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REVIEW

# Macrophage-Derived Exosomes as Advanced Therapeutics for Inflammation: Current Progress and Future Perspectives

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**Abstract:** The development of numerous diseases is significantly influenced by inflammation. Macrophage-derived exosomes (M-Exos) play a role in controlling inflammatory reactions in various conditions, including chronic inflammatory pain, hypertension, and diabetes. However, the specific targets and roles of M-Exos in regulating inflammation in diseases remain largely unknown. This review summarizes current knowledge on M-Exos biogenesis and provides updated information on M-Exos' biological function in inflammation modulation. Furthermore, this review highlights the functionalization and engineering strategies of M-Exos, while providing an overview of cutting-edge approaches to engineering M-Exos and advancements in their application as therapeutics for inflammation modulation. Finally, multiple engineering strategies and mechanisms are presented in this review along with their perspectives and challenges, and the potential contribution that M-Exos may have in diseases through the modulation of inflammation is discussed.

**Keywords:** inflammation, macrophage-derived exosomes, biological functions, inflammation modulation, engineering strategies

## Introduction

Inflammation is a natural biological response dominated by defense in local tissues to stimulation by pathogens, injured cells, chemicals, or physical trauma, the cardinal symptoms of which are typically characterized by redness, swelling, heat, pain, and dysfunction.[1] Inflammation can maintain homeostasis by repairing damaged tissues and organ function, increasing the number of leukocytes, and enhancing metabolism.[2,3] If inflammation is not promptly addressed, it will lead to a series of chronic inflammatory diseases (eg, diabetes, rheumatoid arthritis, cancer, inflammatory bowel diseases (IBD), cardiovascular disease, and neurologic system disorders).[4–6] Conventional drugs approaches to inflammation include steroids, glucocorticoids, nonsteroidal anti-inflammatory drugs or inhibitors targeting specific pro-inflammatory cytokines.[4,7,8] However, the aforementioned medicines have limited effect on inflammation regulation and cannot completely prevent disease progression. In addition, long-term administration of these medications can cause gastrointestinal adverse effects (eg nausea, vomiting) and osteoporosis, hyperglycemia.[9–11] Therefore, promising treatment approaches with durable efficacy and few side effects are urgently needed.

Exosomes, which are nanosized vesicles derived from endocytosis, contain bioactive molecules such as proteins, DNA, and RNA.[12] These vesicles play a crucial role in facilitating material exchange between cells and participate in numerous physiological and pathological processes within the body.[13,14] With the deepening of research on exosomes, a high volume of evidence indicated that exosomes are involved in the regulation of inflammation and different types of

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inflammatory diseases as mediators and potential therapeutic targets.[15] This makes exosomes a promising candidate for inflammation.

Macrophages are generally thought to be terminally differentiated immune cells that develop from monocytes, which originate from hematopoietic stem cells in the bone marrow.[16] After undergoing differentiation steps, monocytes enter the peripheral blood and become circulating monocytes, including inflammatory and resident monocytes.[17] After migrating to tissues, they differentiate into tissue-specific macrophages, including the skeletal system (osteoclasts), central nervous system (microglia), lungs (alveolar macrophages), liver (Kupffer cells), and connective tissue (histiocytes), as well as the spleen, gastrointestinal tract, and peritoneum.[18–20] Within tissues, macrophages are multifunctional cell types that can react to factors in the microenvironment (eg, damaged cells, microbial products).[21,22] Macrophages can display different phenotypes and functions under the influence of changes in surrounding microenvironment factors, which are a heterogenous population with the function of engulfing microbial infections and dead cells to resist pathogen invasion.[23] Depending on their activation status, macrophages can be polarized into different functional phenotypes: classically activated macrophages (M1) and alternatively activated macrophages (M2).[24] Moreover, M2 macrophages can be further subdivided into subsets called M2a, M2b, M2c, and M2d. M1 macrophages are induced by pro-inflammatory mediators like lipopolysaccharide (LPS) and interferon-γ (IFN-γ) to secrete different pro-inflammatory cytokines and chemokines, such as tumor necrosis factor alfa (TNF-α), interleukin 6 (IL-6), interleukin 1alfa (IL-1α), interleukin 1beta (IL-1β), C-X-C motif chemokine ligand 9 (CXCL9).[25] In contrast, M2 macrophages are activated by anti-inflammatory mediators like interleukin 4 (IL-4) and interleukin 13 (IL-13) to release different anti-inflammatory factors, such as transforming growth factor beta (TGF-β), interleukin 10 (IL-10), CC motif chemokine ligand 1 (CCL1), CC motif chemokine ligand 17 (CCL17), C-C motif chemokine ligand 18 (CCL18), and are involved in anti-inflammatory response, tissue repair and remodeling, immunomodulation.[26,27] Among them, M2 macrophages can be further subdivided into four subtypes: M2a, M2b, M2c, and M2d. M2a is induced by IL-4, IL-13, or fungal and parasitic infections; M2b is induced by immune complexes and LPS; M2c is induced by IL-10, TGF-β, glucocorticoids, M2d can be induced by IL-6 and adenosine.[28] Exosomes are key mediators of intercellular communication, which carry biological information on macrophages. They are involved in various inflammatory diseases and consistently regulate the inflammatory microenvironment.[29,30] Also, evidence suggests that the regulation of inflammation by M-Exos is pivotal.[15,31]

As a result, this review aims to provide inspiration for future investigations by summarizing the functions of M-Exos, exploring the approaches for engineering inflammation-related disease applications, and discussing their potential as drug delivery platforms for anti-inflammatory purposes.

## Biogenesis and Composition of Exosomes

Exosomes are a type of nano-sized extracellular vesicle generated from multivesicular bodies (MVBs), generally range in size from 30 to 150 nm in diameter and are enriched in selected proteins, lipids, nucleic acids, and glycoconjugates.[32] Current studies have shown that the invagination of the cytoplasmic membrane forms early endosomes (ESEs), early endosomes (ESEs) are formed by invagination of the cytoplasmic membrane, early endosomes form late endosomes (LSEs) by exchange of material with organelles or interfusion between endosomes and late endosomes. Late endosomes and endosomes produce many ILVs, which then subsequently evolve into multivesicular bodies (MVBs) that are released and eventually form exosomes.[33,34] Exosomes can be released by various cell types such as dendritic cells (DCs), mast cells, and macrophages.[35] This etiologic heterogeneity led to find exosomes in various kinds of biological fluids, including plasma, serum, saliva, cerebrospinal fluid, amniotic fluid, urine, pleural effusion or ascites, and breast milk.[36,37] Exosomes have been confirmed to be vital carriers of unique cargo of protein, lipid, DNA, mRNA, and microRNA (miRNA), which can mediate intercellular communication via these cargo molecules.[38] As vectors for intercellular communication, exosomes from donor cells are internalized by micropinocytosis to fuse with the membranes to release its contents of lipids, proteins and RNAs, inducing subsequent physiological changes in recipient cells.[39,40] As mentioned above, due to these characteristics that exosomes have, exosomes seem to be capable of acting as vehicles for drug delivery to convey their RNA and protein contents.[38,41] In recent years, researchers have discovered that exosomes can also be involved in processes such as the inflammatory response, antigen presentation, pathogen transmission, the immune response, programmed cell death, angiogenesis,

and coagulation.[42–44] Exosomes have been reported to play various roles in the inflammatory response and pathogenesis of inflammatory diseases, such as in inflammatory bowel diseases, exosomes can induce intestinal barrier repair through TSG-6 overexpression.[45] Another study also showed that Schwann cell-derived exosomes containing MFG-E8 can play an anti-inflammatory role by modifying macrophage/microglial polarization, and attenuate inflammation via the SOCS3/STAT3 pathway after spinal cord injury.[46]

Different models show the involvement of different proteins, which are usually highly abundant in exosomes and play crucial roles in cargo sorting and wrapping of exosomes. These proteins usually involve tetraspanins (CD9, CD63, CD37, CD81 or CD82), as well as heat shock proteins (HSP70), Rab family proteins Tsg101 and Alix, which can be used to confirm the presence of exosomes.[47,48] There is also a class of specific proteins, which are only derived from cells and tissues, such as tetraubiquitin, MHC class II, transferrin receptor, etc.[49,50] Moreover, the biogenesis of exosome is regulated by multiple factors (eg, cell type, contact inhibition)[51] and changes in pathological states (eg, diabetes and neuronal degeneration)[52] can affect the yield and content of exosomes, which increases the feasibility of exosome content as a biomarker for disease diagnosis and prognosis. Based on the characteristics and prospects of exosomes as new potential diagnostic and therapeutic tools, current isolation methods for exosomes are also constantly evolving, including ultracentrifugation, affinity-based capture technology, filtration, chromatography, precipitation, and microfluidics.[37,53] However, these technologies have some limitations. For example, the ultracentrifugation method is time-consuming and at high cost of special equipment,[54] other molecules co-precipitated with exosomes in the precipitation method may be contaminated, causing the purity of exosomes to be questioned.[55] Overall, in the future, it is necessary to develop and investigate exosome isolation strategies that are widely recognized or can be used as the gold standard to promote the realization of their applications.

Taken together, exosomes have been implicated in a broad range of biological processes and play essential roles in many facets of human health and disease, including development, immune regulation, inflammation, cancer progression and metastasis, and neuronal communication. Therefore, based on their potential to manipulate drug delivery, the specific targeting and homing nature,[56,57] exosomes can be considered as "professional transporters and messengers" to systemic level. Since exosomes protect their cargo from being degraded by enzymes in the circulation, all of the above offer a potential approach for disease diagnosis and assessing therapeutic efficacy of specific drugs. Biogenesis and composition of exosomes are presented in (Figure 1).

## The Biogenesis, Biological Functions, and Applications of M-Exos

The formation of M-Exos is mainly derived from three stages: exosome biogenesis; sorting of cargo into exosomes; exosome release.[58] Different phenotypes of M-Exos have different biological information, resulting in different executive functions. And their formation is precisely regulated by a process that involves multiple proteins.[21] Until now, current research has dug deeper into three types of M-Exos, including unpolarised M0 macrophage-derived exosomes (M0-Exos) , polarised M1-Exos and M2-Exos.[59] So far, there is still no clear biomarker for distinguishing M1-Exos from M2-Exos. Furthermore, in macrophages, the amphisomes (amphisomes are hybrid organelles produced through the fusion of endosomes with autophagosomes within cells) would not fuse with the lysosome, which causes exosomes to be released in greater quantities.[60] The amount of secretion with M-Exos has different effects on the outcome of different diseases, which also reflects the influence of distinct cell types and diverse cellular environments in governing exosome secretion (Figure 2).

