to primarily compromised therapeutic effects.[373] For instance, during the MSC-based treatment of myocardial infarction, only a tiny portion of MSCs was found capable of reaching the injury site (infarcted heart), with most of the transplanted cells circulated to undesired tissue locations. Although a small portion of MSCs home to the injury site, their conversion into the target functional cell type (cardiomyocytes) is often inefficient. Still, functional restoration of the heart is observed at the early stage of MSC transplantation, faster than one would expect the transdifferentiation into cardiomyocytes to take place. Based on these findings, it was reasonable to believe that MSC-derived soluble factors, rather than cell replacement, drive tissue regeneration, at least in the initial stages. Conditioned media derived from MSCs undergoing hypoxia were further used to treat cardiac infarction, which provides more direct evidence of EV-mediated tissue regeneration functions. So far, this strategy has been widely applied to and tested in acute kidney injuries and a variety of other diseases using in vitro and in vivo models.[374]

Later on, studies have further confirmed that such paracrine effects are usually mediately through nanoscale EVs. Among them, the exosome is the particularly critical one.[375] The paracrine effects also depend on the biomolecules transferred during the formation of EVs, including proteins (e.g., growth factors), mRNAs, miRNAs, DNAs, and lipids.[376] Leveraging the EV-mediated paracrine effects for stem cell-free cell therapies has clear advantages, especially considering the long-existing concerns on the biosafety of stem cells after transplantation, including apoptosis and uncontrollable differentiation of stem cells, as well as cardiac arrhythmia.[377] Regenerative medicine based on EV derived from MSCs has made significant advancements in this regard, especially with the development of immortalized MSC cell lines accompanied by large-scale fabrication and isolation of exosomes.[362] However, the ability to precisely target injury locations in stem cell therapies is often compromised when the EVs were applied instead. This can lead to off-target effects and compromise the therapeutic efficacy during tissue regeneration. Another challenge remains on the reproducible production of EVs with similar compositions of biomolecules, which is vital in the clinical translation of EV-based cell therapies.[378]

EVs have been employed for immunotherapy, given their inherent properties in modulating immune responses during the circulation in blood.[379] One such example is demonstrated by Raposo et al. on the development of an EV-based cancer vaccine. In their pioneering study, exosomes secreted by human B lymphocytes were found to envelop major histocompatibility complex (MHC) molecules, restricting immune responses from T cells.[13] In parallel, exosomes secreted by murine dendritic cells were isolated in order to suppress tumor growth in vivo, and such anti-cancer effects depend on T cells.[380] The field of EV-based immunotherapies has rapidly grown afterward, with breakthrough discoveries in extracellular vesicle-mediated presentation, transfer, inhibition of antigens, as well as augmentation of immune functions in recent years.[381] For example, the role of EVs in activating immune systems and enhancing antigen presentation has been confirmed both in vitro and in vivo in murine animal models.[66] Through this, EVs were found to stimulate macrophages toward pro-inflammatory phenotypes further, secreting inflammatory cytokines, including tumor necrosis factor (TNF) as well as interleukin 1beta (IL1b). In responses to the augmented proinflammatory factors, NK cell functions that were activated by the T cells showed a better survival rate.[382] There have been many other demonstrations on EV-mediated immunotherapies as well. Notably, the immunosuppressive characteristics of EVs can vary depending on their sources and tissues. For instance, autoimmune diseases that originate from unselective activation of the immune system would require efficient immunosuppression solutions. A critical target for the immunosuppression-based treatment of autoimmune diseases is FASL-mediated cell apoptosis.[383] Systemic treatment of exosomes in vivo was observed to induce FASL-mediated T cell apoptosis highly selectively that could further reduce the proliferation of T cells, activate NK cells, the differentiation of DC cells, and limit the expansion of regulator T cells.[384] One of the critical biomolecules responsible for triggering such immunosuppressive effects was IL10 cytokines in exosomes.[385] The effects of exosomes on the modulation of

immune cell functions are currently actively exploited to treat various other diseases. However, the fates of exosomes in vivo as well the detailed mechanisms on how exosomes recognize specific immune cell types remain to be further investigated.

5.2. EV as Drug Delivery Platforms

Another important and potentially exciting therapeutic application of EVs is delivering RNAs and DNAs to various cell types.[3] One of the pioneering works by the Ratajczak group isolated EVs from cancer cells and stem cells. The mRNAs inside these EVs were effectively transferred to different cell types, such as monocytes and HSCs, respectively.[6,386] Consequently, phenotypic changes occur in monocytes and HSCs, showing activated angiogenesis, likely mediated by mRNAs responsible for regulating the PI3K-AKT pathway.[387] This observation is strongly supported by later studies on the existence of high levels of RNAs inside EVs, and human exosomes administered in murine animal models resulted in the expression of human genes in mouse cells.[3] These reports provide strong support for the therapeutic potential of EV-mediated RNA transfer for various applications, including tissue regeneration, antiviral therapies, immunotherapies, and cancer therapies. More detailed applications have been summarized and categorized in a tissue/organ-specific manner in (**Table 2**).

Gene therapy that relies on the delivery of RNAs and DNAs has been conventionally achieved by different viral vehicles, including lentivirus, adenovirus, and retrovirus.[388] However, viral delivery of genetic materials has raised concerns about their biosafety in human clinical trials.[389] In this regard, nonviral delivery vectors, such as nanoparticles and cationic polymers, have been applied to enhance the bioavailability of RNAs and DNAs and have shown promising outcomes in manipulating gene expression in target cells.[390] Still, the foreign nature of nonviral vectors can lead to immune activation. EVs could offer a few advantages as they can be derived from patient cells, showing high biocompatibility, immunological inertness, and excellent capability to cross critical biological barriers such as the BBB for targeting the central nervous systems.[391]

One of the initial efforts on EV-mediated delivery of exogenous RNAs was reported in 2010, where miRNA-150 enriched EVs were derived from THP-1 monocytes and were used to manipulate recipient cells' gene expression.[392] This work is also evidenced by the successful transfection of murine animal models with miRNA-143 after systemic injection of monocyte-derived, miRNA-143 enriched EVs.[393] Besides miRNAs, shRNAs, and mRNAs, exogenous siRNAs have been delivered into recipient cells by exosomes. One of the first reports on this is from the Wood group, where dendritic cells were plasmid-transfected to express Lamp2(lysosomal-associated membrane protein 2) with rabies virus glycoprotein (RVG) fused as a surface receptor for brain targeting. The exosomes harvested from the transfected dendritic cell were loaded with siRNA targeting GAPDH as a proof-of-concept using electroporation techniques. This siRNA-loaded exosome was found to efficiently penetrate the BBB and accumulate in the mouse brain with minimal toxicity observed. Most importantly, they confirmed the robust manipulation of gene expression in brain cells.[25] Their approach to exosome-mediated siRNA delivery into the brain is auspicious for treating brain diseases, especially considering the apparent lack of a target-specific delivery strategy.

Exosome-mediated delivery of siRNA, miRNA, and DNAs has been shown in other tissue regeneration and cancer treatment applications as well. Although still at their initial stage, current reports have strongly supported the excellent promise of EV-based delivery of RNAs and DNAs for therapeutic applications. The combined delivery of nucleic acids with small molecules, synthetic proteins, and nanoparticles has been exploited for improving the outcome of EV-mediated gene transfer.[394] By harvesting synergies among different therapeutic modalities and taking advantage of EVs as a delivery platform, accelerated tissue regeneration, enhanced antitumor efficacy, and improved immunomodulation have been demonstrated in the brain, liver, blood, muscle, and other organs. However, the successful clinical translation of EV-mediated biomolecule delivery would require significant efforts to address several critical barriers. For example, the accurate characterization of interactions between EVs with different therapeutic cargos remains challenging given the complexity and heterogeneity of EVs.[132] In Addition, most of the current therapeutic applications of EVs have focused on modulating non-adherent immune cells, while the accumulation and regeneration in solid tissue have shown less efficiency. This could be attributed mainly to the lack of ability to control the biodistribution of EVs in vivo. Furthermore, it is crucial to gain more fundamental insights on the fates of EVs after delivery in vivo. High-resolution tracking of EVs may provide promising solutions, but their integration into current EV studies has not been commonly adopted. Successfully addressing these challenges would enable the broader applications of EV-based delivery of therapeutic molecules in treating various tissue diseases and injuries.

5.3. MNMs as Facilitators for EV-Based Drug Delivery

Addressing the critical challenges in EV-based drug delivery, including i) accurate characterization of therapeutic molecules inside EVs; ii) effective control over the biodistribution of EVs with selective tissue targeting; iii) high-resolution tracking of EVs in vivo, MNMs have shown great promise and provided effective solutions. For characterizing EVs, MNMs functionalized with targeting ligands can selectively harvest EVs of interest, based on the combination of their surface receptors, as detailed in Section IV. The biomolecules inside EVs can also be separated and characterized in species and sequence-specific manners using MNM-based biosensors. For regiospecific control of EVs after their systemic administration in vivo, MNMs, once integrated into EVs, can facilitate the targeted delivery into target tissues requiring repairment by using an external magnetic field.[395] The magnetic field can facilitate the endocytosis and penetration across the cell membrane during their in vitro and in vivo delivery of MNM-integrated EVs, either by increasing the local concentration of EVs or through mechanically actuated integrin clustering and shearing.[396,397] Moreover, MNMs offer high contrast in vivo imaging modalities,

Table 2. A literature summary of state-of-the-art MNM-facilitated and EV-based therapeutic applications.

