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Extracellular vesicles as therapeutic modulators of neuroinflammation in Alzheimer's disease: a focus on signaling mechanisms

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the accumulation of amyloid-beta (Aβ) plaques and tau tangles, which contribute significantly to neuroinflammation, a central driver of disease pathogenesis. The activation of microglia and astrocytes, coupled with the complex interactions between Aβ and tau pathologies and the innate immune response, leads to a cascade of inflammatory events. This process triggers the release of pro-inflammatory cytokines and chemokines, exacerbating neuronal damage and fostering a cycle of chronic inflammation that accelerates neurodegeneration. Key signaling pathways, such as nuclear factor-kappa B (NF-κB), Janus kinase/signal transducer and activator of transcription (JAK/STAT), mitogen-activated protein kinase (MAPK), and phosphoinositide 3-kinase/protein kinase B (PI3K/Akt), are involved in regulating the production of these inflammatory mediators, offering potential therapeutic targets for AD. Recently, extracellular vesicles (EVs) have emerged as a promising tool for AD therapy, due to their ability to cross the blood–brain barrier (BBB) and deliver therapeutic agents. Despite challenges in standardizing EV-based therapies and ensuring their safety, EVs offer a novel approach to modulating neuroinflammation and promoting neuroregeneration. This review aims to highlight the intricate relationship between neuroinflammation, signaling pathways, and the emerging role of EV-based therapeutics in advancing AD treatment strategies.

Keywords Alzheimer's disease, Neuroinflammation, Extracellular vesicles, Signaling pathways, Therapeutic targets

Introduction

Alzheimer's disease (AD) affects millions worldwide, placing an increasing burden due to the prolonged need for care and the lack of curative treatments. Recent research has revealed a connection between gut microbiota imbalance and AD pathogenesis [1], along with the potential of stem cell therapy to reduce pathology and improve cognitive function [2]. Furthermore, studies on the impact of inflammatory processes on AD progression [3, 4] and the role of extracellular vesicles (EVs) derived from astrocytes in AD [5] provide novel therapeutic avenues. These

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findings form a crucial foundation for developing new treatment approaches targeting AD.

Neuroinflammation plays a pivotal role in AD pathology. Recent studies indicate a strong association between the activation of microglial cells and astrocytes and the development of AD, highlighting several potential therapeutic targets [6]. Specifically, microglial responses to amyloid-beta (Aβ) are linked to an increased risk of AD, yet their activation can also have detrimental effects on neurons. Astrocytes, essential for brain function, can lead to neurodegeneration and synaptic retraction when impaired, leading to AD-related cognitive deficits [7]. Furthermore, exogenous substances such as spermidine have been shown to reduce neurotoxic soluble Aβ and mitigate AD-related neuroinflammation by activating autophagy [8]. These findings suggest that modulating the inflammatory responses of microglial cells and astrocytes is an effective strategy against AD.

EVs have emerged as promising drug delivery systems for central nervous system (CNS) diseases, particularly AD, due to their biocompatibility and ability to cross biological barriers such as the blood–brain barrier (BBB). Recent research has demonstrated the significant potential of utilizing EVs for brain-targeted drug delivery. Studies have shown that combining naturally sourced EVs with drug-loaded nanoparticles, such as grapefruit EVs with doxorubicin-loaded heparin-based nanoparticles [9], and structurally engineered droplet drugs using fruit-derived EVs [10], can greatly enhance the anti-tumor effects on glioblastoma, highlighting the vast potential of EVs in brain-targeted drug delivery. Furthermore, modifications and functionalization of EVs have opened new pathways for their use as therapeutic agents for brain disorders [11, 12], underscoring their promising applications in the diagnosis and treatment of neurodegenerative diseases [13, 14].

Traditional AD therapies face challenges such as limited efficacy and drug delivery barriers. Nanotechnology presents an innovative approach to overcoming these challenges, particularly through systems based on EVs, which can enhance drug utilization, provide precise targeting, and reduce side effects [15–17]. For instance, nano drug delivery systems have shown potential in enhancing the efficacy of NRF2 modulators in cancer treatment [15], and the use of nanoparticles has also presented new possibilities for AD treatment [16, 17]. Furthermore, the development of controlled drug release systems offers a new direction for achieving more effective drug delivery [17]. These studies underscore the importance of optimizing drug delivery through nanotechnology, paving the way for new approaches to the treatment of neurodegenerative diseases such as AD.

In recent years, scientists have started exploring the use of EVs as a natural nanocarrier for targeting neuroinflammation in AD. Studies indicate that EV-based therapeutic strategies can effectively modulate inflammatory response in AD, protect neurons from damage, and slow disease progression [18–20]. For example, specific natural products such as 1,6-O, O-diacetylbritannilactone (OABL) have demonstrated potent anti-inflammatory activity and the ability to traverse the BBB, offering a novel approach against AD [19]. Additionally, research has identified certain compounds like dihydrotestosterone (DHT) that can inhibit lipopolysaccharide (LPS)-induced neuroinflammation, showcasing a protective effect on neuronal cells [20]. These findings highlight the potential of utilizing EVs and specific natural products as a therapeutic strategy for AD, providing crucial scientific evidence for the development of new treatment methods.

While the application of EVs as nanocarriers for combating neuroinflammation in AD is still in its early stages, they are considered a promising approach for AD treatment due to their unique biocompatibility, targeting abilities, and capacity to cross the BBB. With an increasing understanding of EVs' mechanisms and advancements in bioengineering and nanotechnology, EV-based therapeutic strategies hold the potential to provide AD patients with more effective and safer treatment options [21–23]. Furthermore, the development of smart EVs, capable of responding to specific signals at the disease site and offering therapeutic feedback, opens up new avenues for the next generation of nanomedicine [24]. Despite facing challenges in commercialization, the prospects of EVs in disease treatment remain promising [25, 26].

Mechanisms of Neuroinflammation and Its Association with AD

AD and Neuroinflammation: An Intricately Interwoven Relationship

New research has uncovered the intricate intertwining relationship between AD and neuroinflammation, emphasizing the role of autophagy [27], the Aβ pathway [28], the involvement of microglial cells in the neuroinflammation process [4], and the impact of abnormal aggregation of tau protein on neuroinflammation [29, 30]. These findings offer fresh insights into the pathology of AD and provide a scientific basis for the development of novel therapeutic strategies targeting neuroinflammation.

The abnormal accumulation of Aβ triggers a complex neuroinflammation process in AD, leading to a further decline in cognitive function. Studies indicate that the continuous aggregation of Aβ surpasses the handling

capacity of microglial cells and astrocytes, causing their overactivation and release of a large number of inflammatory mediators such as cytokines, chemotactic factors, and reactive oxygen species, therefore damaging neurons and synapses [4, 27, 28]. Recent research advancements suggest that intervention strategies targeting this pathological process, like enhancing the clearance of Aβ through promoting the autophagy pathway [29–31] or inhibiting the progression of AD by regulating microRNA expression [6, 32, 33], demonstrate therapeutic potential. Furthermore, the exploration of novel biomarkers and their correlation with Aβ and tau protein pathology offers new insights for the development of early diagnostic and therapeutic strategies [19, 34, 35]. Hence, understanding and modulating this intricate neuroinflammation network is crucial for achieving disease intervention in AD treatment.

The development of AD is closely associated with the abnormal aggregation of tau proteins, leading to the formation of neurofibrillary tangles. This not only damages the neuronal transport system but also activates immune cells, triggering a neuroinflammatory response [4, 27, 28]. Recent studies have indicated that microglial cells play a crucial role in AD-related neuroinflammation, as their activation exacerbates the abnormal aggregation of Aβ and tau proteins, creating a vicious cycle [29–31]. Furthermore, research has highlighted that targeted therapy against microRNA-485-3p can inhibit the progression of AD [32], while the measurement of plasma amyloid β42/40 ratio offers a new diagnostic possibility for AD [33]. Additionally, studies have shown that specific anti-inflammatory drugs and strategies regulating the autophagy process hold the potential to slow down the pathological progression of AD [6, 19, 34]. These recent research findings provide a crucial scientific basis for a deeper understanding of the pathogenic mechanisms of AD and the development of novel therapeutic strategies.

Neuroinflammation in AD exhibits duality, serving as both a natural response to pathological changes in AD and potentially exacerbating the progression of the disease. Studies suggest that modulation of the autophagy process may offer new therapeutic strategies for treating AD [27], while research targeting microglial cells has revealed their central role in the neuroinflammation pathway [4, 29]. Additionally, targeting microRNA-485-3p has shown promise in halting the progression of AD [32], and the detection of plasma amyloid β42/40 ratio presents a novel method for early diagnosis of AD [33]. These research findings underscore the importance of a comprehensive understanding of the neuroinflammation mechanisms in AD for the development of new therapeutic strategies.

Current research is exploring the modulation of neuroinflammation in AD through the use of anti-inflammatory drugs and the development of targeted therapies that directly act on specific inflammatory signaling pathways, offering new hope for treating AD. The exploration of these strategies underscores the necessity of a thorough understanding of the complex relationship between AD and neuroinflammation, paving the way for the development of more effective treatment methods [4, 27, 28]. While these studies provide new directions for AD treatment, the complexity of AD necessitates a more precise understanding of these mechanisms to achieve more effective intervention measures [29–31].

Triggering neuroinflammation: the key roles of microglial cells and astrocytes

In AD, the overactivation of microglial cells and astrocytes exacerbates neuroinflammation, leading to the release of inflammatory mediators that damage neural cells [6, 19, 36]. The interplay between these cells plays a crucial role in maintaining the neurovascular unit, underscoring the importance of considering their roles in AD treatment [37–39]. Recent studies indicate that by modulating the activity of these cells, novel targets for AD treatment can be identified, such as intervention in the signaling pathways of microglial cells and astrocytes, which holds promise for alleviating neuroinflammation in AD [40–42].

Microglial cells, as the primary immune cells of the brain, exhibit high sensitivity to Aβ aggregation, attempting to clear Aβ through phagocytosis. However, in AD, their phagocytic function is impaired, exacerbating inflammation and neuronal damage [6, 19, 36]. In this process, the activation of microglial cells and the release of inflammatory mediators become critical factors in the progression of AD [37–39], emphasizing the potential of targeting microglial cells to modulate their activity and alleviate neuroinflammation in AD [40–42].

Astrocytes in AD respond to the accumulation of Aβ and abnormal aggregation of tau proteins by releasing inflammatory mediators in their activated state, exacerbating the inflammatory response and impairing their ability to support neuronal functions [6, 19, 39]. This shift not only promotes inflammation but also hampers the ability of astrocytes to provide nutritional support and regulate neurotransmitter uptake, further compromising neuronal health [37, 38, 42].