Studies have confirmed that M-Exos play critical functions in the treatment of diseases including cancer, atherosclerosis, diabetes, heart disease, and inflammation.[61–65] It has been reported that all types of M2-Exos can reduce the severity of IBD, among which M2b-Exos (M2b macrophage-derived exosomes) have the best effect.[66] Recent studies have confirmed that M-Exos can be used as drug delivery tools, gene or protein delivery vehicles. Consequently, they have a significant impact on the diagnosis, prevention, and treatment of future diseases (Figures 3 and 4).[21]

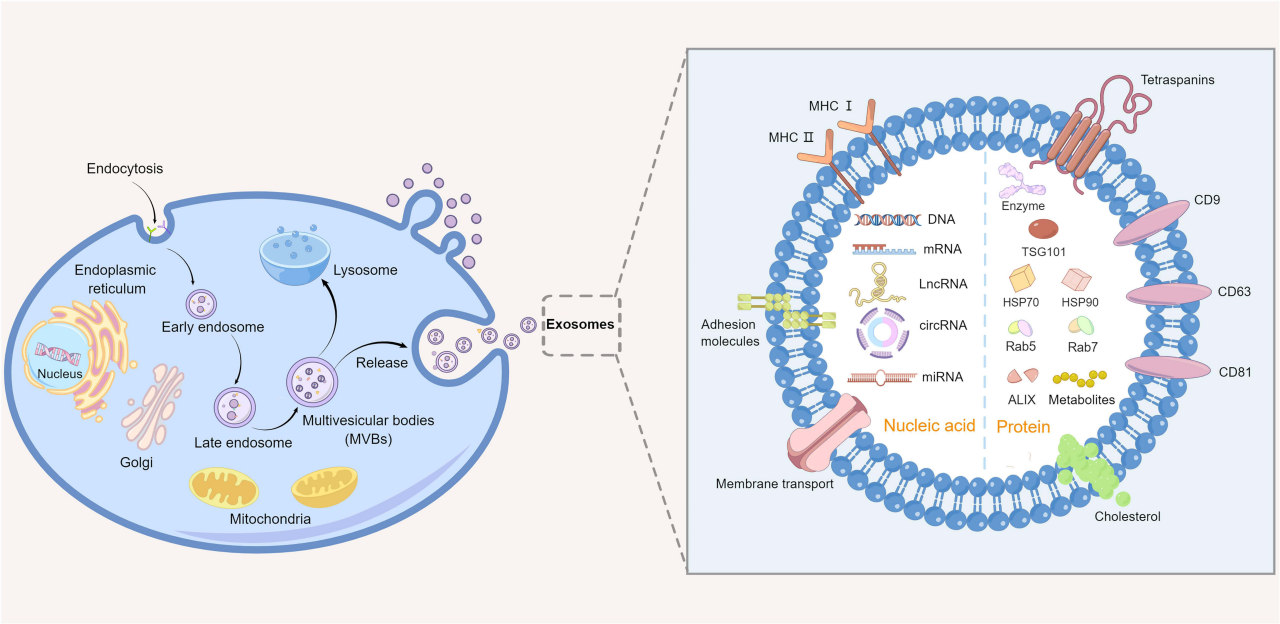


**Figure 1** Biogenesis and composition of exosomes. By Figdraw.
**Notes**: Early endosomes are formed by invagination of the cytoplasmic membrane, which gradually transform into late endosomes. Late endosomes evolve into MVBs by secreting luminal vesicles. In addition to fuse with lysosomes, the remaining vesicles fuse with the cell membrane and are released to the extracellular space, namely exosomes.
**Abbreviation**: MVBs, multivesicular bodies.

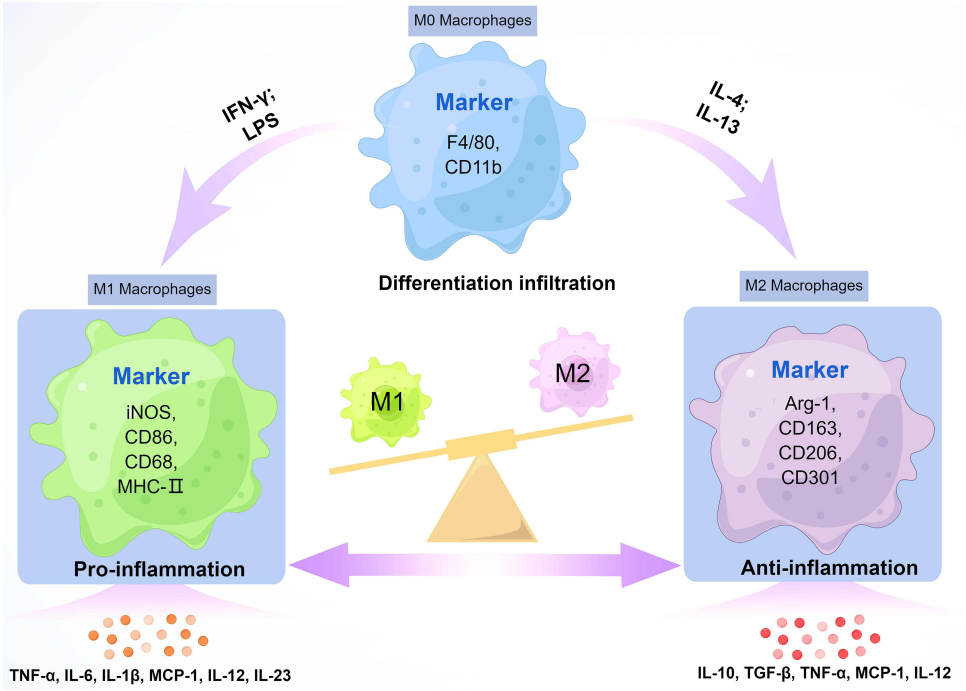


**Figure 2** Different typologies of macrophages and their cell expression markers. By Figdraw.

## M-Exos Diagnostic Value and Biomarkers to Monitor Inflammation Progression

Recent research has demonstrated the involvement of M-Exos in the pathological and physiological processes of inflammation and related diseases and have been studied as a potential diagnostic and prognostic markers of

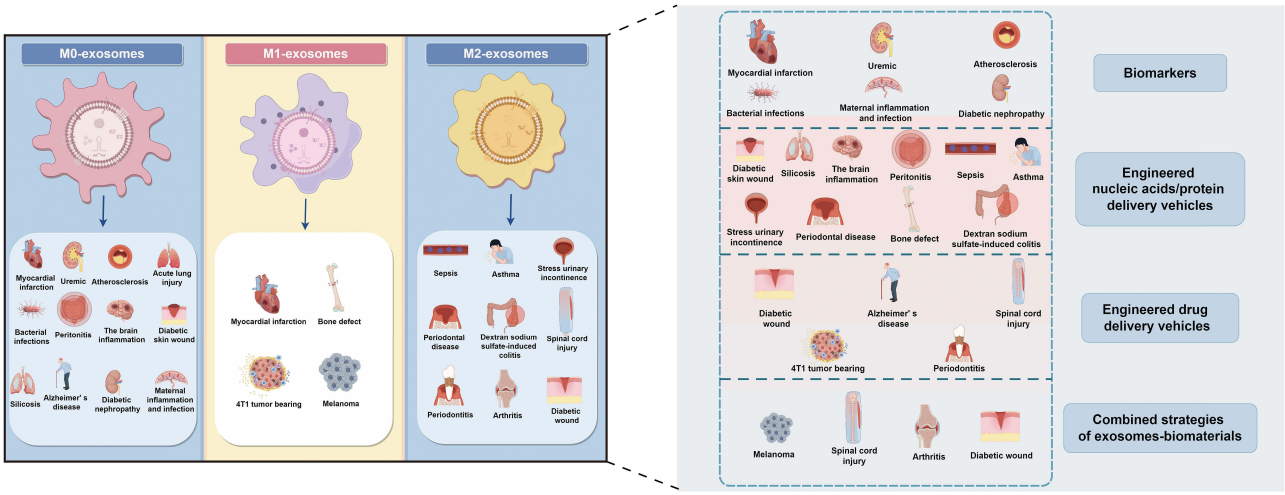


**Figure 3** Related diseases involving exosomes derived from different macrophages. By Figdraw.

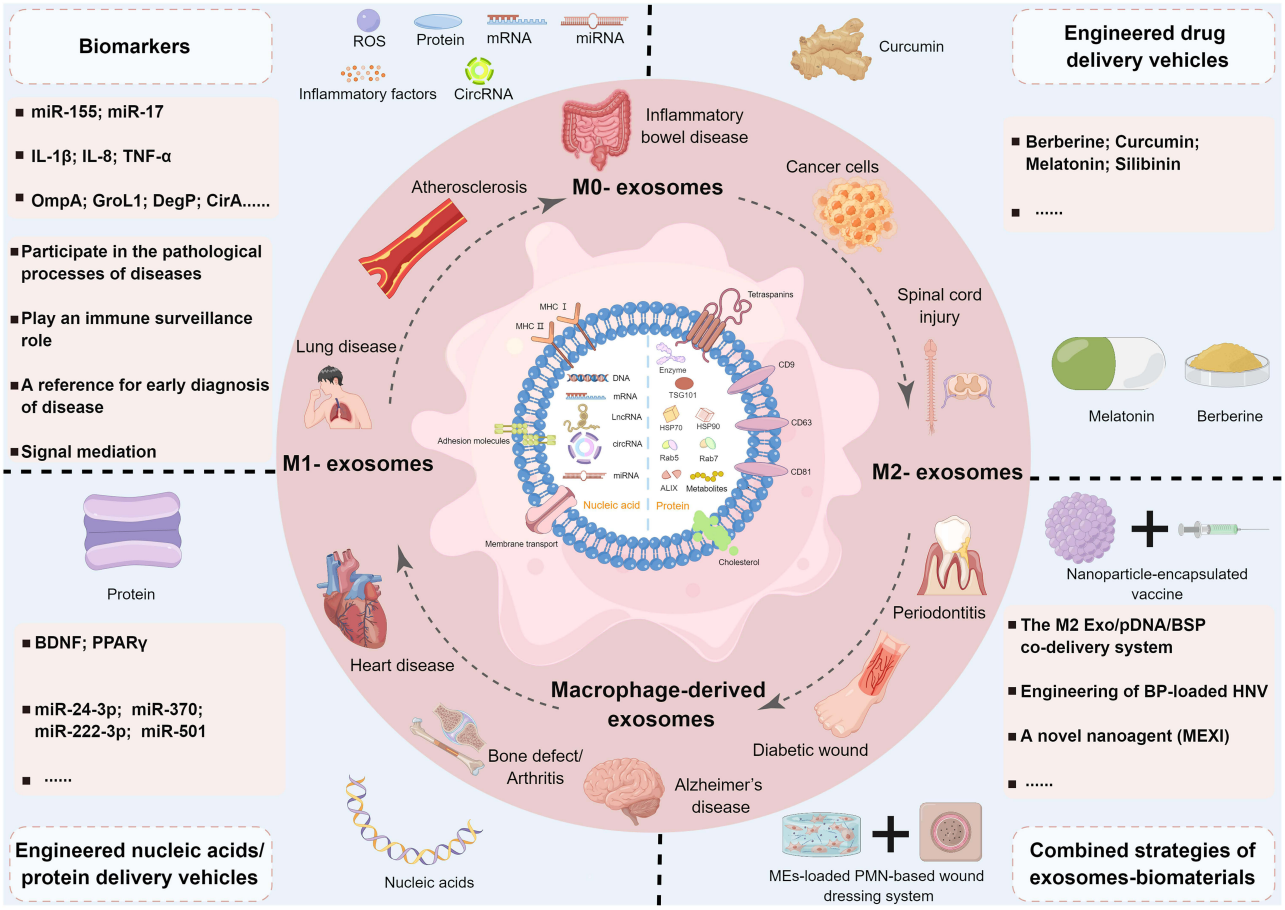


**Figure 4** The biogenesis, biological functions, and applications of macrophage-derived exosomes. By Figdraw.

**Notes**: This schematic demonstrates that exosomes can serve as biomarkers (eg miR-155,[67] miR-17,[68] IL-1β,[69] TNF-α[70]), nucleic acid and protein delivery vehicles (eg BDNF,[71] PPAR-γ,[72] miR-24-3p,[73] miR-370[74]), drug delivery vehicles (eg berberine,[75] curcumin,[76] melatonin,[77] silibinin[78]) and the combined strategy of exosome biomaterials (eg The M2 Exo/pDNA/BSP co-delivery system,[79] engineering of BP-loaded HNV[80]) play a role in diagnosis and treatment of diseases.