MNM	Size/shape	Surface functionalities	EV types sample source	Disease type	Therapeutics	MNM functions	Refs.
Fe_3O_4	5 nm NPs	DSPE-PEG with RGD	Exosome Hepatocellular carcinoma	Hepatocellular carcinoma	Glucose oxidase/ Fenton reaction	MRI/releasing iron ions for Fenton reactions	[446]
Iron oxide	Nanoraspberry	Transferrin	Exosome Mouse serum	Metastatic lung tumor	Dox	Metastases-targeting/ T cell-infiltration inducer	[416]
SPION	5 nm	Polyhistidine	EVs iPSC-derived	Acute kidney injury/ heart ischemic and reperfusion injury	Magneto-EV	MRI tracking	[369]
SPION	10 nm	CPP/CTNF-α	Exosome MSCs	B16F10, melanoma tumor	TNF-α	Magnetic targeting	[449]
Fe_3O_4	Caffolds	HA ceramics	Exosome Osteoclast	Osteoporosis		Triggering cell signaling, promoting osteoblast activity	[456]
SPION	93 nm NPs	Transferrin	Exosome Mice serum	U87, subcutaneous tumor	Dox/miRNA21 inhibitor	Promoting tumor delivery of exosomes	[450]
SPION	<60 nm NPs		Exosomes MSCs	Cutaneous wound	Exos	Magnet-guided navigation	[459]
Fe_3O_4	20 nm	Streptavidin/folate	THP-1 macrophage	Tumor	Dox	Isolation, targeting, and delivery	[460]
Iron oxide	25–30 nm nanocubes	Rhodamine B RITC/ PEG	Exosome-mimetic nanovesicles MSCs	Cardiovascular diseases	Proteins and RNAs/ ROS	Generating ROS/ up-regulating HIF1-mediated GF expression/ augmenting NVs retention	[177]
SPION	8 nm NPs	Carboxylate chitosan/transferrin	Exosome Serum	Type 2 diabetes mellitus	BAY55-9837	Pancreas targeting/ therapeutic delivering	[397]
Fe_3O_4	SMNC	Carboxylated / transferrin	Exosome Blood	H22/4T1 tumor	DOX	pH-responsive binding and targeting	[401]
Fe_3O_4	NPs	PEG transferrin	Exosome Blood	H22 tumor	DOX	Rapid isolation of exosomes/tumor targeting	[461]
SPION	8 nm	Citrate coated	Exosome THP1/SVEC4-10/ mMSC	Macrophage activation and migratory	SPION/Exos	Amplify the immunoregulatory properties of EVs	[407]
Iron oxide	12 nm		Exosome-mimetic nanovesicles IONP-treated MScs	Spinal cord injury	Growth factors	Magnet-guided navigation/ iron ions activating JNK c-Jun signaling	[408]
(RGD–GNP)MNC	34 ± 7 nm nanocages	Citrate capped	Macrophage	Regulating the adhesion and polarization of macrophages	Mechanical force	Reversible magnetic nanocaging	[399]
SPION	NPs		Exosome MDA-MB-231	Breast cancer	DFO/ionizing radiation	Tracking exosomes in vivo	[462]
Au–iron oxide	71 nm	Tumor-derived extracellular vesicle coated	Exosome 4T1/SKBR3/HepG2	Breast cancer	Anti-miR-21/DOX/ photothermal	MR imaging and photothermal effect	[398]
Magnetic NP		Streptavidin modified	Exosome Macrophages	Cancer therapy	DOX	Magnetic separation/ tumor targeting	[395]
Ag_2Se@Mn QDs	1.8 nm		Microparticle OSCC patient peripheral blood	Oral squamous cell carcinoma	siRNA	MPs tracing and tumor targeting	[400]

Table 2. Continued.

MNM	Size/shape	Surface functionalities	EV types sample source	Disease type	Therapeutics	MNM functions	Refs.
Iron oxide	8 nm	Citrate coated	Exosome HUVEC		mTHPC photosensitizer drug	Manipulating and sorting EVs by magnetic forces	[463]
SPION		Carbohydrate coated	Exosome MSCs	PC3/HeLa	Hyperthermia/ mRNA yCD::UPRT	Tumor-targeting/ magnetic-mediated hyperthermia/MRI	[404]
Magnetic NP		Nef peptides	Exosome Nef-gfp-transfected microglia (CHME-5)	HIV pathogenesis in the CNS	Nef peptides	Delivery of Nef peptides/reducing microglia release of Nef exosomes	[464]
SPION	10 nm	Transferrin	Exosome Mice serum	Murine hepatoma	DOX	Magnetic targeting	[178]
SPION		Carboxydextran coated	Exosome hMSCs	Colon cancer	Ferucarbotran	Iron ions inducing endosomal recycling	[465]
IONP	10 nm		Exosome mimetics MDA-MB-231	Orthotopic breast tumor	DOX	Magnetic extrusion/ drug delivery	[452]
Iron oxide	12 nm	PEGylated	Nanovesicles MSCs	Ischemic stroke	MSC Exos/angiogenic factors	Magnetic navigation/ iron ions promoting anti-inflammatory response	[409]
Magnetic MSNs		Indocyanine green (ICG)	Exosome Drug-resistant *S. aureus* BW15 and BWMR26	Drug-resistant *Staphylococcus aureus* infection	ROS/photothermal/ antigens	Efficient transportation of EVs/ in vivo traking	[466]
SPION		Carboxylated, A33 antibody	Exosome LIM1215	Colorectal cancer	DOX	Targeted delivery	[396]
SPION	30 nm, 60 nm	Carboxydextran–carboxymethyl dextran	Exosome	Neuroregeneration	Chemotherapeutic agents	Affecting morphology of primary hippocampal neurons	[467]

particularly MRI, for tracking EVs in vivo.[369,398] Doping and hybridization of MNMs with different elements have also led to the development of multimodal imaging when the higher resolution imaging of EVs is required.[399,400] Lastly, although not widely demonstrated in EV-based drug delivery systems yet, the incorporation of MNMs into synthetic nanovesicles has enabled stimuli-responsive of cargos in a regiospecific manner as well.[399,401] Therefore, integrating MNMs with EVs for drug delivery applications is an ongoing research topic that can strongly affect EV-based therapeutic applications.

Although EVs have surface receptors that can recognize immune cells or other groups of cells, relying on their innate ability to target specific tissues and cells beyond the immune systems has been proven challenging. Conjugation of active targeting ligands, as detailed in the example of RVG-conjugated exosome-based brain targeting, has been a well-adapted strategy to improve its tissue targeting capability.[402] Still, multiple biological barriers during the circulation and penetration of EVs necessitate multiple targeting strategies combined for efficiently guiding EVs carrying therapeutic molecules to target tissues. Magnetic targeting that leverages the magnetic responsiveness of MNMs, especially superparamagnetic nanoparticles (SPIONs) that can drive vesicles in an external magnetic field, has been considered a reliable approach for enhancing the regiospecificity of varying types of drug delivery platforms, including EVs.[403] The MNMs, engineered with their magnetism, often in small sizes, can be integrated into EVs either by transfecting the host cells with magnetic nanoparticles or fusing magnetic nanoparticles with already formed EVs by electroporation described in Section 3. Decoration of EVs with MNMs through surface receptor binding has also yielded magnetic field-responsive EVs.[178] However, this strategy is less adapted for drug delivery applications, as the therapeutic effects from surface receptors of EVs are largely compromised. The exposure of MNMs to immune systems can cause immunoreactions. Once reaching the target site, EVs functionalized with magnetic nanoparticles can be triggered to release the therapeutic molecules. The triggered drug release can be mediated through both physical and biochemical signals. For example, local magnetic hyperthermia remotely induced by an alternating current magnetic field has been widely used for the controlled release of cargos in synthetic vesicles such as liposomes.[404] The drug release from EVs can be mediated by biochemical stimuli as well. For instance, diseases are often associated with significant alterations in microenvironment factors that can destabilize EVs. Tumors, for example, are characterized by rich ROS and acidic pH in their extracellular spaces that can trigger the release of liposomal cargos.[401] Similarly,

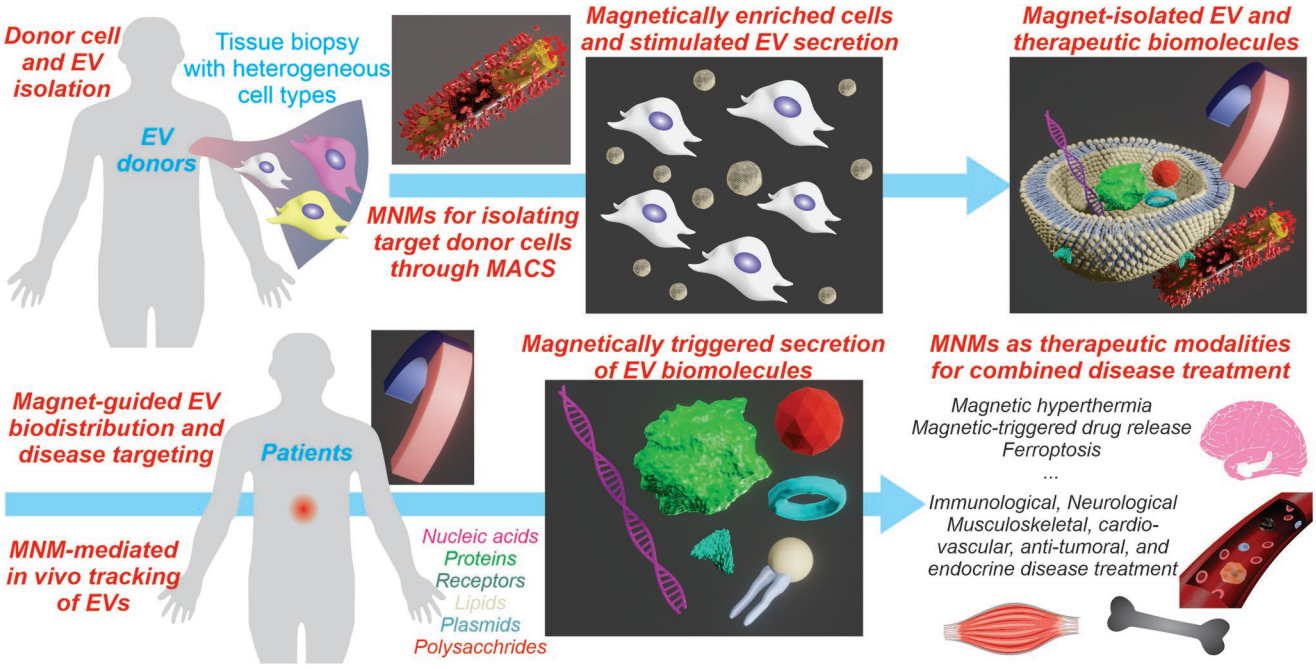

Figure 11. Design principles and workflow of MNM-facilitated delivery of EVs for therapeutic applications.

inflammation is often accompanied by an increased expression of matrix metalloproteinases leveraged for the stimuli-responsive release of RNAs, DNAs, and proteins inside the EVs.[405] This stimuli-release of cargos at target tissue sites, combined with the magnetic targeting strategy, can address the challenge of nonspecific delivery in EV-based drug delivery systems. Additionally, magnetic targeting combined with an active targeting strategy by functionalizing EVs with targeting ligands can serve as a dual-enhanced targeting delivery platform. However, such demonstrations have been lacking to the best of the authors' knowledge.

