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Yin-Yang: two sides of extracellular vesicles in inflammatory diseases

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Abstract

The concept of Yin-Yang, originating in ancient Chinese philosophy, symbolizes two opposing but complementary forces or principles found in all aspects of life. This concept can be quite fitting in the context of extracellular vehicles (EVs) and inflammatory diseases. Over the past decades, numerous studies have revealed that EVs can exhibit dual sides, acting as both pro- and anti-inflammatory agents, akin to the concept of Yin-Yang theory (i.e., two sides of a coin). This has enabled EVs to serve as potential indicators of pathogenesis or be manipulated for therapeutic purposes by influencing immune and inflammatory pathways. This review delves into the recent advances in understanding the Yin-Yang sides of EVs and their regulation in specific inflammatory diseases. We shed light on the current prospects of engineering EVs for treating inflammatory conditions. The Yin-Yang principle of EVs bestows upon them great potential as, therapeutic, and preventive agents for inflammatory diseases.

Keywords Extracellular vesicles, Yin-Yang principle, Engineering extracellular vesicles, Inflammatory diseases

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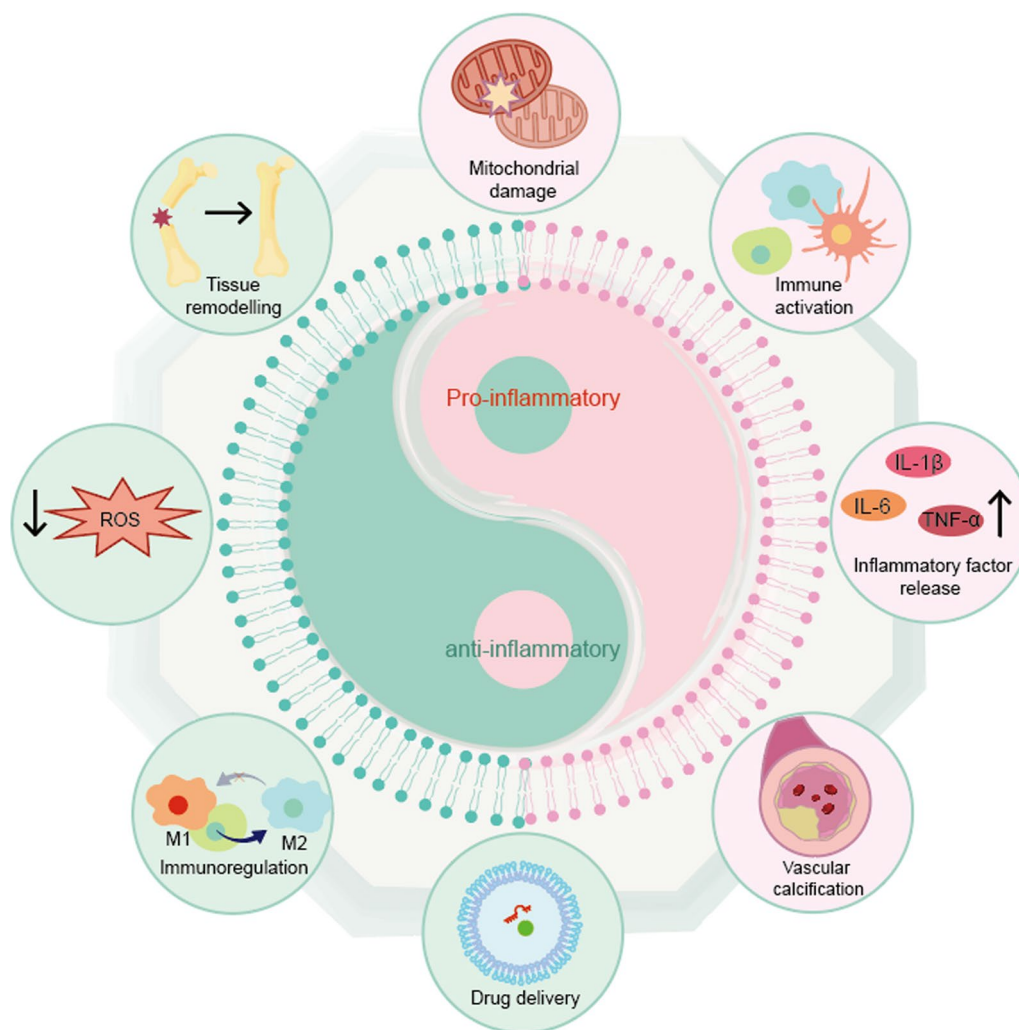binjiang1@eurekaintl.us
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Graphical Abstract

Introduction

Inflammation is a pathophysiological process in which injury and anti-injury factors interact. Inflammation has two sides. Short-term and mild inflammation can help enhance the ability of the immune system to phagocytize, kill and remove pathogens, and promote tissue repair [74], while long-term inflammation can cause irreversible damage to tissues and organs and lead to various diseases or aggravate disease progression [65]. Diseases related to pathological inflammation are also called inflammatory diseases, including osteoarthritis (OA), rheumatoid arthritis (RA), neurodegenerative diseases, atherosclerosis (AS), inflammatory bowel disease (IBD), systemic lupus erythematosus (SLE), etc. [56, 85, 171, 219, 229, 283]. At present, the pathogenesis of most inflammatory diseases is still unclear, which hinders the prevention, diagnosis, and treatment of diseases to a certain extent.

Extracellular vesicles (EVs) are lipid vesicles secreted by cells, and the protein, nucleic acid and lipid components carried on the surface and inside can be delivered to recipient cells and regulate their functions [174]. As one of the main channels of intercellular communication, EVs play a regulatory role in the occurrence and development of inflammatory diseases. Like the yin and yang of Tai Chi, EVs play a dual role of pro-inflammatory and anti-inflammatory in inflammation, with "yang" and "yin" representing these roles, respectively. Matrix vesicles can promote vascular and cartilage calcification, exacerbating AS and OA [184, 278], while mitochondrial vesicles are closely related to diseases characterized by mitochondrial

dysfunction, such as neurodegenerative diseases [121]. In addition, bacteria-derived EVs can disrupt the homeostasis of intestinal flora, activate immune responses, and destroy the intestinal barrier structure, thereby causing or worsening IBD [50]. Therefore, analyzing these cell-derived EVs involved in inflammatory diseases can not only help us understand the pathogenesis of the disease but also enable us to screen the EV cargo for molecules that are highly correlated with disease severity, facilitating efficient disease diagnosis.

Other EVs can reduce inflammation in the body and be used in the treatment of inflammatory diseases. Mesenchymal stem cells (MSCs) and some plant-derived EVs have anti-inflammatory, anti-oxidative, and immune-regulating capabilities. Also, probiotic-derived EVs can protect the intestinal microenvironment [46, 50, 322]. In addition, due to the characteristics of low immunogenicity, tropism to inflammatory sites, and the ability to efficiently cross biological barriers, EVs have unique advantages in the treatment of arthritis, neurodegenerative diseases, and inflammatory diseases such as IBD [34]. However, the anti-inflammatory effect and targeting ability of EVs to inflamed tissues are limited. At present, researchers have tried many methods to enhance the anti-inflammatory properties of EVs (such as hypoxic preconditioning, 3D culture) and targeting capabilities (e.g., genetic engineering, chemical modification, membrane fusion) [104, 146, 251].

EVs can also be used as a drug carrier for the treatment of inflammatory diseases, improving the uptake of drug, the efficiency of crossing biological barriers, and the duration of action [263]. Due to the lack of targeting ability, many small molecule anti-inflammatory drugs or protein antibodies are prone to gather at non-targeted sites, and using EVs to target specific tissues and cells can avoid this off-target effect [59]. EVs can also prevent the degradation of nucleic acid drugs (such as mRNA, ASO, miRNA) in vivo, and are widely used in the delivery of nucleic acid drugs to treat inflammatory diseases [247]. In addition, EVs can be combined with gel scaffolds and microneedles to further improve the stability of EVs in vivo and achieve controlled release for anti-inflammatory therapy [106].

Here, we review the origin of EVs from animal, plant, and bacterial sources and the involvement of EVs in different inflammatory diseases. We also summarize EV-based anti-inflammatory therapies and point out that multi-target, responsive, and intelligent engineered EVs are one of the current development trends of engineered EVs. Finally, we discuss the current opportunities and challenges of EVs in drug delivery for inflammatory diseases, aiming to provide clues for the development of drug delivery strategies based on EVs for the treatment of inflammatory diseases.
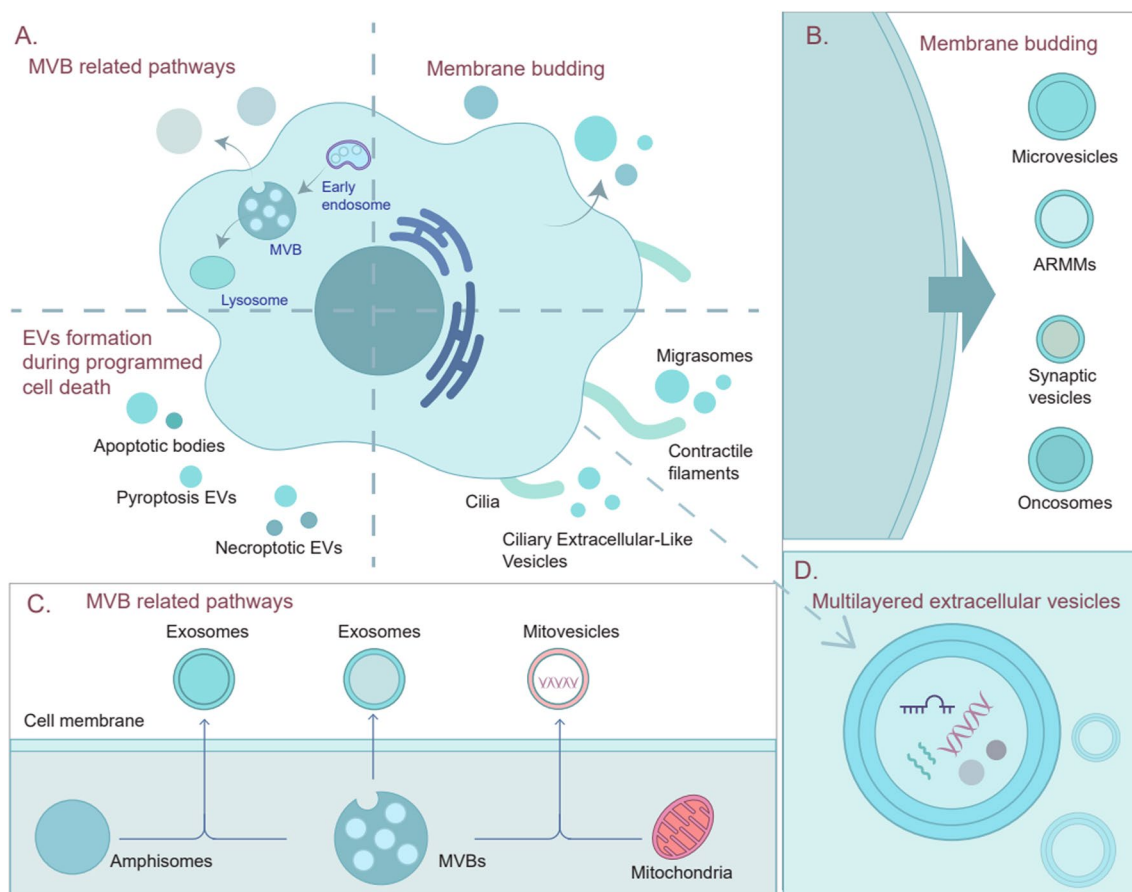
Biogenesis of EVs
Mammalian cell-derived EVs
In mammalian cells, the production process of EVs is divided into several pathways: (1) release to the extracellular environment through the fusion of intracellular multivesicular bodies (MVBs) with the plasma membrane (Table 1, Fig. 1A). In addition to directly fusing with the plasma membrane, MVB can also fuse with the plasma membrane to release EVs after

Table 1 Characteristics of EV types

Types	Source	Intracellular origin	Size	Biomarkers	Refs.
Exosomes	Mammal	Multivesicular bodies	40–150 nm	ALIX, TSG101, Tetraspanins	[223, 308]
Microvesicles	Mammal	Plasma membrane	50–2000 nm	Unknown	[98, 264]
Apoptotic bodies	Mammal	Plasma membrane	50–5000 nm	ANXA5, CASP3	[98, 155]
PEN1-positive EVs	Plant	Unknown	50–300 nm	PEN1	[216]
EXPO-derived EVs	Plant	Exocyst-positive organelle	200–500 nm	Exo70E2	[270]
TET8-positive EVs	Plant	Multivesicular bodies	30–100 nm	TET8	[91]
Outer membrane vesicles	Bacteria	Outer membrane	25–250 nm	ClyA, ompa	[222]
Oncosomes	Mammal	Plasma membrane	1–10 µm	ARF6, V-ATPase G1, CK18, Annexin A1	[98, 179]
Ciliary ectosome	Mammal	Ciliary membrane	30–164 nm	CD166, POR	[180]
Mycomembrane vesicles	Bacteria	Mycomembrane	NA	DtHtaA	[272, 281]
Mitovesicles	Mammal	Mitochondrion	100–200 nm	COX-IV, PDH-E1α, HSP60	[45, 173]
Necroptotic EVs	Mammal	Plasma membrane	150–200 nm	MLKL, RIPK3	[226, 305]
Pyroptotic bodies	Mammal	Plasma membrane	1–5 µm	Cleaved caspase1, GSDMD-N	[203]

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Fig. 1 Biogenesis of mammalian-derived EVs. Panel **A** demonstrates the primary pathways for animal cell-derived vesicle formation: **A** MVB Fusion with Plasma Membrane: The upper left corner of Figure **A** shows MVBs fusing directly or indirectly with the plasma membrane to generate EVs. Some MVBs also result from fusion with lysosomes. Inhibiting the MVB degradation pathway typically increases EV release. **B** Cell Membrane Budding: Cell membrane budding forms various vesicle types. Two specific modes, migratory body and ciliated vesicle formation, are presented in the lower right corner of Panel **A**. These are considered special forms of plasma membrane budding due to the involvement of contractile filaments and cilia, both enclosed by the cell membrane. **C** EVs from Programmed Cell Death: The lower left corner of Figure **A** illustrates three pathways for EV generation during programmed cell death: Apoptotic bodies, Necroptotic EVs, and Pyroptotic bodies. Panel **B** showcases conventional plasma membrane budding-derived EV types, including microvesicles, ARMM, synaptic vesicles, and oncosomes. Unconventional processes, such as hair follicle formation, are omitted. Panel **C**, the central process of EV formation via MVB-related pathways is detailed. MVBs can fuse directly with the plasma membrane or release EVs after fusing with other organelles. Specifically, EVs resulting from mitochondria-MVB fusion are termed mitovesicles, associated with mitochondrial dysfunction and diseases. Panel **D** is a sketch displays atypical EVs with multilayers. These lack the traditional phospholipid bilayer structure, comprising multiple lipid layers

fusion with certain organelles (such as mitochondria, amphisome) [43, 79–81] (Fig. 1C); (2) released directly from the plasma membrane or ciliary membrane [195] (Fig. 1A); (3) produce a large number of EVs during programmed death (apoptosis, pyroptosis, programmed necrosis) [9, 29, 226] (Fig. 1A); (4) release vesicles from the fusion of mitochondria and lysosomes to the plasma membrane [11]; (5) formed by assembly of large domains on the plasma membrane (migratosomes) [314] (Fig. 1A).