inflammation and associated diseases.[65] M-Exos cargoes from different pathological conditions (bacterial infection, viral infection and disease states) may show distinct genomic and proteomic profiles for distinguishing and identifying the diagnosis and development status assessment of different inflammatory stages and different inflammatory diseases, so as to serve as a reference for exploring the pathogenesis of inflammation and related diseases. For instance, mycobacterium avium glycopeptidolipids (GPLs) were found in exosomes released by infected macrophages, resulting in GPLs transfer from infected to uninfected macrophages.[81] Notably, the exosomes released by Toxoplasma gondii-infected macrophages stimulated proinflammatory responses in Toll-like receptors and MyD88 in uninfected macrophages.[70] Proteomic result of M-Exos after infection with live or heat-killed Escherichia coli showed that OmpA, GroL1, DegP, CirA, and FepA are potential biomarkers for exosome-mediated inflammation.[82] Another study demonstrated that the Shiga toxins (Stxs)-containing M-Exos can cause cytotoxicity and inflammation, which leads to cell death in toxin-sensitive cells. Compared with purified Stx2a, exosomes released from Stx2-treated human macrophages contained pro-inflammatory cytokine mRNAs and proteins, resulting in more severe inflammation. The above studies imply that human macrophages may act as a significant role in the exacerbation of renal inflammation.[69] Interestingly, in a study of atmospheric particulate matter (PM), researchers found that the exosomes derived from PM2.5-treated macrophages could stimulate the release of inflammatory cytokine and alter the inflammatory phenotype of recipients via activating the TLR-4-NF-κB signaling pathway, thereby inducing inflammatory responses in mouse lung.[83]

The regulation of inflammation by M-Exos has different features in different homeostasis and disease states. In cell experiments simulating diabetic nephropathy, high glucose-treated M-Exos contained a large amount of IL-1β and iNOS, which could regulate NF-κB p65 signaling pathway in vitro, induce the activation of M1 and the increased release of inflammatory cytokines, leading to the kidney damage.[84] Another similar study reported that high glucose-stimulated M-Exos accumulated more inflammation factors and accelerated kidney injury by promoting NLRP3 inflammasome to be activated and impairing mesangial cell autophagy in diabetic nephropathy. The above studies have highlighted that the pathological process of inflammation will be further aggravated in high glucose state.[85] Furthermore, exosomes can also be used as messengers to monitor the body's inflammatory response to reflect its development process and pathogenesis so that the body can make corresponding adjustments. There are data indicating that exosomes could be the means by which the placenta responds to these non-contact-dependent messages from activated macrophages, which subsequently signaled to the placental unit to facilitate the response to maternal inflammation and infection. In this way, a protective placental immune response might be mediated during pregnancy through the exosomal pathway.[86] Taken together, M-Exos provide a novel mode for maternal-placental messaging.

Noncoding RNA such as miRNA, lncRNA, and circRNA is an important biologically active molecule in exosomes. Additionally, they also contribute significantly to the regulation of the occurrence of inflammatory responses and development of inflammatory diseases. Recent research has illustrated that activated macrophages secreted miR-155-enriched exosomes, which could downregulate suppressor of cytokine signaling 1 expression to induce fibroblast inflammation in the case of cardiac injury.[67] Similarly, another study found that proinflammatory M1-Exos released a large number of high expression of pro-inflammatory exosomes miR-155 after myocardial infarction. miR-155 was transported to endothelial cells, resulting in the downregulation of its novel target genes, including Rac family small GTPase 1 (RAC1), p21 (RAC1)-activated kinase 2 (PAK2), Sirtuin 1 (Sirt1), and protein kinase AMP-activated catalytic Subunit alpha 2 (AMPKα2). While M1-Exos inhibited Sirt1/AMPKα2-endothelial nitric oxide synthase and RAC1-PAK2 signaling pathways by targeting the five molecular genes to reduce the angiogenic capacity of endothelial cells and aggravate myocardial injury.[61] According to this, miR-155 in M-Exos is associated with the inflammation development of various diseases and is an effective diagnostic biomarker. Interestingly, one study has found that exosomal miRNAs such as miR-155-5p, miR-132-3p, miR-1246, miR-210-3p, and miR-330-5p secreted by inflamed macrophages in peripheral tissue might cross the blood–brain barrier and activate microglia and astrocytes to produce proinflammatory cytokines, thereby inducing neuroinflammation and contributing to the progression of Parkinson's disease (PD).[87] In conclusion, M-Exos exert significant functions in immune surveillance, signal mediation and promotion of disease progression in the pathogenesis and pathological process of inflammation and related diseases. We have tabulated M-Exos-related biomarkers from recent studies as useful references for the detection of inflammation and its associated diseases (Table 1 and Figure 5).

**Table 1** Biomarkers in M-Exos with Inflammation and Related Diseases

| M-Exos-Biomarkers | Expression Levels | Animal Type/ Cell Type | Exosomes Source | Disease Model | Possible Biological Effects | Exosomes Surface Markers | Reference |
|---|---|---|---|---|---|---|---|
| *microRNAs (miR)* | | | | | | | |
| miR-155 | Upregulation | Mir-155$^{-/-}$ mice and littermate WT mice in C57BL/6 background | Bone-marrow-derived macrophages were isolated from the femurs and tibias of adult mice | Myocardial Infarction model | Macrophage-derived mir-155-containing exosomes inhibited cardiac fibroblast proliferation by downregulating Son of Sevenless 1 expression and promoted inflammation by decreasing Suppressor of Cytokine Signaling 1 expression. | Alix | [67] |
| miR-155 | Upregulation | Mice Wild type (WT) C57BL/6J mice; Rab27a−/− and aSMase−/− mice on a C57BL/6 background | The human acute monocytic leukemia cell line THP-1 | Myocardial infarction model | The proinflammatory M1-like-type macrophages released an extensive array of proinflammatory exosomes miR-155 after myocardial infarction, which depressed Sirt1/AMPKα2–endothelial nitric oxide synthase (eNOS) and RAC1–PAK2 signaling pathways by simultaneously targeting the five molecule nodes, aggravated myocardial injury. | Alix, CD63, CD81, CD9 | [61] |
| miR-155 | Upregulation | miR-155$^{-/-}$ mice in a C57BL/6 background and wild-type C57BL/6 mice | Mouse macrophage line RAW 264.7 | Uremic model | MiR-155-enriched exosomes secreted by infiltrated macrophages exacerbated cardiomyocyte pyroptosis and uremic cardiomyopathy changes by directly targeting FoxO3a in uremic mice, thereby amplifying Inflammatory reactions | Alix, CD9, CD63 | [64] |

(*Continued*)


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**Table 1** (Continued).

| M-Exos-Biomarkers | Expression Levels | Animal Type/ Cell Type | Exosomes Source | Disease Model | Possible Biological Effects | Exosomes Surface Markers | Reference |
|---|---|---|---|---|---|---|---|
| miR-16-5p | Upregulation | ApoE-/- mice | Mouse macrophage line RAW 264.7 | Atherosclerosis model | Exosomal miR-16-5p from macrophages promotes inflammatory response via downregulating SMAD7 expression, leading to aggravate AS progression | CD9, CD81 | [88] |
| miR-155-5p | Upregulation | C57BL6/J mice | Mouse macrophage line RAW 264.7 | Parkinson's disease model | Exosomal miR-155-5p from inflamed macrophages transduced inflammation in peripheral tissue into the brain, thus induce neuroinflammation and cause the progression of Parkinson's Disease. | CD9, CD63 | [87] |
| miR-17 | Downregulation | Wistar rat | The human acute monocytic leukemia cell line THP-1 | Hypertensive model | Ang II-stimulated macrophage-derived exosomes upregulated the expression of ICAM1 and PAI-1, and contained the downregulation of miR-17 levels, which indicated that inflammation of endothelial cells under hypertensive conditions may be partially caused by macrophage-derived exosomes | HSP90, HSC70, CD63, CD9 | [68] |
| *mRNA* | | | | | | | |
| IL-1β, IL-8 | Upregulation Upregulation | The human proximal tubule epithelial cell line (HK-2) and the human retinal pigment epithelial cell line (ARPE-19) | The human acute monocytic leukemia cell line THP-1 | Cytotoxicity and inflammation model (eg, toxin dissemination and exacerbation of inflammation in the kidney) | Exosomes released from Stx2-treated human macrophages contained pro-inflammatory cytokine mRNAs and proteins, induced more severe inflammation. | Alix | [69] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Cell Surface Molecules and Cytokines* | | | | | | | |
| TNF-α, IL-12 | Upregulation<br>Upregulation | BALB/c and C57BL/6 mice; $TLR2^{-/-}$ (C57BL/6 background) and $TLR4^{-/-}$ (BALB/c background) mice; $MyD88^{-/-}$ mice (C57BL/6 background) | Bone marrow–derived macrophages were isolated from mice; The mouse macrophage cell line J774; The human acute monocytic leukemia cell line THP-1 | Exosomes released from macrophages infected with intracellular pathogens stimulate a proinflammatory response model in vivo (Mycobacterium tuberculosis; Mycobacterium bovis BCG) | Exosomes isolated from M bovis BCG– and M tuberculosis–infected macrophages, when injected intranasally into mice, stimulate TNF-α and IL-12 production as well as neutrophil and macrophage recruitment in the lung. | CD86, CD81, LAMP1, LAMP2, MHC II | [70] |
| IL-6, IL-8 | Upregulation, Upregulation | Placenta samples | The human acute monocytic leukemia cell line THP-1 | Maternal inflammation and infection model | Macrophage exosomes induced release of proinflammatory cytokines by the placenta, facilitating responses to maternal inflammation and infection, and thereby preventing harm to the fetus. | Alix, CD81 | [86] |
| TNF-α | Upregulation | Balb/c mice | Alveolar macrophage | Acute lung injury model | Macrophage-derived exosomes in BALF were the major secretors for early pro-inflammatory cytokines, which might activate neutrophils to produce a variety of pro-inflammatory cytokines and IL-10 | TSG101, Alix, CD81, CD63, CD9 | [65] |
| *Proteins* | | | | | | | |
| OmpA, GroL1, DegP, CirA, FepA | Not reported | Macrophages | Mouse macrophage line RAW 264.7 | Bacterial infections model (Live Escherichia coli) | Exosomes from macrophages infected with live Escherichia coli can induce the secretion of proinflammatory factors by uninfected macrophages. | Integrin-β1, HSP90, CD63, CD9 | [82] |
| L-plastin, coronin-like protein, pyruvate kinase, actin-related protein 3 (Arp3), HSP90β, vimentin | Downregulation, Downregulation, Downregulation, Upregulation, Downregulation, Upregulation | Macrophages | U937 human monocytic cell line | Exposure to calcium oxalate monohydrate (COM) crystals model | Exosomes treated with calcium oxalate monohydrate crystals induced production of monocyte proinflammatory cytokine IL-8, accompanied by six significantly altered proteins | HSP70, Rab5, Rab7 | [89] |

(*Continued*)


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**Table 1** (Continued).