Another crucial aspect of MNM that facilitated the delivery of EVs is their innate ability to provide MRI-based tracking of vesicles in vivo. There are multiple advantages of MNMs-enhanced MRI over other imaging methods for tracking EVs, including achieving high resolution and deep tissue penetration, noninvasiveness, and improved soft tissue imaging.[406] MRI is also widely used in clinical applications, facilitating the study of fates of EVs after delivery to patients in clinical trials.

Given their clear advantages, EVs integrated with MNMs have been widely applied for the delivery of biomolecules to enhance tissue targeting, minimize systemic side effects, and track the delivery processes in varying types of tissues and organs, including immune systems, neurological systems, as well as musculoskeletal systems (**Figure 11**).[177,407–409] We will overview the exemplary studies for each tissue type to explain the general design principles in applying magnetic nanomaterial-hybrid EVs for therapeutic applications.

5.4. MNMs as Facilitators for EV-Based Immunomodulators

EVs such as exosomes are involved in various cellular processes that regulate immune responses. For example, EVs produced after MHC I and MHC II-mediated antigen presentation were found to activate T cells (CD4 and CD8 positive T cells).[410] Another mechanism that EVs can stimulate T cells is the transfer of antigens to antigen presentation cells (APCs) such as dendritic cells.[66] EVs naturally exist in human bodies and were also found to have immunosuppressive effects by activating regulatory T cells. Therefore, EV-mediated immunomodulation is highly dynamic and widely exists in nature. Leveraging this unique capability of EVs can lead to effective immunomodulatory therapeutics. However, because of the high heterogeneity of EVs, it has been challenging to precisely predict and control the therapeutic outcome once injected in vivo. For instance, both immunostimulatory and suppressive exosomes can exist in the same solution, even if they are derived from the same cell or tissue origins. Their effects on immunomodulation can be counteractive, compromising their therapeutic effects in vivo. Another challenge has been the lack of spatiotemporal control over the release or presentation of immunomodulatory biomolecules from the EVs. For example, exosome-mediated immunotherapy has been applied for cancer treatment.[411] However, a universal increase of immunostimulatory signaling may not be desired for cancer patients. As such, there is a clear need for innovative approaches to overcome these heterogeneity barriers of EVs to facilitate their therapeutic applications.

MNMs can isolate specific types of EVs through immunoaffinity isolation and endow the trackable, magnetic field-controllable delivery of EVs to control their biodistribution once administrated in vivo effectively. In addition, magnetic hyperthermia, typically triggered by AMF, has also been widely used for the remotely triggered release of drugs in nanomedicine.[412] MNMs, iron oxide nanoparticles specifically, have also been reported to stimulate EV secretion in vitro and in vivo, which can be leveraged for the rapid production of EVs.[404] Given these clear advantages of MNMs, there has been an immense

interest in developing MNM-hybrid EVs for immunomodulatory applications, including immunotherapies in cancer treatment, as well as anti-inflammation in stroke treatment.[409] However, this field is at an early stage, and we will overview a few examples of how MNM facilitated in vivo immunomodulation for treating cancer and other diseases.

Immunotherapy has recently emerged as a promising biological treatment of cancer and has been typically enabled by administering immune checkpoint inhibitors, T-cell therapies, antibodies, or vaccines.[23,414,414] Although the means of realizing immunotherapy varies, T lymphocytes, as one of the most critical cell types targeting the infiltrating metastatic cancer cells, have shown enormous potential to regulate tumor metastasis.[415] Nevertheless, the intrinsic tumor heterogeneity significantly limits the efficacy of immune cell infiltration, which includes the T lymphocytes. This leads to a drastic decrease in immunotherapy-based treatment of solid tumors and protects cancer cells from toxic effects from immune cells. To this end, Hu et al. reported a magnetic exosome-based multifunctional cancer immunotherapy platform for simultaneously boosting T cell infiltration, targeting cancer metastasis, and delivering anticancer drugs (**Figure 12**). Specifically, exosomes that envelop multiple ultrasmall sized magnetic nanoparticles inside the lipid bilayers, were derived by first synthesizing iron oxide nanoparticle aggregates in the shape of "nanoraspberry (RB)" using a hydrothermal reaction, then conjugating the iron oxide nanoparticle with transferrin, which supposedly binds to the surface receptor of exosomes. Sonication of transferrin-conjugated iron oxide nanoparticles with exosomes, derived from mouse serums, resulted in the fusion and formation of magnetic exosomes containing the nanoraspberry-shaped magnetic nanoparticles. The combination of nanoraspberry-shaped magnetic nanoparticles endowed the magnetic isolation of exosomes. Injection of magnetic exosomes significantly changed the biodistribution compared to bare magnetic nanoparticles, with reduced accumulation of nanoparticles in the liver 24 h postinjection. This is likely due to the existence of surface receptors and targeting ligands on the surfaces of exosomes. To track the fate of magnetic exosomes injected in vivo, both fluorescent dyes and quantum dots were used to label exosomes. However, there are concerns about their interferences with the targeting ligand on the exosomes. Using in vivo fluorescence imaging, magnetic exosomes were reported to effectively accumulate at the metastatic tumor sites in a mouse model, which was speculated to originate from the nanoparticle-induced endothelial leakiness (NanoEL) effect. To prove the magnetic exosome-mediated NanoEL effect, a metastatic melanoma cell-based microfluidic chip was employed. The different nanoparticles were flowed through the chip to investigate their effects on the endothelial cell permeability on the chip based on the penetration of dextran-conjugate dye after treatment. Interestingly, a higher induction of endothelial leakage was consistently observed in magnetic exosomes compared to exosomes alone. The differences in densities were considered the primary reason that accounts for the differential induction of NanoEL. This enhanced penetration of magnetic exosome was further confirmed in a multicellular tumor spheroid (MTS) assay and in vivo in a murine cancer model. Most importantly, such magnetic exosome-based NanoEL effects induced the recruitment of T cells into the solid tumor in a more efficient manner, leading to enhanced immunotherapy. Additionally, when exposed to an AMF, the nanoraspberry shaped magnetic nanoparticles effectively converted magnetic energy into thermal energy and further stimulated T cell activation at the tumor sites. This, combined with the magnetic exosome-facilitated delivery of anti-cancer drugs, resulted in an overall tumor suppression efficiency of 98% in just 20 d. This study demonstrates the truly multifunctional role of MNMs in facilitating exosome-mediated immunomodulatory therapies.[416]

There are other ways that MNMs can potentially facilitate EV-based immunomodulatory applications as well. For example, Powis et al. used magnetic nanoparticles to track and monitor microvesicles secreted by immune cells, providing fundamental insights into EVs' cellular production in the immune system.[324] Nie et al. also suggest that iron oxide nanoparticles, after being uptaken by immune cells, can stimulate their generation of exosomes in vivo, which may indirectly alter immune cell-secreted exosomes.[417] MNM-based exosome delivery has been applied for modulating macrophage and microglial cells as well. For example, magnetic exosomes derived from MSCs, after being injected in vivo in a murine stroke model, facilitated functional recovery with a significant reduction of inflammatory responses.[418]

5.5. MNMs as Facilitators for EV-Based Treatment of Neurological Disorders

Developing effective strategies for sustained and targeted delivery of therapeutics into the CNS and PNS has been a long-sought task in tissue engineering. The BBB and the dynamic neuroinflammatory signaling in the CNS make the targeted delivery particularly challenging.[391,419] Stem cell therapies, especially MSC and neural stem cell (NSC) replacement therapy, have been considered as one of the promising approaches for treating neurological disorders such as ischemic stroke, traumatic brain injury, and spinal cord injury. However, their clinical success is still limited, despite many previous attempts.[420] The promise of EV-mediated stem cell-free delivery of biomolecules into neurological systems, including CNS and peripheral nervous systems, is based on their ability to cross the BBB, and target neuroinflammatory cells and release anti-inflammatory and neuroprotective factors.[421] MNMs can facilitate the targeting process during EV-mediated drug delivery and provide imaging modalities to help clinicians understand the brain through MRI and other imaging modalities.[422] In addition, a magnetic hyperthermia-based release of therapeutics from synthetic vesicles has enabled spatiotemporal control over brain activities.[423] Therefore, MNMs can play a truly multifunctional role in the EV-based treatment of neurological disorders.

One of the first and so far the most investigated EVs for treating neurological disorders is MSC-derived exosomes. MSC transplantation has been applied to treat various types of CNS diseases and disorders for over four decades. MSC transplantation promises to deliver paracrine factors for restoring a healthy CNS microenvironment continuously.[424] However, in a systemic MSC secretome study, it was found that only exosomes can recapitulate the therapeutic effect of MSC transplantation.[425]