MVB related pathways

The MVB-associated pathway is the main pathway for the formation of EVs (Table 1, Fig. 1A). Early endosomes formed during endocytosis eventually evolve into MVBs containing ILVs loaded with various cargoes. After MVB formation, there are several destinations: (1) directly fusing with the plasma membrane to release EVs [80, 195]; (2) after fusion with mitochondria, MVBs rich in mitochondrial components fuse with the plasma membrane and release mitochondrial vesicles (mitovesicles) [44];

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(3) reaching the plasma membrane after fusion with amphisome and releasing EVs [80]; (4) fusing with lysosomes and degrading internal components [58]. In many pathological environments (such as tumors, inflammation, neurodegeneration), the autophagic activity of cells is inhibited, and more MVBs will fuse with the plasma membrane to generate EVs instead of being degraded in lysosomes.

Membrane budding

A variety of EVs can be produced by budding from the plasma membrane [38] (Table 1, Fig. 1B). Microvesicles (MVs) are one of the subgroups of EVs that have been widely studied, and the particle size is usually 150–1000 nm (except the particle size of arresting domain-containing protein 1-mediated microvesicles (ARMMs) is less than 150 nm) [32, 38, 274]. The increase in the number of MVs and changes in their composition have been shown to be associated with the pathogenesis of autoimmune diseases, tumors, neurodegenerative diseases, obstructive sleep apnea, coagulation disorders, and many other diseases [4, 88, 89, 178, 256]. Cells can also produce EVs such as oncosomes and synaptic vesicles by budding through the plasma membrane (Fig. 1) [16, 126].

Ciliary ectosomes can be formed by membrane budding on the surface or tip of cell cilia (Table 1, Fig. 1A). Ciliated vesicles have been shown to be distinctly different from cytoplasmic EVs in terms of protein composition, suggesting that ciliated vesicles possess many unexplored functions [180]. Studies have shown that ciliary extracellular-like vesicles (cELVs) play an important role in maintaining the physiological functions of the body. Inhibition of cELV protein expression in zebrafish leads to cystic kidneys, hydrocephalus, situs inversus, and cardiogenic edema [181]. Other studies have shown that ciliated vesicles play an important role in cell mitosis [200]. Cilia are widely present in various parts of the human body (such as respiratory bronchi, sperm cells, fallopian tubes, and the brain), and studying the specific functions of ciliated vesicles in the human body is helpful for understanding cilia-related diseases [176]. While the majority of extracellular vesicles (EVs) exhibit the classic phospholipid bilayer structure, it's important to acknowledge that a subset of EVs may display atypical morphology, characterized by multilayered lipid arrangements. These unique EVs deviate from the traditional bilayer configuration, encompassing multiple lipid layers instead as shown in the Fig. 1D.

EV formation during programmed cell death (PCD)

Cells produce apoptotic bodies with 100–5000 nm diameters during apoptosis [9, 98] (Table 1, Fig. 1A). Apoptotic bodies carry organelles, histones, cytokines, and nucleic acids, and participate in regulating inflammation, immune response, bone homeostasis, tumorigenesis, and liver fibrosis [102, 153, 321, 323]. Clearance of apoptotic bodies and apoptotic cells triggers inflammation in the body and leads to a series of diseases [47]. Adiponectin and autophagy (ATG)-related proteins are involved in regulating the clearance of apoptotic cells and apoptotic bodies and inhibiting the inflammatory response [238, 249]. In addition, during the process of cell pyroptosis, EVs (pyrotic bodies) similar in size to apoptotic bodies are produced, and the function of pyroptotic bodies remains to be further studied [29].

In addition to apoptosis and pyroptosis, a large number of EVs are also released during programmed necrosis [226]. The size of necroptotic EVs is between 100 and 200 nm [226], containing a large amount of mixed lineage kinase domain-like kinase (MLKL) and receptor-interacting serine/threonine-protein kinase 3 (RIPK3) [78, 311]. MLKL and RIPK3, in addition to triggering programmed necrosis and leading to the release of damage-associated molecular patterns (DAMPs) and the increase of pro-inflammatory cytokines, also mediate the formation of necroptotic EVs. MLKL is required for EV formation, and RIPK3 positively regulates the release of EVs containing phosphorylated-MLKL [78, 311]. The release of phosphorylated-MLKL-containing EVs may be a mechanism by which cells protect themselves from necroptosis [305].

Plant-derived EVs

Currently, it is believed that three pathways are involved in the release of plant vesicles: (1) fusion of MVBs with the membrane [6, 62, 186]; (2) fusion of intraluminal vesicles (ILVs)-containing vacuoles with the plasma membrane [62, 186]; (3) vesicle release mediated by exocyst-positive organelles (EXPOs) [215] (Table 1). Studies have shown that TETRASPANIN 8 (TET8)-positive EVs may form through MVB-membrane fusion [27]. After knocking out TET8, the number of EVs released by Arabidopsis was significantly reduced, suggesting that TET8 plays an important role in EV formation [158]. Through whole-cell electron tomography, Cui et al. found that small vacuoles (SVs) in Arabidopsis root cells were derived from MVB fusion and contained ILVs similar to MVBs, and SVs could fuse with the plasma membrane to release EVs [42]. In addition, EXPO, as a distinct double-membrane organelle in plants, can also release EVs (single-membrane EXPO) with a diameter of 200–500 nm and a single-membrane structure characterized by Exo70E2 by fusion with the plasma membrane [270]. Recent studies have shown that the generation pathway of PEN1-positive vesicles may not depend on MVB, but further investigation is needed to determine whether it relies on EXPO or other pathways [83].

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Bacterial-derived EVs

Gram-negative bacterial-derived vesicles are divided into two types: outer membrane vesicles (OMVs) and outer-inner membrane vesicles (O-IMVs) (Table 1). OMVs are formed by (1) budding of the bacterial outer membrane; (2) accumulation of lipids; (3) peptidoglycan fragments; (4) misfolded proteins in the periplasmic space; (5) accumulation of pseudomonas quinolone signaling (PQS); (6) lipid components in the outer leaflet [50, 255]. Several studies have suggested that OMV release may serve as a protective mechanism for Gram-negative bacteria to remove abnormally accumulated lipids or misfolded proteins. O-IMVs are mainly released during bacterial autolysis [50, 255, 259]. Phage infection results in bacterial autolysis and the production of large amounts of EVs [189, 259]. In some extreme environments, such as sucrose fatty acid ester treatment, exposure to cold shock, starvation, hypoxia, bacteria autolyse and release O-IMVs [50].

Compared to that of Gram-negative bacteria, fewer studies are performed on EVs derived from Gram-positive bacteria. Currently, it is believed that EVs derived from Gram-positive bacteria via membrane budding and bacterial autolysis. The process of membrane budding is regulated by the fluidity of the bacterial plasma membrane and the permeability of the peptidoglycan layer. In addition, the degree of PGN cross-linking on the cell wall

of the Gram-positive bacteria determines the number and particle size of the EVs released into the extracellular environment [50]. A new type of membrane budding was found in Dietzia sp. DQ12-45-1b, a Gram-positive bacteria that can secrete chorismate membrane vesicles [272] (Table 1). Chorismic acid membrane vesicles, characterized by DtHtaA, can mediate the acquisition and transport of heme molecules in microorganisms in a low-iron environment, which benefits the sharing of heme in microbial communities [272].

Isolation, characterization and storage of EVs
EVs isolation

Traditional vesicle separation methods mainly fall into the following categories: ultracentrifugation, density gradient centrifugation, ultrafiltration, TFF, size exclusion chromatography (SEC), and methods based on immunoaffinity (Fig. 2). Ultracentrifugation is simple to operate and is the most widely used method. However, ultracentrifugation is time-consuming and cannot separate impurities of similar size to the vesicles [116]. Density gradient centrifugation separates molecules with different sedimentation coefficients using sucrose solutions of varying densities, allowing for higher purity vesicles, but it is difficult and time-consuming to operate. Ultrafiltration involves filtering vesicles through ultrafiltration membranes, a simple and less time-consuming
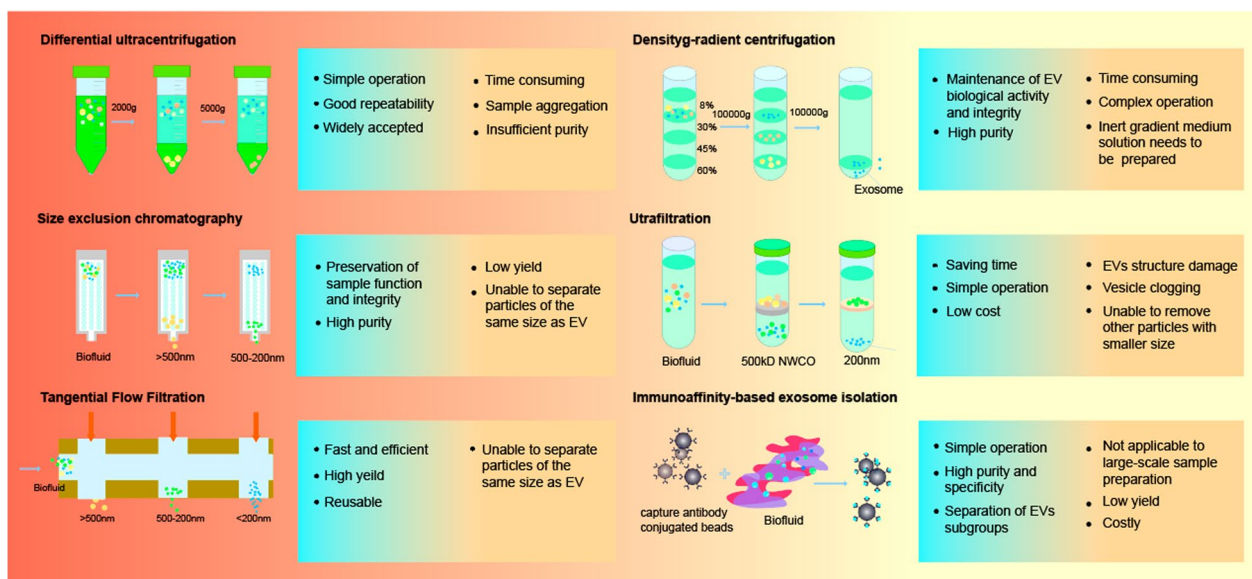
Fig. 2 Methods for separation of extracellular vesicles. Extracellular vesicle (EV) isolation and purification employ a diverse array of techniques, broadly classified by their underlying principles. Differential centrifugation, encompassing ultracentrifugation and density gradient centrifugation, leverages variations in sedimentation rates under centrifugal force. Size-based separation, including ultrafiltration, size exclusion chromatography (SEC), and tangential flow filtration (TFF), exploits differences in EV size and hydrodynamic radius. Lastly, immunoaffinity-based methods, such as immunoprecipitation, target specific surface antigens on EVs using antibodies. Each method presents unique advantages and limitations, necessitating careful consideration based on the specific research or diagnostic application

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method that does not require expensive equipment [309]. However, the shear forces during filtration may damage the vesicle structure [246], and ultrafiltration cannot distinguish vesicles of the same size from impurities.

SEC separates vesicles containing micropores from other large molecules, with larger molecules unable to enter the micropores and being flushed out preferentially, while vesicles can penetrate the micropores and are flushed out last. This method can obtain vesicles of higher purity without damaging their original structure during the separation process, but the final yield is lower and expensive equipment is required [71]. Tangential flow ultrafiltration separates nanoparticles of different sizes by tangential flow at different velocities [129], resulting in higher sample yield, but it cannot distinguish particles of sizes similar to vesicles [296]. Immunoprecipitation based on antibody capture captures vesicles with antibodies binding to vesicle membrane proteins, obtaining high purity and specificity, although this method may affect the original function of the vesicles [296].

In addition, the combination of the above methods can also be used to improve the purity and yield of vesicle separation. When selecting specific methods, it is necessary to consider the type and source of the sample, operational feasibility and equipment conditions, research purposes, and economic aspects, and make choices based on different conditions and requirements. In general, existing separation purification methods still face difficulties in achieving large-scale, high-purity vesicle preparation. Therefore, exploring and innovating vesicle separation methods also contribute to the promotion and popularization of extracellular vesicles.

EVs characterization

EVs are typically characterized by morphology, nanoparticle size, lipid composition, and exosomal protein markers [75, 118, 163, 175, 230, 239, 245, 280]. Transmission electron microscopy (TEM) is currently commonly utilized to characterize the morphological features of EVs. TEM can observe platelet-shaped structures of EVs. Furthermore, by counting EVs and background impurities under TEM, the concentration and purity of EV samples can be preliminarily observed [239].

Currently, the main methods used to detect the size of EVs' nanometer particles are Nanoparticle Tracking Analysis (NTA) and Dynamic Light Scattering (DLS) [163, 167, 303]. DLS particle size detection has the advantages of being fast and simple. However, since the intensity of scattered light is proportional to the sixth power of the particle size, larger particles will scatter light more intensely [141, 245, 310]. Therefore, for samples with a wide size distribution, the average particle size results tend to skew towards larger particles [141, 245, 310]..

The working principle of NTA is to irradiate a suspension of particles in solution with a concentrated laser beam through a glass prism [75, 118, 163, 310]. It detects the intensity of light scattered by each particle, observes and images the Brownian motion of nanoparticles in the solution [75, 118, 310]. By tracking and analyzing the Brownian motion of particles and using the Stokes–Einstein equation, it calculates the particle size of nanoparticles. The concentration is determined based on the number of particles [75, 118, 310].

In addition to size and morphology, various techniques can be used to analyze the lipid composition of EVs [72, 205, 236]. Lipidomics involves comprehensive analysis of lipid molecules, providing insights into the lipid composition and diversity of EV membranes [72, 205, 236]. Techniques such as mass spectrometry and nuclear magnetic resonance spectroscopy are commonly employed for lipidomic analysis [17, 182]

Proteomic analysis is another important method for EV characterization [230, 280]. By identifying and quantifying proteins present in EVs, researchers can understand their cargoes and potential functions. Mass spectrometry-based proteomics is the most commonly used technique for EV proteomic analysis [187, 230].

Western blot (WB) can be used to detect protein markers of EVs. For EVs derived from mammalian cells, the expression levels of proteins such as CD61, CD9, and CD81 are typically examined [41, 300]. Specific marker proteins need to be detected for certain subtypes of EVs. For instance, when characterizing apoptotic bodies, the expression level of CASP3 needs to be evaluated [97]. Furthermore, standardized protein markers for EVs originating from bacterial and plant cells are currently lacking. Identifying protein markers for EVs derived from bacterial and plant cells will assist researchers in characterizing these EVs.