| M-Exos-Biomarkers | Expression Levels | Animal Type/ Cell Type | Exosomes Source | Disease Model | Possible Biological Effects | Exosomes Surface Markers | Reference |
|---|---|---|---|---|---|---|---|
| L-lactate dehydrogenase B chain, 14–3-3 protein beta/alpha, 14-3-3 protein epsilon, Amyloid-like protein 2, Annexin A2, Annexin A5, CD44 antigen, CD82 antigen, Filamin-A, HSP 90-beta, Immunoglobulin epsilon receptor subunit gamma, Integrin beta-2, Osteopontin, Plasminogen activator inhibitor 2, Plastin-2, Prelamin-A/C, Protein S100-A6, Tropomyosin alpha-3 chain, Tropomyosin alpha-4 chain | Downregulation, Downregulation, Downregulation, Downregulation, Upregulation, Downregulation, Downregulation, Not reported, Downregulation, Upregulation, Downregulation, Upregulation, Upregulation, Upregulation, Downregulation, Downregulation, Upregulation, Downregulation, Downregulation | Madin-Darby canine kidney (MDCK) cell line | U937 human monocytic cell line | Progressive inflammation in kidney stone disease model (Inflammatory response to calcium oxalate monohydrate crystals) | Exosomes derived from COM-treated macrophages released intravesicular contents into the renal interstitium and triggered renal tubular cells to secrete greater level of IL-8, which induced neutrophil migration into the renal interstitium, thereby worsening tissue inflammation. | Rab5, HSP90 | [90] |
| IL-1β, iNOS | Upregulation, Upregulation | C57BL/6 mice | Mouse macrophage line Raw264.7 | High glucose-treated model | High glucose-treated macrophage-derived exosomes could regulate NF-κB p65 signaling pathway to induce the activation of M1 macrophages and increase the release of inflammatory cytokines, leading to the kidney damage and inflammation | CD63, TSG101 | [84] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| TLR-4,<br>MYD88,<br>IκBα,<br>the p65 subunit [P65(N)] of NF-κB,<br>TNF-α,<br>IL-1β | Upregulation,<br>Upregulation,<br>Downregulation,<br>Upregulation,<br>Upregulation,<br>Upregulation | C57BL/6 mice | The human acute monocytic leukemia cell line THP-1 exposed to PM2.5 | Lung inflammation model | Exosomes derived from PM2.5-treated macrophages could activate the TLR-4-NF-κB signaling pathway to release inflammatory cytokine and alter the inflammatory phenotype of recipients, thereby inducing the inflammatory responses of lung in mice. | CD63,<br>TSG101 | [83] |
| NLRP3 | Upregulation | C57BL/6 mice | Mouse macrophage line Raw264.7 treated by high glucose (HG) | Diabetic nephropathy (DN) model | High glucose-treated macrophage-derived exosomes increased the inflammatory levels and accelerated kidney injury by promoting activation of NLRP3 inflammasome and autophagy deficiency of mesangial cells in diabetic nephropathy. | TSG101,<br>CD63, CD9 | [85] |
| Others | | | | | | | |
| glycopeptidolipids (GPLs) | Upregulation | Macrophages | Mycobacterium avium-infected macrophages (The mouse macrophage cell line J774) | Bacterial infections model (Mycobacterium avium-infection) | Exosomes released from infected macrophages contain mycobacterium avium glycopeptidolipids could induce a proinflammatory response, which is dependent on TLR2, TLR4, and MyD88. | LAMP1,<br>LAMP2,<br>MHCI,<br>MHCII,<br>CD81,<br>CD86,<br>HSP70 | [81] |
| Reactive oxygen species (ROS) | Upregulation | C57BL/6 WT mice and gp91$^{phox-/-}$ mice | Alveolar macrophage | Hemorrhagic shock (HS) model (Post-hemorrhagic shock lung inflammation) | Exosomes released from alveolar macrophage activated by hemorrhagic shock mainly induced NADPH oxidase-derived ROS production inside PMNs and promoted necroptosis, which exacerbated post-HS lung inflammation | CD63 | [91] |
| Functional enzymes for leukotriene biosynthesis | Upregulation | Macrophages | Human monocyte-derived macrophage | Not reported | Exosomes from macrophages can contribute to inflammation by participation in LT biosynthesis and granulocyte recruitment. | CD63,<br>CD81,<br>CD86,<br>HLA-DR | [92] |


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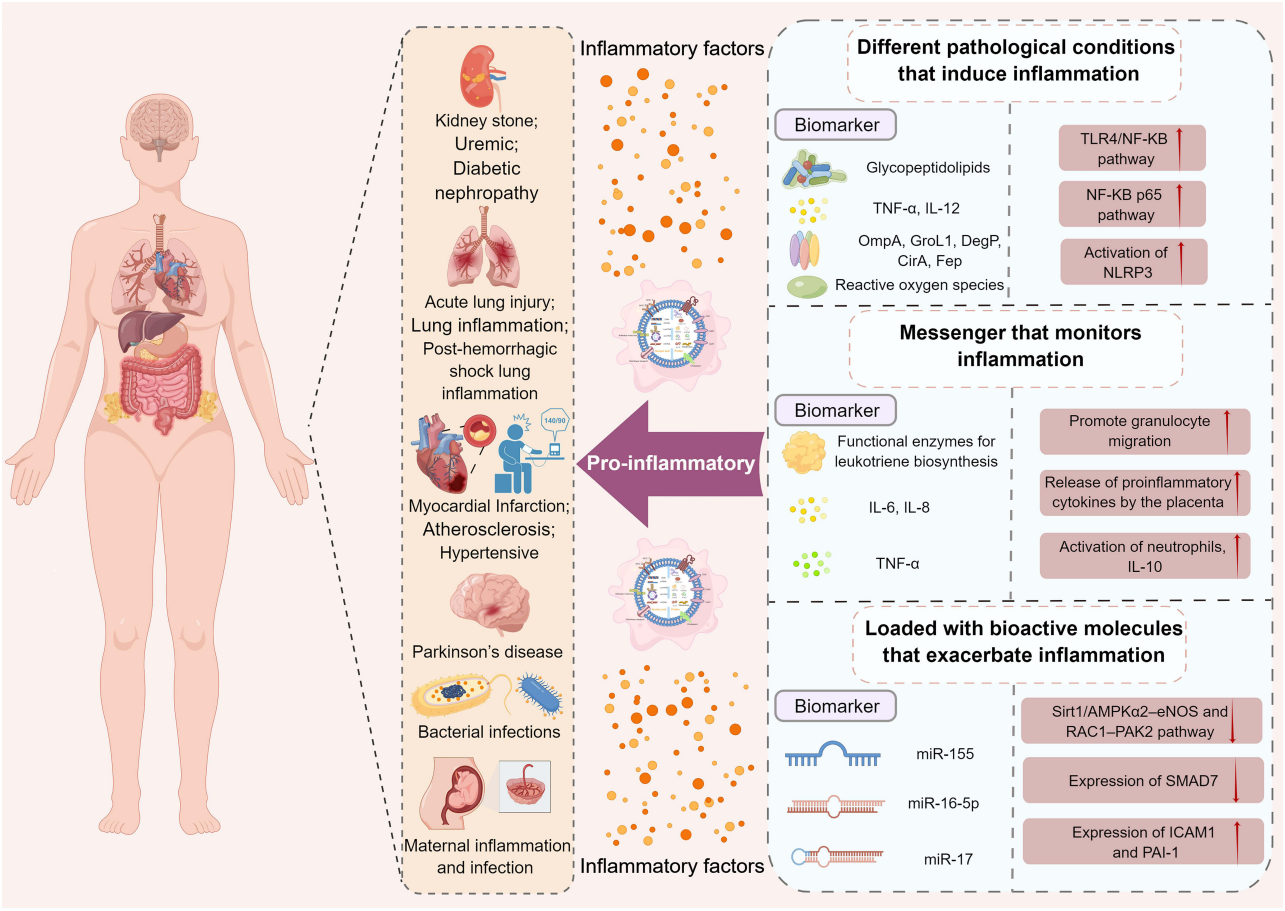


**Figure 5** Macrophage-derived exosomes-related biomarkers from recent studies as useful references for the detection of inflammation and its associated diseases. By Figdraw.

# Advanced Therapeutic Strategies for M-Exos to Improve Inflammation in Diseases

## Immunomodulatory and Anti-Inflammatory Effects of M-Exos in Inflammation

The infiltration of inflammatory cells has important functions in various diseases, the modulation of immune function may help delay inflammation progression.[93] Conventional approaches adopt inhibiting inflammatory cytokines and driving the conversion of dominant phenotype from M1 to M2 to ameliorate inflammation.[94–96] A study has shown that M-Exos could possess anti-inflammatory properties by suppressing the release of pro-inflammatory enzymes and cytokines. Additionally, M-Exos were shown to promote diabetic wound healing by speeding up the process of angiogenesis and re-epithelialization.[97] Noteworthy, besides the mentioned inhibition of inflammation methods, the present study found that peripheral M-Exos (PM-Exos) triggered microglial autophagy by suppressing PI3K/AKT/mTOR signaling pathway, promoted the polarization of anti-inflammatory type microglia and stimulated the anti-inflammatory properties. This also suggested that PM-Exos possess immense potential in promoting repair after spinal cord injury.[98] Nevertheless, clinical applications of unmodified M-Exos are limited due to insufficient targeting capabilities (Supplementary Table 1).

## Engineered M-Exos as Therapeutic Agents for Inflammation

Although M-Exos have shown certain anti-inflammatory effects, previous studies indicated that the therapeutic efficiency of exosomes might be restricted by some intrinsic limitations, including short half-life, low specific organ retention, low targeting efficiency, and low production yield.[99] Hence, M-Exos must be functionalized using advanced engineering

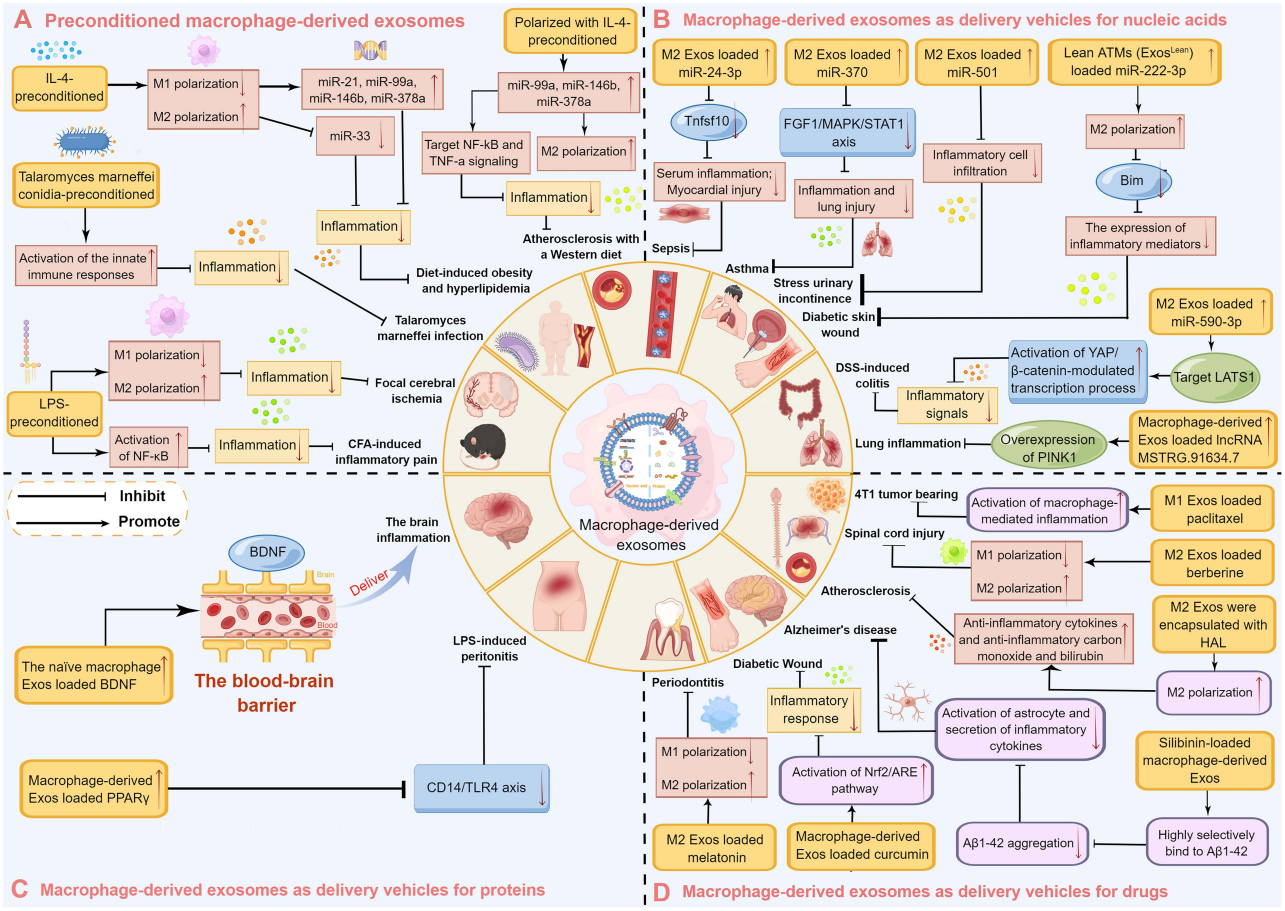


**Figure 6** Advanced therapeutic strategies for engineering macrophage-derived exosomes to improve inflammation in diseases. By Figdraw.
**Notes**: Arrows indicate activation or induction, and T-bars indicate inhibition. (**A**) Macrophage cells can be preconditioned with pathological stimuli to generate M-Exos with desirable profiles that are beneficial for relieving inflammation. In addition to the mentioned M-Exos precondition methods, the anti-inflammatory capacity of M-Exos can also be increased by preconditioning with proinflammatory cytokines. (**B**–**D**) The purposeful design of engineered exosomes make exosomes an ideal functional carrier for delivering nucleic acids, proteins and drugs to disease lesions.