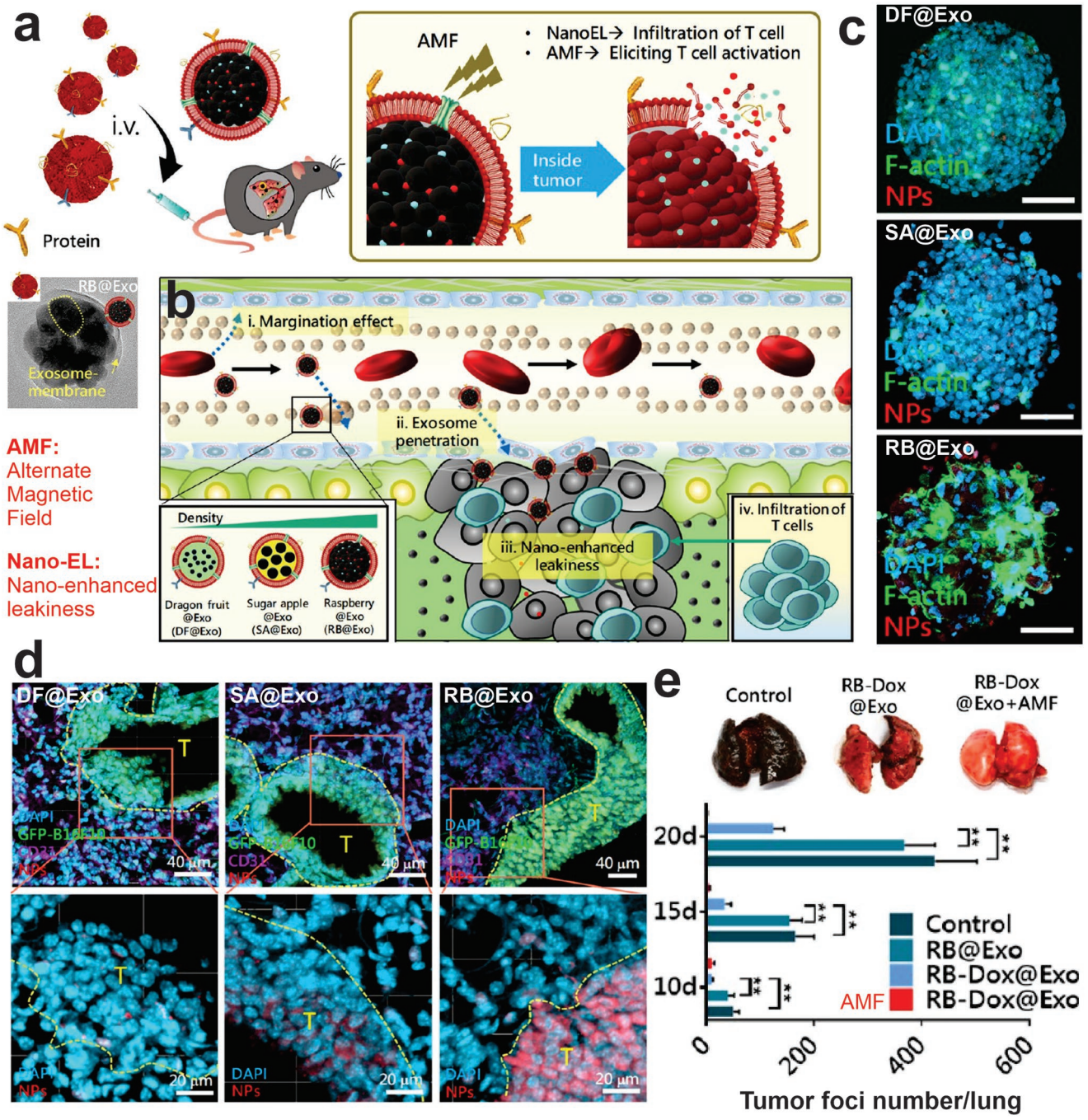

Figure 12. MNM-mediated nanoparticle-induced extracellular leakiness (nanoEL) for enhanced delivery of EVs into tumor sites for immunotherapy. a,b) The mechanism of MNM-induced nanoEL and how it facilitates the delivery of EVs and immunotherapy. Design principles and workflow of MNM-enabled isolation and detection of biomolecules in EVs. The magnetic raspberry-like nanostructure was named RB. Briefly, in lung cancers, metastasis is related to densities of EVs. Therefore, enhanced tumor penetration was achieved by magnetic nanoparticle-encapsulated EVs that were further used to induce infiltration of T cells. c) Confirmation of nano-EL in RB-exosome compared to the two control EVs with lower densities using tumor spheroid models. d) Confirmation of nano-EL in RB-exosome compared to the two control EVs with lower densities in vivo. CD31 stains tumor vascular structures. e) Enhanced tumor treatment by magnetic RB-loaded EV (RB@exosome) combined with an alternating magnetic field, which triggers the release of growth factors inside the EV. Adapted with permission.[416] Copyright 2021, American Chemical Society.

A few more recent studies have then confirmed the critical role of MSC-derived exosomes for enhancing the recovery of rodents from CNS injuries such as stroke and TBI.[426,427] In these pioneering studies, therapeutic effects of intravenous (IV) injection MSC-derived exosomes for treating stroke have been strongly supported their outcome of promoting motor function recovery, spatial learning ability enhancement, and pattern separation perseveration. Investigating brain injury using

large animal models such as sheep and pigs, has also supported that IV injection of MSC-derived exosomes can reduce seizure frequencies.[428] These findings were further translated into primate animal models with traumatic brain injury. For instance, the Rhesus monkey-based TBI model was recently treated with MSC-derived exosomes, which resulted in a significant recovery evidenced by a restoration of grasping pattern, often a sign for a high level of motor function control in both primates and humans.[429] Altogether, with their therapeutic effects validated in multiple animal models, MSC-derived exosomes are rapidly emerging as a promising treatment for neurological disorders, especially CNS injuries.

EVs harvested from different cell types other than MSCs have also been applied to remodel the CNS microenvironment and treat neurological disorders (**Figure 13**). For example, one study that directly compared the therapeutic outcomes of EVs derived from MSCs and NSCs demonstrated that NSCs more significantly decreased the infarct volume of mice with ischemic stroke.[430] This further led to better neurological function restoration. Another large animal ischemic stroke model injected NSC-derived exosomes into pig brain 2 h after stroke was found to decrease the lesion size 24 h after injury. However, at 84 d after stroke, although some neurological outcomes were observed, the lesion did not significantly differ in size between the NSC-exosome-treated and control conditions.[431] Epithelial cell-derived EVs have also been tested for brain protection as well. For example, in an ischemia-reperfusion injury model, exosomes harvested from endothelial cells effectively protected neurons in vitro.[432] Therefore, EVs derived from other cell types are also under active testing for treating neurological disorders, especially ischemic stroke.

A particularly notable feature of EVs for neurological disorder treatment is their ability to cross the BBB and effectively deliver therapeutics into the brain. Both in vitro and in vivo studies have confirmed that administration of EVs derived from stem cells can enhance the plasticity of neurons, remodel white matter, facilitate oligodendrocyte differentiation of endogenous stem cells, and improve neurogenesis after brain injuries.[433] However, it has been unclear whether EVs can cross the BBB or deliver trophic factors that permeate the BBB to realize their therapeutic effects. These critical questions were answered by a cohort of studies, including intranasal administration of dye-labeled stem cell-derived exosomes into animal models. These dye-labeled vesicles were found to exist in the animal brain after injection.[26] MSC-derived exosomes have also been labeled with gold nanoparticles to facilitate CT imaging, confirming the reliable delivery of EVs into CNS across BBB through intranasal delivery mechanisms.[434]

Although EVs demonstrate several clear advantages for treating neurological disorders, their selective delivery into the injury sites remains a critical challenge. For example, while exosomes can cross the BBB, they have also been distributed into various other organs, including the liver, kidney, heart, and muscle. Their wide biodistribution compromises the therapeutic effect and can lead to undesired effects on other organs. Therefore, efforts have been made to enhance the targeted delivery of exosomes into the ischemic brain. For instance, the expression of neuron-targeting peptides such as Arg-Gly-Asp (RGD) peptides or rabies viral glycoprotein (RVG) on the surface of exosomes has led to more efficient targeting of the brain in vivo.[435] However, this type of targeting is often at the cellular level instead of the tissue level. Therefore, when administrated systemically, targeted exosomes into the brain remain a significant challenge. MNMs allow the efficient targeting of nanovesicles into specific locations through the manipulation of magnetic fields. In this regard, there has been tremendous interest in integrating MNMs into EVs to facilitate their targeting toward the brain.

Kim et al. synthesized and applied MSC-derived magnetic EVs to achieve targeted treatment of ischemic stroke with both higher efficiency and better tissue selectivity. The magnetic EVs were prepared by first incubating polyethylene glycol-functionalized iron oxide nanoparticles with MSCs in the cell culture, followed by magnetic isolation of the EVs in the media. Using gene analysis techniques such as quantitative real-time polymerase chain reaction (qRT-PCR), they found even with the uptake of magnetic nanoparticles alone, MSCs showed an effective upregulation of a variety of neurotrophic factor-related genes, including Ang-1, HGF, VEGF, bFGF, TGF-β1, PDGF, TGF-β3, NGF, GDNF, BDNF, and NT3, which are all crucial genes responsible for angiogenesis, antiapoptosis, neuroprotection, axonal growth, and anti-inflammation. This is likely because of the stimulation of Jun and JNK pathways due to the degradation of iron oxide and the release of iron ions after MSCs uptake the magnetic nanoparticles. Most importantly, magnetic field-guided delivery of the magnetic nanoparticle-labeled EVs into the brain with ischemic stroke was further confirmed using a rat model. An external magnetic field was placed through a magnet fixed on a 3D-printed helmet mold that fits into the mouse brain. In vivo biodistribution study on the magnetic exosome administrated by IV suggested that while still most exosomes circulated into the liver, a significantly higher (nearly threefolds) amount of exosomes accumulated in the rat brain 24 h after the injection when magnetic field-guided delivery was applied. The enhanced targeting effects of magnetic exosomes further reduced inflammation, gliogenesis, and increased neurogenesis in the ischemic stroke animal models compared to animals administrated with magnetic exosomes but without a magnetic field. In this way, the authors suggested that magnetic EVs have enhanced therapeutic potential for treating ischemic stroke.[409]

Magnetic nanoparticle-labeled EVs have been applied to treat other neurological disorders as well. In their earlier work, Kim et al. have also derived magnetic exosomes from MSCs and used a magnetic field to facilitate the treatment of spinal cord injury (SCI).[408] SCI is a complex condition that often results from trauma, such as car accidents. The pathology of SCI often involves massive cell death, bleeding, high concentration of calcium ions in their acute phase, followed by inflammation, axonal loss, scarring, and cyst formation in a more chronic phase.[436] MSCs and NSCs have been used to treat SCI, and there is evidence that exosomes derived from stem cells are partially responsible for the therapeutic outcomes.[437] However, substantial safety concerns exist, especially on the FDA's direct implantation of stem cells into the SCI sites. Exosomes can provide an effective means for avoiding the implantation of stem cells while harvesting the therapeutic effects of stem cells. Indeed, previous reports have attempted the systemic injection