EVs storage

Three main methods are for storing EVs: freezing, freeze-drying, and gel storage. Freezing is the most common method for EV storage. Research has shown that after storing at 4 ℃ for one day, the quantity of EVs decreases sharply, and after 28 days of storage at − 20 ℃, their function significantly changes. However, after storing for 28 days at − 80 ℃, the size, quantity, or function of EVs hardly changes [265]. Other studies have indicated that after storing at 4 ℃ for one month, exosome marker proteins are almost completely degraded, while after storing at − 80 ℃ for one month, a large amount of marker proteins can be detected in EV samples [278]. Currently, − 80 ℃ freezing is considered the gold standard for maintaining EV integrity and function over longer periods [265,

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278]. Gel storage may be more suitable for maintaining EV stability during short-term storage or transportation.

The use of freeze-drying and microneedle long-term storage of EVs has shown potential but further research and development are needed for practical application on a larger scale [40, 51, 295]. These methods have potential advantages in maintaining EV function and stability without the need for expensive cold chain storage conditions [51, 295]. However, challenges such as structural damage to EVs during freeze-drying and the scalability of soluble microneedles need to be addressed [40, 191]. Future research should focus on optimizing these technologies and exploring other innovative methods for long-term EV storage.

Emerging roles of EVs in inflammatory diseases

EVs carry a diverse cargo of molecules, including proteins, lipids, and nucleic acids, that can either promote or suppress immune responses. EVs can transport anti-inflammatory molecules like cytokines (IL-10, TGF-β) and growth factors, which inhibit immune cell activation and promote tissue repair, leading to reduced inflammation [26]. Conversely, EVs can also carry pro-inflammatory molecules like cytokines (IL-1β, TNF-α), DAMPs, and PAMPs, which activate immune cells, enhance antigen presentation, and promote inflammation. This dual nature of EVs makes them crucial regulators of immune homeostasis. Here we delve the role of EVs in multiple inflammatory diseases.

The role of EVs in arthritis
EVs in rheumatoid arthritis

RA is a chronic, inflammatory joint disease characterized by hyperplasia of synovial membrane and destruction of joint structures. There are three independent but interacting pathological stages in RA joints: the asymptomatic stage within the tolerance range, the acute inflammatory stage when the synovial tissue is inflamed, and the chronic inflammatory stage that causes severe deformity and irreversible damage to multiple joints [282]. As a medium of intercellular communication, EVs derived from synovial tissue in RA patients can promote the release of inflammatory factors by transferring miRNAs, proteins, and lncRNAs, aggravating joint inflammation and leading to the destruction of joint structures.

The enhanced osteoclast formation and resorption induced by receptor activator of nuclear factor-kappa B ligand (RANKL) in synovial fluid is a significant feature of RA patients [1], and studies have shown that the number of exosomes in the synovial fluid of RA patients and the expression level of RANKL in exosomes are significantly increased, and can significantly induce osteoclast differentiation [231]. EVs have also been shown

to transmit IL-6R between cells, and IL-6R may lead to enhanced bone resorption and chronic inflammation after binding to IL-6 [8, 239]. In addition, MVs secreted by rheumatoid synovial fibroblasts have proteoglycanase activity and may be involved in the degradation of extracellular matrix (ECM) in joints [163]. Another study showed that the expression level of programmed cell death 1 (PD-1), citrullinated protein, amyloid A (AA), lymphatic endothelial hyaluronan receptor 1 (LYVE-1), and toll-like receptor 3 (TLR3) fragments in blood-derived EVs of RA patients was significantly increased [175, 245]. The expression of the co-inhibitory receptor PD-1 is a sign of T cell exhaustion, and the citrullinated protein can promote the release of pro-inflammatory factors and initiate the pro-inflammatory response [75, 245]. Therefore, EVs derived from the blood and synovial fluid of RA patients carrying a variety of proteins and protein fragments promote the development of RA inflammation and the destruction of articular cartilage and bone.

In addition to the proteins or fragments transported by EVs, a large number of studies have shown that various RNAs carried by EVs also play an important role in the occurrence and development of RA inflammation. For example, miR-let-7b promotes conversion of macrophages into pro-inflammatory M1-type macrophages in RA patient EVs [118]. In addition to a variety of miRNAs, lncRNAs such as lncRNA NEAT1, lncRNA Hotair, and lncRNA HOTTIP are also highly expressed in EVs derived from RA patients [230, 280, 303]. The up-regulation of lncRNA NEAT1 can promote the migration, invasion and secretion of inflammatory cytokines of RA fibroblast-like synoviocytes (FLSs) [280], while LncRNA Hotair can promote the migration of macrophages and up-regulate the expression of matrix metalloproteinase-2 (MMP-2) and MMP-13, aggravating the inflammation of RA [230]. In addition, lncRNA HOTTIP promotes the development of RA inflammation by regulating the expression of miR-1908-5p and STAT3 [303].

In addition to proteins and RNAs, the lipid components of EVs can initiate the inflammatory response in RA. A large amount of reactive oxygen species (ROS) in the RA microenvironment oxidizes the lipid components of EVs, and oxidized EVs activate the inflammatory response by stimulating TLR4 [167]. The role of EV lipid components in RA inflammation needs further research.

Studies have shown that the composition of EVs (such as proteins, miRNAs) can change significantly under pathological conditions [141, 310]. The lipid bilayer membrane of EVs protects miRNAs from rapid degradation and improve the sensitivity of miRNAs amplification [236]. In addition, it is easier to collect EVs since they are widely present in human body fluids [141, 310]. Therefore, EVs show great promise as useful biomarkers

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in the diagnosis of diseases. For example, the expression of miR-221-3p in blood-derived EVs from RA patients was eightfold higher than that in normal human EVs [205, 280], while the expressions of TLR3 and RANKL in EVs from RA patients were sixfold and fivefold higher, respectively, than those in the control group [175]. These differentially expressed RNAs and proteins in RA can be potential biomarkers.
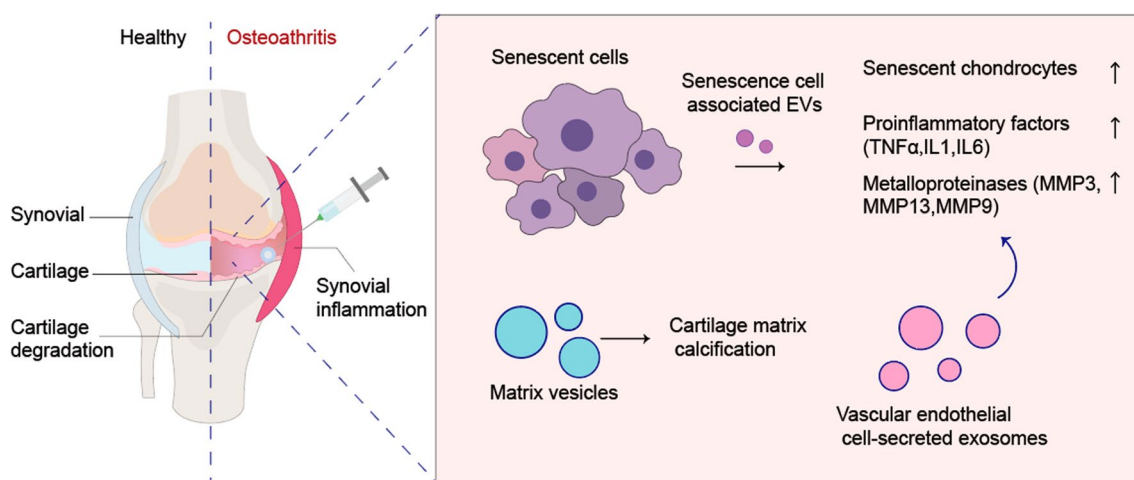
EVs in osteoarthritis

OA is a chronic degenerative bone joint disease characterized by articular cartilage damage and periarticular bone hyperplasia [72]. The incidence of OA increases with age, and there are more than 300 million OA patients worldwide [17]. During the progression of OA, the upregulated expression of inflammatory factors such as tumor necrosis factor-alpha (TNF-α), IL-6 and IL-1β stimulates the production of MMPs and a disintegrin and metalloprotease with thrombospondin motifs (ADAMTS), promotes the apoptosis of chondrocytes and the degradation of extracellular matrix, leading to the destruction of articular cartilage [182]. EVs can exacerbate the inflammatory response and cartilage damage of OA by promoting the release of inflammatory factors and proteases, inducing cell senescence, and participating in pathological calcification of cartilage [41, 97, 187, 265, 278, 295, 300].

 Studies have shown that synovial fluid exosomes from OA patients can promote the release of inflammatory cytokines, chemokines, and metalloproteinases (IL-1β, CCL8, CCL15, MMP13, MMP10, etc.) [51].

Another study showed that articular cartilage secretes more exosome-like vesicles in the damaged state, and these exosome-like vesicles from osteoarthritic chondrocytes enhance the production of mature IL-1β in macrophages and promote the progression of OA disease [187]. In addition, exosomes derived from vascular endothelial cells can reduce the ability of chondrocytes to resist oxidative stress by inhibiting autophagy and p21 expression, resulting in increased cellular ROS content and induction of chondrocyte apoptosis [300].

The development of OA is closely related to the increase in the number of senescent cells (SnCs) in joint tissues [41] (Fig. 3). Extracellular proteases, proinflammatory cytokines, and chemokines secreted by SnCs can further damage cartilage and aggravate OA inflammation. Senescent chondrocytes isolated from OA patients were able to secrete more EVs than nonsenescent chondrocytes [97]. These SnCs-derived EVs suppress cartilage ECM deposition in healthy chondrocytes and induce the transition of adjacent cells to a senescent state [97]. Another study showed that small EVs (sEVs) released from chondrocytes of OA patients contained high levels of connexin 43 (Cx43), which was three times greater than that of normal human chondrocyte sEVs [265]. Exosomal Cx43 could induce senescence phenotypes in chondrocytes, synoviocytes, and bone cells, and promote the formation of an inflammatory and degenerative joint environment by secreting senescence-associated secretory phenotype (SASP) molecules (IL-1, IL-6, and MMPs, etc.) [265].

Fig. 3 The role of EVs in the pathogenesis of knee osteoarthritis. The left half of the figure illustrates the structural features of a healthy knee compared to a knee affected by osteoarthritis, while the right half summarizes the role of EVs in the progression of knee osteoarthritis. EVs can elevate the levels of inflammatory factors and metal matrix proteases within joints, thereby exacerbating synovial inflammation and cartilage degradation. Furthermore, EVs secreted by senescent cells can promote the senescent phenotype in other chondrocytes through paracrine effects. Additionally, matrix vesicles contribute to the worsening of OA by fostering calcification of the cartilage matrix

Pathological calcification of cartilage is an important factor leading to OA and promoting the progression of OA. The "bone-cartilage" interface of the normal human knee joint is an ideal soft-hard interface model, which can ensure the effective transmission of force and avoid stress concentration during millions of cyclic loadings. Damage to the ultrastructure of the "bone-cartilage" interface tissue often leads to OA. In the early stage of OA, "bottom-up" pathological calcification will occur in the osteocartilage interface tissue [278]. With the development of OA, the sandwich calcification structure at the calcified cartilage will fuse, and the calcified cartilage area will thicken and invade the upper layer of cartilage [278]. Studies have shown that this pathological calcification may be attributed to matrix vesicle nucleation in the early stages of OA [278] (Fig. 3). In addition, microtubule-associated proteins 1A/1B light chain 3B (LC3)-positive calcified EVs released from mineral-containing autophagosomes have also been shown to cause pathological calcification and degeneration of cartilage, aggravating OA progression [295].

EVs in neuroinflammation

Neuroinflammation in the central nervous system is associated with neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD); it is also a ubiquitous feature of the diseases, including stroke, traumatic brain injury (TBI), and spinal cord injury (SCI) [40, 96, 128, 136, 191]. Inflammation of the peripheral nerves can lead to pain and impairment of sensory and motor function. The number of nerve cell deaths caused by neuroinflammation in AD is at least 10 times higher than that caused by Aβ and neurofibrillary tangles, highlighting the crucial role of neuroinflammation in neurological diseases [172]. EVs can transmit pro-inflammatory factors, misfolded proteins, and lipid components, promoting the degeneration of the nervous system. Additionally, autophagy and lysosomal dysfunction, as well as mitochondrial damage, will further promote the release of EVs carrying α-synuclein (SNCA), Aβ and other proteins, as well as EVs carrying mitochondrial DNA (mtDNA) and other mitochondrial components, thereby aggravating nervous system inflammation and nerve damage [11, 24, 45, 81, 177, 202, 234, 261].

EVs in neurodegenerative diseases

EVs transport proteins to mediate neurodegenerative diseases   Abnormal aggregation and deposition of proteins (such as Tau, Aβ, SNCA and other proteins) are histopathological hallmarks of neurodegenerative diseases [232]. Abnormal protein aggregation can activate the immune system, inducing inflammation. The inflammatory response can further aggravate abnormal protein aggregation and lead to neuron death [232]. Many studies have shown that EVs carry and transport Tau, Aβ, α-synuclein, and other proteins to mediate the pathological process of neurodegenerative diseases, such as AD and PD. In addition, EVs from patients with neurodegenerative diseases also carry many pro-inflammatory factors. IL-6, TNF-α, and IL-1β in EVs from patients with AD and PD are significantly elevated [28, 289]. Moreover, the composition of EVs in an inflammatory environment is more easily taken up by neuronal cells, a process that may lead to the further dissemination of EVs carrying Tau, Aβ proteins, and pro-inflammatory factors to healthy cells in the brain [307].

Autophagy lysosomal pathway dysfunction regulates EV secretion and exacerbates neurodegenerative diseases   The autophagy lysosomal pathway (ALP) is critical for the survival of neurons and the maintenance of nervous system homeostasis through the clearance of abnormal protein aggregates and damaged organelles [140, 170, 320]. Abnormal protein aggregation caused by lysosomal dysfunction is an important pathogenesis of neurodegenerative diseases such as AD, PD, and HD [131]. The study found that the formation rate of Aβ oligomers was 7900 times higher than that in the interstitial pH (PH = 7.3) when the pH was between the pH of the lysosome and the pH of the endosome (PH = 4.8), which indicates that the endosome/lysosome system may be an important site for the assembly of Aβ oligomers related to pathological mechanisms [221].

MVB fuses with the plasma membrane to release EVs and fuses with lysosomes to be cleared by the ALP system. Impairment of ALP system function usually leads to an increased release of EVs carrying misfolded proteins, and this process may be one of the important mechanisms by which EVs are involved in neurodegenerative diseases [177]. Many studies have shown that inhibiting autophagy can promote the release of EVs containing SNCA, Aβ, and other proteins [24, 81, 177]. In addition, exosomes carrying Tau protein can induce increased lysosome permeability after fusion with lysosomes and promote the leakage and accumulation of Tau protein in lysosomes to the cytoplasm [202]. This study shows that this mechanism seems to be universal, that is, the exosome-induced increase in lysosomal permeability is independent of the source of exosomes and whether the exosomes carry Tau protein, which also suggests that this property of exosomes may promote the accumulation of other proteins in the cytoplasm in neurodegenerative diseases other than AD [202].