techniques and carry out the targeted modification of exosomes to address these issues and increase their potency and efficiency as inflammation therapeutics. In the following sections, we give a brief overview of the present modification methods and efficacy of engineered M-Exos in different inflammation and related diseases models (Supplementary Table 2 and Figure 6).

## Preconditioned M-Exos for Inflammation Therapy

Preconditioned M-Exos can be utilized for the treatment of inflammation. In general, macrophage cells can be preconditioned with pathological stimuli to generate M-Exos with desirable profiles that are beneficial for relieving inflammation (Supplementary Table 2). A previous study demonstrated that the stimulation of RAW 264.7 cells with LPS increased the levels of inflammatory chemokines and RNAs in exosomes. The NF-κB signaling cascade was activated, causing a shift towards anti-inflammatory gene transcription later in inflammation progression.[100] Lipopolysaccharides (LPS)-stimulated M-Exos attenuated paw swelling and thermal hyperalgesia associated with complete Freund's adjuvant (CFA)-induced inflammatory pain.[101] Similarly, Zheng et al found that LPS-stimulated M-Exos prevented cerebral ischemia via shifting microglial functional polarity from M1 toward an anti-inflammatory M2 phenotype, which revealed the anti-inflammatory and neuroprotection effects of LPS-Ex.[102] Additionally, a recent study revealed that Talaromyces marneffei infected M-exos would trigger the innate immune responses to control inflammation, which were mainly manifested in promoting the activation of ERK1/2 and autophagy, the release of IL-10 and TNF-α in human macrophages.[103]

In addition to the mentioned M-Exos precondition methods, the anti-inflammatory capacity of M-Exos can also be increased by preconditioning with anti-inflammatory cytokines. For instance, IL-4 polarized human M-Exos (THP1-IL4-exo) promoted macrophages towards the anti-inflammatory phenotype and reprogramed energy metabolism via upregulating miR-21/99a/146b/378a while suppressing miR-33. Besides this, the above intervention also might reprogramme the inflammatory signaling of circulating Ly6Chi monocytes.[104] Bouchareychas et al indicated that exosomes secreted from naive bone marrow-derived macrophages (BMDM) contained anti-inflammatory miR-99a/146b/378a, which was upregulated in the case of BMDM polarized with IL-4. These exosomal microRNAs inhibited inflammation via targeting NF-kB and TNF-α signaling and promoted M2 polarization in recipient macrophages. Moreover, this study has suggested that exosomes secreted from IL-4-induced M2-like macrophages could be used to improve inflammatory diseases such as atherosclerosis in mice.[105] As a result, rather than adopting sophisticated engineering methods, the application of exosomes derived from these preconditioned macrophages may offer a simple route for the treatment of inflammation.

## M-Exos as Delivery Vehicles for the Treatment of Inflammation

Exosomes are highly complex vesicles, there are many physiological and pathological functions involved in the molecular information of each exosome. As their bilayer structure and the capacity of cargo transfer, it is possible for exosomes to serve as natural carriers of therapeutic agents and prevent them from degradation in the body.[106–108] Currently, techniques for loading cargoes into exosomes can be divided into endogenous loading including genetic modification of exosome donor cells, and exogenous loading including coincubation, electroporation, sonication of drugs with isolated exosomes.[109–113] M-Exos can be loaded with a wide variety of therapeutics, including nucleic acids, proteins, and drugs. Engineered M-Exos with promoted therapeutic properties and organ targeting efficacy can be gained from gene-modified donor cells, such as the overexpression or knockdown of gene or protein. For example, after septic mice were injected miR-24-3p-modified M2-Exos or siRNA of Tnfsf10, the levels of miR-24-3p decreased and the levels of Tnfsf10 increased in the myocardium. In short, M2-Exos-derived miR-24-3p could improve cardiac function and reduce serum inflammation in the myocardial tissue through reducing Tnfsf10 levels.[73] Li et al have reported a strategy that M2-Exos could relieve lung injury and inflammation, and delay asthma progression through carrying miR-370 and inhibiting the FGF1/MAPK/STAT1 axis.[74] And the latest research proved that lean adipose tissue M-Exos induced M2 polarization in vivo and in vitro, upregulated miR-222-3p level and decreased the expression of Bim to inhibit the levels of inflammatory mediators and accelerate diabetic wound healing. This also implied that the exosomes had a positive regulatory role in macrophage polarization.[114] A previous study demonstrated that M2-Exos improved inflammatory cell infiltration and contributed to the recovery of damaged pubococcygeus muscle in stress urinary incontinence models, in which M2-Exos miR-501 might have the potential as a therapeutic drug target for treating diseases caused by muscle injury.[115] Similarly, recent studies also have shown that a possible mechanism by which M2-Exos promote osteoprotection may be through the transport of IL-10 mRNA to BMSCs and BMDM, thereby upregulating the expression of the IL-10 cytokine. Subsequently, activation of the IL-10/IL-10R pathway modulated cell differentiation and prevented bone loss in murine periodontitis. The findings provided a novel therapeutic strategy for periodontitis.[116] In another study, in the early stage of inflammation, M1-Exos may promote osteogenesis in BMSCs via miR-21a-5p. This study contributes to the understanding of the regulatory role of M1-Exos miRNAs on BMSCs and has tremendous potential in designing effective strategies to improve regenerative outcomes and optimize fracture treatment.[117] Moreover, genetic modifications can enhance the targeting capability of M-Exos. A recent study showed that M2-Exos miR-590-3p attenuated DSS-induced mucosal injury and reduced inflammation via regulating LATS1 and the transcription of YAP/β-catenin. This discovery would potentially open up new avenues for treating ulcerative colitis in clinical practice.[118] LncRNA is a major component transported by exosomes in many inflammatory diseases. A research result on the pathogenesis of silicosis illustrated that M-Exos lncRNA MSTRG.91634.7 inhibited fibroblast activation by targeting PINK1, and overexpression of PINK1 suppressed silica-induced lung inflammation and fibrosis in mice. This also revealed that the modification of M-Exos targeted genes played a major role in the regulation of inflammation.[119] In summary, these studies suggest that genetic engineering is an effective approach for improving the targeting function and therapeutic effects of M-Exos.

Proteins, as important regulatory molecules, act as a pivotal function in the treatment of inflammation. Growing evidence has demonstrated that genetically engineered M-Exos containing therapeutic proteins were used for treating

inflammation. For instance, Yuan et al further proved that following intravenous administration, naïve macrophage (Mf) exosomes could across the blood-brain barrier and deliver the cargo protein of brain-derived neurotrophic factor (BDNF) to the inflamed brain. To summarize, these findings have important implications for utilizing Mf-derived exosomes as natural nanocarriers for delivering therapeutic proteins to the brain to treat diseases of the central nervous system.[71] Noteworthy, Meng et al demonstrated that exosomal PPARγ derived from macrophages could serve as a mediator of intercellular communication to inhibit LPS-induced peritonitis by negatively modulating the CD14/TLR4 axis.[72]

In addition, M-Exos can also be used for delivering drugs to improve the therapeutic index. Wang et al used M1-Exos as Paclitaxel (PTX) carriers to the preparation of a nano-formulation (PTX-M1-Exos), and subsequently used the sonication method to formulate a drug delivery system. They found that M1-Exos could boost the antitumor activity of paclitaxel through triggering macrophage-mediated inflammation.[120] Recent studies have demonstrated that M2-Exos were encapsulated with an FDA-approved hexyl 5-aminolevulinate hydrochloride (HAL) by using electroporation technology. After systemic administration, the engineered M2-Exos exerted inflammatory tropism and anti-inflammatory effects through surface-bound chemokine receptors as well as released anti-inflammatory cytokines from anti-inflammatory M2 macrophages. Additionally, the encapsulated HAL could promote the production of anti-inflammatory carbon monoxide and bilirubin to improve the anti-inflammatory ability and relieve atherosclerosis, which would open an expanding new avenue for atherosclerosis therapeutics.[121] Researchers designed M2-type primary peritoneal macrophages exosomes as a drug carrier for berberine (Exos-Ber), which could deliver the drug to the injured spinal cord across the BBB. Exos-Ber could suppress iNOS and increase CD206 to diminish inflammation such as TNF-α, IL-1β via inducing the polarization of macrophages/ microglia from M1 phenotype to M2 phenotype. Overall, Exos-Ber is a promising anti-inflammatory strategy for spinal cord injury.[75] Similarly, Li et al have reported a strategy that curcumin-loaded M-Exos (Exos-cur) could not only reduce ROS in vitro, regulate mitochondrial membrane potential, and alleviate inflammation, but also suppress the inflammatory response via activating the Nrf2/ARE pathway, promote angiogenesis, and accelerate wound healing process of diabetic rats in vivo.[76] Also, engineered M2- exosomes loading with melatonin (Mel@M2-exos) could target inflammatory regions and lead to a macrophage reprogramming from M1 to M2 type, which relieved chronic inflammation and improved periodontitis. This confirms that Mel@M2-exos is a promising treatment for periodontitis.[77] Innovatively, Huo et al designed biomimetic silibinin-loaded M-Exos (Exos-Slb), which could highly selectively bind to Aβ1-42 and inhibit Aβ1-42 aggregation to diminish astrocyte activation and inhibit secretion of inflammatory cytokines. This also inspired researchers to use Exos-Slb as an innovative strategy for multifunctional drug delivery systems for AD treatment in the future.[78]

Taken together, the limitations of exosomes such as low targeting efficiency, and low concentration of functional molecules will be solved through the purposeful design of engineered exosomes, which will make exosomes an ideal functional carrier for delivering genetic materials and drugs to disease lesions (Tables 2, 3 and Figure 6).[99]