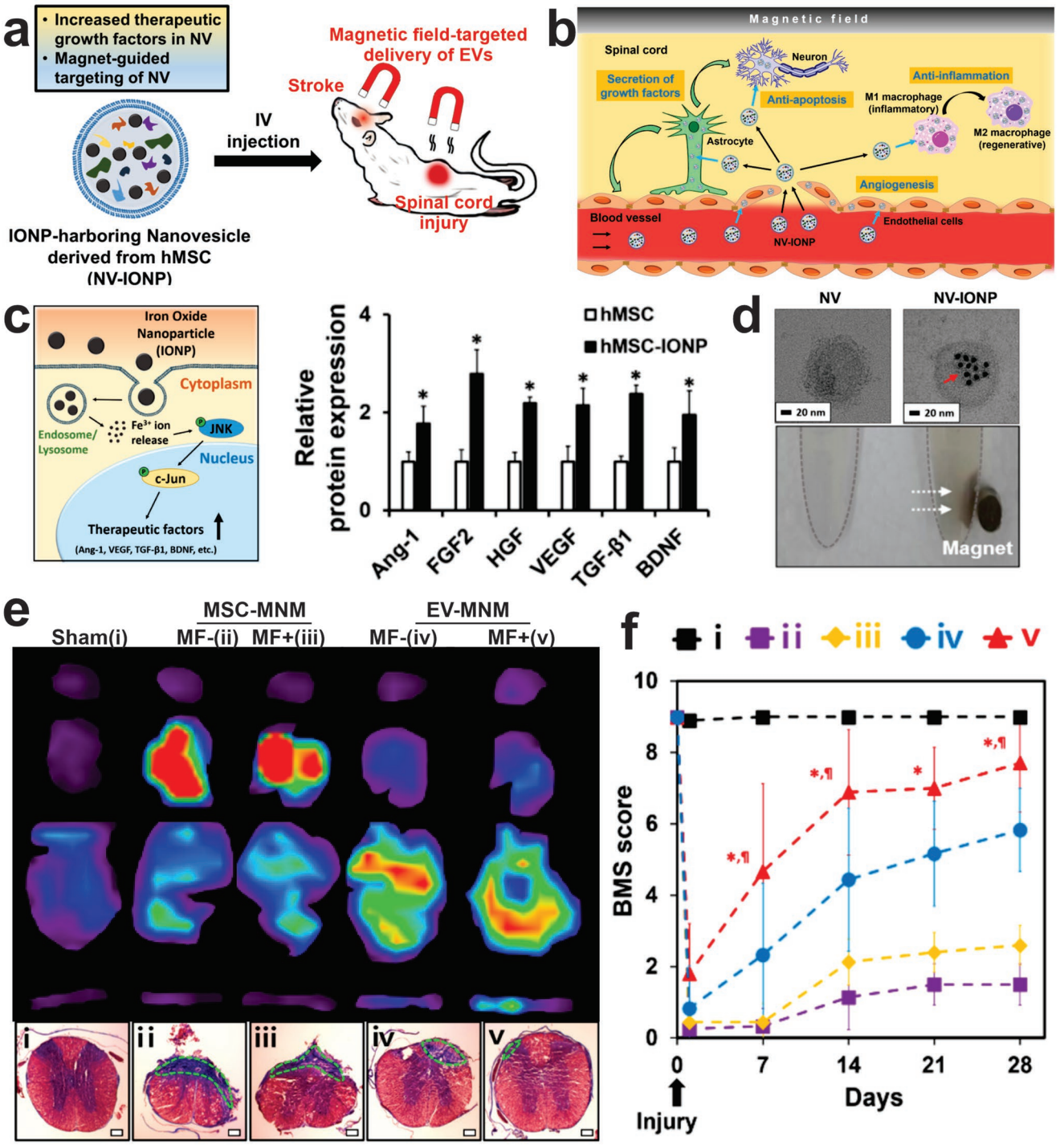

Figure 13. MNMs as facilitators for EV-based treatment of neurological disorders (spinal cord injury and stroke). a) A schematic diagram illustrating the working principles of MNM-EV for enhanced localization and treatment of spinal cord injury and stroke. b) Mechanisms of EV-facilitated treatment of neurological disorders. c) A schematic diagram illustrating the MNM-stimulated EV secretion from MSCs through iron-mediated pathways. d) TEM images showing the structure of MNM-EV (upper panel) and magnetic isolation of EVs. e) In vivo biodistribution of MSC-derived MNMs (upper panel) and treatment outcome at sites of SCI (lower panel, from Masson's trichrome stain). Scale bar: 100 µm. f) Summary graph showing the enhanced functional recovery (28 d from injury) from the combined treatment of MNM-EV and magnetic field-induced localization at the SCI sites of injured mice. $n = 10$ animals per group, $^{*}p < 0.05$ versus (ii), and $¶p < 0.05$ versus (iii). Images adapted with permission.[408] Copyright 2018, American Chemical Society.

of exosomes, which induced functional recovery of animals with SCI.[438] However, to enhance the in vivo efficacy of exosomes, it is crucial to enhance their targeting capability. Therefore, the same magnetic EV (iron oxide nanoparticle labeled and MSC-derived EVs) was applied and delivered to a rat SCI model, and the targeted delivery was achieved by applying a magnetic

field to the SCI site. This magnetic targeting strategy effectively increased neuron survival, endothelial cell angiogenesis, and anti-inflammation through macrophage polarization toward the M2 type.[408] Collectively, these effects have led to enhanced functional outcomes in the SCI animal models, compared to magnetic exosomes without magnetic guidance.

The above-mentioned examples provide a general idea of applying magnetic nanoparticle labeled EVs to treat neurological disorders. However, this field is still in its early stage of development. Other critical neurological disorders such as Alzheimer's disease, Parkinson's disease, depression, and epilepsy, as well as peripheral nervous system diseases and injuries, have not been treated by magnetic EVs yet, despite the likelihood of enhanced therapeutic outcome compared to non-targeted delivery of EVs. Another vital development direction would be the more reliable labeling of EVs in vivo, as most current work has used lipophilic labeling dyes that may fuse with host tissues and cell membranes and may not always track the original EVs.

5.6. MNMs as Facilitators for EV-Based Drug Delivery into Heart Tissues

As the most common cause of human death globally, cardiovascular diseases remain a significant challenge to treat despite various breakthroughs that have been made in the field.[439] Some of the most notable advancements of therapeutics for treating cardiovascular diseases include cell therapies. However, transplanted stem cells' accumulation and survival rate at the diseased heart tissue are still quite low, limiting their therapeutic efficacy during clinical applications.[440] More recently, paracrine actions were the main pathway for stem cell-based treatment of cardiovascular diseases.[441] In particular, EVs that carry beneficial paracrine factors have been used as cell-free therapies with significantly improved outcomes in treating cardiovascular diseases in animal models.[442] However, owing to their fast blood flow and circulation rates, it has been challenging to control the biodistribution of EVs to accumulate at the ischemic milieu of the heart tissues.

Jin et al. developed a stimuli-responsive magnetic core silica shell nanoparticle-hybrid exosome to control exosomes' biodistribution and enhance their therapeutic effects for in vivo treatment of myocardial infarcted animals (**Figure 14**).[381] The stimuli-responsive magnetic core silica–shell nanoparticle was named a "vesicle shuttle" based on its functions on accumulating exosomes at the injured tissue sites. Their nanoparticle is constructed from an iron oxide core, which allows for heart tissue targeting by manipulating magnetic fields, a silica shell, which acts as biocompatible anchoring sites for surface functionalization, and an acidic pH-cleavable poly(ethylene glycol) ligand conjugated to the silica shell as well two different antibodies. One of the antibodies (CD63 antibody) on the poly (ethylene glycol) binds explicitly to exosomes carrying the proper surface receptors, and the other antibody (anti-MLC antibody) targets surface receptors located on the injured cells. The antibody-conjugated core–shell magnetic nanoparticle was then evaluated for in vivo biodistribution using myocardial infarcted rats. It was observed that nanoparticles effectively accumulated at the injured heart tissues under a defined magnetic field. Most importantly, it was observed that the core–shell magnetic nanoparticles simultaneously capture the circulating exosomes in the bloodstream of the rats with myocardial infarction during the circulation. In this way, endogenous circulating exosomes were successfully captured, accumulated in situ at the infarcted heart tissues through core–shell magnetic nanoparticles. Using this strategy, both enhanced angiogenesis and cardiac functions in animals with myocardial infarction were realized in rat and rabbit models.[443]

There have been attempts to facilitate the exogenous exosome-based cardiovascular disease treatment using MNMs as well.[444] Although endogenous exosomes cause minimal concerns on immune rejection, the number of endogenous exosomes may not be sufficient for an optimal therapeutic outcome. Exogenous exosomes, in this regard, can be harvested from multiple donors and purified in a more reproducible manner. Using a similar strategy that they developed to treat ischemic stroke and spinal cord injury, Kim et al. applied iron oxide nanoparticle-hybrid exosomes for the selective accumulation at the cardiac injury sites and enhanced the treatment of myocardial infarction.[409] Also, the release of iron ions during the formation of magnetic exosomes boosted administration of magnetic nanoparticle-hybrid exosomes in combination with a cardiac targeting magnetic field enhanced a variety of therapeutic effects in the repair of rat infarcted myocardium, including reduced cell the number of secreted exosomes significantly through their previously established Jun and JNK pathways. The apoptosis, decreased fibrosis, and suppression of inflammation, as well as promoted angiogenesis, ultimately contributed to an enhanced function improvement in their long-term study.[177] These findings altogether suggest an excellent potential of MNM-facilitated delivery of both endogenous and exogenous exosomes for treating myocardial infarction. However, the mechanism of how MNM-hybrid EVs facilitate cardiac repair remains poorly understood. For example, which type of biomolecules inside exosomes are responsible for cardiac repair? The fates of magnetic nanoparticles after the tissue repair also have not yet been well studied. Also, developing nanoparticles with higher magnetism may facilitate their translation into clinical applications, considering significant larger body shielding effects in human patients.