The excessive activation of microglial cells and astrocytes and the release of inflammatory mediators in AD are not confined solely to regions with abnormal aggregation of Aβ and tau proteins but can also impact other areas of the brain through mechanisms such

as cytokine release. This spread of the inflammatory response disrupts neuronal communication, impairs synaptic function, and exacerbates cognitive decline [6, 19, 36]. Modulating the inflammatory responses of these cells is crucial in developing treatment strategies for AD [37–39]. These findings not only reveal the crucial roles of microglial cells and astrocytes in AD but also provide a scientific basis for developing new strategies to treat AD, particularly those aimed at reducing the excessive activation of these cells and inhibiting the production of inflammatory mediators.

The deleterious dance of inflammatory mediators

In the progression of AD, inflammatory mediators play a crucial role by engaging in cell communication, activating immune cells, and directly causing damage to neurons. These inflammatory factors, such as interleukins (IL) and tumor necrosis factor-α (TNF-α), significantly increase in the brain of AD patients, thereby driving disease advancement [43–45]. Recent studies have demonstrated that targeting these inflammatory mediators can alleviate AD-related neuroinflammation, showing therapeutic potential. For instance, agonists targeting the TNF receptor 2 have been shown to improve cognitive function in AD mouse models by mitigating neuropathological damage, thus exhibiting neuroprotective effects [46–48]. Furthermore, natural compounds like flavonoids and boswellic acid have shown beneficial anti-inflammatory and neuroprotective effects in AD [49–51]. Therefore, a comprehensive exploration of the role of inflammatory mediators in AD is not only essential for a better understanding of the disease mechanism but also provides a scientific basis for the development of novel treatment strategies.

The latest research on the role and therapeutic potential of inflammatory mediators in AD indicates that Sirtuin 3 protects elderly mice from anesthesia/surgery-induced cognitive decline by inhibiting neuroinflammation in the hippocampal region [43]. Furthermore, studies have linked peripheral inflammatory markers TNF-α and CCL2 to the severity of Parkinson's disease, underscoring the role of inflammation in neurodegenerative diseases [44]. Additionally, flavonoids as potential anti-inflammatory molecules have shown therapeutic potential in alleviating AD-related neuroinflammation [45]. These findings not only enhance our understanding of the inflammatory mechanisms in AD but also provide a scientific basis for the development of new therapeutic strategies.

Current research indicates that blocking the transmission of inflammatory signals by developing antibodies or small molecule inhibitors targeting specific inflammatory mediators can effectively reduce the production or effects of inflammatory mediators, thereby protecting neurons from damage [46–48]. Additionally, modulating the activity of microglial cells and astrocytes to decrease the release of inflammatory mediators by inhibiting their overactivation is also an effective strategy for alleviating AD-related inflammatory damage and neurodegeneration [49–51]. These studies have paved the way for new approaches in the treatment of AD and provided a scientific basis for developing novel therapeutic methods.

Utilizing endogenous regulatory mechanisms to control inflammatory responses, particularly by promoting the natural resolution of inflammation through enhancing resolution pathways signaling, has emerged as a significant direction in AD research [52–54]. This approach not only aids in restoring the homeostasis of neural tissue but also presents opportunities for the development of new therapeutic interventions. A profound understanding of the specific mechanistic roles of inflammatory mediators in AD and their interactions with other pathological processes is crucial for the successful development and application of these therapeutic strategies.

In summary, the role of inflammatory mediators in AD is highly complex, as they serve not only as disease markers but also as potential therapeutic targets. Current research suggests that precise regulation of these inflammatory mediators may offer more effective treatment options for AD patients to slow down or halt disease progression [43, 53, 54]. This encompasses the development of antibodies or small molecule inhibitors targeting specific inflammatory mediators and utilizing endogenous regulatory mechanisms to control inflammatory responses, promote the natural resolution of inflammation, and restore neural tissue homeostasis.

Exploring the abnormal activation of signaling pathways and the regulatory mechanisms of inflammation in AD as the basis for therapeutic targets

In the pathological progression of AD, the abnormal activation of multiple signaling pathways plays a crucial role in the development of the disease. Specifically, the imbalanced activation of pathways such as NF-κB, JAK/STAT, MAPK, and PI3K/Akt in AD promotes the excessive production of inflammatory mediators, exacerbates the inflammatory response of immune cells, and poses a threat to the survival of nerve cells. Research has found that miR-135a-5p, through the Rock2/Add1 signaling pathway, mediates memory and synaptic damage in an AD mouse model [55]. Resveratrol prevents tau pathology and neuroinflammation, demonstrating therapeutic potential in an AD mouse model [56]. Targeting specific molecules within these signaling

pathways can lead to the development of new drugs to regulate or inhibit the production of inflammatory mediators, alleviate the overactivation of immune cells, protect nerve cells from damage, and thus slow down the progression of AD.

In the progression of AD, the abnormal activation of the NF-κB pathway, triggered by Aβ aggregation, leads to the overactivation of microglial cells and astrocytes, resulting in increased production of inflammatory cytokines such as TNF-α, IL-1β, and IL-6. These factors further promote inflammatory responses, damaging neighboring neurons. miR-135a-5p mediates memory and synaptic damage in an AD mouse model through the Rock2/Add1 signaling pathway [55], while resveratrol shows therapeutic potential in preventing tau pathology and neuroinflammation in an AD mouse model [56]. Targeting specific molecules within these signaling pathways can facilitate the development of new drugs to regulate or inhibit the production of inflammatory mediators, reduce the excessive activation of immune cells, protect nerve cells from damage, and thereby decelerate the progression of AD.

The NF-κB pathway is a key signaling pathway involved in regulating inflammation in AD. Triggered by Aβ aggregation, the pathway leads to the overactivation of microglial cells and astrocytes, activating NF-κB, thereby increasing the production of inflammatory cytokines such as TNF-α, IL-1β, and IL-6. This cascade of events further stimulates the inflammatory response, resulting in damage to neurons [55–57]. Additionally, research on the JAK/STAT pathway indicates its significant role in modulating immune cells and inflammatory responses. In AD, the activation of the JAK/STAT pathway can enhance the inflammatory response of microglial cells, promote the release of inflammatory mediators, exacerbate neuroinflammation, and contribute to neuronal damage [58–60]. These research advancements provide a new theoretical foundation and potential therapeutic targets for regulating and treating inflammation in AD.

The abnormal activation of the MAPK and PI3K/Akt pathways in AD has attracted widespread attention due to their impact on neurons and the underlying regulatory mechanisms. A study on miR-135a-5p in an AD mouse model, which mediates memory and synaptic damage through the Rock2/Adducin1 signaling pathway [55], revealed the role of miRNAs in modulating neuroinflammation and neuroprotection via these pathways. Another research demonstrated the protective role of Rutin in an AD mouse model by preventing tau protein pathology and neuroinflammation [56]. Furthermore, the role of melatonin in regulating the treatment of type 2 diabetes and AD [57] underscores the significance of hormones and metabolic regulation in AD, hinting at the crosstalk of these pathways in disease management. These studies provide insights that interventions targeting the MAPK and PI3K/Akt pathways in AD must consider not only their direct effects on neuroprotection and neuro damage but also their interactions with other signaling pathways, such as miRNAs, hormonal regulation, and metabolic processes. These findings offer new perspectives and targets for AD therapy.

One of the pathological features of AD is the dysregulation of inflammatory processes, with multiple signaling pathways playing a crucial role in this process. Recent studies have found that miR-135a-5p, by modulating the Rock2/Add1 signaling pathway, mediates memory and synaptic damage, highlighting the regulation of miR-135a-5p levels as a potential therapeutic strategy for AD [55]. Additionally, Rutin, through inhibiting the aggregation of tau protein and reducing neuroinflammation, has demonstrated the potential to improve cognitive function in an AD model [56]. Melatonin, with its antioxidant and anti-inflammatory effects, has been shown to delay the progression of AD in individuals with type 2 diabetes, indicating melatonin as a potential approach for treating AD [57]. These studies not only reveal the dysregulated activation of signaling pathways and the mechanisms of inflammation in AD but also provide a scientific basis for the development of new therapeutic strategies.

EVs: an innovative delivery system at the forefront of AD therapy

EVs have emerged as a groundbreaking therapeutic strategy with significant potential in overcoming the BBB and targeting AD treatment. Recent studies have demonstrated that engineered EVs can efficiently deliver therapeutic molecules to the brain, leading to a marked improvement in AD-related symptoms [9, 61, 62]. Furthermore, research indicates that by customizing the surface properties of EVs, their targeting specificity towards specific brain cells can be enhanced, further boosting treatment efficacy [10, 63, 64]. Despite existing challenges in the production, purification, and clinical application of EVs, these studies have provided valuable insights and directions for developing novel AD treatment strategies [14, 65, 66]. By further optimizing the design and functionality of EVs, it is promising to achieve more precise and effective treatment approaches for AD in the future.

Recent studies have demonstrated that EVs can effectively alleviate depressive symptoms induced by chronic unpredictable stress (CUS) through the delivery of specific biological molecules, such as circDYM, showing potential in treating major depressive disorder

(MDD) [61]. Furthermore, a bioinspired drug delivery system combining grapefruit exosomes and heparin-based nanoparticles has significantly enhanced the efficacy of anti-glioma treatment, proving the efficacy of receptor-mediated intercellular interactions and membrane fusion in traversing the BBB/blood–brain tumor barrier (BBTB) and enhancing drug delivery efficiency [9]. For the treatment of ischemic stroke, EVs containing mitochondria have been developed with dual modifications to enhance their ability to cross the BBB and precisely target the ischemic area, thereby reducing brain damage and improving treatment effectiveness [62]. These studies not only showcase the potential of EVs as therapeutic delivery systems but also highlight their application prospects in treating AD and other neurodegenerative diseases.

Challenges and prospects of EVs in the treatment of AD

EVs have emerged as a novel strategy for treating AD by leveraging their unique biological functions and ability to cross the BBB, offering an unprecedented therapeutic approach. Recent studies have demonstrated that utilizing high-resolution imaging techniques and innovative EV labeling strategies can deepen our understanding of the biological behavior of EVs in vivo and their potential applications in AD therapy [67]. Furthermore, the enhanced therapeutic effects of MSC-EVs on AD have been showcased through a self-triggered release hydrogel inspired by comparative proteomics, highlighting a significant improvement in the clinical applicability of EVs through intelligent hydrogel design [68]. Summaries of the latest advancements in MSC-EVs for AD therapy, as well as their specific mechanisms, have been provided, along with discussions on various methods to optimize MSC-EV functionality such as gene editing, altering stem cell culture conditions, and peptide modifications, emphasizing the therapeutic potential of MSC-EVs as a cell-free treatment strategy and the clinical application challenges they face [69]. Further research has introduced a simplified "one-material" strategy utilizing Ti4+-modified magnetic graphene oxide composite (GFST) to enrich and identify phosphoproteins in serum-derived EVs (SEVs), presenting a new approach for drug target discovery based on abnormal kinase and substrate transport of SEVs, with implications for cancer monitoring [70].