## Different Loading Capacities of M-Exos in Nucleic Acids, Proteins and Drugs

Currently, there are many methods for loading nucleic acids, proteins, and drugs into exosomes, including endogenous loading and exogenous loading.[122] Among them, miRNA is generally loaded into exosomes by chemical transfection, fusion expression, and electroporation. mRNA is generally loaded into exosomes by cell overexpression, electroporation, fusion expression, and nanoporation, and proteins are mostly loaded into exosomes by electroporation, chemical transfection, ultrasound, fusion expression, and other methods for loading exosomes, and for drugs, exosome loading methods include electroporation, co-incubation, ultrasound and other methods. Various substances have different loading strategies depending on the purpose of delivery.[123,124] This review summarizes the loading methods of M-Exos in nucleic acids, proteins, and drugs. The loading of nucleic acids and proteins mainly relies on transfection methods, while the loading methods of drugs are mainly ultrasound, electroporation, and co-incubation. Among them, the loading capacity of M-Exos in nucleic acids is mostly not clearly defined. It only describes the injection volume of exosomes containing relevant nucleic acids, while the loading capacity of M-Exos in proteins and drugs is clearly described. Therefore, the specific loading of miRNA, mRNA, etc. in exosomes requires further exploration of quantifiable objective indicators as a loading reference. Since drugs have pharmacokinetics, drug uptake time experiments, and a large number of basic and clinical studies on various packaging materials, their loading capacity and loading efficiency already have a mature

**Table 2** M-Exos as Engineered Nucleic Acids/Protein Delivery Vehicles for the Treatment of Inflammation

| Exosomes Source | Animal Type/Cell Type | Disease Model | Modification Method | Route of Administration | Exosomes Dose | Isolation Method | Therapeutic Effects | Mechanism of Action | Exosomes Surface Markers | References |
|---|---|---|---|---|---|---|---|---|---|---|
| M2 macrophages from the peritoneal lavage fluid (Primary cell) | C57BL/6 mice | Sepsis models | Loaded miR-24-3p; Lentivirus transfection | Tail vein injection | 0.4 pmol/μL; Not reported (NR) | UC | Serum inflammation↓<br>Cardiomyocyte apoptosis in the myocardial tissue↓<br>Myocardial injury↓ | Expression of miR-24-3p↑<br>Expression of Tnfsf10↓ | TSG101, CD63, CD81 | [73] |
| M2 macrophage from the bone marrow cells (Primary cell) | BALB/c mice | Asthma models | Loaded miR-370; Lentivirus transfection | NR | 20 μg; continuous 3 days | UC | Inflammation↓<br>Lung injury↓<br>Asthma progression↓ | Expression of miR-370↑<br>Expression of the FGF1/MAPK/STAT1 axis↓ | CD9; CD63; TSG101 | [74] |
| Lean adipose tissue macrophage isolated from mouse visceral adipose tissues | B6.BKS(D)-Leprdb/J (db/db) mice | The diabetic skin wound models | Loaded miR-222-3p; Transfection | Subcutaneous injection | 100 μg per 100 μL of ExosLean was injected at 4 injection sites (25 μL per site) around the wounds.; Once a day for 5 days | Exosome quick extraction solution | M2 macrophages polarization↑<br>The expression of inflammatory mediators↓<br>Promote diabetic wound healing↑ | Expression of miR-222-3p↑<br>Expression of Bim↓<br>Expression of TNF-α, CD86↓<br>Expression of CD206↑ | HSP70, TSG101, CD63 | [114] |
| M2 macrophages | C57BL/6J mice | Stress urinary incontinence models | Loaded miR-501; Transfection | Locally injected around the pubococcygeal muscle | 5ug/mL (in vitro); Once | UC | Inflammatory cell infiltration↓<br>Therapeutic effect on damaged pubococcygeal muscle↑<br>Pubococcygeal muscle regeneration↑ | Expression of miR-501↑<br>Expression of YY1↓ | CD9; TSG101 | [115] |
| Reparative M2-like macrophages from bone marrow derived macrophage (BMDM) (Primary cell) | C57BL/6 mice | Periodontal disease model | Loaded IL-10 mRNA | Locally injected | 100ug/mL; Once | UC | Bone loss in murine periodontitis models↓<br>Osteogenesis↑Osteoclastogenesis↓ | Expression of IL-10 cytokines↑<br>Activation of the cellular IL-10/IL-10R pathway↑ | TSG101; CD9 | [116] |
| RAW264.7 cells | Primary murine bone mesenchymal stem cells (BMSCs) | Bone defect model | Loaded miR-21a-5p; Lentiviral transfection | Add to the osteogenic medium used to culture BMSC | 1 μg/mL; Once | The complete exosome isolation reagent (Invitrogen, Carlsbad, CA, United States) | Osteogenesis of BMSCs at the early stage of inflammation↑ | Expression of miR-21a-5p↑ | CD63; CD81 | [117] |

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|---|---|---|---|---|---|---|---|---|---|---|
| The human monocytic cell line (THP-1) | C57BL/6 mice | Dextran sodium sulfate (DSS)-induced colitis model | Loaded miR-590-3p; Transfection | Intraperitoneal injection | 50 μg/mouse; Four times | UC | Inflammatory signals↓<br>DSS-induced mucosal damage↓<br>Epithelial repair↑ | Expression of miR-590-3p↑<br>Expression of pro-inflammatory cytokines including TNF-α, IL-1β, and IL-6↓<br>Expression of LATS1↓<br>Activation of YAP/β-catenin-modulated transcription process↑ | CD63; CD81 | [118] |
| The human monocytic cell line (THP-1) | C57BL/6 mice | Silicosis model | Loaded lncRNA MSTRG.91634.7; Adenovirus transfection | NR | NR | Blood Pure ExoSolution reagent | Lung inflammation and fibrosis by overexpressing PINK1↓<br>Fibroblast's activation↓ | Expression of lncRNA MSTRG.91634.7↑<br>Expression of PINK1 of the target gene of lncRNA MSTRG.91634.7↑ | Alix, CD9, CD63 | [119] |
| RAW264.7 cells | CD-1 mice | The brain inflammation model | Loaded BDNF; Mix exosomes and BDNF in 10 mM phosphate buffer (pH 7.4). The binding of BDNF (isoelectric point 9.99) with the negatively charged exosomes (zeta potential −18 mV) by electrostatic and polysaccharide interactions formed a BDNF-exosome complex (ExoBDNF) | Jugular vein injection | 0.538 ± 0.315%ID/g brain in inflamed brain; Once | UC | Brain inflammation↓ | Expression of exosome-formulated BDNF (ExoBDNF) in the brain-inflamed mice↑ | Alix, TSG101 | [71] |
| RAW264.7 cells | BALB/C mice | LPS-induced peritonitis model | Loaded PPARγ; Electrotransfection | Intraperitoneal injection | 3 mg/kg; 24h | UC | LPS-induced peritonitis↓ | Expression of PPARγ↑<br>Expression of the CD14/TLR4 axis↓<br>Expressions of IL-6, TNF-α↓ | CD63, HSP90 | [72] |


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**Table 3** M-Exos as Drug Delivery Vehicles for the Treatment of Inflammation

| Exosomes Source | Animal Type/ Cell Type | Disease Model | Modification Method | Route of Administration | Exosomes Dose | Isolation Method | Therapeutic Effects | Mechanism of Action | Exosomes Surface Markers | References |
|---|---|---|---|---|---|---|---|---|---|---|
| RAW264.7 cells | BALB/c mice | 4T1 tumor bearing model | Paclitaxel-M1-exosomes nano-formulation (PTX-M1-Exos); Slight sonication method | Intravenous injection | 5 mg/kg; every 3 days. | UC | The polarization of macrophages (M1-Exos up-regulated the expression of inflammatory cytokines iNOS, IL-6, and IL-12 and down-regulated the expression levels of IL-4 and IL-10. M2-Exos down-regulated the expression levels of IL6, TNF-α, IL-12 and up-regulated the expression of IL4 and IL-10 M2 phenotypes of macrophages)↑ Anti-tumor effects↑ | Expression of caspase-3↑ Expression of pro-inflammatory Th1-type cytokine↑ | TSG101, CD63, Alix | [120] |
| RAW264.7 cells | ApoE deficient ($ApoE^{-/-}$) mice | Atherosclerosis model | M2 macrophage-derived exosomes (M2 Exo) were encapsulated with an FDA-approved hexyl 5-aminolevulinate hydrochloride (HAL); Electroporation | Intravenous injection | 5.5 mg/kg HAL-equivalent and 9 mg/kg M2-equivalent; Five weeks | UC | Anti-inflammatory carbon monoxide and bilirubin↑ Anti-inflammation effects↑ M2 macrophages polarization↑ Atherosclerosis↓ | Expression of pro-inflammatory cytokines including MMP-10, IL-6, TNF-α and interleukine-1β (IL-1β) ↓ | CD63 | [121] |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| M2-type primary peritoneal macrophages (Primary cell) | C57BL/6J mice | Spinal cord injury (SCI) model | M2-type primary peritoneal macrophages exosomes loaded berberine (Exos-Ber);<br>Ultrasonic method and the incubation drug loading method | Tail vein injection | 5 mg/kg;<br>Treat at the same time point every day for 7 days | UC | Anti-inflammatory effect↑<br>Macrophages/ microglia M1 polarization↓<br>Macrophages/ microglia M2 polarization↑<br>Anti-apoptotic effect↑<br>The motor function↑ | Expression of the M1 protein marker iNOS↓<br>Expression of the M2 protein marker CD206↑<br>Expression of inflammatory and apoptotic cytokines (TNF-α, IL-1 β, IL-6, Caspase 9, Caspase 8)↓ | CD9;<br>CD63;<br>TSG101 | [75] |
| RAW264.7 cells | Sprague −Dawley rats | Diabetic Wounds model | Exosomes derived from macrophages loaded with curcumin (Exos-cur);<br>40 μg/mL curcumin was added to the culture medium of RAW264.7 cells, and then the cells were cultured under this condition for 24 h. Exos-cur were then isolated by ultracentrifugation | Subcutaneous injection | 200 μL (curcumin content in 200 μL of Exos-cur is 20 μg/mL);<br>Once a day at the same time point for 21 days | UC | Inflammatory response↓<br>The production of ROS↓<br>Improve the mitochondrial membrane potential↑<br>Cell oxidative damage↓<br>Oxidative stress↓<br>Promote angiogenesis and wound healing process↑ | Activation of Nrf2/ARE pathway↑<br>Expression of TNF-α, IL-6, IL-1β, and MMP-9↓ | CD81, CD9, TSG101, Alix | [76] |

(*Continued*)


https://doi.org/10.2147/IJN.S449388

**Table 3** (Continued).

| Exosomes Source | Animal Type/ Cell Type | Disease Model | Modification Method | Route of Administration | Exosomes Dose | Isolation Method | Therapeutic Effects | Mechanism of Action | Exosomes Surface Markers | References |
|---|---|---|---|---|---|---|---|---|---|---|
| RAW264.7 cells | SD rats | Periodontitis model | Melatonin-loaded engineered M2 macrophage-derived exosomes (Mel@M2-exos); Cyclic ultrasonication | Topical injection | 200 μL; Every two days for 2 weeks | UC | Improved periodontitis↑ Promote macrophage repolarization↑ The excessive endoplasmic reticulum stress (ER stress) and unfolded protein response (UPR)↓ Promote periodontal regeneration↑ | Expression of CD86↓ Expression of CD206↑ | HSP70, CD63, TSG101 | [77] |
| RAW264.7 cells | C57BL/6 mice | Alzheimer's disease (AD) model | Silibinin-loaded macrophage-derived exosomes (Exo-Slb); Add silibinin (Slb) to macrophages and incubate for 24 h, and obtain Exo-Slb suspension by ultracentrifugation | Tail vein injection | 100uL; Once a day for 7 consecutive days | UC | Astrocyte activation↓ the secretion of inflammatory cytokines↓ Astrocyte inflammation-mediated neuronal damage↓ Improvement of cognitive deficits↑ | Aβ aggregation↓ Inflammation-mediated activation of the NF-κB pathway↓ Expression of proinflammatory cytokines TNF-α, IL-6 and IL-1β↓ NF-κB pathway-related proteins including P-P65/ P65 and P-IκBα/ IκBα↓ | Alix; CD63 | [78] |