Mitochondrial vesicles in aging and neurodegenerative diseases   Mitochondrial dysfunction is a recognized

hallmark of aging and neurodegenerative diseases such as Down syndrome disease and Alzheimer's disease, and mitochondrial vesicles containing mitochondrial components may be involved in various neurodegenerative diseases [44, 45]. Mitochondrial vesicles are a type of EV distinct from exosomes and microvesicles. They have consistent morphological characteristics (for example, double membrane and electron density) with mitochondrial origin. They are rich in mitochondria-related proteins, such as COX-IV, PDH-E1α, and HSP60. Their lipid distribution is similar to mitochondria but lack the marker proteins of microvesicles and exosomes [45]. The generation of mitochondrial vesicles may occur through the fusion of intracellular mitochondrial-derived vesicles (MDVs) with MVBs, which are then released into the extracellular space through the plasma membrane. The composition of mitochondrial vesicles, includingproteins and mtDNA, may be altered in pathological conditions, suggesting their important role in mitochondria-related diseases [45]. Recent studies have shown a significant increase in the number of mitochondrial vesicles in brain tissue and the content of mtDNA derived from mitochondrial vesicles in DS animal models. In addition, the level of vesicle mRNA was found to decrease. The authors hypothesized that mitochondrial vesicles may activate microglia to promote the development of neuroinflammation [44, 45].

Flunarizine (FNZ), as a calcium ion channel inhibitor, is widely used in the treatment of vertigo, migraine, epilepsy, and peripheral vascular disease. However, FNZ may also cause Parkinson's disease [11]. Studies have shown that FNZ can induce mitochondrial dysfunction and significantly reduce the number of mitochondria in cells. Mechanistically, FNZ can induce the fusion of mitochondria and lysosomes in nerve cells to produce a new type of organelle structure called mitolysosome, which is then released through a VAMP2/STX4-dependent way, ultimately leading to a decrease in the total amount of mitochondria [11]. This study elucidates the pathway by which vesicles participate in mitochondrial clearance and reveals a novel mechanism of drug-induced parkinsonism.

The lipid composition of EVs in neurodegenerative diseases and neuroinflammation The lipid composition of EVs plays an important role in neurodegenerative diseases and neuroinflammation. The fibrosis of α-synuclein is closely related to the pathogenesis of synucleinopathy, and the lipid membrane of EVs has been shown to promote α-synuclein fibrillation [261]. The ability of EVs to promote α-synuclein fibrillation was greatly reduced after treatment with methanol or a combination of methanol and sarsaparyl alcohol, which disrupted the lipid membrane ultrastructure of EVs [261].

A study comparing the lipid composition of EVs derived from the brains of Alzheimer's patients with that from normal human brain tissue showed that the content of docosahexaenoic acid, which has anti-inflammatory functions, was reduced by two-fold in AD brain-derived EVs (BDEVs) [234]. In addition, the exosomes released by neurons when the lysosomal function is abnormal are rich in lipid components such as sphingolipids and phospholipid bis (monoacylglycerol) phosphate (BMP). BMP, which usually exists in vesicles inside lysosomes, can be used as a candidate biomarker for neurodegenerative disease-related lysosomal dysfunction, but the role of these lipid components in neurodegenerative diseases remains to be further studied [99].

Overall, these studies highlight the importance of EVs and their lipid composition in neuroinflammation and neurodegenerative diseases. Manipulating EVs and their cargo could potentially be a therapeutic strategy for these conditions. However, further research is needed to fully understand the mechanisms and potential therapeutic applications of EVs in neuroinflammation and neurodegenerative diseases.

EVs in stroke

Stroke can lead to local inflammatory responses caused by neuronal cell death and DAMPs release [225], which can then spread to cause global inflammation. One mechanism through which is the release of pro-inflammatory mediators (C1q, C3a and C3b) carried by exosomes in the blood of elderly individuals. These exosomes can worsen stroke progression by inducing microglial phagocytosis [313]. Additionally, an increase in prion protein (PrP) content has been observed in EVs derived from brain tissue after stroke. These PrPs can affect the uptake of EVs by neurons, possibly playing a role in intercellular communication following stroke [20].

EVs have shown promise as a diagnostic and prognostic tool for stroke. Blood-derived ex-miRNAs have been used to distinguish different stroke subtypes, such as intraparenchymal hemorrhage (IPH), aneurysmal subarachnoid hemorrhage (SAH), and ischemic stroke caused by cerebrovascular occlusion [108]. Additionally, the mRNA content of blood-derived EVs has been found to reflect the inflammatory response and recovery processes after stroke [22]. High levels of endothelial MVs (EMVs) and leukocyte-derived MVs (LMVs) have also been associated with poorer cardiovascular outcomes in stroke patients, suggesting their potential use in risk prediction [92]. These findings highlight the potential of EVs as biomarkers in stroke diagnosis and prognosis.

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The role of EVs in acute injury of nervous system

EVs, specifically plasma EVs, have been found to play a role in the acute injury of the nervous system, particularly in SCI and TBI. These injuries can impact the number of plasma EVs, the expression profile of microRNAs, and the proteome characteristics [73, 113].

EVs in SCI Research has identified differentially expressed proteins and miRNAs in individuals with SCI and TBI, indicating the involvement of EVs in these conditions [57, 214, 260]. Additionally, individuals with SCI have a higher risk of cardiovascular disease compared to those without spinal cord injury. Studies have shown that circulating microbubbles in SCI patients do not affect cell inflammation and oxidative stress but can contribute to the development of AS and thrombosis in the vascular endothelium. This suggests that circulating microbubbles may play a role in the cardiovascular pathogenesis following SCI [57, 214].

EVs in TBI   Severe TBI can result in the release of autonomic and inflammatory mediators into the bloodstream, leading to dysfunction in various organs [123]. In the context of TBI, the expression of a specific inflammation-related microRNA called miR-142-3p in plasma EVs has been found to significantly increase. MiR-142-3p has been shown to promote the secretion of TNF-α from human microglia, a type of immune cell in the brain, and induce the differentiation of human astrocytes into a pro-inflammatory phenotype. This suggests that EVs may play a role in the neuroinflammatory response following TBI through the action of miR-142-3p [122]. Further research is needed to fully understand the mechanisms by which EVs and miR-142-3p contribute to the neuroinflammatory response and potential therapeutic interventions.

Patients who have experienced TBI may exhibit abnormal coagulation function. Those with coagulant TBI have been found to have a significantly higher mortality rate (ninefold) and a greater risk of adverse outcomes (30-fold) compared to patients with noncoagulant TBI [18]. Research has shown that BDEVs following traumatic injury can express procoagulant anionic phospholipids and tissue factor, leading to a systemic hypercoagulable state that can rapidly progress to consumptive coagulopathy [254, 317]. During acute TBI, a large number of exogenous tenase complexes are formed on BDEVs, indicating that EVs can act as platforms for initiating and propagating extrinsic coagulation, resulting in the consumption of coagulation factors and platelets and ultimately leading to consumptive coagulopathy [53].

Studies have demonstrated that drugs targeting the membranes of EVs expressing anionic phospholipids or drugs that enhance EV clearance can prevent coagulopathy and significantly improve the survival rate of mice with severe TBI. This further supports the important role of EVs in coagulation following TBI and brain trauma. These findings highlight the potential for EV-targeted therapies to mitigate coagulation abnormalities and improve outcomes in TBI patients [53, 324].

EVs are involved in peripheral nervous system inflammation

Studies have demonstrated that EVs play a crucial role in peripheral nervous system inflammation by promoting the activation and polarization of pro-inflammatory macrophages. The delivery of up-regulated miR-23a and miR-21-5p by EVs to macrophages can activate the NF-κB pathway and promote the polarization of M1 macrophages, which are known to be pro-inflammatory [227, 313]. Inhibition of miR-21-5p expression can reduce neural hypersensitivity and the recruitment of inflammatory macrophages [227]. Overall, these studies highlight the importance of EVs in the crosstalk between neurons and immune cells in peripheral nervous system injury and inflammation.

The role of EVs in inflammatory bowel disease

EVs play a significant role in the development and progression of IBD. IBD is characterized by chronic inflammation in the gastrointestinal tract, leading to symptoms such as abdominal pain, diarrhea, and weight loss [109]. During the pathogenesis of IBD, various factors contribute to the disruption of intestinal homeostasis. This includes enhanced intestinal barrier permeability, activation of immune cells, and the production of pro-inflammatory cytokines. EVs derived from endothelial cells and intestinal microbes are involved in maintaining intestinal homeostasis and regulating immune responses [125, 127, 132, 193].

Effect of endothelial cell-derived EVs on IBD

Endothelial cell-derived EVs have been shown to have a beneficial effect on IBD. Specifically, A33$^+$ Li-EVs, which are EVs secreted by endothelial cells, have been found to alleviate IBD-related symptoms. These EVs induce the production of regulatory T cells (Tregs), which help to suppress inflammation and maintain immune tolerance. A33$^+$ Li-EVs also inhibit the proliferation of CD4$^+$ T cells and the activation of dendritic cells (DCs), which are both involved in promoting inflammation in IBD [103].

The activation of epithelial cell adhesion molecule (EpCAM), a cell surface protein, is crucial for the specific distribution of A33$^+$ Li-EVs in gastrointestinal organs. Loss of EpCAM activity can weaken the protective effect of endothelial cell-derived EVs on the gut, potentially contributing to the exacerbation of IBD [103].

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Another protein, gasdermin-D (GSDMD), has been implicated in promoting the release of EVs containing IL-1β from endothelial cells, thereby aggravating the inflammatory response in IBD [161]. GSDMD is significantly upregulated in the intestinal epithelial cells (IECs) of IBD patients. It promotes the secretion of IL-1β through the NEDD4-dependent EV release pathway in response to caspase-8 inflammasome activation. Knockout of GSDMD has been shown to alleviate IBD-related symptoms in animal models, suggesting that it may be a potential therapeutic target for IBD [23].

Overall, endothelial cell-derived EVs have the potential to modulate immune responses and alleviate inflammation in IBD. Understanding the specific mechanisms by which these EVs exert their effects may lead to the development of targeted therapies for this chronic inflammatory disease.

Influence of intestinal microbia-derived EVs on IBD

Intestinal microbia-derived EVs have been shown to play a role in the pathogenesis of IBD. EVs derived from intestinal microbia can directly interact with the intestinal epithelial cells and modulate their functions.

Harm the structure of the intestinal barrier   EVs derived from certain microorganisms in the gut, such as Escherichia coli and Ekmansia muciniphila, play a role in maintaining intestinal homeostasis and preserving the integrity of the epithelial barrier. On the other hand, EVs produced by other microorganisms can harm the structure of the intestinal barrier, leading to inflammation and disease. For instance, EVs derived from Fusobacterium nucleatum can stimulate macrophages to release pro-inflammatory factors such as TNF-α and IFN-γ, thereby disrupting the intestinal barrier [157, 165]. This disruption not only allows LPS-positive bacterial extracellular vesicles (BEVs) to enter the bloodstream and trigger inflammatory responses but also potentially affects pancreatic islet function through the delivery of microbial DNA [68, 258]. Furthermore, studies have demonstrated that EVs derived from fecal samples of patients with non-alcoholic steatohepatitis (NASH) can decrease the expression of tight junction proteins involved in the formation of the intestinal barrier, leading to liver damage and inflammation. These findings suggest that timely clearance of pathogenic EVs and restoration of intestinal barrier function could be effective strategies for treating IBD and its complications.

Cross the intestinal barrier to modulate the immune response   In addition to affecting the structure and function of the intestinal barrier, gut microbiota-derived vesicles can also cross the intestinal barrier to modulate the immune response of immune cells. The studies suggest

that BEVs and OMVs can regulate host immune function in a cell-specific manner. However, under pathological conditions such as IBD, this regulatory pattern is altered.

One study, using single-cell RNA sequencing and host-microbial protein–protein interaction network, predicted the influence of BEV proteins on different immune cells [76]. The results indicated that BEVs can regulate immune cells in a cell-specific manner, and this regulation is altered in IBD conditions. Another study focused on Bacteroides polymorpha OMVs and found that they can promote the release of IL-10 from regulatory DCs, which helps regulate intestinal homeostasis. However, this regulatory process is altered in IBD patients, leading to reduced IL-10 production [55].

The reasons for these changes in the regulation of immune cells by BEVs and OMVs under pathological conditions are not fully understood. The author speculates that gene mutations may play a role in these changes. Some studies have suggested that gene mutations can block the response of immune cells to OMVs and promote the process of immune anti-inflammatory response [36]. Further research is needed to explore the mechanisms underlying these changes and the role of gene mutations in modulating the immune response to gut microbiota-derived vesicles.

The role of EVs in atherosclerosis
EVs aggravate inflammation and atherosclerosis

EVs aggravate inflammation and AS by promoting the spread of inflammation, cardiovascular calcification, and thrombus formation [10, 149]. Atherosclerotic plaque-derived EVs can trigger intimal inflammation and induce arterial wall thickening and lumen narrowing. Inhibition of EV release can attenuate AS formation and reduce macrophage infiltration [198]. EVs derived from monocyte/platelet preparations can activate endothelial cells to produce IL-6 and activate human atherosclerotic plaques [190]. Platelet-derived EVs can transfer platelet adhesion receptors to monocytes, promoting their recruitment to vascular endothelial cells and potentially contributing to thrombosis and vascular inflammation [35].

Smoking, fatty liver, obesity, and other factors have been found to be highly correlated with the onset of AS. Nicotine, found in cigarettes, can promote endothelial dysfunction through EV-miRNAs and aggravate AS by promoting oxidative stress and the release of EVs, which can lead to vascular calcification [199, 269]. Hepatocyte-derived EVs under conditions of steatosis (fatty liver) have also been shown to promote endothelial inflammation and AS via miR-1 [100, 101]. Dysregulation of sphingolipid metabolism, specifically in thoracic adipose tissue (ThAT) secretome in obesity, has been found to play a significant role in

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AS. Adipocyte-derived EVs in obese individuals release sphingolipids that can regulate vascular redox status and contribute to advanced AS. High plasma concentrations of certain sphingolipids have been independently associated with an increased risk of cardiac death [3]. These findings highlight the importance of these factors and their impact on AS development and progression.

Several EV components have been identified as potential biomarkers for different atherosclerosis-related pathological conditions. These biomarkers include miR-23a-3p, complement component 1r, complement component 1 s protein, and Rap1 carried by large EVs derived from patients with metabolic syndrome [14, 198]. These biomarkers can be used to assess the presence and severity of atherosclerosis, as well as to predict prognosis and guide therapeutic interventions. Additionally, circulating cardiogenic CD172a$^+$ EVs have shown promise as prognostic biomarkers for aortic stenosis. Overall, the identification and characterization of EV biomarkers provide valuable insights into the pathogenesis and management of atherosclerosis-related conditions [7].

Matrix vesicles (MVs) provoke vascular calcification and atherosclerosis

Matrix vesicles (MVs) mediate the formation of microcalcifications in vascular calcification and AS. These MVs are derived from various cell types, including macrophages, smooth muscle cells, and valvular interstitial cells. They aggregate and form tiny calcifications through different pathways, contributing to the development of cardiovascular diseases [2].

To study the formation of microcalcifications, researchers seeded calcified EVs in a collagen hydrogel model that mimics the fibrous cap of atherosclerotic plaque or fibrotic valve tissue. They observed the entire process of microcalcification formation within 3–5 days [93]. This study is significant as it is the first to demonstrate the formation of microcalcifications at the single EV level, providing insights into the mechanisms underlying vascular calcification and atherosclerosis.