5.7. MNM as Facilitators for EV-Based Cancer Therapies

Both MNMs and EVs have been widely applied for anticancer applications. As detailed in previous sections, MNMs can magnetically target tumor tissues in vivo, inducing magnetic hypothermia for triggering cancer apoptosis and delivering anticancer reagents in a magnetically triggered manner. In addition, as one of the most common compositions of MNMs, iron oxides can degrade into iron ions in the acidic cytoplasm of cancer cells, catalyze Fenton's reaction, and induce ferroptosis.[445,446] Furthermore, MNMs endows high-resolution MRI and other imaging modalities for cancer diagnosis and prognosis.[447] Similarly, exosomes have also been reported for various cancer applications, which can be found in a few excellent reviews. Briefly, exosomes can either recruit immune cells for

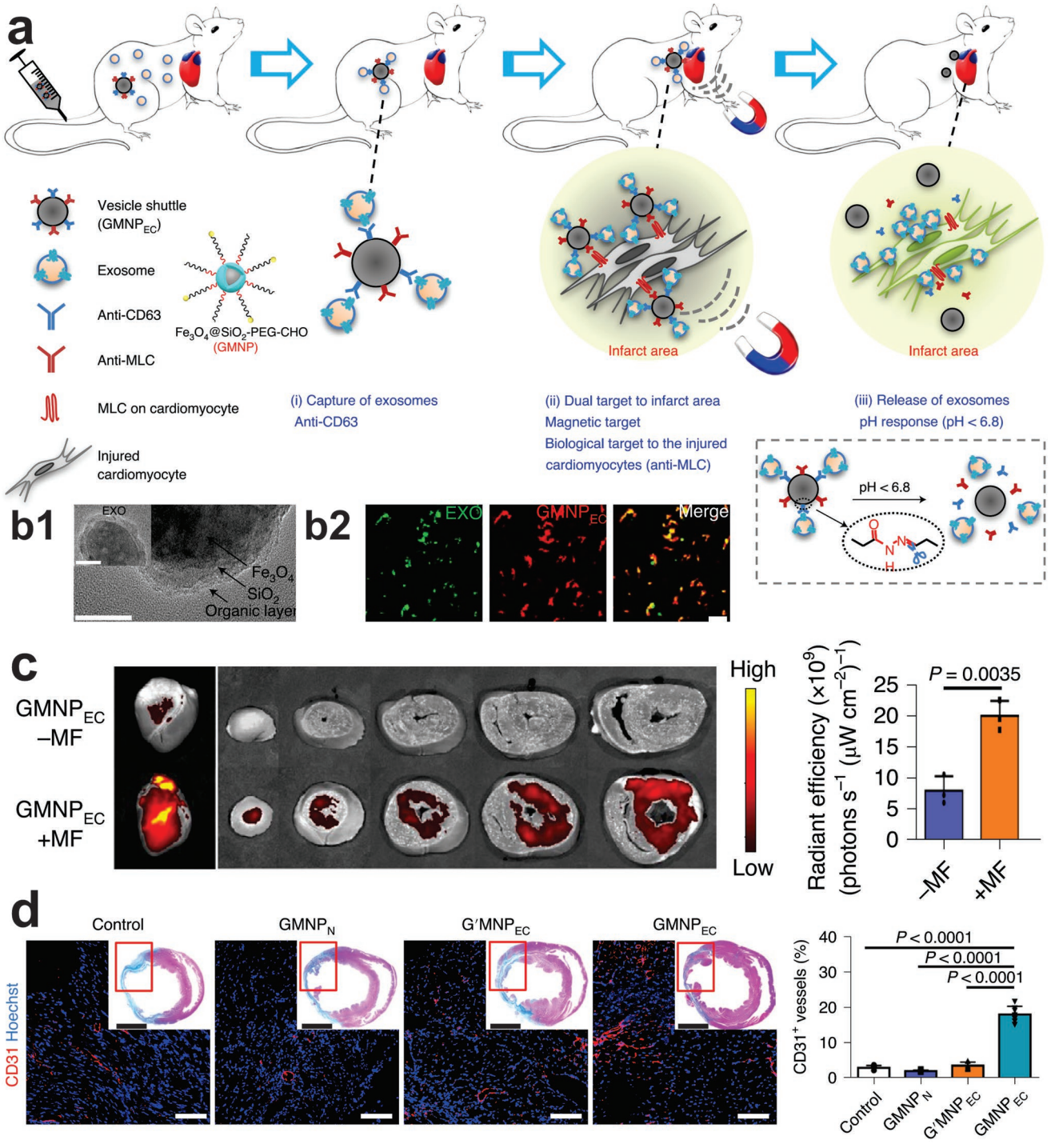

Figure 14. MNMs as facilitators for EV-based drug delivery into heart tissues. a) A schematic diagram showing the concept of MNM-mediated EV shuttle for treatment of cardiac infarct. MNMs (GMNP: surface-grafted magnetic nanoparticles) conjugated with CD63 and anti-MLC antibodies injected into the bloodstream can target EVs. Following this, the MNM-EV will selectively degrade and release cytokines at sites of infarct with a low pH < 6.8 and under a magnetic field. Thus, targeted delivery of MNM-EVs can be achieved. b) TEM (b1) and confocal (b2) images of MNM-EV. c) In vivo biodistribution MNMs with or without magnetic field (MF). d) Enhanced treatment by MNM-EV in combination with MF-mediated targeted delivery of EVs to infarct sites. Images adapted with permission.[443] Copyright 2020, Nature Publishing Group.

cancer-killing or directly deliver biomolecules such as TNF protein and miR-143 to induce apoptosis.[394] The surface ligands of EVs can be engineered to target specific deliver cargos to cancer cells as well. Moreover, synthetic anticancer drugs, such as doxorubicin, paclitaxel, cisplatin, and curcumin, have all been loaded into exosomes for cancer therapeutic applications.[396,416]

Despite the huge potential, few works have aimed to harvest the synergistic therapeutic effect by integrating MNMs and EVs. The unique advantages of using magnetic nanoparticle-hybridized EVs may include magnetic field-guided isolation, purification, and accumulation of EVs, as well as magnetic hyperthermia triggered release of exosomal cargos.[404] There have been many demonstrations and therapeutic applications of MNM-hybrid liposomes, which further support the excellent potential and can inspire the development of magnetic EVs for cancer applications.[448] Representative reviews on the topic of magnetic liposomes for cancer applications have been cited here (Table 2), and we will overview a few exemplary reports on how MNMs could facilitate EV-based anticancer applications.

In the work by Rao et al., MSC-derived exosomes were hybridized with SPIONs and functionalized with cell-penetrating peptide (CPP, KETWWETWWTEWSQPKKKRKV) for the target-specific delivery of TNF into cancer cells for cancer therapy. As the first cytokine exploited for cancer biotherapy, TNF suffers from its side effects in clinical applications. Despite a few breakthroughs in nanoparticle-based TNF delivery systems, improving the target-specificity while ensuring a high cancer-killing efficacy remains an ongoing challenge. Exosomes with excellent stability in blood circulation, the appropriate size range for tumor targeting, and minimal immune reactions could overcome some of the barriers in TNF delivery for cancer nanomedicine. Chitosan-coated SPIONs with enhanced colloidal stability and binding toward TNF were incorporated into MSC-derived exosomes to enhance their cancer-targeting capability through magnetic field-guided delivery. A CPP was functionalized on the surface of exosomes for better penetration into tumor tissue and cellular uptake by cancer cells. Both CPP and TNF were expressed in the MSCs through gene transfection, which leads to the formation of CPP- and TNF-containing exosomes. Notably, the CPP and SPION-containing exosomes inhibited tumor growth significantly better than protein delivery alone or exosome alone groups in a murine melanoma cancer model.[449] Similar strategies have also been developed by using magnetic nanoparticles to hybridize with blood-derived exosomes with transferrin receptors for cancer-specific delivery of miRNA21 or doxorubicin.[178,450] There have been interests in using plant cell-derived exosomes, such as oleosomes, to deliver anticancer reagents.[451] These studies show how magnetic exosomes could be engineered for target-specific delivery of anticancer reagents to enhance anticancer therapeutic effects in vivo.

Beyond the target-specific delivery of EVs to cancer sites, another barrier for EV-based anticancer therapies from a bioengineering perspective is the difficulties in the scale-up synthesis of EVs for applications in human patients. Moses et al. developed a magnetic extrusion method for scale-up production of a particular version of EVs, termed "endosome-derived vesicles," and "exosomal mimetics," for delivering anticancer therapeutics. Due to their higher prevalence in cells compared to exosomes, endosomes in cells were labeled with iron oxide nanoparticles through endocytotic mechanisms and collected through magnetic isolation. A nanoporous membrane-mediated extrusion was adapted to separate endosomes from the cells, which could remove the large-sized cell membranes and cell organelles. Compared to EVs with small sizes, the exosomal mimetics with endosomal origin shows comparable performance in delivering a small molecule anticancer reagent, doxorubicin, to the tumor sites in vivo. However, the primary advantage of using their magnetic extrusion approach is to harvest a significantly larger amount of therapeutic vesicles. Specifically, while about 6×10^8, 7×10^7, and 1×10^{10} EVs have been typically obtained from one million cells using ultracentrifugation, size exclusion chromatography, and flow filtration, respectively, the reported magnetic extrusion method shows about 7×10^{10} vesicles per million cells, nearly an order higher yield even compared to the cutting-edge techniques.[452] This high yield and the possibility of scale-up synthesis of exosomal mimetic vesicles would be crucial for clinical trials, where human patients require the administration of a large number of therapeutic vesicles.

Despite these demonstrations on MNM-facilitated exosomal anticancer therapies, most current work has mainly focused on using the vesicles as a delivery system without synergistically combining the anticancer effect of magnetic nanoparticles or exosomes themselves.[453] For instance, the ferroptosis effects of iron oxide nanoparticles, magnetic hyperthermia of MNMs, and the naturally cancer-suppressive exosomes derived from immune cells have not yet been well studied. Therefore, a significant amount of work remains to be done in this field to harvest the therapeutic potential of MNM-hybrid EVs fully.

5.8. MNMs as Facilitators for Other EV-Based Therapeutic Applications

Beyond immunological, neurological, cardiovascular, and cancer applications, there are intense interests in developing MNM-hybrid EV-based therapeutic strategies for treating endocrine diseases, viral infections, and other degenerative diseases as well.[454,455] Many of these explain a similar pattern as described above by combining the targeting capability and stimuli-triggered drug release function of MNMs with the therapeutic functions from the biomolecules of EVs.