**Table 4** M-Exos Loading Capacity for Nucleic Acids/Proteins

| Exosomes Source | Disease Model | Exosome Cargo | Loading Method | Loading Capacity | References |
|---|---|---|---|---|---|
| M2 macrophages from the peritoneal lavage fluid (Primary cell) | Sepsis models | miR-24-3p | Lentivirus transfection | Not reported | [73] |
| M2 macrophage from the bone marrow cells (Primary cell) | Asthma models | miR-370 | Lentivirus transfection | Not reported | [74] |
| Lean adipose tissue macrophage isolated from mouse visceral adipose tissues | The diabetic skin wound models | miR-222-3p | Transfection | Not reported | [114] |
| M2 macrophages | Stress urinary incontinence models | miR-501 | Transfection | Not reported | [115] |
| Reparative M2-like macrophages from bone marrow derived macrophage (BMDM) (Primary cell) | Periodontal disease model | IL-10 mRNA | Not reported | Not reported | [116] |
| RAW264.7 cells | Bone defect model | miR-21a-5p | Lentiviral transfection | Not reported | [117] |
| The human monocytic cell line (THP-1) | Dextran sodium sulfate (DSS)-induced colitis model | miR-590-3p | Transfection | 50 μg/mouse | [118] |
| The human monocytic cell line (THP-1) | Silicosis model | lncRNA MSTRG.91634.7 | Adenovirus transfection | Not reported | [119] |
| RAW264.7 cells | The brain inflammation model | BDNF | Mix exosomes and BDNF in 10 mM phosphate buffer (pH 7.4). The binding of BDNF (isoelectric point 9.99) with the negatively charged exosomes (zeta potential −18 mV) by electrostatic and polysaccharide interactions formed a BDNF-exosome complex (ExoBDNF) | 0.038%ID/g (the percentage of injected dose per gram of brain in the parenchyma fractions) | [71] |
| RAW264.7 cells | LPS-induced peritonitis model | PPARγ | Electrotransfection | 3 mg/kg | [72] |

research basis for reference. Overall, in the future, more in-depth and refined experimental quantitative studies should be carried out for evaluation, and efficiency comparisons between different loading methods should be carried out to provide data reference for the specific loading amounts and loading efficiencies of miRNA and mRNA (Tables 4 and 5).

## Combined Strategies of M-Exos-Biomaterials for Treatment of Inflammation

In addition to the mentioned engineered M-Exos modification methods, current evidence indicates that functional biomaterials have been applied in innovative strategies of M-Exos to improve inflammation and related diseases. Combination of such combined strategies may be a prospective strategy for advanced anti-inflammatory therapy. For example, the study illustrated that M1-Exos might be served as vaccine adjuvants and enhance cancer vaccine via creating a pro-inflammatory microenvironment in the lymph nodes.[125] In another study, the researchers encapsulated a plasmid DNA encoding the anti-inflammatory cytokine interleukin 10 (IL-10 pDNA) and the chemotherapeutic drug betamethasone sodium phosphate (BSP) into the biomimetic vector M2-Exos. The co-delivery system of M2-Exos

**Table 5** M-Exos Loading Capacity for Drugs

| Exosomes Source | Disease Model | Exosome Cargo | Loading Method | Loading Capacity | References |
|---|---|---|---|---|---|
| RAW264.7 cells | 4T1 tumor bearing model | Paclitaxel | Slight sonication method | 5 mg/kg | [120] |
| RAW264.7 cells | Atherosclerosis model | FDA-approved hexyl 5-aminolevulinate hydrochloride (HAL) | Electroporation | 25.14% | [121] |
| M2-type primary peritoneal macrophages (Primary cell) | Spinal cord injury (SCI) model | Berberine | Ultrasonic method and the incubation drug loading method | 5 mg/kg | [75] |
| RAW264.7 cells | Diabetic Wounds model | Curcumin | 40 μg/mL curcumin was added to the culture medium of RAW264.7 cells, and then the cells were cultured under this condition for 24 h. Exos-cur were then isolated by ultracentrifugation | Each Exos-cur containing curcumin 1.5 μg/mL | [76] |
| RAW264.7 cells | Periodontitis model | Melatonin | Cyclic ultrasonication | 35.47% | [77] |
| RAW264.7 cells | Alzheimer's disease (AD) model | Silibinin | Add 50ug/mL silibinin (Slb) to macrophages and incubate for 24 h, and obtain Exo-Slb suspension by ultracentrifugation | 5 mg/mL | [78] |

/pDNA/BSP nanoparticles not only showed better targeting and anti-inflammatory properties, but also alleviated inflammation in joint lesions with the help of the synergistic effect of pDNA and BSP in rheumatoid arthritis (RA). Moreover, this delivery system showed negligible toxicity.[79] Similarly, Zhao et al developed novel hybrid exosome-mimic nanovesicles (HNV) with broad-spectrum anti-inflammatory function for RA treatment by fusing M1 macrophage membranes with M2-Exos-mimicking nanovesicles. The experimental results also demonstrated that the HNV loaded with black phosphorus targeted and accumulated at the inflamed knee joints, which exerted a multimodal rheumatoid arthritis treatment by comprehensively inhibiting inflammation combined with NIR irradiation.[80] Otherwise, Zeng et al designed a novel nanoagent (MEXI) for spinal cord injury (SCI) therapy through conjugating bioactive IKVAV peptides to the surface of M2-Exos by click chemistry method. The engineered exosomes could target the injured site of the spinal cord, and MEXI promoted motor function recovery in SCI mice via reducing macrophage infiltration, decreasing pro-inflammatory factors, and speeding regeneration of damaged nerve tissue.[126] Besides the mentioned exosome encapsulation methods, the M2-Exos-loaded photosensitive hydrogel microneedles (PMN)-based wound dressing system (M2-Exos@PMN) was designed to treat diabetic wounds. The experimental results showed that M2-Exos@PMN suppressed the inflammation and drove angiogenesis to promote diabetic wound healing with the combined action of M2-Exos and the photothermal effects produced by PMN, which was a promising cell-free approach.[127]

Collectively, these results suggest that biomaterial-based M-Exos engineering is an efficient approach for enhanced anti-inflammatory therapy. The combination of various engineering strategies can further enhance the anti-inflammatory potency of M-Exos-biomaterials therapies, which can improve bioavailability, targeting capability and potentiate therapeutic effect (Table 6 and Figure 7).

## M-Exos for Inflammation Therapy in Clinical Trials

To date, even though the number of related in vitro and animal studies is on the rise, and growing evidence has demonstrated that M-Exos are an effective anti-inflammatory strategy, few clinical trials have been conducted associated

**Table 6** Combined Strategies of M-Exos-Biomaterials for Treatment of Inflammation

| Exosomes Source | Animal Type | Disease Model | Combined Strategies | Route of Administration | Exosomes Dose | Isolation Method | Therapeutic Effects | Mechanism of Action | Exosomes Surface Markers | References |
|---|---|---|---|---|---|---|---|---|---|---|
| RAW264.7 cells | C57BL/6 mice | Melanoma model | Exosomes derived from M1-polarized macrophages can be used as a vaccine adjuvant of lipid calcium phosphate (LCP) nanoparticle-encapsulated Trp2 vaccine | Subcutaneous administration | Equivalent to 10 ug of protein; Once | UC | Create a local inflammatory milieu suitable for the Induction of a Th1 adaptive immune response↑<br>Enhance activity of LCP nanoparticle-encapsulated Trp2 vaccine↑<br>Induce a stronger antigen-specific cytotoxic T cell response↑ | The release of a pool of Th1 cytokines↑ | NR | [125] |
| RAW264.7 cells | DBA/1 mice | Collagen-induced arthritis model | The M2 Exo/pDNA/BSP co-delivery system;<br>Encapsulate a plasmid DNA encoding the anti-inflammatory cytokine interleukin 10 (IL-10 pDNA) and the chemotherapeutic drug betamethasone sodium phosphate (BSP) into the biomimetic vector M2 exosomes (M2 Exo) derived from M2- type macrophages | Intravenous injection | The dose of BSP was 0.4 mg/kg and the pDNA was 0.5 mg/kg; Once every three days for a total of five times | UC | Inflammation in joint lesions↓<br>Macrophage polarization from a pro-inflammatory to an anti-inflammatory state↑(M1 macrophages polarization↓ M2 macrophages polarization↑) | Expression of iNOS, IL-12, TNF-α, and IL-1β↓<br>Expression of Arg-1, CD206, IL-10 and IL-4↑ | TSG101; CD63 | [79] |
| RAW264.7 cells | DBA/1J mice | Collagen-induced arthritis model | Engineering of BP-loaded HNV (HNV@BP);<br>The hybrid nanovesicles (HNV) are prepared by fusing an M1 macrophage membrane into exosome-mimic nanovesicles extruded from M2 macrophages. Then an electroporation system is used to introduce black phosphorus nanosheets (BP) into the HNVs. | Intravenous injection | 0.2 mg $kg^{-1}$ HNV in 100 μL of PBS; Twice | UC | Inflammatory reaction↓<br>Anti-inflammation activity↑<br>The pathological symptoms of rheumatoid arthritis (RA) ↓ | Expression of TNF-α and IL-6↓ | NR | [80] |

(*Continued*)


https://doi.org/10.2147/IJN.S449388

**Table 6** (Continued).

| Exosomes Source | Animal Type | Disease Model | Combined Strategies | Route of Administration | Exosomes Dose | Isolation Method | Therapeutic Effects | Mechanism of Action | Exosomes Surface Markers | References |
|---|---|---|---|---|---|---|---|---|---|---|
| RAW264.7 cells | C57BL/6 mice | Spinal cord injury (SCI) model | A novel nanoagent (MEXI); Conjugate bioactive Acryl-Gly-Ill-Lys-Val-Ala-Val (IKVAV) peptides to the surface of M2 macrophage-derived exosomes by click chemistry method | Tail vein injection | $1.0\times10^{11}$ Particles/mL, 100 μL; Once | UC | Infiltration of macrophages↓<br>Pro-inflammatory factors↓<br>The regeneration of injured nervous tissues↑<br>Motor functional recovery of SCI↑<br>Repolarization of macrophages and differentiation of neural stem cells (NSCs)↑<br>M2 macrophages polarization↑ | Expression of pro-inflammatory cytokines TNF-α, IL-6 and IL-1β↓<br>Expression of anti-inflammatory cytokine IL-10↑<br>Expression level of M1-like macrophage marker iNOS↓<br>Expression level of M2-like macrophage marker Arg1↑ | TSG101; CD9 | [126] |
| RAW264.7 cells | Sprague Dawley rats | Diabetic wound model | The M2 macrophage-derived exosomes (MEs)-loaded photosensitive hydrogel microneedles (PMN)-based wound dressing system (MEs@PMN); Encapsulate MEs in the needle tips and polydopamine (PDA) nanoparticles in backing layer | Dressing application | 100 μg/mL; Two weeks | UC | Inflammation↓<br>M1 macrophages polarization↓<br>M2 macrophages polarization↑<br>Promote angiogenesis and diabetic wound healing↑ | Expression of TNF-α, iNOS↓<br>Expression of CD206↑ | TSG101, CD9 | [127] |