Studies have shown that platelet-derived growth factor-BB and TNF-α can enhance the release of microvesicles, which in turn promotes the calcification process. Calcium stress further promotes the mineralization of MVs by increasing the expression of Annexin A6 (AnxA6) in MVs derived from vascular smooth muscle cells [110, 111]. Additionally, ANXA1 was found to be significantly increased in MVs, and knockdown of ANXA1 reduced EV aggregation and microcalcification formation [210]. These findings highlight the crucial role of EVs, particularly MVs, in vascular calcification.

Atherosclerosis alters EV biological activities

Recent studies have shown that EVs secreted by cardiomyocytes, which are specialized cells in the heart, can influence blood pressure under hemodynamic stress [164, 204]. These EVs contain a receptor called angiotensin II type I receptor (AT1R), which has been found to have both beneficial and detrimental effects on cardiovascular health.

On one hand, AT1R EVs may have the ability to lower blood pressure in response to hemodynamic stress [201]. On the other hand, they may also promote the generation of reactive oxygen species (ROS) or trigger pro-inflammatory responses, leading to further damage to the cardiovascular system. The effects of AT1R EVs seem to depend on the presence of AT1R itself [25]. Another study has shown that the uptake of EVs by endothelial cells, which line the inner surface of blood vessels, is significantly increased in the presence of low shear stress or oscillatory shear stress (OSS) [203]. This enhanced uptake of EVs may be attributed to the occurrence of oxidative stress in endothelial cells induced by low shear stress [203].

These findings provide new insights into the role of EVs in the development of AS under conditions of low shear stress. They also suggest that EVs could be utilized in the development of nano-drug delivery systems for targeted therapy in vivo. By understanding and manipulating the biological activities of EVs, it may be possible to develop novel strategies for the prevention and treatment of atherosclerosis.

The role of EVs in systemic lupus erythematosus (SLE)

EVs play a significant role in the pathogenesis of SLE. SLE is characterized by an abnormal immune response where the body's immune system mistakenly attacks its own tissues and organs [119]. The nucleic acids, nucleosomes, and lipid components present in EVs can act as self-antigens and induce autoimmunity [262]. These self-antigen-containing EVs can also participate in the formation of immune complexes, which are aggregates of self-antigens and antibodies. Immune complexes can deposit in various tissues and organs, leading to inflammation and damage.

Apoptotic cells and their associated EVs contribute to SLE

In SLE, there is a defect in the clearance of apoptotic cells and their associated EVs. This leads to the accumulation of self-antigen-containing EVs, which can trigger an immune response and contribute to the development of autoimmunity [15, 82, 194, 217, 262].

The presence of apoptotic bodies and the transfer of self-antigens to these bodies during apoptosis are

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important factors in SLE development. Research has shown that the formation of apoptotic bodies and the transfer of self-antigens occur early in the disease process, before other typical signs of apoptosis, such as phosphatidylserine exposure or DNA degradation [206, 262]. This suggests that antibodies containing self-antigens are formed in the early stages of SLE.

Apoptotic bodies in the blood of SLE patients can stimulate plasmacytoid dendritic cells (PDCs) and myeloid dendritic cells (MDCs) to produce pro-inflammatory cytokines such as IL-6, TNF-α, and IFN-α. These cytokines contribute to the inflammatory response seen in SLE. Additionally, apoptotic bodies can promote a type of neutrophil cell death called NETosis, which further contributes to inflammation. During endothelial cell apoptosis, large apoptotic bodies containing precursor and mature forms of interleukin-1 alpha (IL-1 α) are released. IL-1α can induce monocytes to secrete chemokines, which attract immune cells to the site of inflammation. This can lead to local neutrophil infiltration and exacerbate the inflammatory response in SLE [15].

DNASE1L3 is an enzyme that is responsible for removing chromatin and DNA fragments from the surface of microparticles released by apoptotic cells. When DNASE1L3 is lacking or its activity is reduced, these DNA fragments and chromosomes are not cleared efficiently, leading to their accumulation [228]. This accumulation can trigger the production of antibodies against DNA and chromosomes, leading to immune inflammation and the development of SLE. Studies have shown that reduced DNASE1L3 activity is observed in more than 50% of SLE patients with nephritis [82]. These patients also have a significant presence of circulating microparticles containing cfDNA, which has a high affinity for autoantibodies. Additionally, specific antibodies against DNASE1L3, such as HMGB1, carried by these autoantibodies contribute to the decreased activity of DNASE1L3 and the accumulation of cfDNA [82].

Exosomes from SLE patients promoting the inflammatory response

Exosomes from SLE patients have been implicated in promoting the inflammatory response. These exosomes can activate Toll-like receptor 7 (TLR7) by delivering miRNAs, leading to the secretion of IFN-α from human plasmacytoid DCs. This activation of TLR7 and subsequent IFN-α secretion aggravates the inflammatory response in SLE [217]. Moreover, T cell-derived EVs from SLE patients have been found to exhibit increased expression of eosinophil cationic protein (ECP). These EVs containing ECP can promote the production of autoantibodies and the secretion of IFNγ, thereby

inducing inflammatory responses in animal models [37]. These studies demonstrate that different components of EVs, such as exosomes and T cell-derived EVs, contribute to the autoimmune and inflammatory processes in SLE through various pathways. Furthermore, it has been demonstrated that inhibiting inflammation in SLE patients significantly reduces the levels of endothelial EVs, suggesting that EVs also play a role in the inflammatory response of SLE and their levels can be modulated by inhibiting inflammation [194].

EVs as candidate biomarkers in the diagnosis and assessment of SLE

The up-regulation of proteins such as BPI and ECP in T cells and T cell-derived exosomes of SLE patients suggests their potential as disease biomarkers. These proteins can be used to assess disease activity and may help diagnose SLE [37, 194]. Additionally, the presence of mitochondrial components in large microparticles (mitoMPs) in SLE patients' blood was positively correlated with disease activity, anti-dsDNA antibodies, and pro-inflammatory cytokines. This suggests that mitoMPs could potentially be used as biomarkers to assist in determining the onset of SLE [200]. Furthermore, circulating microparticles in SLE patients with nephritis contained abundant cell-free DNA (cfDNA) that had a high affinity for autoantibodies. Although total plasma cfDNA levels were normal, this specific cfDNA in microparticles could serve as a biomarker for nephritis in SLE patients [82]. In summary, EVs, particularly apoptotic bodies, play a significant role in the development of SLE and have the potential to be targeted therapeutically. Additionally, they can be explored as candidate biomarkers to aid in diagnosing and assessing SLE.

Therapeutics

The anti-inflammatory properties of MSCs, immune cells, plant, and bacterial-derived EVs make them promise for treating inflammatory diseases. EVs have several advantages over cell therapy, including their safety profile and ability to cross biological barriers efficiently. EVs are composed of lipid bilayers, allowing them to effectively traverse biological barriers such as the blood–brain, intestinal, and placental barriers. Moreover, EVs possess chemotactic abilities, allowing them to migrate to inflammatory sites. This property makes them attractive as drug-delivery vehicles for treating inflammatory diseases (Fig. 4). Their natural composition and ability to mimic the functions of parent cells make them biocompatible and less likely to elicit immune responses. Additionally, EVs are stable in circulation and have prolonged half-lives, allowing for sustained release of therapeutic cargo.
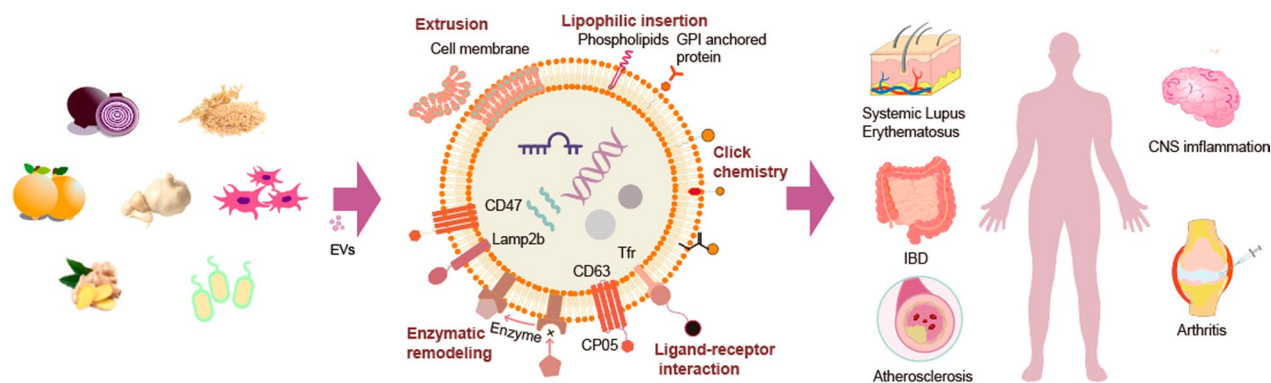
Fig. 4 Engineering EVs from different sources for the treatment of inflammatory diseases. The left portion illustrates the common sources of EVs currently employed in treating inflammatory diseases and for drug delivery. These sources include MSCs, bacteria, and plants (such as oranges, ginger, oats, garlic, etc.). Various common modification methods for EVs are introduced in the middle section of the image. These methods encompass genetic engineering, enzyme remodeling, receptor-ligand interactions, click chemistry, extrusion, and lipophilic group insertion. Each of these techniques corresponds to specific proteins or chemical groups. These modification methods make it possible to attach specific targeting groups to EVs and load them with various drugs, including nucleic acids, small molecules, and protein drugs. Following these engineering processes, EVs are poised for targeted therapy in various inflammatory diseases, as depicted in the right portion of the figure

EVs in Arthritis treatment

EVs in RA treatment

Anti-inflammatory effect Mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) have emerged as a promising alternative to MSCs for the treatment of RA. MSC-EVs have been shown to possess similar therapeutic effects as MSCs in controlling the development of RA and relieving inflammation. They have been found to effectively prevent the development of joint lesions and systemic damage associated with RA.

One advantage of MSC-EVs over MSCs is their lower immunogenicity. Allograft survival of MSCs is often limited due to immune rejection [306]. However, MSC-EVs have been found to have lower immunogenicity, making them a more suitable option for clinical application.

Furthermore, MSC-EVs carry various miRNAs that have been shown to promote cartilage repair and inhibit the secretion of inflammatory factors such as nitric oxide (NO), IL-1β, and TNF-α. These miRNAs can effectively inhibit joint and synovial inflammation, providing additional therapeutic benefits for RA. Clinical trials involving MSC-EVs in the treatment of RA are currently underway, further highlighting the potential of MSC-EVs as a viable therapeutic option for this inflammatory disease (Table 2).

In summary, MSC-EVs have shown great promise in the treatment of RA, with similar therapeutic effects as MSCs but with lower immunogenicity. The miRNAs carried by MSC-EVs contribute to cartilage repair and the inhibition of inflammatory factors, providing additional benefits for RA treatment. Further research and clinical trials are needed to fully explore the potential of MSC-EVs in RA therapy.

EVs as drug delivery vehicles In addition to the direct anti-inflammatory effect, EVs can also be used as drug delivery vehicles to alleviate the side effects of RA therapeutic drugs in vivo. M1 macrophages cause chronic inflammation, joint and synovial injury in RA patients by releasing inflammatory cytokines (TNF-α, IL-1β, IL-6, etc.) and inflammatory mediators (prostaglandins and chemokines, etc.) [243, 306]. Therefore, regulating the macrophage phenotype is an effective way to treat RA. IL-4 and IL-10 can convert macrophages into the anti-inflammatory M2 type to reduce the inflammatory response of RA, but the lack of targeting leads to drug toxicity at non-treatment sites, which limits the clinical application of these drugs. Macrophage-derived EVs have the ability to target macrophages, and the use of macrophage-derived EVs to deliver the aforementioned drugs can effectively target macrophages and induce their differentiation into the M2 type, thereby alleviating joint synovial inflammation [114, 137, 240].

Engineering EVs in RA treatment Engineering EVs can enhance their targeting ability and anti-inflammatory effects in the context of RA. Metabolic glycoengineering (MGE) is a technique that involves modifying the surface of EVs by introducing specific sugars, such as azide-functionalized dextran sulfate (DS). By using click chemistry, DS can be attached to the surface of MSC-Exo, resulting in engineered EVs that efficiently target macrophages. These engineered EVs, known as DS-MSC-Exo, have been shown to have a higher targeting efficiency compared to unmodified MSC-Exo. Importantly, the dose of DS-MSC-Exo required for RA treat-

Table 2 The role of exosomal miRNAs of MSCs in arthritis inflammation

Source of EVs	Contains	Diseases	Functions	Animal model	Refs
BMSCs	miR-150-5p	RA	Inhibiting synoviocyte hyperplasia and angiogenesis	Mouse	[33]
MSCs	miR-146a	RA	Increasing Treg cell populations and anti-inflammatory cytokines	Mouse	[244]
BMSCs	miR-34a	RA	Inhibiting abnormal RA-FLS growth and RA inflammation	Rat	[285]
ADSCs	let-7b-5p, miR-24-3p	RA	Attenuating cartilage injury via promoting M2 macrophage polarization	Mouse	[118]
SMSCs	miR-31	OA	Alleviating cartilage damage and inflammation in knee joints	Mouse	[271]
AT-MSCs	miR-100-5p	OA	Maintaining cartilage homeostasis via inhibition of mTOR	Mouse	[286]
BMSCs	miR-135b	OA	Attenuating cartilage injury via promoting M2 macrophage polarization	Rat	[275]
MSCs	miR-135b	OA	Promoting chondrocyte proliferation by regulating Sp1	Rat	[276]
BMSCs	miR-136-5p	OA	Inhibiting cartilage degeneration by targeting ELF3	Mouse	[30]
BMSCs	miR-216a-5p	OA	Promoting the proliferation, migration, and inhibiting apoptosis of chondrocytes by targeting JAK2	Rat	[213]
BMSCs	miR-361-5p	OA	Alleviating OA damage and inflammation by targeting DDX20	Rat	[242]
SMSCs	miR-155-5p	OA	Enhancing ECM secretion of osteoarthritic chondrocytes	Mouse	[281]
MSCs	miR-92a-3p	OA	Inhibiting cartilage degradation by targeting WNT5A	Mouse	[168]
Synovial MSCs	miR-140-5p	OA	Enhancing cartilage tissue regeneration	Rat	[241]
Urine-derived stem cells	miR-140-5p	OA	Enhancing cartilage regeneration and subchondral bone remodeling	Rat	[161]
ADSCs	miR-486-5p	OA	Alleviating chondrocyte apoptosis and regulating macrophage polarization	Rat	[279]
BMSCs	miR-206	OA	Relieving inflammation and promoting osteoblast differentiation by reducing Elf3	Mouse	[90]

ment is significantly lower than that of MSC-Exo, indicating their enhanced therapeutic potential [306].

Another approach is the use of PEGylated hyaluronic acid (PHA)-decorated EVs. PHA can be efficiently synthesized using orthogonal chemistry and then attached to the surface of EVs. PHA-EVs have been shown to effectively target RA tissues due to the high affinity of PHA for CD44, a receptor overexpressed in inflamed joints. Additionally, the attachment of PHA to EVs prolongs their circulation time in the bloodstream, further enhancing their therapeutic potential [150].