One of the initial studies to combine MNMs with EVs in endocrine applications is demonstrated by Rao et al., where magnetic exosomes were used to deliver BAY55-9837 for treating Type II diabetic mellitus. BAY55-9837 has extremely short retention in the blood, with a typical half-life around 20 min during circulation. Using the SPION-labeled exosome-based approach, there is a significant enhancement of the half-life of BAY55-9837 by about 27 folds. In addition, the drug was also effectively targeted to pancreatic β-cells in a murine diabetic model in vivo. Through this, magnetic exosomes combined with magnetic field-guided delivery into the pancreas increased insulin levels, decreased glucose concentrations, and reduced body weight of diabetic mice, compared to all control groups.[397] The applications of MNM-facilitated exosomal therapeutics are less investigated in musculoskeletal systems, with very little work reported so far, which is probably because of their cell- and extracellular matrix–dense tissue environment. One study used magnetic nanoparticle-functionalized hydroxyapatite (HA) scaffold to promote the proliferation and stimulate the secretion of exosomes from osteoblasts through Rho kinase-mediated pathways.[456] This can be considered as

a less direct way to use MNMs for improving exosome-based therapeutics. Some research works attempted to use MNM to isolate biomarkers from the respiratory system, but the therapeutic application of MNM-hybrid EVs remains explored.[457,458] Given the unique advantages of MNMs in overcoming the heterogeneity barriers of EV-based biological applications, there is plenty of room to investigate their applications in various diseases and study the in vivo cell–cell communications mediated by EVs.

6. Challenges and Future Directions

6.1. Subpopulations of EVS

Although how MNMs could facilitate overcoming challenges associated with EV heterogeneity has been discussed extensively in previous sections, recent reports have directed that a simple categorization of origin, content, function, and size heterogeneity may not be sufficient to define EV heterogeneity.[64,468–471] For example, while EVs derived from different cell types are known to show significant molecular signatures, new studies suggest the biogenesis of EVs within each cell type heavily depends on the microenvironment as well as the physiological states, adding to a new degree of complexity in the analysis and application of EVs.[472,473] Previously, EVs have been categorized into two subgroups, microvesicles derived from the budding plasma membrane, and exosomes generated from multivesicular bodies and released from the exocytic pathways. Recently, EVs have been further divided into subpopulations of large, medium-sized, and small EVs based on sizes and centrifugation speeds used in isolation; and subpopulations of CD63, CD9, and CD81 positive and others, depending on their immunoreactivity. So far, most studies on EVs have focused on the biological functions instead of the origins of EVs.[64] Therefore, which subpopulation of vesicles is actually dictating the fates of target disease cells remains largely unclear. This can be further complicated by the continuous addition of different descriptors into the current EV pool. For example, apoptotic bodies, migrasomes that mediate the transportation of cytoplasmic contents from multiple vesicles when cells migrate; as well as ARRDC1 (arrestin domain-containing protein 1)-mediated microvesicles, or ARMMs that are uniform in size (around 50 nm) and shown to extrude from the plasma membrane through a process mimicry of virus budding in ARRDC1 and ESCRT (endosomal sorting complex required for transport protein)-dependent manner, have all been discovered and associated with regulation of cell behaviors.[71,474–477] Subpopulations of EVs with different components could also originate from the different sorting machinery involved during the biogenesis of EVs. For instance, ESCRT has subtypes including ESCRT I, II, and III, and EVs associated with different ESCRT types can envelop distinct biomolecules.[71] In addition, EVs can be formed independently from ESCRT-based pathways, and the subpopulations can also be further specified during the lysosomal degradation stages.[72] Because of the high complexity of subpopulations of EVs, current protocols reported for recovery of EVs from liquid biopsy samples or cell culture supernatants have primarily resulted in a combination of heterogeneous subpopulations of EVs that include vesicles without clear identifications.[478] This can be further compounded by the overlapping of sizes, morphologies, and compositions of vesicles derived from different subpopulations. Without advanced isolation techniques, the understanding of the roles and functions of EVs in cell–cell communications would be incomplete. The ability to generate uniform subpopulations of EVs could also facilitate precision diagnostics and personalized therapeutics based on EVs.

To circumvent these hurdles, future development of MNM-facilitated and EV-based theranostic systems can be focused on the following directions. First, it would be essential to integrate novel mechanisms associated with EV targeting as well as the biogenesis of EVs into the surface functionalization design of MNMs with higher biological complexities. In the field of EV biology, there have been regular updates on novel mechanisms of how cells bind to and communicate with specific types of EVs, based on which there has been a more explicit and more thorough description and classification of EVs. It would be critical to incorporate new targeting ligands to increase the complexity of MNMs for targeting subpopulations of EVs.[29,67,479,480] Most updated discoveries and classifications of EVs can be found in several EV databanks, including Vesiclepedia (http://microvesicles.org/), ExoCarta (http://www.exocarta.org/), and EVpedia (http://evpedia.info/). Multiplex targeting based on the conjugation of ligands targeting multiple surface receptors of a specific subpopulation of EVs can also increase the complexity of MNMs. The second direction could be a combination of MNMs with various types of omics technologies to analyze EVs at the single vesicular level.[469] This direction is inspired by the rapid evolution of single-cell omics technology that has facilitated personalized medicine in the past decade.[481,482] Although the International Society for Extracellular Vesicles (ISEV) endorsed the categorization of EVs based on the differential speeds of ultracentrifugation, this categorization may not be sufficient to distinguish outstanding biomarkers behind the different sub-populations of EVs with similar densities. To address limitations of physical method-based separation and analysis of EV subpopulations, single vesicle analysis (SVA) techniques have been recently developed with several advantages by offering characterization of EV at a single vesicular level.[483–486] For example, one recent discovery on the critical role of the T2SS-like family of proteins in the selective loading of cargos into EVs in the microorganism *Shewanella vesiculosa* is successfully revealed by SVA.[482] MNMs would be particularly useful for label-based SVA. Specifically, label-based SVA methods include high-resolution flow cytometry, fluorescent microscopy, fluorescence resonance energy transfer (FRET), super-resolution microscopy, excitation-patterning methods, digital droplet PCR, and SERS.[469] There have been early attempts to combine these SVA approaches with MNM-based EV isolation methods, especially microfluidics-based systems.[313] However, few of them really demonstrate the profiling of subpopulations from heterogeneous samples. Given that magnetic bead-assisted parallel single-cell gene expression sequencing (MAPS-seq) has become a popular method for single-cell analysis, there would be plenty of room for the development of MNM-based SVA techniques for a better understanding of the biology behind subpopulations of EVs. Similarly, MNM-based EV isolation may

also facilitate the multiomics analysis of EVs through a more efficient enrichment of subpopulation of EVs. The third direction would be in vivo tracking and real-time imaging of subpopulations of EVs using MNMs.[487] Due to the small sizes, EVs have been mostly studied after separation from biofluids. Despite their broad applications in theranostics, these studies performed on purified populations of EVs can have limitations when the dynamics, biodistribution, and trafficking of EVs are of interest.[488] Therefore, there has been tremendous interest in developing real-time and high-resolution monitoring techniques combined with novel EV labeling and detection systems, to provide reliable methods for studying EVs in vivo at a single vesicle level without disrupting their physiological environments. MNMs with MRI, MRET (magnetic resonance energy transfer), or GMR properties, therefore, may represent unique toolsets for answering questions pertinent to EV biology and therapeutics owing to the large inertness of biological fluids and the human body to magnetic fields, as compared to other tracking and imaging methods such as fluorescence-based, electrical, and optical imaging methods.[197] However, the relatively low resolution of MRI (a few hundred microns) makes it particularly challenging to track individual EVs. Therefore, engineering MNMs through crystallization control, doping, or size tuning to achieve high magnetosensitivity would substantially facilitate the field of EV imaging. This process could be partially facilitated by learning from the recent development of single-cell MRI tracking techniques that are undergoing clinical translation.

To summarize, the diverse subpopulations of EVs represent a major challenge and an excellent opportunity for EV-based theranostic applications. MNMs that can enhance the efficiency of immunocapture, the precision of EV isolation, facilitate single vesicular analysis and multiomics techniques, and enable in vivo real-time imaging of EVs would offer promising approaches to address the critical problems associated with the heterogeneous populations of EVs.

6.2. Challenges of Clinical Translation

As discussed in Sections 2 and 5, EVs have several clear advantages as cell-free cell therapeutics over traditional synthetic carriers for drug delivery. According to the FDA website, this can be reflected by the rapid increase of clinical trials, with over 200 ongoings (www.clinicaltrials.gov). Disease types that can be treated by EVs, according to various preclinical studies, are also broad, covering cardiovascular, neurological, endocrine, immunological, and cancer-related diseases. In particular, EVs for drug delivery attracted intensive attention from both academia and industry, which have been well covered by previous reviews.[489] A table that summarizes representative clinical trials of EV-based drug delivery applications can be found in **Table 3**.

Despite the clear advantages over conventional drug carriers, clinical translation of EVs still encountered significant barriers. Among them, two of the most critical hurdles that can be potentially addressed or mitigated by MNMs are: i) immune reactions toward exogenous EVs and the associated toxicity; and ii) large-scale manufacturing.

6.2.1. Immune Responses toward EVS

Although EVs are widely reported as biocompatible carriers because of their mammalian cell origin, there are huge gaps between in vitro and in vivo conditions. Most tests on the IV injection of EVs derived from bovine milk and blood-cell-derived EVs into rodents (e.g., mouse) resulted in no significant adverse effects, except for a report on potential transfusion-associated acute lung injury.[233] However, when the EV has oncogenic origins, including stem cell-derived angiogenic EVs that are widely applied for cell-free tissue engineering, EVs have potential side effects in promoting tumor growth when there is already a primary tumor in the mouse.[490] This has been a significant concern in clinical translation. EVs have also been shown to be capable of carrying pathogenic factors, including tumor-associated and major histocompatibility class receptor-presented peptides to induce immune system activation.[66] This could be another undesirable factor, as drug delivery carriers should have minimum immunogenicity. Other delivery routes, including intraperitoneal and oral administration of EVs, have not resulted in noticeable side effects and immune reactions.[131] However, considering that EVs used for current in vivo applications are often combinations of heterogenous populations of sub-type vesicles, different batches of EVs must be rigorously examined in terms of immune reactions and biocompatibility, using in vitro, 3D cell culture, and in vivo (including rodent and non-human primate) models, before they could be translated for clinical applications. However, a safer way to minimize potential side effects from EVs is to use autologous EVs or use a large pool of EVs with rigorous quality control and characterization when autologous EVs are not available.[491] The latter approach is widely pursued in MSC-based clinical trials. Nevertheless, donors who are cancer patients, or have significant risks from cancer, must be more carefully examined.