EVs have shown significant potential in the treatment of AD, yet their application still faces a series of challenges. Recent research advancements shed light on several key points: firstly, the application of high-resolution imaging techniques contributes to a deeper understanding of EVs' behavior in vivo and their potential therapeutic applications [67]. Secondly, self-triggered release hydrogels have been found to enhance the therapeutic effects of MSC-EVs for AD treatment, with this strategy inspired by comparative proteomics holding promise for increasing the clinical applicability of EVs [68]. Furthermore, research on the latest developments and specific mechanisms of MSC-EVs in AD therapy, along with strategies for optimizing MSC-EV functionality such as gene editing and peptide modifications, provides new perspectives and methods for cell-free treatment strategies [69]. Lastly, the development of a simplified "one-material" strategy utilizing GFST to enrich and identify phosphoproteins in SEVs opens new directions for drug target discovery and cancer monitoring [70]. These advancements indicate that although the application of EVs in AD therapy presents challenges, with the development of innovative technologies and methods, overcoming these obstacles and achieving their clinical application is within reach (Fig. 1).

EVs as drug delivery systems

General characteristics and sources of EVs

EVs, including natural exosomes, microvesicles, apoptotic bodies, and engineered vesicles modified through genetic engineering or chemical modification, are important tools for intercellular communication. EVs have various sources, including MSCs, neural stem cells (NSCs), immune cells (e.g., macrophages, microglia), blood cells, and engineered cells (e.g., HEK293 cells, fibroblasts) [71–73]. In particular, utilizing the biosynthesis process of EVs for bioengineering modifications allows for the effective loading of therapeutic proteins or nucleic acids into EVs, which can be used to treat various diseases [74–76]. Recent studies have revealed the important roles of EVs in regulating immune responses, promoting tumor progression, and drug delivery [77–79], deepening our understanding of EVs as intercellular communication tools and showcasing their potential in clinical therapeutic applications.

Engineered strategies for crossing the BBB and achieving precision targeted delivery to the CNS using EVs as natural nanocarriers

Although the BBB has long been a barrier to nanomedicines reaching the CNS, research advancements in BBB penetration show that various nanotechnologies, specific brain-targeting peptides, and modified viral vectors can effectively facilitate drug delivery across the BBB. For example, studies have shown that specifically modified adeno-associated viral vectors can achieve BBB penetration by targeting carbonic anhydrase IV and LY6C1 [80]. Another study revealed a novel mechanism by which IL-17 crosses the BBB and triggers neuroinflammation, which holds potential value

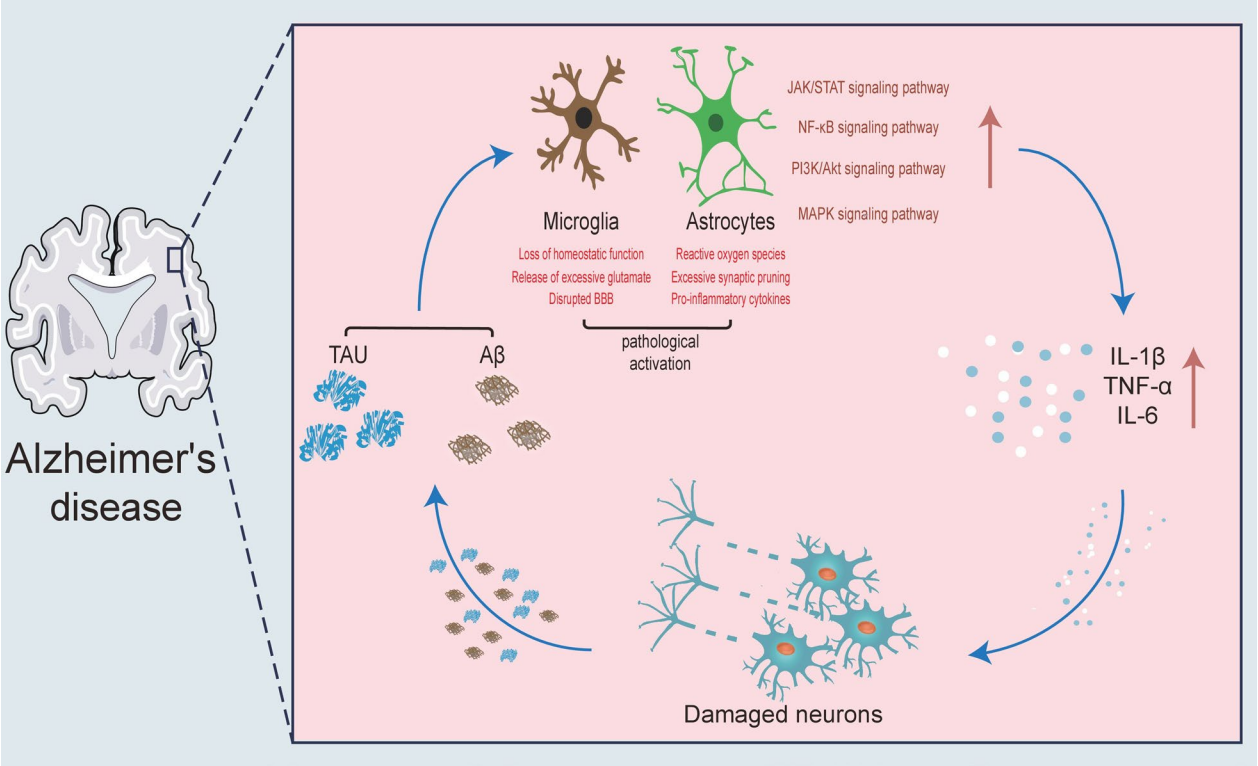

Fig. 1 Revealing the interweaving relationship between Alzheimer's disease and neuroinflammation, and the research progress of new approaches for extracellular vesicle therapy (created by Biorender)

for treating chronic migraines [81]. The application of nanoparticle technology in neuro-oncology drug delivery also shows the ability to cross the BBB [82]. These studies provide new therapeutic strategies for treating neurodegenerative diseases like AD, showcasing the potential of utilizing biotechnology and nanotechnology to improve drug delivery efficiency and overcome BBB limitations, thus providing new strategies and directions for treating AD and other neurological diseases.

Recent studies have not only emphasized the innovative application of EVs in crossing the BBB but also demonstrated the potential for engineered EVs to deliver neurotrophic factors or specific antibodies for AD directly to the brain [80–82]. For example, engineered neural cell-derived EVs expressing specific proteins have successfully targeted overexpressed proteins in the AD brain, carrying drugs that effectively improve AD pathology and cognitive function [83]. Another study developed dual-targeting nanoparticles, enhancing the efficacy and tumor selectivity of cancer immunogene therapy [22]. Additionally, a nanostructure specifically targeting cholinergic neurons, combined with a mitochondrial therapy strategy, improved neural function and slowed disease progression in an AD model [84]. These advancements not only demonstrate the feasibility of EVs as therapeutic delivery systems but also provide new strategies and directions for developing treatments for neurological diseases. In particular, in-depth research into the biosynthesis and secretion mechanisms of EVs has provided new strategies for using these natural nanoparticles to treat neurodegenerative diseases like AD [74–76].

Progress in targeted delivery of therapeutic molecules using EVs for AD treatment

EVs, as nano-scale communication systems, show unique therapeutic potential in neurodegenerative diseases like AD. Studies have shown that EVs play a role in regulating inflammatory responses, promoting neural cell repair and regeneration, and providing new therapeutic avenues for treating AD and other neurodegenerative diseases. By engineering EVs and incorporating surface-specific proteins and ligands, precise targeted delivery can be achieved. For example, neuron-derived EVs engineered with Fe65 protein successfully encapsulated corynoxine-B, improving cognitive abilities and pathology in AD mice [83]. Using lipid-dendrimer-calcium phosphate nanoparticles, dual-targeted immunogene therapy against tumors was achieved [22]. A nanostructure designed to target

cholinergic neurons, combined with a mitochondrial therapy strategy, improved neural function and slowed pathological progression in AD models [84]. Additionally, the use of clickable albumin nanoparticles for pre-targeted drug delivery provided combination immunotherapy for tumors overexpressing PD-L1 [85]. More in vivo studies show that miRNA delivered by EVs can regulate pathological processes within neural cells, reducing Aβ production or promoting its clearance [86–88], alleviating AD progression in animal models. Furthermore, EV-delivered antibodies targeting Aβ aggregates in the brain show potential for promoting Aβ clearance [89–91]. Other studies have shown that EV-delivered neuroprotective factors like BDNF promote the survival and regeneration of damaged neural cells [92–94]. These studies not only demonstrate the effectiveness of engineering strategies in enhancing therapeutic targeting and reducing side effects but also highlight challenges in translating these technologies into clinical applications, such as efficiently and stably loading drugs and controlling the production and purification of exosomes.

Different types and sources of EVs also show varying potential for treating diseases, including AD. Exosomes and microvesicles exhibit significant differences in composition and function, presenting different challenges for their application in AD treatment [95–97]. Exosomes typically form and release through intracellular budding, while microvesicles form through outward budding of the cell membrane [98–100]. Exosomes, due to their smaller size and complex molecular composition, have an advantage in crossing the BBB and targeting damaged neural cells. They can effectively carry therapeutic molecules like RNA and proteins, directly impacting the pathological processes of AD [98–100]. In contrast, microvesicles, due to their larger size and more direct formation mechanism, may be more effective in carrying larger or more complex drug payloads. However, their larger size could limit their ability to cross the BBB, a challenge that needs to be overcome in AD treatment applications [95–97]. Specific types of EVs can target specific pathological features of AD, such as Aβ aggregation and tau protein alterations, by carrying corresponding therapeutic molecules (e.g., antibodies, small-molecule inhibitors, or RNA interference molecules). Engineered EVs can be designed to carry antibodies against Aβ or RNA interference molecules that reduce tau protein phosphorylation, directly targeting AD's pathological mechanisms [101, 102]. These advancements offer new perspectives for the application of EVs in AD treatment while also pointing out challenges that need further research and overcoming.