Furthermore, ROS-responsive DC-derived exosomes can be constructed using a lipid insertion method. These exosomes are designed to respond to ROS, which are elevated in RA. Upon exposure to ROS, these engineered exosomes activate immune regulation and alleviate RA inflammation [130].

EVs in OA treatment

EVs can effectively penetrate the cartilage matrix for drug delivery and OA treatment [250]. MSC-EVs from different tissue and body fluid sources, such as fat, synovium, umbilical cord, bone marrow, and urine, have been widely used to reduce OA inflammation and promote cartilage repair (Table 2). Recently, antler stem cell-derived exosomes were also shown to attenuate inflammation and cellular senescence and promote cartilage regeneration

in a mouse model of OA [134]. Additionally, the use of biomaterial scaffolds can help to prolong the release time of EVs, allowing for sustained and controlled delivery of therapeutic cargoes [66, 294].

EVs as a drug delivery system for the treatment of OA EVs can be utilized as a drug delivery system for the treatment of OA. By engineering EVs to carry specific therapeutic cargo, such as Wnt3a [250] or sleep-related circRNA3503 [220], it is possible to enhance their therapeutic effects on OA. This targeted delivery of therapeutic cargo through EVs can help reduce symptoms of OA and promote cartilage repair.

In addition, the development of hybrid exosomes, which are prepared by co-extruding liposomes and exosomes, can further enhance their drug-loading capacity and stability. These hybrid exosomes have been shown to efficiently deliver cas9 plasmid to knock out MMP13, a protein associated with joint inflammation in OA [148].

Two clinical trials using MSC-derived EVs to treat bone and joint diseases are currently underway (e.g., NCT05060107, NCT05261360, Table 5). Although the specific mechanisms by which EVs traverse the extracellular matrix and the anti-inflammatory mechanisms targeting osteoarthritis remain limited, further research on these mechanisms will help advance the development of EV-based drug delivery systems for the clinical application of treating OA.

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Engineering EVs in OA treatment   Engineering modifications of EVs can greatly improve their targeting and penetration abilities to cartilage, thereby enhancing their drug delivery capacity and anti-inflammatory effects in OA treatment.

One approach involves modifying the surface of MSC-derived EVs with εPL-PEG-DSPE through a lipid intercalation method. This modification alters the surface charge of EVs and enhances their penetration into cartilage, improving their ability to deliver therapeutic cargo to the affected area [63]. Genetic engineering techniques can also be utilized to enhance the targeting ability of EVs. For instance, the chondrocyte-targeting peptide CAP can be fused with the surface lamp2b protein of EVs. This modification enhances the ability of EVs to specifically target OA chondrocytes, increasing the efficiency of drug delivery to the desired cells [147].

Furthermore, genetic engineering can be used to modify the surface of EVs loaded with the chondrogenic factor KGN. By introducing the E7 peptide on the surface of KGN-loaded EVs, termed E7-Exo-KGN, these engineered EVs can specifically target MSCs and induce their chondrogenic differentiation. This

approach holds promise for alleviating OA by promoting cartilage regeneration [293].

EVs in CNS inflammatory diseases
EVs in neurodegenerative disease therapy
Mammalian cell-derived EVs   Neural stem cells (NSCs) have shown promise in treating neurodegenerative diseases due to their ability to differentiate into various neural cell types and secrete neuroprotective factors (Fig. 5). EVs derived from NSCs contain these therapeutic factors and can be used as a cell-free alternative for therapy. These EVs can promote nerve regeneration, reduce neuroinflammation, and decrease protein deposition, slowing down neurodegenerative disease progression.

MSC-derived EVs from sources such as fat and bone marrow have also shown potential in treating neurodegenerative diseases (Fig. 5). They have been found to promote nerve regeneration, reduce neuroinflammation, and enhance the clearance of toxic protein aggregates [39, 218].

Astrocytes, a type of glial cell in the brain, play important roles in maintaining neuronal health and function (Fig. 5). EVs derived from astrocytes have demonstrated
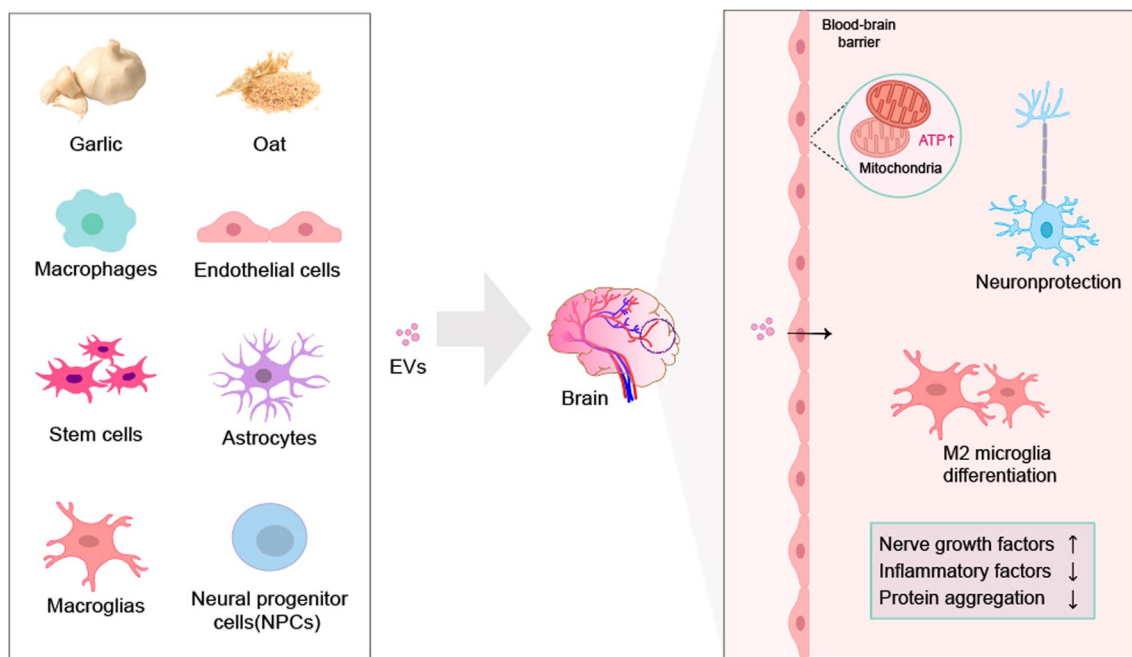
Fig. 5 Treatment of CNS inflammatory diseases with EVs. As shown in the figure, the upper section presents the common sources of EVs currently utilized for the treatment of CNS inflammatory diseases and drug delivery. These sources encompass macrophages, microglia, MSCs, as well as EVs derived from oats and garlic. In the lower portion of the figure, the specific effects of EVs on the central nervous system after crossing the blood–brain barrier are summarized. These effects primarily involve the promotion of nerve growth factor secretion, reduction in pro-inflammatory factor secretion, and inhibition of protein deposition, such as Aβ and alpha-synuclein. Specifically, EVs from macrophages, microglia, MSCs, oats, and garlic can target inflammatory sites and foster the differentiation of M2 microglial cells, which have anti-inflammatory properties. Meanwhile, EVs derived from neural progenitor cells enhance post-stroke blood–brain barrier integrity, thus improving stroke-related hindbrain injuries. Additionally, endothelial cell-derived EVs can alleviate cerebral ischemia by enhancing brain endothelial cell mitochondrial function and increasing intracellular ATP levels

neuroprotective effects by inhibiting neurodegeneration and synapse loss, restoring mitochondrial function, and promoting the clearance of amyloid-beta (Aβ) plaques, a hallmark of Alzheimer's disease [49, 133, 196].

Microglia, the brain's resident immune cells, also release EVs that can be harnessed for therapy (Fig. 5). These microglia-derived EVs have been shown to promote the breakdown of Aβ plaques through the action of miR-124-3p targeting Rela, providing neuroprotective effects [70].

Currently, there are 4 clinical studies investigating the use of exosomes for the treatment of central nervous system-related diseases, including Alzheimer's disease, stroke, neuroprotection in low-birth-weight infants, and the treatment of diseases such as depression, anxiety, and dementia. For example, a study using MSC-Exo to deliver miR-124 for stroke treatment has demonstrated the clinical potential of exosome-mediated nucleic acid delivery in disease therapy (Table 5).

Plant-derived EVs   In addition to EVs derived from mammalian cells, plant-derived EVs have also shown promise in alleviating inflammation in the central nervous system [237, 292] (Fig. 5).

Plant-derived EVs, such as garlic exosome-like nanoparticles (GaELNs) [237] and oat exosome-like nanoparticles [292], have shown promise as potential drug delivery carriers for targeting the brain and alleviating inflammation in neurodegenerative diseases. These plant-derived EVs have been shown to be able to enter the mouse brain and be taken up by microglial cells, leading to beneficial effects such as the inhibition of inflammatory cytokine expression and the alleviation of brain inflammation. Plant-derived EVs have the advantage of being more abundant and easier to obtain compared to mammalian cell-derived EVs, making them a potentially valuable resource for drug delivery in the future.

Targeting peptides-modified EVs   Targeting peptides-modified EVs have shown great potential for enhancing the therapeutic effects of EVs and the drugs they deliver. By genetically engineering the surface of EVs with neuron-specific RVG peptides, targeted therapy for neurodegenerative diseases like Parkinson's and Alzheimer's can be achieved (Table 3). For instance, RVG-targeted exosomes loaded with BACE1 siRNA were able to significantly reduce BACE1 mRNA levels and total β-amyloid 1–42 levels in the brains of Alzheimer's mice, without causing any immune response [5]. Additionally, RVG-exosomes loaded with shRNA minicircles have shown the ability to clear α-synuclein aggregates, offering a potential long-lasting treatment for Parkinson's disease [94]. These studies highlight the promising application of targeted EVs in the field of neurodegenerative disease therapy.

EVs with multiple functions and targeting capabilities   These advancements in engineering EVs with multiple functions and targeting capabilities have greatly

Table 3 Engineering EVs for the treatment of CNS inflammatory diseases

Source of EVs	Modified group	Loading cargo	Disease	Animal Type	Refs
NSCs	PDGFRα	Bryostatin-1	Demyelination	Mouse	[287]
HEK-293 T	RVG	mRNA	Parkinson's disease	Mouse	[120]
HEK-293 T	RVG	Aptamers	Parkinson's disease	Mouse	[209]
DCs	RVG	**α**-Syn shRNA	Parkinson's disease	Mouse	[95]
DCs	RVG	shRNA minicircles	Parkinson's disease	Mouse	[94]
DCs	RVG	Curcumin/ siRNA	Parkinson's disease	Mouse	(Izco et al., 2023b)
MSCs	SA-P/SA-RVG29	Curcumin/miR-133b	Parkinson's disease	Mouse	[197]
DCs	RVG	BACE1 siRNA	Alzheimer's disease	Mouse	[302]
BM-MSCs	RVG	miR-124	Ischemia	Mouse	[297]
HEK-293 T	RVG	cricSCMH1	Ischemic stroke	Mouse/ Monkey	[298]
BM-MSCs	c (RGDyK)	Curcumin	Ischemic stroke	Mouse	[253]
BM-MSCs	c (RGDyK)	miR-210	Cerebral ischemia	Mouse	[315]
HEK293Ts	RBP	AMO181a-chol	Ischemic stroke	Rat	[117]
NPCs	RGD	miRNAs	Cerebral ischemia	Mouse	[252]
HucMSCs	CD73	CD73	Spinal cord injury	Mouse	[312]
Macrophages	NGF	Curcumin	Spinal cord injury	Mouse	[313]

RBP RAGE-binding-peptide, *NPCs* Neural progenitor cells, *NSCs* Neural stem cells, *HucMSCs* Human umbilical cord mesenchymal stem cells, *NGF* Nerve growth factor

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expanded their potential as nanotherapeutics for neurodegenerative diseases like Parkinson's disease (PD).

One approach involves loading EVs with superparamagnetic iron oxide nanoparticles (SPION), PPS–PEG-curcumin, and miR-133b, and modifying them with SA-P/SA-RVG29. This multifunctional EV system allows for tracking, targeting, gene therapy, and small molecule therapy for PD treatment [197].

Another strategy involves loading curcumin/ANP and siRNA (siSNCA) into RVG-exosomes to create a "nanoscavenger" that targets the brain and responds to ROS. This nanoscavenger can inhibit the formation of α-synuclein aggregates and abnormal immune activation, improving PD symptoms [156].

Additionally, a "one-to-one" approach combines MSC-derived exosomes with "artificial modules" like NO-driven nanomotors to cross the blood–brain barrier and clear inducible nitric oxide synthase (iNOS), ROS, and α-synuclein aggregates [273]. This approach demonstrates the potential of incorporating artificial modules into EVs to enhance their therapeutic capabilities.

Overall, these advancements in engineering EVs with multifunctionality and responsiveness offer new avenues for developing targeted and effective therapies for neurodegenerative diseases.

EVs in stroke therapy

EVs derived from stem cells and other cell types   Studies show that various types of stem cell-derived EVs, as well as EVs derived from other cell types, have the potential to alleviate inflammation and promote recovery in stroke. These EVs can exert their therapeutic effects through different mechanisms, such as restoring blood flow, regulating immune cells, reducing inflammation, promoting neuronal recovery, and enhancing cerebrovascularization [124, 268].

Stem cell-derived EVs, such as those derived from MSCs, embryonic stem cells, and induced pluripotent stem cells, have shown promising results in promoting neurological recovery and relieving post-stroke inflammation and brain infarction. They achieve these effects by expanding regulatory T cell populations, promoting blood–brain barrier repair, and restoring normal physiological functions in neurons [12, 124].

Furthermore, EVs derived from microglia, astrocytes, neural progenitor cells, and endothelial cells have also demonstrated efficacy in alleviating inflammation and brain damage in stroke [86, 142, 144, 207, 313]. Microglia-derived EVs inhibit glial scar formation, promote oligodendrocyte precursor cell differentiation, and enhance oligodendrogenesis, leading to stroke recovery [137, 138, 141–144, 207]. Astrocyte-derived EVs and neural

progenitor cell-derived EVs improve post-stroke brain injury by restoring the structure and function of the corpus callosum and enhancing the integrity of the blood–brain barrier, respectively [86, 313]. Recently, cerebral endothelial cell (CEC)-derived EVs have been shown to promote axonal outgrowth and improve mitochondrial function in brain endothelial cells by transporting mitochondria under ischemic conditions. Notably, these CEC-derived EVs significantly increase the ATP levels of endothelial cells during the process of mitochondrial transport [54, 319].

EVs loaded with nucleic acid drugs for stroke therapy   EVs can be loaded with a variety of nucleic acid drugs for stroke therapy. Some examples include protein, curcumin, circRNA, miRNA, and anti-microRNA oligonucleotide (AMO) (Table 3). These nucleic acid drugs can be encapsulated within the EVs and delivered to the ischemic sites for therapeutic effects. For example, AMO181a-chol-loaded exosome linked to RAGE-binding-peptide (RBP-Exo), when administered nasally, have shown targeting ability to ischemic sites and significant anti-inflammatory effects in stroke therapy [117]. Additionally, the delivery of circular RNA SCMH1 through RVG-EVs has demonstrated post-stroke recovery effects in both mouse and monkey stroke models. These findings highlight the potential of EVs as carriers for nucleic acid drugs in stroke therapy [296].