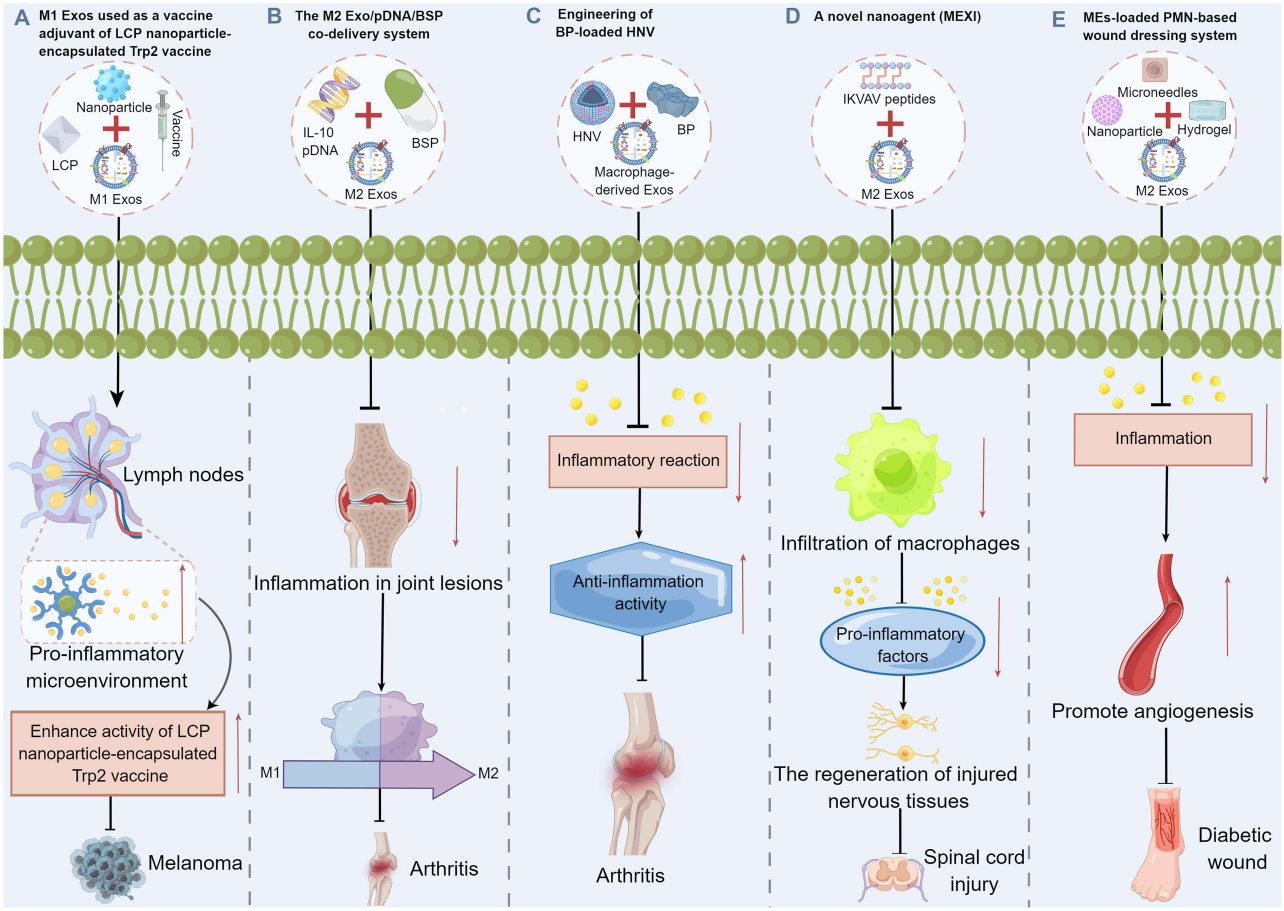


**Figure 7** Combined strategies of macrophage-derived exosomes-biomaterials for treatment of inflammation. By Figdraw.

with direct inflammation. The majority of them are still in the early stages, clinical evaluations of M-Exos remain limited, and the current state is still far from clinical applications. At present, there are extremely few previous studies regarding inflammation-related clinical trials. In a clinical study, twenty silicosis patients and twenty-nine health workers were recruited to collect peripheral blood to isolate serum exosomes. According to high-throughput sequencing, researchers found that the expression of M-Exos lncRNA MSTRG.91634.7 was reduced in silicosis patients. Subsequent animal experiments showed that the target protein PINK1 of lncRNA MSTRG.91634.7 was involved in inhibiting silica-induced lung inflammation in mice.[119] However, the study did not mention the blinding method of the clinical trial and the rigor of the experimental design, and the relevant clinical design still needs to be further improved. Overall, current evidence indicates that there are still some practical problems that needed to be taken into account and addressed before further translation of M-Exos into clinical applications: (1) The lack of standardized isolation methods, safety and dosage for clinical use. (2) Safe production and quality control criteria of M-Exos. (3) Feasible scheme for mass production of M-Exos. (4) The gold standard for the characterization and quantitation of M-Exos. (5) The Advanced technology need to be further optimized for the co-delivery of drugs and genes in clinics. (6) Implementation of standardized, large-scale, multicenter clinical trials. (7) Monitoring and evaluation of adverse reactions in patients.

## Conclusion and Future Perspectives

Inflammation is an alternating process, so it is important to consider the role of M-Exos from a dynamic point of view.[21] Recent studies provide evidences that the advantage of specific biomarkers of exosomes endow a method for the identification of specific diseases, and the heterogeneity of exosomes sizes and contents can reflect the state and types of source.[128] Hence, it can be seen that exosomes can provide a useful bank of biomarkers for diagnostics of a variety of

diseases.[129] Accumulating evidence indicates that M-Exos exhibit distinct genomic and proteomic features in inflammatory responses in different pathologic conditions such as bacterial infection, virus infection, and disease states. The nature of miRNAs, lncRNAs and circRNAs in exosomes determines the function of exosomes in inflammation and their role as biomarkers, which is helpful for evaluating and diagnosing the development of inflammation and inflammatory diseases, and exploring their specific pathogenesis.[130] In terms of functional therapeutic, M-Exos can be engineered for immunomodulation and generate content that contributes to inflammatory resolution. Moreover, due to the good biocompatibility, accurate targeting, low toxicity and immunogenicity of M-Exos, they can be used as delivery vehicles by virtue of advanced engineering techniques.[106,131] M-Exos are capable of selectively delivering proteins, nucleic acids and drugs to the site of inflammation through the interaction between their surface-antibody or modified ligand with cell surface receptors. This will increase the targeting effect and decrease the dose used, which will facilitate the translation of exosomes towards clinical application.[132] Although research on exosomes as carriers is in the early stages, the powerful targeted delivery ability of M-Exos has been well documented. All in all, engineered M-Exos are an emerging player in the field of inflammation and have great potential for diagnosis, therapy, and clinical translation.

Nevertheless, M-Exos still faces a number of challenges before it can be widely used in clinical practice. For future studies in this field, several directions may be helpful: (1) There is currently no standardized method for isolating exosomes to get high-purity and high-yield exosomes. And existing research progress shows that the methods for exosome separation, quantification and characterization vary greatly. Consequently, a unified protocol for exosome isolation, purification and storage needs to be further explored.[133,134] (2) Exosomes are unstable, tend to aggregate, and have a short half-life. Their long-term effects still need to be studied with the help of the continued evolving advanced technologies. (3) Controversy still exists in the dosage, measurement standards, and administration routes of exosomes. Limited pharmacokinetic research has been conducted on the utilization of M-Exos as vehicles for drug delivery. Thus, follow-up studies still need to clarify the specific distribution, survival time and metabolism of M-Exos in vivo through pharmacokinetic studies and toxicological studies. From the above, determining the optimal dose, time and route of injection about M-Exos is of great significance for enhancing clinical efficacy and minimizing the side effects.[135] (4) The difficulty in isolating subtypes of M-Exos, low drug loading efficiency, complicated preparation steps, and the low yield of specific exosome subtypes limits their application as targeted drug delivery systems. Strategies for isolating specific subtypes of M-Exos and methods for analyzing exosomal contents with high sensitivity are needed to identify the origin of specific exosome subtypes, thereby improving exosome production efficiency. (5) Most previous studies about inflammation have focused on the therapeutic effects of M-Exos on miRNAs, but proteins, lncRNAs, circRNAs and some other bioactive molecules may also participate in the function of exosomes. Therefore, more attention should be paid to other bioactive molecules present in M-Exos.[136] (6) Current research on the anti-inflammatory effects of M-Exos primarily relies on small animal models, such as mice or rats, with limited studies conducted on large animal models. Given the immense variation in genetics and physiology between humans and small animals, these models are unlikely to fully mimic the pathology of human patients.[137] As a result, future research requires standard and large animal models that better mimic human inflammation and related diseases in this field. (7) With regard to clinical application, drug delivery vehicles based on M-Exos can deliver targeted drugs to designated locations with low cost, minimal or no immune response or toxicity. However, in order to achieve the best clinical effect and safety, there are still some technical issues such as route of administration, injection rate, and frequency of transplantation that need to be resolved. Considering the above factors and concerns, regulators and stakeholders should collaborate to develop quality control (QC) standards for M-Exos and safety specifications for evaluating clinical trials and approving exosome-related products.[138] This is helpful for exosomes to shift from bench top science to clinical area, and advance the clinical diagnosis and treatment.[132,139,140] Besides this, more large-scale, multi-sample, multi-center, and long-term follow-up studies are needed to validate the effectiveness and safety of M-Exos in the treatment of clinical inflammation and related diseases. In the future, it can be foreseen that with the development of biotechnology and the significant improvement of emerging methodologies, M-Exos may achieve large-scale production with good stability. Consequently, this development will pave the way for novel prospects in clinical applications, specifically in the diagnosis and therapy of inflammation and related diseases (Supplementary Figure 1).

## Abbreviations

M-Exos, macrophage-derived exosomes; IBD, inflammatory bowel disease; M1, classically activated macrophages; M2, alternatively activated macrophages; MVBs, multivesicular bodies; ESEs, early endosomes; LSEs, late endosomes; DCs, dendritic cells; MSCs, mesenchymal stromal cells; miRNA, microRNA; LPS, lipopolysaccharide; IFN-γ, interferon-γ; IL-4, interleukin 4; IL-13, interleukin 13; M0-Exos, unpolarised M0 macrophage-derived exosomes; M1-Exos, polarised M1 macrophage-derived exosomes; M2-Exos, M2 macrophage-derived exosomes; Stxs, Shiga toxins; PM, particulate matter; RAC1, Rac family small GTPase 1; PAK2, p21-activated kinase 2; Sirt1, Sirtuin 1; AMPKα2, protein kinase AMP-activated catalytic Subunit alpha 2; PD, Parkinson's disease; COM, calcium oxalate monohydrate; PM-Exos, peripheral macrophage-derived exosomes; HS, hemorrhagic shock; ROS, reactive oxygen species; PMNs, polymorphonuclear neutrophils; BALF, bronchoalveolar lavage fluid; ALI, acute lung injury; CFA, complete Freund's adjuvant; BMDM, bone marrow-derived macrophages; Mf, naïve macrophage; PTX, Paclitaxel; HAL, hexyl 5-aminolevulinate hydrochloride; Exos-cur, curcumin-loaded M-Exos; Mel@M2-exos, M2-exosomes loading with melatonin; Exos-Slb, silibinin-loaded M-Exos; BDNF, brain-derived neurotrophic factor; LCP, lipid calcium phosphate; BSP, betamethasone sodium phosphate; RA, rheumatoid arthritis; HNV, hybrid exosome-mimic nanovesicles; SCI, spinal cord injury; PMN, photosensitive hydrogel microneedles.

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## Disclosure

The authors report no conflicts of interest in this work.

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