Specific mechanisms of EV-mediated regulation of neuroinflammation

An in-depth exploration of the specific signaling pathways through which EVs influence neuroinflammation is crucial for a comprehensive understanding of the cellular and molecular mechanisms of EV-mediated neuroinflammation regulation in AD. Recent studies show that therapeutic molecules delivered by EVs can directly target damaged neural cells, thus regulating the pathological processes of AD at multiple levels. For example, studies have found that microglial cells play an important role in neuroprotection by regulating the growth and integrity of myelin in the CNS [86]. The loss of TDP-43 and ALS risk SNPs drive the mis-splicing and depletion of UNC13A, further revealing potential molecular mechanisms in neurodegenerative diseases [87]. Further research has shown that myelin dysfunction promotes the deposition of Aβ in AD models [88], providing new perspectives for targeting EVs to deliver therapeutic molecules.

Additionally, EVs, as membrane-bound structures that transport cargo between cells in vivo [103], have been shown to contain high levels of Aβ when isolated from cerebrospinal fluid (CSF), which can stimulate Aβ aggregation in cultured neurons [104] and animal models [105]. Tau aggregates are another major hallmark of AD [106], and EVs isolated from the CSF of AD patients have been shown to contain tau, which can be transmitted to neurons where they induce tau aggregation [107–109].

EVs regulate inflammation in AD through various receptors. For instance, EVs can modulate Toll-like receptors (TLRs) expression and activation, promoting or inhibiting inflammation [110]. Tumor necrosis factor receptors (TNFRs) can also be influenced by cytokines in EVs, altering signaling and affecting the degree of inflammation. Furthermore, EVs may regulate interleukin receptor (ILR) signaling pathways by transferring cytokines such as IL-1 and IL-6 [111]. NF-κB, MAPK, and JAK-STAT pathways also play significant roles in EV regulation. Certain molecules in EVs (e.g., miRNAs and proteins) can regulate inflammation by promoting or inhibiting NF-κB activation [112]. NF-κB plays a key role in immune responses and inflammation, and its activation is closely linked to inflammatory responses in neurodegenerative diseases, including AD. EVs can affect MAPK signaling pathways by transmitting specific signaling molecules (e.g., cytokines, miRNAs), impacting cell proliferation, differentiation, and stress response, thus contributing to neuroinflammation and neuronal damage in AD [113]. These mechanisms are critical for advancing EV-based therapeutic research in AD.

Challenges and opportunities in translating EV-based therapies into clinical applications

Despite the positive outcomes of these studies, there are still many challenges in applying EVs for targeted delivery to neural tissues, such as improving targeting specificity, enhancing BBB penetration, and optimizing production and purification processes. For instance, the heterogeneity of EVs, challenges in their recognition and characterization, and their exact mechanisms under specific physiological and pathological conditions still require further investigation [101, 102]. Potential off-target effects are a major issue in EV applications, and improving EV targeting through the development of engineered EVs with enhanced targeting capabilities is a significant challenge for future translation. Ensuring the stability and bioactivity of the cargo, as well as scaling up production and purification of therapeutic EVs, is also a key focus for future research.

Due to the use of different isolation and purification methods across laboratories, there is a lack of standardized production protocols. Additionally, when EVs are used as therapeutic agents, accurate quantification of EVs is crucial. Various methods based on particle count, size, and total protein, lipid, or nucleic acid content have their limitations and unique challenges, making it difficult to determine which method is superior [114]. Researchers are actively exploring cell expansion strategies, culture conditions, and bioreactors to improve EV yield [115]. The dosage and administration method for EV delivery remain significant challenges. A recent systematic review on EV-based portable organ therapy reported EV dosages ranging from 10^5 to 10^12 particles, with extreme variations in administration routes, including intravenous, intra-arterial, and organ-specific injections. This heterogeneity currently prevents meaningful meta-analyses and practical application in large organ models [116].

Lastly, safety is critical when considering the clinical application of EVs. Immunogenicity, immunotoxicity, and potential carcinogenicity are considered safety concerns [117]. Balancing potential benefits and risks requires careful consideration. Future directions should focus on using EVs for early diagnosis and exploring combination therapies involving EVs and other treatment modalities to better harness the potential of EVs in AD treatment.

Recent advances suggest that by gaining a deeper understanding of the biological properties and functional differences of EVs, as well as developing advanced technologies and methods, these challenges can be partly overcome. For example, studies have found significant differences in the composition and functions of EVs from different sources, providing new strategies for precision therapy [98–100]. Additionally, improved separation and characterization techniques can more effectively identify and purify EVs with therapeutic potential, providing new tools for treating neurodegenerative diseases like AD [95–97]. Further research shows that understanding the role of EVs in intercellular communication can lead to the development of new therapeutic methods that utilize EVs to directly target diseased tissues, thereby improving therapeutic efficacy [101, 102]. In conclusion, although there are challenges in applying EVs to AD treatment, ongoing research holds promise for overcoming these obstacles through optimization of targeting specificity, delivery efficiency, and production processes, leading to their widespread clinical application (Fig. 2).

EVs: exploring frontier therapeutic strategies against AD neuroinflammation

Application and challenges of EVs in AD treatment

EVs play a crucial role in maintaining the health and functionality of the nervous system as fundamental tools for intercellular communication. These small membranous structures have the ability to traverse the BBB, making them potent candidates for exploring novel therapeutic strategies for CNS disorders. Particularly in neurodegenerative diseases such as AD characterized by neuroinflammation, the role of EVs has sparked significant interest among researchers [118–120]. Recent studies have revealed that EVs can modulate the inflammatory environment by delivering anti-inflammatory molecules or intervene in the expression and aggregation of Aβ and tau proteins by transporting specific RNA molecules, thereby influencing multiple aspects of the AD pathological processes [26, 121, 122].

AD, as a neurodegenerative disorder characterized by neuroinflammation, has drawn significant interest among researchers regarding the role of EVs within it. The pathological features of AD encompass neuronal damage, the formation of Aβ plaques, and the abnormal aggregation of tau proteins, accompanied by evident neuroinflammation. Studies have demonstrated that EVs can impact various aspects of this pathological process, such as modulating the inflammatory environment by delivering anti-inflammatory molecules or intervening in the expression and aggregation of Aβ and tau proteins by transporting specific RNA molecules [118–120]. Furthermore, utilizing EVs as nanocarriers to combat AD-related neuroinflammation not only showcases their ability to traverse the BBB but also leverages their biocompatibility and low immunogenicity [26, 121, 122].

By engineering modifications, the drug-loading capacity of EVs can be further optimized. For instance, utilizing gene editing techniques to induce source cells to produce EVs rich in specific therapeutic molecules or physically and chemically loading drugs directly into

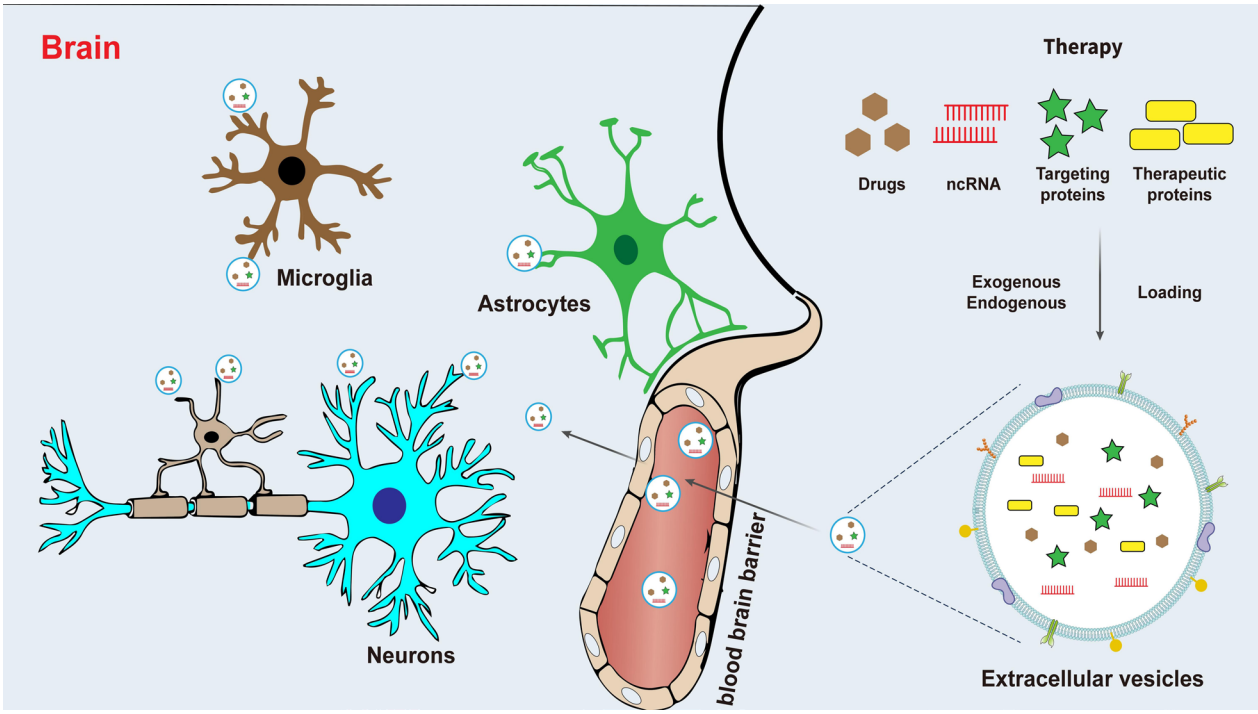

Fig. 2 Extracellular vesicles: Challenges and prospects for targeted treatment of Alzheimer's disease (created by Biorender)

EVs [123–125]. The development and application of these strategies offer new insights and approaches for the treatment of neurodegenerative diseases like AD. However, despite the significant potential displayed by EVs in anti-AD research, there are numerous challenges to their application. These challenges include precise control of EVs' targeting capabilities, enhancing drug loading efficiency, and ensuring the safety and effectiveness of the treatment process [126–128]. To overcome these hurdles, researchers are dedicated to developing more efficient EV modification techniques. This includes leveraging specific ligands or antibodies to enhance the targeting capabilities of EVs and optimizing the production and purification processes of EVs to enhance their quality and consistency.

Cutting-edge strategies for innovating the treatment of AD neuroinflammation

EVs have shown significant potential in AD treatment due to their unique biocompatibility, low immunogenicity, and ability to traverse the BBB. Particularly, EVs derived from stem cells, such as stem cell-derived EVs (SC-EVs), have been proven to promote regeneration of age-related tissue loss and function, treat both central and peripheral age-related conditions, and inhibit cellular aging, thereby reversing various age-related diseases and functional impairments [129]. Furthermore, EVs derived from MSCs demonstrate the potential to enhance synaptic remodeling mediated by microglial cells for aging recovery [130].