Magnetic EVs in stroke therapy   The use of magnetic nanoparticles in engineering EVs has shown promise in enhancing their targeting ability and therapeutic effects in stroke therapy. These magnetic EVs can be obtained through various methods such as co-incubation, mechanical extrusion, or receptor-ligand interaction. The presence of magnetic nanoparticles allows these EVs to cross the blood–brain barrier and target the treatment site with the guidance of a controllable magnetic field. Compared to unmodified EVs, magnetic EVs demonstrate superior targeting ability [107, 115, 314, 316]. Additionally, the magnetic field can be used to separate these EVs directly, eliminating the need for time-consuming ultracentrifugation.

One example of magnetic EVs in stroke therapy is the use of MSC-derived EVs containing iron oxide nanoparticles (IONP). These magnetic EVs have been shown to effectively target ischemic brain injury sites in mice under the influence of a magnetic field. They exhibit the ability to inhibit neuroinflammation and promote tissue recovery. In fact, magnetic NVs (MNVs) accumulated 5.1 times more at the brain injury site compared to MSC-EVs alone. Moreover, the presence of IONP in MSCs increases the expression of growth factors and enhances

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the anti-inflammatory, anti-apoptotic, and angiogenesis-promoting effects of MSC EVs [115].

These findings highlight the potential of magnetic EVs in improving the targeting ability and therapeutic efficacy of EV-based stroke therapies.

EVs in spinal cord injury therapy

EVs derived from cells   Studies suggest that EVs derived from various cell types have the potential to alleviate inflammation and promote the repair of spinal cord injury (SCI). For example, EVs released by MSCs can be specifically taken up by macrophages at the site of SCI injury and upregulate the expression of tight junction proteins of the blood–brain barrier/blood-spinal cord barrier, which promotes SCI recovery [159, 185].

Furthermore, the content of ubiquitin-specific peptidase 29 (USP29) in EVs released from MSCs pretreated with melatonin was found to be significantly increased. These USP29-containing EVs were able to regulate microglia/macrophage polarization and reduce inflammation in SCI [160].

In addition to MSC-EVs, EVs derived from blood cells, umbilical vein endothelial cells, Treg cells, astrocytes, NSCs, and dental pulp stem cells have also been shown in recent studies to alleviate SCI inflammation and promote spinal cord injury repair [100, 137, 152, 192, 208, 211, 212, 290, 318]. Overall, these findings suggest that EVs derived from various cell sources have therapeutic potential in the treatment of SCI by modulating inflammation and promoting tissue repair.

EVs-loaded hydrogels for SCI repair   The use of EV-loaded hydrogels for SCI repair is an emerging field of research [84]. Compared to systemic administration, hydrogel-loaded EVs have several advantages, including prolonged residence and release time in nerve tissue, enhanced tissue repair, and anti-inflammatory effects.

One approach to enhance the retention of EVs at the site of SCI is through the modification of hydrogels with laminin-derived peptides. These peptides can react with integrins on the surface of EVs, further enhancing their retention at the site of injury [139]. Another strategy is the use of hydrogel microneedle arrays to load 3D-cultured MSC-derived EVs. 3D culture significantly improves the anti-inflammatory efficacy of MSC-EVs and promotes tissue recovery. The microneedle array ensures the sustained release of MSC-EVs at the site of injury [79].

Conductive hydrogels, which have electrical transmission properties similar to natural neural tissue, can also be utilized for SCI repair. These hydrogels can promote the recruitment and differentiation of NSCs after SCI.

When combined with MSC-EVs, conductive hydrogels show promise as a potential therapy for SCI [60].

EVs as a targeted drug delivery system for SCI treatment   EVs have shown great potential in delivering therapeutic drugs to the site of spinal cord injury (SCI). Macrophage-derived exosomes, for example, can be loaded with drugs like Berberine to inhibit the inflammatory response after SCI [69]. Similarly, MSC-Exo loaded with specific siRNA can be administered nasally and home to the spinal cord lesion, improving motor and sensory function [77].

Engineered EVs, such as CD73 modified EVs and nerve growth factor (NGF)-coupled EVs, have also been investigated for SCI treatment [312, 313]. CD73 on the surface of engineered EVs can help scavenge excess ATP in the inflammatory environment following SCI, reducing inflammation and promoting tissue repair. NGF-coupled EVs, on the other hand, can release NGF at the site of spinal cord injury, promoting SCI recovery by stimulating nerve regeneration.

EVs in IBD treatment

Mammalian cell-derived EVs in IBD treatment

Intestinal epithelial cells (IECs) play a crucial role in maintaining intestinal immune balance, and their released EVs have been found to have therapeutic potential in IBD. IEC-EVs have been shown to induce regulatory T cells, which help suppress excessive immune responses and inflammation in the gut, leading to the relief of IBD symptoms [103].

In addition to IEC-EVs, exosomes released by immune cells have also been investigated for their potential in IBD treatment [13, 48]. For example, exosomes derived from M2 macrophages have been shown to alleviate intestinal mucosal injury and inhibit inflammatory responses in IBD models [48]. This effect is mediated by the transfer of miR-590-3p, which helps regulate inflammatory pathways and promote tissue repair [291].

Furthermore, exosomes released by DCs have been found to play a role in promoting an anti-inflammatory environment in the gut. DC exosomes can transfer miR-146a, which helps promote the differentiation of intestinal macrophages into an anti-inflammatory phenotype, thus alleviating colitis [13].

These findings highlight the potential of immune cell-derived exosomes as therapeutic agents for IBD, as they can modulate immune responses and promote anti-inflammatory effects in the intestine. Further research is needed to explore the mechanisms of action and optimize the use of these exosomes for clinical applications in IBD treatment.

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Mechanisms of MSC-EVs in alleviating IBD MSC-EVs have shown great potential in alleviating IBD by modulating various aspects of the immune response and gut microbiota.

One key mechanism is the ability of MSC-EVs to reshape the phenotype of intestinal macrophages. These EVs can induce the polarization of macrophages towards an anti-inflammatory phenotype, characterized by reduced secretion of pro-inflammatory cytokines like IFN-γ, IL-1β, IL-6, and TNF-α, and increased secretion of the anti-inflammatory cytokine IL-10 [154]. This shift in macrophage phenotype helps to dampen the excessive immune response and inflammation in the gut, thereby alleviating IBD symptoms.

Furthermore, MSC-EVs have been found to inhibit the differentiation of Th17 cells, which are known to play a role in promoting inflammation in IBD. By suppressing Th17 differentiation, MSC-EVs help to reduce the production of pro-inflammatory cytokines such as IL-17A and IL-22, thereby attenuating gut inflammation [299].

In addition to their immunomodulatory effects, MSC-EVs can also restore intestinal homeostasis by influencing the composition of gut microbes. Studies have shown that MSC-EVs can enhance the growth of beneficial bacteria and restore the diversity of the gut microbiota, leading to a more balanced microbial community and reduced inflammation in the intestine [188].

Overall, MSC-EVs hold great promise as a therapeutic approach for IBD due to their ability to modulate immune responses, inhibit Th17 differentiation, and restore gut microbiota composition. Further research is needed to fully understand the underlying mechanisms and optimize their clinical application.

Gut flora-derived EVs
EVs secreted by intestinal microbes in maintaining intestinal homeostasis and regulating immune responses Findings have highlighted the important role of EVs in mediating the crosstalk between intestinal microbes, immune cells, and the host [50, 55, 76, 127, 258]). The EVs derived from intestinal microbes have the ability to activate B cells and DCs, leading to the production of IgA and the maintenance of intestinal homeostasis [112, 277]. They can also promote the production of anti-inflammatory cytokines and regulatory T cells, further regulating the intestinal immune system (Durant et al., 2020b) [224]).

Furthermore, the composition of the intestinal microbiota can influence the production and function of EVs. For example, EVs derived from high-protein-fed microbiota have been shown to induce IgA response by activating Toll-like receptor 4 [277]. This suggests that dietary factors can modulate the production and function of EVs, thereby impacting the host-microbe interactions in the intestine.

EVs released by intestinal microbes in restoring intestinal barrier function and inhibiting inflammation EVs released by certain intestinal microbes, such as Clostridium butyricum and Akkermansia muciniphila, have the potential to restore intestinal barrier function and inhibit inflammation in IBD patients [52, 64, 304]. By increasing the expression of mucin proteins and TJ proteins (Claudin1, Claudin2, Claudin3, Zo-1), these EVs can enhance the integrity of the mucus layer and tight junctions, which are crucial for maintaining the barrier function of the intestine [166].

Furthermore, these EVs can also suppress the production of proinflammatory markers (LPS, IL-6, and TNF-α) and cytokines, while promoting the secretion of anti-inflammatory cytokines (IL-10, IL-13) [166, 277]. This dual effect on the immune system helps to modulate the inflammatory response and reduce inflammation in the intestines.

Overall, the EVs released by specific intestinal microbes have the potential to be used as therapeutic agents for IBD by restoring barrier function, inhibiting inflammation, and promoting intestinal homeostasis. However, further research is needed to fully understand the mechanisms underlying these effects and to develop safe and effective EV-based therapies for IBD.

Intestinal microbial-derived EVs in regulating the composition of the gut microbiome and promoting intestinal homeostasis Studies have indicated that intestinal microbial-derived EVs have the potential to regulate the composition of the gut microbiome, which is important for maintaining intestinal homeostasis and preventing IBD [135, 266]. By selectively promoting the growth of beneficial bacteria and reducing the abundance of pathogenic bacteria, these EVs can help restore a healthy gut microbiome and alleviate intestinal inflammation.

One specific example is Akkermansia muciniphila-derived EVs, which have been shown to selectively promote the proliferation of beneficial bacteria while reducing the abundance of pathogenic bacteria in the gut microbiota. This selective modulation of the microbiome can contribute to the restoration of intestinal homeostasis and the prevention or treatment of IBD [277]. Similarly, EVs derived from Clostridium butyricum have been found to greatly increase the diversity of gut microbes in a murine model of colitis induced by dextran sulfate sodium (DSS). This increased microbial diversity is associated with improved gut health and reduced inflammation [166].

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Overall, these findings suggest that intestinal microbial-derived EVs have the potential to regulate the composition of the gut microbiome and promote intestinal homeostasis, making them promising candidates for the treatment of IBD. However, further research is needed to fully understand the mechanisms by which these EVs modulate the gut microbiome and to optimize their therapeutic potential.

Plant-derived EVs

Recent studies have shown the potential of plant-derived EVs as novel therapeutic agents for IBD. The unique cargo of mRNA, miRNA, lipids, and proteins found in these EVs can modulate intestinal homeostasis and exert anti-inflammatory and antioxidant effects (IL-10, IL-6, HO-1) (Table 4) [301–308].

One interesting example is ginger-derived ELNs, which have been shown to activate the AHR pathway and induce IL-22 production, leading to the inhibition of

Table 4 Plant-derived EVs for colitis models

Source of EVs	Administration route	Loading Cargo	Function	Animal model	Refs
Mulberry bark	Oral	NA	Inhibiting intestinal inflammation via the AhR/COPS8 pathway	Mouse	[233]
Ginger	Oral	NA	Regulating the composition, metabolites, growth, and localization of gut microbiota	Mouse	[248]
Ginger	Oral	NA	Anti-inflammation, regulating the gut microbiota, promoting M2 macrophage differentiation and restoring damaged intestinal epithelial barrier	Mouse	[183]
Grape	Oral	NA	Promoting intestinal stem cell proliferation	Mouse	[105]
Grapefruit	Oral	MTX	Reducing the production of proinflammatory cytokines in colonic macrophages	Mouse	[267]
Ginger	Oral	NA	Anti-inflammation and anti-oxidant	Mouse	[31]
Ginger	Oral	Infliximab	Inhibiting TNF-α and blocking the NLRP3 inflammasome	Mouse	[169]
Tea leaves	Oral	NA	Inhibiting the inflammatory bowel responses, restoring disrupted colonic barriers and enhancing the diversity and overall abundance of gut microbiota	Mouse	[310]
Grapefruit	Oral	NA	Enhancing the anti-inflammatory capacity of intestinal macrophages	Mouse	[183]

Table 5 EV-based therapy that currently under clinical trial source with ClinicalTrial.gov

Disease	Source of EVs	ClinicalTrial. gov identifier	Administration route	Phase	Estimated enrollment	Year started
Alzheimer disease	MSC	NCT04388982	Intranasal administration	12	9	2020
Ischemic stroke	MSC	NCT03384433	Intravenous injection	12	5	2019
Inflammatory Bowel Disease	Ginger	NCT04879810	Oral administration	N	90	2018
Complex Anal Fistula	Placenta MSC	NCT05402748	Intrafistula injection	12	80	2022
Colon cancer	Plant	NCT01294072	Oral administration	1	35	2011
Primary gastric cancer and colorectal cancer	HEK293	NCT05375604	Intravenous injection	1	30	2022
Crohn disease	BMSC	NCT05130983	Intravenous injection	1	10	2022
Ulcerative Colitis	BMSC	NCT05176366	Intravenous injection	1	10	2022
Psoriasis	MSC	NCT05523011	External application	1	10	2022
Dystrophic epidermolysis bullosa	BMSC	NCT04173650	External application	1	10	2023
Dystrophic epidermolysis bullosa	MSC	NCT04173650	External application	12	10	2023
Osteoarthritis	MSC	NCT05060107	Intra-articular injection	1	10	2021
Degenerative Meniscal Injury	SF-MSC	NCT05261360	Intra-articular injection	2	30	2022
Acute respiratory distress syndrome	BMSC	NCT05127122	Intravenous injection	12	81	2023
COVID-19 related ARDS	BMSC	NCT05354141	Intravenous injection	3	400	2022
bronchopulmonary dysplasia	BMSC	NCT03857841	Intravenous injection	1	3	2021
COVID-19 related ARDS	BMSC	NCT04493242	Intravenous injection	2	120	2020
COVID-19	BMSC	NCT05125562	Intravenous injection	2	30	2021
Post-Acute COVID-19 and Chronic Post-COVID-19 syndrome	BMSC	NCT05116761	Intravenous injection	12	60	2021

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colitis in mice. This pathway is known to play a critical role in regulating immune responses in the gut, and its activation by ginger-derived EVs may contribute to the anti-inflammatory effects observed [248].

Furthermore, plant-derived EVs have also been found to regulate the composition, metabolism, growth, and localization of intestinal microorganisms [248]. This suggests that these EVs have the potential to modulate the gut microbiome, which is known to play a crucial role in IBD pathogenesis.

Overall, the use of plant-derived EVs as therapeutic agents for IBD holds great promise. However, further research is needed to fully understand the mechanisms by which these EVs exert their effects and to optimize their therapeutic potential. Additionally, clinical trials are required to evaluate the safety and efficacy of plant-derived EVs in humans with IBD.