6.2.2. Large-Scale Manufacturing

Another critical challenge for the clinical translation of EVs is the difficulties associated with reproducible large-scale production of EVs, mainly due to the EVs' complexity and heterogeneity, including heterogeneous sizes, batch-to-batch variations, and biological differences from different donors that frequently occur in the manufacturing process.[492–494] These issues are often not common in synthetic carriers, thereby leading to additional difficulties in clinical translation.[494] Through proper engineering of the cells used in EV production, there have been several platforms developed for generating therapeutic EVs, including i) natural EVs that can be harvested from native or genetically modified cells; ii) hybrid EVs that are loaded with drugs, nanoparticles, or other molecules; iii) EV-like liposomes. Future translation of MNM-hybrid EVs will majorly fall into the second category.[489] In terms of regulation, the MNM-EV hybrid would require approval of both the MNM and EV for the clinical translation (at the level of Phase II and above). Although magnetic nanoparticle has been previously approved as imaging contrast agent, and as a treatment for anemia, their approval for other disease treatment would still require

Table 3. EV-based ongoing clinical trials. Source: clinicaltrials.gov.

NCT Number	Status	Name	Disease	EV type
NCT01159288	Phase 2	Trial of a vaccination with tumor antigen-loaded dendritic cell-derived exosomes	Lung cancer	Dendritic cell exosomes
NCT01668849	Phase 2	Extracellular vesicle infusion therapy for severe COVID-19	COVID-19	Grape exosomes
NCT04493242	Phase 2	Extracellular vesicle infusion therapy for severe COVID-19	COVID-19	Bone marrow EVs
NCT04602442	Phase 2	Safety and efficiency of method of exosome Inhalation in COVID-19 associated pneumonia	COVID-19	MSC exosomes
NCT04544215	Phase 1/2	A clinical study of mesenchymal progenitor cell exosomes nebulizer for the treatment of pulmonary infections	Bacterial infections	MSC exosomes
NCT04491240	Phase 1/2	Evaluation of safety and efficiency of exosome inhalation in SARS-CoV-2 associated pneumonia	COVID-19	MSC exosomes
NCT04213248	Phase 1/2	Effect of UMSCs derived exosomes on dry eye in patients with cGVHD	Dye eye	MSC exosomes
NCT03384433	Phase 1/2	Allogenic mesenchymal stem cell derived exosome in patients with acute ischemic stroke	Cerebrovascular disorders	MSC exosomes
NCT04388982	Phase 1/2	Safety and efficacy evaluation of allogenic adipose MSC-exosomes in patients with Alzheimer's disease	Alzheimer's disease	ADMSC exosomes
NCT04602104	Phase 1/2	A clinical study of mesenchymal stem cell exosomes nebulizer for the treatment of ARDS	Acute respiratory distress syndrome	MSC exosomes
NCT04276987	Phase 1	A pilot clinical study on inhalation of mesenchymal stem cells exosomes for the treatment of severe novel coronavirus pneumonia	COVID-19	MSC exosomes
NCT03437759	Phase 1	MSC-Exos promote healing of MHs	Macular holes	MSC exosomes
NCT04313647	Phase 1	A tolerance clinical study on aerosol inhalation of mesenchymal stem cells exosomes in healthy volunteers	Not applicable	MSC exosomes
NCT04270006	Phase 1	Evaluation of adipose derived stem cells exosomes in treatment of periodontitis	Periodontitis	ADMSC exosomes
NCT04173650	Phase 1	MSC extracellular vesicles in dystrophic epidermolysis bullosa	Dystrophic epidermolysis bullosa	MSC EVs
NCT02565264	Phase 1	Effect of plasma derived exosomes on cutaneous wound healing	Ulcer	Exosome-rich plasma
NCT04389385	Phase 1	COVID-19 specific T-cell derived exosomes	COVID-19	T-cell exosomes
NCT01668849	Phase 1	Edible plant exosome ability to prevent oral mucositis associated with chemoradiation treatment of head and neck cancer	Head and neck cancer, oral mucositis	Grape exosomes
NCT03608631	Phase 1	iExosomes in treating participants with metastatic pancreas cancer with KrasG12D mutation	Pancreatic cancer	MSC exosomes
NCT01294072	Phase 1	Study Investigating the ability of plant exosomes to deliver curcumin to normal and colon cancer tissue	Colon cancer	Plant exosomes

significant preclinical and clinical trials.[495] However, in terms of process design, MNM-hybrid EVs that allow for more efficient isolation during the manufacturing, and enable in vivo monitoring of the EVs can facilitate quality control and scale-up fabrication. Nevertheless, biologics including nucleic acids and proteins encapsulated inside EVs still add an additional level of complexity, as they are typically produced from living organisms. Therefore, cell type, cell passage, EV harvesting frequency, and the manufacturing processes of MNMs and biologics should all be rigorously controlled with a high level of quality control. Although many of the good manufacturing practices previously established for biologics production could be translated into EV-based therapeutics, the size, content, and origin heterogeneity, and complexity of EVs must be specially considered. Some additional controls could include characterizations on EV size, contents, concentrations, and nucleic acid sequences to ensure the final EV product can meet the pre-defined quality measures on EVs' physicochemical, immunological, and functional properties. As detailed in previous sections, MNMs hybridization can partially overcome these challenges, as it provides additional modalities of quality control with higher efficiency in terms of isolation. However, MNMs themselves, once form hybrids with EVs, add additional complexity into the system, and their quality control must be rigorously performed. Still, potential solutions to these issues can be learned from the past success and failures in the field of nanomedicine.[496] In this way, the clinical potential of EVs and MNM-hybrid EVs can be maximized.

7. Conclusions

Extracellular vesicles are vital mediators for cell–cell communications and convey various biomolecules, including nucleic

acids, proteins, polysaccharides, and lipids. These diverse biomolecules have inspired a variety of therapeutic applications. However, the heterogeneities of sizes, compositions, tissue origins, and cell origins have presented various research opportunities and challenges to using EVs for biomedical applications. MNMs that have been broadly used for isolation, purification of biomolecules in biosensors, magnetic mode imaging modalities, and in vitro and in vivo treatment of varying diseases can offer several clear advantages to help overcome the heterogeneity challenges in EV-based applications. In this review, we first briefly covered the biological context of EVs, the biomolecules and functions of EVs, and the strategies of engineering MNMs for biomedical applications. Then we engaged these two topics into dialogue by focusing on the central question of "how MNMs can be engineered to overcome the size, composition, and origin heterogeneities to facilitate the diagnostic and therapeutic applications of EVs." Recent research has focused on MNM-based capturing, isolation, purification, and concentration of EVs to optical, magnetic, fluorescent, electric, and electrochemical biosensors for EV-based diagnostic applications to achieve sensitive, selective, reproducible, and rapid detection of biomolecules inside EVs. Using this information obtained from MNM-based biosensors, multiplex detection of biomarkers for precision medicine and noninvasive monitoring of diseases or biological applications have been investigated. Most current work has been dedicated to using MNMs combined with magnetic fields for the tissue-specific delivery of EVs for therapeutic applications. The property of MNMs to respond to alternate magnetic fields and release heat has also been applied for the triggered release of cytokines inside EVs for more targeted disease treatment. Other advantages of MNMs, including iron ion-stimulated EV secretion, MRI-based in vivo tracking of EVs, and nano-EL, have also facilitated EVs' therapeutic applications in various disease types, including immunological, neurological, cardiovascular disorders, and other conditions. However, only a few examples of MNM-facilitated EV therapeutic applications have been established for each direction, indicating a large room for further development of therapeutic and diagnostic applications.

Moving forward, it is crucial to apply MNM-based EV biosensors to answer important biological questions. For example, neurological disorders have often been challenging to monitor at their early stage due to the BBB. While more invasive methods, such as brain biopsy, can determine early-stage neurological diseases, it is not preferred by doctors or patients. EVs that can pass through the BBBs can potentially provide a solution for sensitive, noninvasive, and early-stage detection of biomarkers pertinent to neurological disorders. MNMs can enhance sensitivity and selectivity and allow multiplex detection for higher accuracy. However, currently, no direct studies are suggesting a potential to innovate. In addition, it is also essential to establish a vigorous standard to confirm the performance and reproducibility of MNM-based EV biosensors. Current literature has suggested inconsistency in the reported detection limits due to different sample sources and impurities.

There is plenty of room for MNM-based EV therapeutic applications as well. Both MNMs and EVs have a high potential for clinical translation, given that many clinical trials are concentrated on MNMs and EVs. However, despite clear advantages highlighted in the current review, very limited demonstrations on MNM-facilitated EV therapeutic applications, suggesting this field is still at an early stage of research. Given that cell-specific and tissue-specific drugs would be desired in most diseases, magnetic field-guided EV delivery could be broadly applicable to in vivo applications. However, their translation into human bodies would require significant innovation to develop MF devices for deep tissue penetration. Similar concerns apply to the AMF-triggered drug release. It would be more practical for the field to start with MNM-facilitated, EV-based treatment of diseases and injuries closer to skin surfaces, and then explore the future treatment of internal organ disorders. Nevertheless, proof-of-concept demonstrations on MNM-facilitated EV therapeutics in the in vivo systems would still pave the road for the next generation of cell-free therapy, as well as precision tissue engineering. The combination of diagnostic and therapeutic functions of MNM-EV systems would further provide promising solutions to various applications, including image-guided therapies and other theranostic applications.

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Conflict of Interest

The authors declare no conflict of interest.

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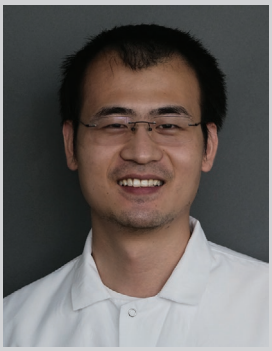

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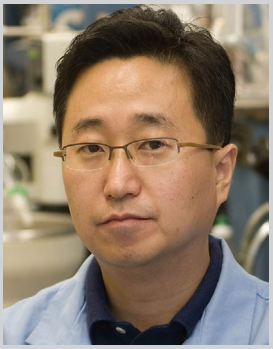

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