Research on AD neuroinflammation has revealed that combined treatment with low-temperature therapy and EVs can ameliorate neurodevelopmental disorders resulting from neonatal hypoxic-ischemic brain injury, demonstrating the potential for early-stage AD neuroinflammation treatment [131]. Additionally, animal model experiments have shown that EVs effectively reduce microglial cell activation, decrease levels of inflammatory factors, and slow down the deposition of Aβ and tau proteins, thus improving cognitive function [132].

Despite this, challenges facing the use of EVs in anti-AD neuroinflammation therapy include how to scale up the production of EVs with consistency and high purity, ensure the long-term safety of their usage, and enhance the delivery efficiency and precision of therapeutic molecules. Future research will focus on tackling these issues to advance the application of EVs in the treatment of AD and other neurodegenerative disorders [133].

Innovative strategies for combatting AD neuroinflammation

In the research on addressing neuroinflammation associated with AD, the utilization of EVs has opened

up a new avenue for therapy, demonstrating unique advantages as a naturally occurring nano-sized drug delivery system. These advantages include good biocompatibility, low immunogenicity, and the ability to cross the BBB [134]. Scientists have developed various strategies to load therapeutic molecules into EVs, aiming to enhance therapeutic efficacy and reduce potential impacts on non-target cells [119]. Among these strategies, direct loading involves physically or chemically encapsulating therapeutic molecules inside EVs, surface modification entails anchoring therapeutic molecules on the surface of EVs through chemical or biological methods, and genetic engineering methods involve modifying the source cells of EVs to naturally carry specific therapeutic molecules [135].

Selecting the most suitable EV drug-loading strategy requires careful consideration of the nature of the therapeutic molecules, the desired therapeutic effects, and the types of target cells [136]. With advancements in nanotechnology and molecular biology, the development of EV drug-loading strategies is becoming increasingly refined and efficient [137]. These innovative strategies provide new hope for the treatment of neurodegenerative diseases like AD, heralding significant breakthroughs in the fields of precision medicine and targeted therapy in the future [138]. Current research has demonstrated the potential of using EVs to deliver therapeutic molecules to inhibit AD-related neuroinflammation. Experimental findings in animal models show that EVs after treatment can effectively reduce the activation of microglial cells, lower levels of inflammatory factors, and slow down the deposition of Aβ and tau protein, thus improving cognitive function [139].

Despite the great promise shown by EVs in the treatment of anti-AD neuroinflammation, there are still many challenges to overcome, including how to mass-produce EVs with consistency and high purity, ensure the long-term safety of their use, and further enhance the efficiency and precision of delivering therapeutic molecules. As research progresses, it is expected that EVs will play an increasingly important role in the treatment of neurodegenerative diseases like AD.

Exploring the application of EVs in the treatment of AD: experimental models and case studies analysis

EVs serve as crucial mediators of intercellular communication, demonstrating unique potential in the treatment of neurological diseases. Recent studies have unveiled the roles of EVs in crossing the BBB, promoting neural regeneration, and mitigating neuroinflammation, particularly showcasing significant applications in the treatment of AD. Through engineered modifications, EVs can be loaded with therapeutic biomolecules such as miRNA, antibodies, or small molecule drugs, directly targeting specific neural disease pathways. For instance, by loading EVs derived from curcumin-treated sulfide nanoparticles, they can effectively traverse the BBB, accumulate in the brain, and alleviate endoplasmic reticulum stress, thus restoring cognitive function in AD models [140]. Furthermore, utilizing nanoparticles constructed with poly(lactic-co-glycolic acid) (PLGA) and lactoferrin (Lf) has not only facilitated efficient delivery of the membrane-bound phosphodiesterase inhibitor curcumin but also significantly improved memory and learning abilities while reducing amyloid deposition in an AD mouse model [141].

Aside from directly delivering drug molecules, EVs can also exert therapeutic effects by modulating inflammatory responses in the neural environment and promoting neuroprotective mechanisms. For instance, endogenous EVs regulate the activity of microglial cells, reducing the release of pro-inflammatory cytokines to combat neuroinflammation triggered by AD [142]. These findings offer a fresh perspective on the application of EVs in the treatment of neurological disorders, especially in facilitating drug passage through the BBB and enhancing the efficacy of therapeutic molecules in the brain.

In conclusion, EVs, as highly tunable biological mediators, exhibit multimodal mechanisms of action in the treatment of neurological disorders, particularly AD, providing new strategies and directions for future disease treatments. With further research, it is reasonable to believe that leveraging the unique biological properties of EVs in conjunction with advanced biotechnologies will lead to the development of more effective therapeutic approaches for neurological disorders.

Preclinical advancements of EVs in the treatment of AD

Recent advances in understanding the pathological mechanisms of AD have led to the maturation of strategies utilizing EVs for the delivery of therapeutic molecules. Table S1 presents a summary of preclinical studies on EV-based therapies for AD. A study revealed that the administration of a combination of metabolic activators (CMA) through EVs significantly enhances cognitive function in AD patients, which is correlated with a notable improvement in plasma levels of proteins and metabolites associated with NAD + and glutathione metabolism [143].

Furthermore, research conducted in 3xTg-AD model mice demonstrated that high-frequency repetitive transcranial magnetic stimulation (rTMS) can ameliorate cognitive deficiencies in AD mice by modulating the PI3K/Akt/GLT-1 axis, reducing hippocampal Aβ1-42 levels, and improving oxidative stress and glucose

metabolism [144]. Studies on vitamin D supplementation also suggest that a 12-month regimen of vitamin D supplementation enhances cognitive function in elderly AD patients and reduces blood biomarkers associated with Aβ [145].

Another study focused on drug repurposing strategies revealed that the CDK4/6 inhibitor abemaciclib mesylate can improve spatial and recognition memory in 5xFAD mice by modulating dendritic spine density and neuroinflammatory responses, inhibiting Aβ accumulation, and suppressing tau protein phosphorylation through the reduction of DYRK1A and/or p-GSK3β levels [146]. Research on high-dose Omega-3 and Omega-6 polyunsaturated fatty acids, along with antioxidant vitamin supplementation, indicates a significant improvement in cognitive function and functional ability in elderly individuals with mild cognitive impairment (MCI) [147].

In summary, these studies not only confirm the effectiveness of EVs for delivering therapeutic molecules in suppressing neuroinflammation, protecting neurons, and enhancing cognitive function but also provide essential scientific evidence for potential future clinical applications.

Future prospects and challenges of EVs in the treatment of AD

EVs have emerged as an innovative delivery system for the treatment of AD, showcasing significant progress in preclinical studies. Delbreil et al. pointed out that, despite the potential demonstrated by nanotechnology in AD therapy, there are currently no nanotechnology products on the market, highlighting the challenge of translating these technologies into practical treatment modalities [148]. The potential of nanolipids and nanostructured lipid carriers (NLC) in delivering drugs from the nasal cavity to the brain emphasizes the importance of optimizing nasal-brain delivery to enhance therapeutic outcomes [149]. Séguy et al. examined how drugs on the market and drug repositioning can be enhanced in terms of their bioavailability and/or selectivity through nanocarrier systems to improve treatment efficacy [150].

Research by Hu et al. has shown significant progress in the use of nanomedicine for the treatment of AD, demonstrating the success of various nanocarriers such as polymer nanoparticles, liposomes, solid lipid nanoparticles, dendritic nanoparticles, biomimetic nanoparticles, and magnetic nanoparticles in developing novel therapeutic strategies [151]. Additionally, Moreira et al. have examined the application of dendrimeric macromolecules and their derivatives as multifunctional nano-therapeutic agents in AD treatment, emphasizing the unique drug delivery capabilities of dendrimeric macromolecules and their inherent anti-amyloidogenic properties [152].

Although EVs have shown immense potential in AD treatment research, they still face several challenges, including the large-scale production of high-purity and consistent EVs, precise control of EVs' targeted delivery processes, and the assessment of the long-term safety and stability of EVs. Future research should explore more targeting strategies, including surface modifications, genetic engineering technologies, and the use of EVs to deliver new therapeutic molecules, such as the CRISPR/Cas9 system for gene editing, or to develop multifunctional EVs to achieve therapeutic effects targeting multiple pathological processes in AD. Through technological innovation and in-depth research, we anticipate overcoming current challenges to translate EVs, this cutting-edge technology, into effective therapeutic agents for AD (Fig. 3).

The application of innovative technologies and methods in EVs research

Modification and drug loading of EVs

Recent research has highlighted the significant potential of EVs as a novel therapeutic tool in combating AD. Modifying and loading EVs with drugs through various methods have led researchers to make significant advancements in enhancing their therapeutic efficiency and targeting capabilities. The application of gene editing techniques, notably the CRISPR/Cas9 system, has opened new avenues for precisely controlling the therapeutic molecules carried by EVs, enabling them to act on specific pathological features of AD. While the CRISPR/Cas9 technology holds promise for correcting gene errors in AD, the challenge lies in developing safe and effective delivery systems to translate CRISPR-based therapies from the lab to clinical applications [153], as discussed by Hanafy et al. Moreover, the development of nanotechnology has paved the way for leveraging EVs as drug-delivery systems. Delbreil et al., through a critical analysis of the past decade's research on nanomedical therapies for AD, have pointed out the immense potential of nanotechnology in AD treatment despite existing challenges. They suggest that through methods like gene editing, gene modulation, and vaccines, nanotechnology holds promise for the treatment of AD [148]. Chacko et al. have comprehensively evaluated the development of in vitro and in vivo models using CRISPR-Cas9 in AD research and treatment, emphasizing its capability to identify and validate potential therapeutic targets for AD [154].