Engineered EVs

Engineered EVs have shown potential as therapeutic agents for IBD. For example, MSC-EVs overexpressing PD-L1 through lentiviral transfection can inhibit immune cell activation and alleviate ulcerative colitis in mice (Fang [291]). Similarly, BMSCs-derived exosomes modified with Heme Oxygen-1 (HO-1) can reduce inflammatory damage in intestinal epithelial cells by regulating HMGB3 expression through miR-200b [235].

To prolong the treatment time of MSC-Exo in the intestinal tract, researchers have developed a sequential wrapping approach using sodium alginate (SA) hydrogel microspheres, gelatin layer, and a pH-sensitive thin Eudragit FS30D (EFS) shell. This engineered EV system, known as EAGE, has shown better anti-inflammatory effects and promotes tissue recovery in an IBD model [67]. In addition, EVs from HEK293T modified with DNA Aptamer can also be used to treat IBD [313].

A gene transfection technology using lentivirus-mediated gene delivery has been developed to produce extracellular vesicles derived from programmed cell death ligand 1-positive (PD-L1+) mesenchymal stem cells (MSCs), termed MSC-sEVs-PD-L1, for modulating the local immune microenvironment of affected tissues in autoimmune diseases [291]. MSC-sEVs-PD-L1 have demonstrated the ability to regulate various activated immune cells towards an immunosuppressive state in vitro. Moreover, in murine models of ulcerative colitis induced by dextran sulfate sodium (UC) and psoriasis induced by imiquimod, MSC-sEVs-PD-L1 exhibited superior homing to inflamed tissues compared to PD-L1 + MSCs. The use of MSC-sEVs-PD-L1 can reshape the inflammatory ecosystem in the local immune environment, demonstrating therapeutic efficacy in UC and psoriasis mouse disease models.

Plant-derived engineered EVs can also serve as drug carriers for IBD therapy. For instance, nanovesicles derived from grapefruit have shown specific absorption by intestinal macrophages. Grapefruit-derived EVs can be utilized as a drug delivery system to target intestinal macrophages and deliver anti-inflammatory drugs like methotrexate for colitis treatment [267]. Similarly, ginger-derived exosomes (GE) can target the colon for the delivery of anti-TNF-α antibodies (infliximab) after oral administration. GE can enhance the stability of INF-loaded large mesoporous silicon nanoparticles (LMSN) in the gastrointestinal tract, improving the efficacy of INF in treating IBD [169].

These examples highlight the potential of engineered EVs for targeted drug delivery and enhanced therapeutic effects in IBD. However, further research is needed to optimize these approaches and evaluate their safety and efficacy in clinical settings.

EVs in atherosclerosis treatment
Engineering EVs to enhance the delivery of atheroprotective miRNAs

Studies have suggested that EVs can be used as therapeutic agents in the treatment of AS. By delivering specific miRNAs, EVs can modulate various cellular processes involved in AS, such as endothelial cell pyroptosis, inflammatory response, and plaque formation.

One potential approach is to engineer EVs to overexpress or enhance the delivery of atheroprotective miRNAs. For example, EVs derived from KLF2-transduced or shear-stress-stimulated endothelial cells can deliver miR-143/145 to smooth muscle cells [87], inducing an atheroprotective phenotype. Similarly, macrophage-derived EVs containing miR-199a-5p [145], miR-99a/146b/378a [19], or dendritic cell-derived EVs containing miR-203-3p can inhibit AS-related phenotypes by targeting specific genes or pathways involved in AS progression [151]. Another approach is to use EVs derived from MSCss, which have shown promising therapeutic effects in AS. MSC-derived EVs can inhibit the expression of Junction adhesion molecule A (JAM-A), a protein involved in atherosclerotic plaque formation, through the delivery of miR-145. This can help reduce plaque formation and promote plaque stability [301].

Combining the regenerative properties of MSC exosomes with stent placement   Combining EVs with stents for AS treatment is a promising approach. Liposome-encapsulated Pro-efferocytic MSC exosomes can enhance the efficacy of stents by promoting the removal of apoptotic cells and preventing restenosis. Restenosis, the re-narrowing of blood vessels after stent placement, is a common complication in AS treatment [325].

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The use of Pro-efferocytic MSC exosomes in stents helps to enhance the clearance of apoptotic cells, which are known to contribute to plaque progression and inflammation. By promoting the removal of these cells, the risk of restenosis can be significantly reduced. In an atherosclerotic rat model, the implantation of scaffolds containing Pro-efferocytic MSC exosomes led to a significant reduction in the cardiovascular risk factor Lp-PLA2 in pathological vessels [325]. This approach not only addresses the mechanical aspects of AS treatment with stents but also targets the underlying pathological processes involved in plaque formation and progression. By combining the regenerative properties of MSC exosomes with stent placement, the overall effectiveness of AS treatment can be improved.

Further research is needed to evaluate the long-term effects and safety of this approach in preclinical and clinical studies. However, the combination of EVs with stents holds great potential in revolutionizing the treatment of AS and reducing the risk of restenosis.

EVs loaded with anti-inflammatory drugs   EVs can be loaded with anti-inflammatory drugs and targeted to atherosclerotic plaques, enhancing the therapeutic effects of these drugs in reducing plaque inflammation. For example, platelet-derived EVs loaded with the NLRP3 inflammasome inhibitor MCC950 have been shown to target atherosclerotic plaque sites and effectively inhibit plaque inflammation [166]. Similarly, M2 macrophage exosomes loaded with hexyl 5-aminolevulinate hydrochloride (HAL) can alleviate AS symptoms through their anti-inflammatory properties [284].

In addition, EVs can be modified to improve their targeting capabilities. Liposomes modified by apoptotic bodies have been shown to be more effective in targeting atherosclerotic plaques and exerting anti-inflammatory effects compared to unmodified liposomes when delivering drugs like pioglitazone [288].

These studies highlight the advantages of using EVs for drug delivery in AS treatment. EVs can deliver drugs directly to the site of plaque inflammation, maximizing their therapeutic effects while minimizing systemic side effects. Further research is needed to fully explore the potential of EVs as drug delivery vehicles in AS treatment.

EVs delivering nucleic acid drugs   EVs have shown great potential in delivering nucleic acid drugs for AS treatment. Nucleic acid drugs, such as mRNA, can be loaded into EVs to improve their stability and protect them from rapid degradation in the body.

One study demonstrated that Ldlr mRNA-enriched exosomes can efficiently deliver mRNA to recipient cells and restore LDLR protein expression. This is important because functional loss mutations of the LDL receptor lead to the accumulation of LDL cholesterol, which worsens AS. By restoring LDLR expression, these exosomes can enhance the clearance of LDL cholesterol and reduce AS-related symptoms in animal models [144]. Another study used exosomes to deliver engineered IL-10 mRNA to target plaque site macrophages. IL-10 is an anti-inflammatory cytokine that can help reduce plaque inflammation. By delivering IL-10 mRNA using exosomes, the stability of the mRNA is improved, leading to a better anti-inflammatory effect compared to using the mRNA alone [21].

These findings highlight the potential of using EVs as carriers for nucleic acid drugs in AS gene therapy. EVs can protect and deliver these drugs to the desired target cells, enhancing their therapeutic effects and improving AS treatment outcomes.

EVs in SLE treatment

One study showed exosomes derived from umbilical cord blood mesenchymal stem cells (UC-MSCs) can reduce the expression of KLF13 by increasing the level of miR-19b in peripheral blood mononuclear cells (PBMCs) of SLE patients, thereby regulating Th17/Treg homeostasis and inhibiting the expression of inflammatory factors such as TNF, IL-6, and IL-17 [257]. Approaches using EVs derived from other MSCs such as AD-MSCs, gingival MSCs, and synovial MSCs can also be used in the treatment of SLE by regulating immune cells and exerting anti-inflammatory effects [162].

Furthermore, engineered EVs can be loaded with immunomodulatory drugs or small molecules to enhance their therapeutic effects in SLE. For example, EVs derived from MSCs can be loaded with dexamethasone, a glucocorticoid with anti-inflammatory properties, and delivered to the target cells to suppress inflammation and immune dysregulation in SLE [61].

Overall, engineered EVs hold great potential for the treatment of SLE by modulating immune responses, suppressing inflammation, and inducing immune tolerance. As of now, the FDA has not approved any exosome products for general medical use. However, there are many clinical trials which may track the details from the ClinicalTrial.gov as shown in Table 5.
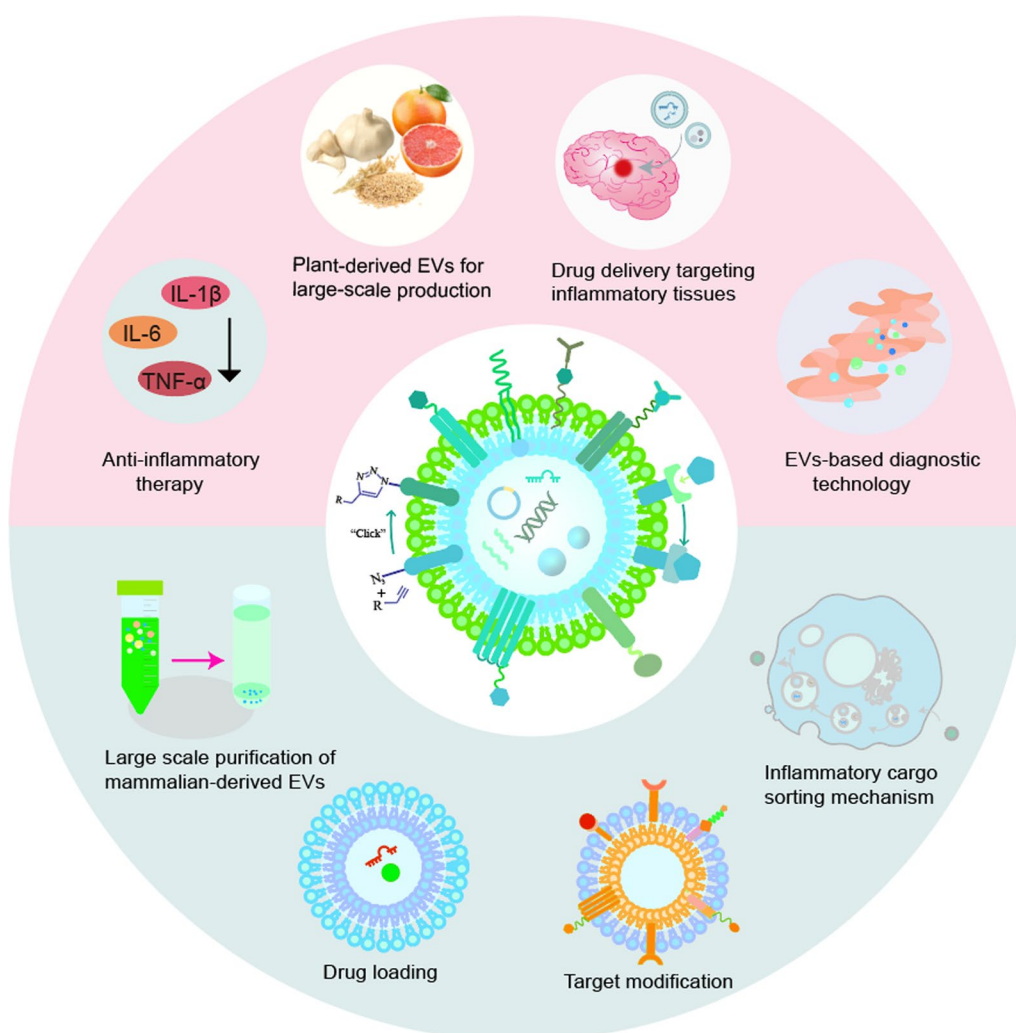
Conclusion and perspectives

EVs have been found to play a dual role in inflammation. On one hand, they can contribute to the progression of inflammatory diseases by promoting processes such as calcification, mitochondrial damage, cell senescence, and activation of autoimmunity. These pro-inflammatory effects are often observed in

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EVs derived from damaged or stressed cells. On the other hand, certain types of EVs have been shown to have anti-inflammatory properties (Fig. 6). For example, immune cell-derived EVs can regulate immune responses and modulate inflammation. MSC-derived EVs have been found to have immunomodulatory and regenerative effects, promoting tissue repair and reducing inflammation. EVs derived from plants and probiotics have also shown anti-inflammatory properties and can help mitigate damage caused by inflammation. In essence, the role of EVs in inflammation can be likened to the concept of yin and yang in Tai Chi, where "yin" corresponds to the pro-inflammatory effect of EVs and "yang" corresponds to their anti-inflammatory effect.

Another area of research that requires further investigation is the role of EVs in the resolution of inflammation. While it is known that EVs derived from certain cell types, such as macrophages, can promote the resolution of inflammation, the specific mechanisms and factors involved in this process are not fully understood. Identifying these mechanisms and factors could lead to the development of new therapeutic strategies for promoting inflammation resolution and preventing chronic inflammation. Furthermore, the heterogeneity of EV

Fig. 6 Conclusion and future prospective. EVs are vital for cell communication and play a key role in inflammatory diseases. They can either worsen inflammation by contributing to processes like vascular calcification and autoimmunity or alleviate it through immune regulation and tissue repair. EVs derived from immune cells, MSCs, plants, and probiotics show potential for treating inflammation. However, questions remain about their mechanisms and side effects. Plant and milk-derived EVs are cost-effective but need further study. Using EVs for drug delivery has promise but faces challenges like unstable loading methods and isolation limitations. Stable loading and modification techniques are needed. High-purity, large-scale, sterile EV isolation remains a challenge. In summary, EVs offer new avenues for diagnosing and treating inflammatory diseases, with potential breakthroughs in their use as drug carriers

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populations is a challenging aspect to consider. EVs can vary in terms of size, cargo composition, and surface markers, which can influence their pro-inflammatory or anti-inflammatory properties. Developing standardized methods for EV isolation and characterization will be crucial for accurately assessing their role in inflammation and for comparing results across different studies.

Additionally, the use of EVs as therapeutic agents for inflammatory diseases requires careful consideration of their delivery methods. While systemic administration of EVs is a common approach, it may result in off-target effects and limited accumulation at the site of inflammation. Therefore, the development of targeted delivery systems, such as functionalizing EVs with specific ligands or engineering EV-producing cells to express homing peptides, is necessary to enhance their specificity and efficacy. Finally, the regulatory and ethical considerations surrounding EV-based therapies need to be addressed. As with any new therapeutic approach, there will be regulatory requirements and ethical considerations that need to be navigated. It is important to establish clear guidelines and regulations for the development, production, and clinical use of EV-based therapies to ensure their safety and efficacy.

Overall, the field of EVs is rapidly expanding, and there is growing interest in their potential applications in the diagnosis, treatment, and drug delivery of inflammatory diseases. The diverse cargo and communication capabilities of EVs make them attractive candidates for therapeutic interventions, and ongoing research is focused on optimizing methods for EV isolation, drug loading, and modification to enhance their efficacy and specificity. However, there are still several challenges that need to be addressed before EV-based therapies can be widely implemented. These include the standardization of isolation and characterization methods, scalability of production, and safety concerns regarding potential off-target effects or immune responses (Fig. 6). Despite these challenges, the potential of EVs in the field of inflammatory diseases is promising, and further research and development in this area are expected to lead to significant advancements in the coming years.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Competing interests
The authors declare no competing interests.

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