In addition to gene editing techniques, direct drug-loading strategies for EVs are also advancing. Usman et al. have described a novel approach utilizing EVs

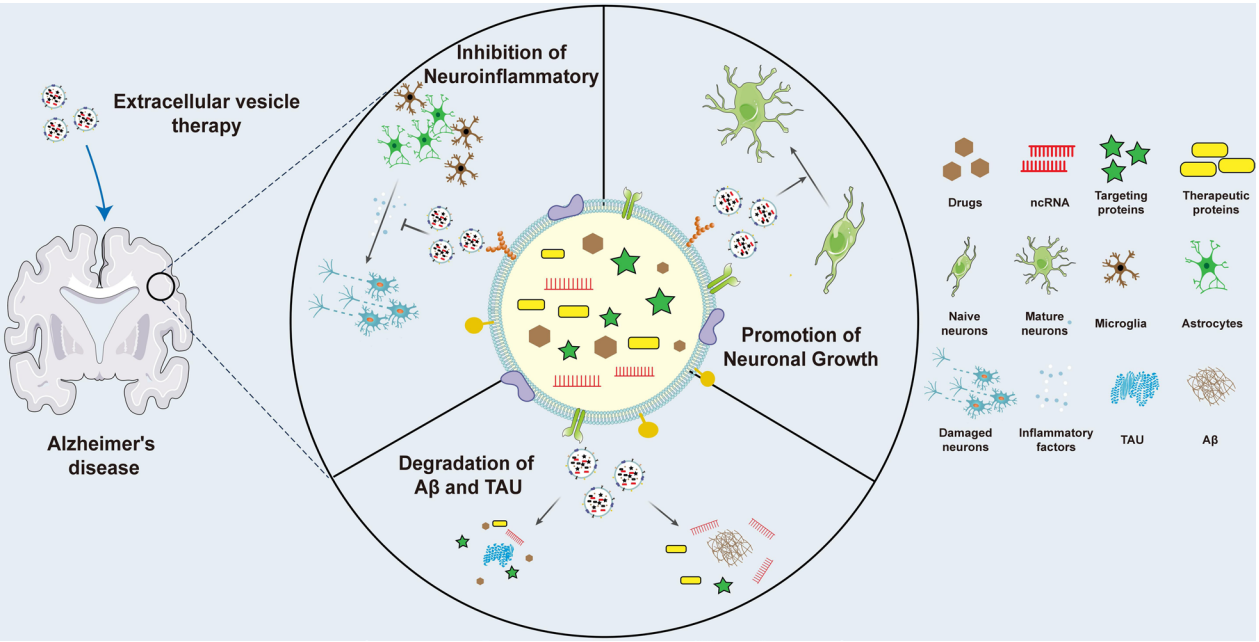

Fig. 3 Extracellular vesicles: a new frontier and challenge in the treatment of Alzheimer's disease (created by Biorender)

derived from human red blood cells (RBCEVs) as RNA drug delivery tools. This method has demonstrated efficient microRNA inhibition and CRISPR-Cas9 gene editing abilities without significant cytotoxicity [155]. Furthermore, Osteikoetxea et al. have developed a new method by fusing Cas9 with EV-sorting partners through reversible dimerization, effectively loading CRISPR-Cas9 into EVs and achieving high-efficiency gene editing in HEK293 and HepG2 cells [156].

In conclusion, with a deeper understanding of EVs' structure and functions, coupled with advancements in bioengineering, physics, and chemistry techniques, the prospects for the use of EVs in treating AD and other neurodegenerative diseases are expanding. These innovative technologies not only provide robust support for the clinical translation of EVs but also bring new hope for future disease treatments.

Revolutionary applications of single-cell technology

Single-cell technology, particularly single-cell RNA sequencing (scRNA-seq), has exhibited unprecedented potential in exploring research fields of AD and related neuroinflammation. Recent studies utilizing single-cell transcriptome analysis have revealed dynamic changes and distinct transcriptional activation states in microglial cells during the AD pathological process. Srinivasan et al., through single-nucleus RNA sequencing of frontal cortex samples from AD patients, identified unique transcriptional activation patterns in age-associated microglial cells, including upregulation of APOE, a factor that may play a crucial role in AD development [157]. Additionally, Morabito et al. conducted multi-omics single-nucleus studies on 191,890 nuclei from late-stage AD, uncovering cell type-specific disease-related candidate cis-regulatory elements and their target genes. This provides a fresh perspective on understanding the cis-regulatory relationships of AD risk loci in specific cell types [158].

Furthermore, Jiang et al., through a systematic comparison of single-cell transcriptome data from AD patients and normal controls, developed an integrated database named scREAD. This is the first database dedicated to managing all existing scRNA-Seq and snRNA-Seq datasets from postmortem human brain tissues of AD patients and AD pathological mouse models. scREAD offers comprehensive analysis results for 73 datasets, including control chart construction, cell type prediction, identification of differentially expressed genes, and recognition of cell type-specific regulatory networks [159]. These recent research advancements not only pave new pathways for the clinical application of AD but also provide new hope for treating neurodegenerative diseases such as AD.

Comprehensive application of multi-omics methods

In the field of AD research, various omics approaches, such as proteomics, lipidomics, and transcriptomics, have provided a comprehensive understanding of the

role of EVs in AD. Su et al.'s study revealed the cellular and molecular mechanisms of meningeal immunity in CNS homeostasis and dysfunction using a novel single-cell omics method, challenging previous viewpoints and offering fresh perspectives on potential therapeutic targets [160]. Furthermore, Liu et al. discovered that SerpinA3N derived from astrocytes promotes neuroinflammation and seizures by activating the NF-κB signaling pathway, presenting a new target for exploring neuroinflammation-based treatment strategies [161]. Maïer et al. investigated the neuroinflammatory response following acute ischemic stroke and refined the spatial organization of the pathological biological processes leading to clinical outcomes in IS patients using clinical imaging and single-cell omics techniques [162].

Clark et al.'s study, utilizing integrated multi-level cerebrospinal fluid (CSF) omics, identified numerous interactions associated with AD pathology. Enrichment pathway analysis revealed the overexpression of coagulation, immune response, and extracellular matrix signaling pathways related to AD, laying a scientific foundation for personalized diagnostics and treatment of AD [163]. Lv et al. evaluated the role of Traditional Chinese Medicine in treating vascular cognitive impairment (VCI), exploring the therapeutic potential of Chinese herbal medicine through various molecular mechanisms such as inhibiting oxidative stress, suppressing neuroinflammation, increasing cerebral blood flow, and inhibiting iron deposition [164].

Collectively, these studies demonstrate the significant importance of omics approaches in elucidating the role of EVs in AD, uncovering the involvement of neuroinflammation in AD progression, and providing a scientific basis for developing new diagnostic tools and treatment strategies. These investigations not only enhance our understanding of EVs' biological properties but also offer robust support for identifying novel therapeutic targets, showcasing new directions for future research and treatment strategies in AD.

Advanced techniques for characterizing EVs

The application of EVs in AD treatment research has been increasing, leading to advancements in the precise characterization of EV technology. Traditional techniques such as nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM) have been widely utilized to study the physical and chemical properties of EVs. For instance, NTA technology enables researchers to precisely estimate the size and concentration of EVs, providing a clear image for distinguishing different subtypes of EVs [165, 166]. Moreover, Flow Cytometry (FACS) has emerged as a crucial tool for analyzing surface markers of EVs. Nonetheless, traditional FACS faces challenges due to the small size of EVs. Recent studies have demonstrated that by employing specially designed FACS instruments and high-sensitivity detectors, quantitative analysis of specific proteins or ligands on the surface of EVs can be achieved [167, 168].

Recent developments in novel characterization technologies and methods, such as high-sensitivity Fluorescent Nanoparticle Tracking Analysis (F-NTA) and single-step immunoaffinity assays, have opened up new possibilities for studying EVs. These technologies not only enhance the capability to characterize individual EVs in terms of size and membrane protein expression but also enable the quantitative analysis of hepatocellular carcinoma (HCC)-specific EVs directly from small serum volumes through specific markers like GPC3 and alpha-fetoprotein (AFP) [169, 170].

By integrating the aforementioned technologies, scientists can not only obtain detailed molecular fingerprints of EVs but also uncover their specific mechanisms of action in diseases such as AD. This paves the way for the development of novel therapeutic strategies based on EVs grounded in solid scientific evidence. As EV characterization technologies continue to advance and be applied, our understanding of these small yet powerful particles will deepen, opening up new avenues for future medical research and clinical treatment.

Towards future clinical translation

With the in-depth exploration of the potential of EVs in the treatment of neurological diseases, particularly in the context of AD, new therapeutic strategies and technologies are constantly emerging. Bioengineered nanovesicles (BNVs), comprising endogenous EVs from different cells and artificial nanovesicles, have shown significant roles in drug delivery to the brain by crossing the BBB [64]. Furthermore, studies have found that EVs derived from MSCs, particularly administered non-invasively via intranasal delivery, exhibit immunomodulatory and neuroprotective effects in animal models of AD, alleviating pathological conditions [171].

For CNS diseases, the therapeutic potential of EVs often resembles that of their parent cells and is considered a therapeutic tool that can be used alone or in combination with bioactive molecules to traverse the BBB with low immunogenicity [172]. However, current clinical trials are still in their infancy, facing challenges such as a lack of standardized methods for scaled production, isolation, and characterization, as well as low encapsulation efficiency.

In models of traumatic brain injury (TBI), EVs derived from NSCs, astrocytes, and microglia have demonstrated neuroregenerative properties. However, the therapeutic application of these EVs is not yet ready for clinical translation, requiring rigorous testing of their effects on preventing chronic neuroinflammation and persistent motor and cognitive impairments after acute TBI treatment [173].

Furthermore, the therapeutic potential of EVs in alleviating neuroinflammation after CNS trauma by modulating cell death is being further explored. However, the connections between EVs and different types of cell death in the context of CNS trauma remain incompletely understood, necessitating deeper mechanistic research [174].

As research on the therapeutic use of EVs in CNS diseases continues to advance, especially in areas such as AD and TBI, their potential as future treatment strategies is increasingly recognized. However, achieving their clinical application requires overcoming challenges such as scaled production, safety and biodistribution assessments, as well as collaboration with regulatory agencies [175] (Fig. 4).

Challenges and opportunities in clinical applications

Recent advancements in the research on EVs as nano drug carriers for treating AD have shown significant potential in alleviating neuroinflammation. The distinctive characteristics of EVs, such as their ability to traverse the BBB and deliver therapeutic molecules directly to damaged brain cells and tissues, present a novel strategy for AD treatment. However, the successful translation of EVs from laboratory studies to clinical applications necessitates overcoming a series of challenges and obstacles.

Primarily, one major challenge facing the clinical application of EVs is the scalability of production. Various methods for extracting and purifying EVs have been developed, including differential centrifugation, ultrafiltration, gel filtration, and immunoaffinity purification. Nevertheless, these methods often struggle to meet the demands of large-scale production while preserving the biological activity and consistency of EVs [176–178]. This underscores the need for the development of novel technologies to achieve efficient and scalable production of EVs.

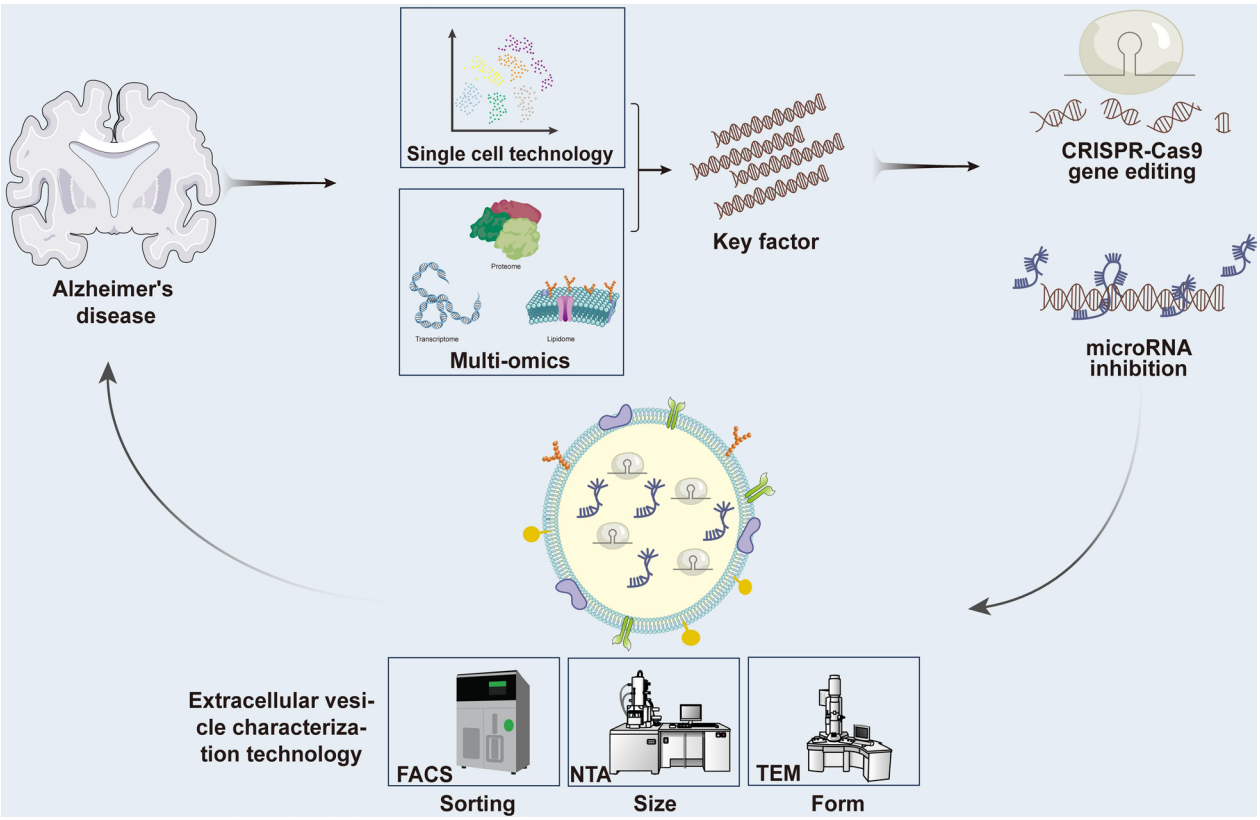

Fig. 4 Extracellular vesicles: Innovative strategies and challenges for Alzheimer's disease treatment (created by Biorender)

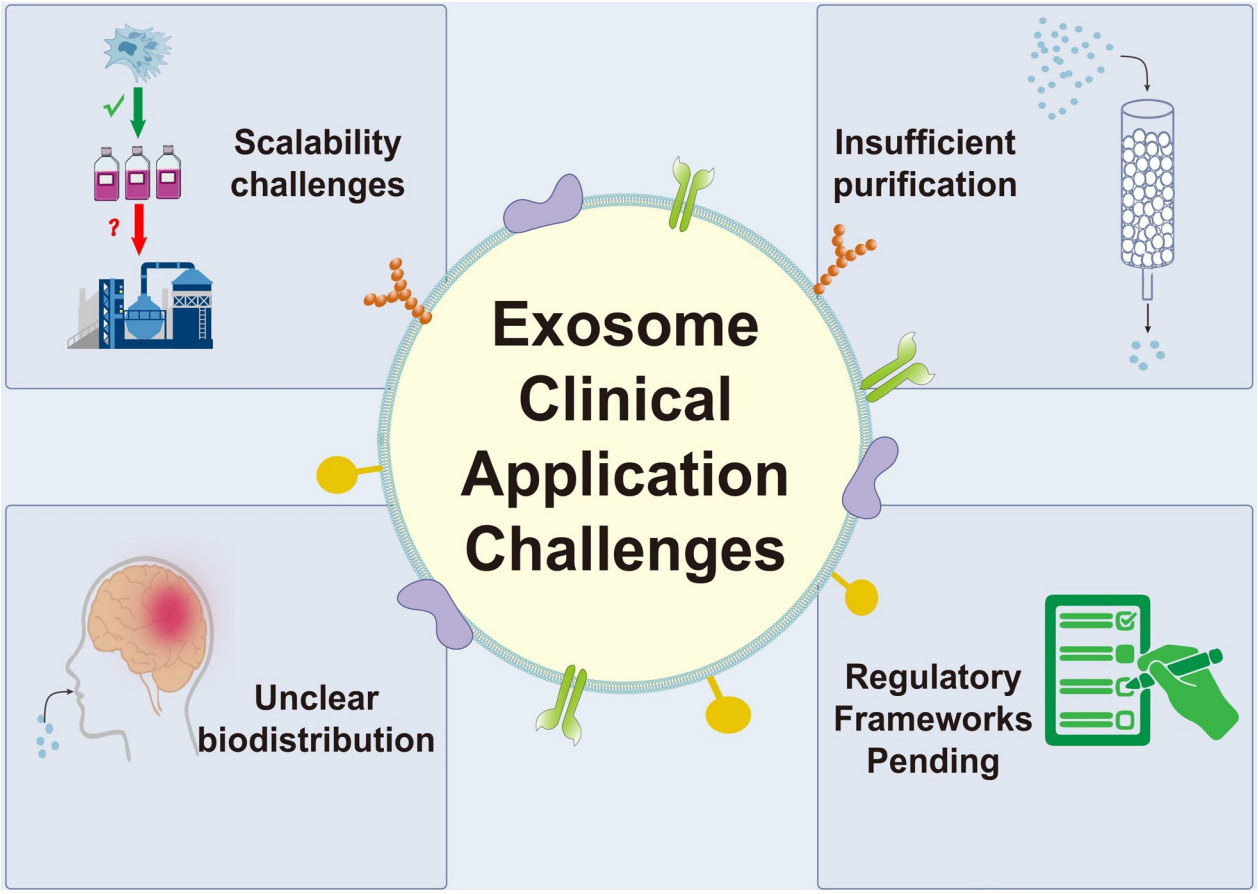

Fig. 5 Extracellular vesicle therapy for Alzheimer's disease: challenges in clinical application, technological innovation, and future opportunities (created by Biorender)

Furthermore, purification poses another critical challenge in the clinical application of EVs. Existing purification techniques often fail to completely eliminate all non-EV components, which could compromise the biological activity of EVs and limit their clinical potential. Consequently, researchers are exploring more efficient and higher-purity EV purification methods, including those based on size and density differences, specific binding of surface markers, and high-resolution separation techniques [179–181].

Moreover, issues related to the biodistribution and safety of EVs must be carefully considered during the clinical translation of EVs. Despite their ability to traverse the BBB and their potential therapeutic effects, the distribution characteristics, metabolic pathways, and long-term safety of EVs in the body remain unclear. Therefore, conducting more preclinical studies, particularly those focusing on the biodistribution and metabolic mechanisms of EVs, is essential for evaluating and ensuring the safety of their clinical application [176, 182].

Lastly, close collaboration with regulatory agencies is key to the successful clinical translation of EVs. As research on EVs progresses, it is essential to establish and update relevant regulatory guidelines and standards to ensure the safety and efficacy of new therapeutic strategies. This includes stringent regulation of the EV production process, guidance on clinical trial design, and standardization of assessments for treatment effects and safety [177, 178, 182].

In conclusion, despite facing numerous challenges, ongoing research efforts and technological innovations offer promising prospects for the clinical application of EVs in treating AD and other neurological disorders. With an increased understanding of EVs at a deeper level and advancements in production technologies, their role as effective nano drug carriers in future healthcare will become increasingly prominent.

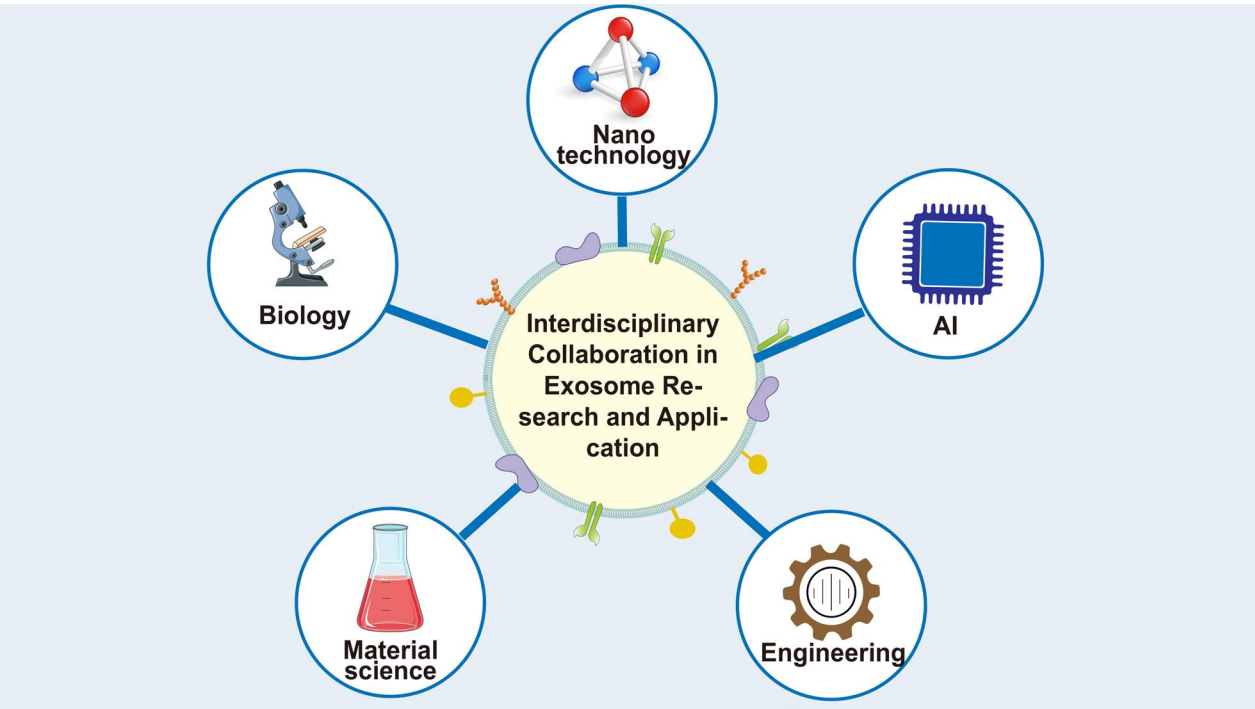

Fig. 6 Innovative applications and challenges of interdisciplinary collaboration driven extracellular vesicles in the treatment of Alzheimer's disease (created by Biorender)

Looking ahead to the future

With the advancement of research on EVs, future studies are expected to focus on the discovery of new therapeutic targets, the development of treatment strategies, and further exploration of the fundamental aspects of EVs. The identification of new therapeutic targets stands out as a crucial area for future research. This endeavor not only holds the promise of providing more precise treatment targets for neurodegenerative diseases like AD but also aids in enhancing our understanding of the pathogenesis of these diseases. By leveraging systems biology and omics technologies, researchers can pinpoint specific molecules and signaling pathways associated with diseases, laying a theoretical groundwork for the targeted delivery of therapeutics via EV drug carriers. Additionally, the development of novel therapeutic strategies based on EVs may be another key focus of future research. This includes genetically engineering EVs to enhance their targeting capabilities, payload capacities, and evasion of immune clearance, thereby improving treatment efficacy and reducing side effects.

In the realm of fundamental research on EVs, future investigations aim to delve further into elucidating the mechanisms underlying the biogenesis, release, and interactions of EVs with cells. These foundational studies not only aid in uncovering the roles of EVs in physiological and pathological processes but also furnish critical scientific bases for the development of novel EV-based therapeutic approaches. Furthermore, exploring the heterogeneity and complexity of EVs may also stand out as a focal point in future endeavors. Understanding the specific functions and mechanisms of action of EVs from different sources and types holds significant importance for leveraging EVs in disease therapeutics.

The application of artificial intelligence (AI) and machine learning (ML) in the design of future EV drug delivery systems shows tremendous potential. AI and ML technologies can handle and analyze large-scale biomedical data, providing robust data support for the design and optimization of EV drug delivery systems. Through AI algorithms, the interaction between EVs and specific disease markers can be predicted, allowing for the enhancement of EVs' targeting capabilities through surface modifications. Additionally, ML methods can be utilized to analyze the distribution and metabolic dynamics of EVs in the body, thus enabling the design of more effective and safer EV drug delivery schemes. By integrating AI and ML technologies, not only can the development process of EV therapeutic strategies be accelerated, but it can also facilitate personalized medicine, offering new

hope for the treatment of neurodegenerative diseases like AD.

In conclusion, with the continuous advancement of technology and deepening research, the future application of EVs in the treatment of neurological diseases is set to become more extensive and profound. The discovery of new targets and treatment strategies, coupled with the application of AI and ML technologies, can further drive the translation of EVs from the laboratory to clinical settings, providing new directions and hope for the treatment of diseases like AD (Fig. 5).

Conclusion

In current scientific exploration, the research progress on EVs as natural nano drug carriers in combating neuroinflammation in AD demonstrates their unparalleled potential and versatility. These minuscule biological molecular complexes, with their unique biocompatibility, low immunogenicity, and ability to traverse the BBB, pave the way for the treatment of neurodegenerative diseases like AD. By loading various therapeutic molecules such as small molecule drugs, RNA, proteins, and other bioactive molecules, EVs can directly target the disease, showcasing tremendous potential in preventing and treating AD. Importantly, EV-based drug delivery systems offer a new perspective for early intervention in the disease, which holds significant importance in delaying or halting the progression of diseases like AD.

Despite demonstrating significant potential in AD treatment research, translating these research findings into clinical therapeutic strategies requires overcoming a series of technical and methodological challenges related to EVs. These challenges include but are not limited to large-scale production, purification, standardization, and safety assessment of EVs. Interdisciplinary collaboration plays a crucial role in this process. Expert teams from various fields, such as biology, materials science, nanotechnology, engineering, and artificial intelligence, can accelerate the development of EV-related technologies and facilitate the discovery and optimization of new treatment strategies through cooperation. For instance, utilizing artificial intelligence and machine learning to optimize the design and functionality of EVs can efficiently identify the most effective EV drug delivery systems with higher precision in a shorter timeframe.

In conclusion, EVs, as natural nano drug carriers combating AD neuroinflammation, not only demonstrate enormous potential in treating complex neurological diseases but also drive the advancement of interdisciplinary research. Future efforts should focus on addressing the challenges faced in the clinical application of EVs and leveraging the advantages of interdisciplinary collaboration to expedite the translation of breakthroughs in EVs-based fundamental research into practical clinical applications. Through these collaborative efforts, we can anticipate witnessing EVs-based therapeutic strategies bringing hope and transformation to patients with AD and other neurological diseases in the near future (Fig. 6).

Supplementary Information

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Additional file 1.

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Author contributions
JH and XZ equally contributed to the manuscript preparation, literature review, and drafting of the manuscript. LK and JG conceptualized the study, supervised the work, and revised the manuscript critically for important intellectual content. All authors reviewed and approved the final manuscript for submission.

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Data availability
All data generated or analyzed during this study are included in this article and/or its supplementary material files. Further enquiries can be directed to the corresponding author.

Declarations

Ethics approval and consent to participate
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References

1. Liu S, Gao J, Zhu M, Liu K, Zhang H-L. Gut microbiota and dysbiosis in Alzheimer's disease: implications for pathogenesis and treatment. Mol Neurobiol. 2020. https://doi.org/10.1007/s12035-020-02073-3.
2. Wang Z-B, Wang Z-T, Sun Y, Tan L, Yu J-T. The future of stem cell therapies of Alzheimer's disease. Ageing Res Rev. 2023. https://doi.org/10.1016/j.arr.2022.101655.
3. Twarowski B, Herbet M. Inflammatory processes in Alzheimer's disease—pathomechanism, diagnosis and treatment: a review. Int J Mol Sci. 2023. https://doi.org/10.3390/ijms24076518.
4. Cai Y, Liu J, Wang B, Sun M, Yang H. Microglia in the neuroinflammatory pathogenesis of Alzheimer's disease and related therapeutic targets. Front Immunol. 2022. https://doi.org/10.3389/fimmu.2022.856376.
5. Li B, Ma Z, Li Z. A novel regulator in Alzheimer's disease progression: the astrocyte-derived extracellular vesicles. Ageing Res Rev. 2023. https://doi.org/10.1016/j.arr.2023.101871.

6. Uddin MdS, Lim LW. Glial cells in Alzheimer's disease: from neuropathological changes to therapeutic implications. Ageing Res Rev. 2022. https://doi.org/10.1016/j.arr.2022.101622.
7. Brett BL, Gardner RC, Godbout J, Dams-O'Connor K, Keene CD. Traumatic brain injury and risk of neurodegenerative disorder. Biol Psychiatry. 2022. https://doi.org/10.1016/j.biopsych.2021.05.025.
8. Freitag K, Sterczyk N, Wendlinger S, Obermayer B, Schulz J, Farztdinov V, et al. Spermidine reduces neuroinflammation and soluble amyloid beta in an Alzheimer's disease mouse model. J Neuroinflammation. 2022. https://doi.org/10.1186/s12974-022-02534-7.
9. Niu W, Xiao Q, Wang X, Zhu J, Li J, Liang X, et al. A biomimetic drug delivery system by integrating grapefruit extracellular vesicles and doxorubicin-loaded heparin-based nanoparticles for glioma therapy. Nano Lett. 2021. https://doi.org/10.1021/acs.nanolett.0c04753.
10. Chen J, Pan J, Liu S, Zhang Y, Sha S, Guo H, et al. Fruit-derived extracellular-vesicle-engineered structural droplet drugs for enhanced glioblastoma chemotherapy. Adv Mater. 2023. https://doi.org/10.1002/adma.202304187.
11. Wang R, Wang X, Zhang Y, Zhao H, Cui J, Li J, et al. Emerging prospects of extracellular vesicles for brain disease theranostics. J Control Release. 2022. https://doi.org/10.1016/j.jconrel.2021.12.024.
12. Lino MM, Simões S, Tomatis F, Albino I, Barrera A, Vivien D, et al. Engineered extracellular vesicles as brain therapeutics. J Control Release. 2021. https://doi.org/10.1016/j.jconrel.2021.08.037.
13. Dar GH, Badierah R, Nathan EG, Bhat MA, Dar AH, Redwan EM. Extracellular vesicles: a new paradigm in understanding, diagnosing and treating neurodegenerative disease. Front Aging Neurosci. 2022. https://doi.org/10.3389/fnagi.2022.967231.
14. Khan NA, Asim M, El-Menyar A, Biswas KH, Rizoli S, Al-Thani H. The evolving role of extracellular vesicles (exosomes) as biomarkers in traumatic brain injury: clinical perspectives and therapeutic implications. Front Aging Neurosci. 2022. https://doi.org/10.3389/fnagi.2022.933434.
15. Sezgin-Bayindir Z, Losada-Barreiro S, Bravo-Díaz C, Sova M, Kristl J, Saso L. Nanotechnology-based drug delivery to improve the therapeutic benefits of NRF2 modulators in cancer therapy. Antioxidants. 2021. https://doi.org/10.3390/antiox10050685.
16. Srivastava S, Ahmad R, Khare SK. Alzheimer's disease and its treatment by different approaches: a review. Eur J Med Chem. 2021. https://doi.org/10.1016/j.ejmech.2021.113320.
17. Adepu S, Ramakrishna S. Controlled drug delivery systems: current status and future directions. Molecules. 2021. https://doi.org/10.3390/molecules26195905.
18. Zipp F, Gold R, Wiendl H. Identification of inflammatory neuronal injury and prevention of neuronal damage in multiple sclerosis. JAMA Neurol. 2013. https://doi.org/10.1001/jamaneurol.2013.4391.
19. Tang J-J, Huang L-F, Deng J-L, Wang Y-M, Guo C, Peng X-N, et al. Cognitive enhancement and neuroprotective effects of OABL, a sesquiterpene lactone in 5xFAD Alzheimer's disease mice model. Redox Biol. 2022. https://doi.org/10.1016/j.redox.2022.102229.
20. Yang L, Zhou R, Tong Y, Chen P, Shen Y, Miao S, et al. Neuroprotection by dihydrotestosterone in LPS-induced neuroinflammation. Neurobiol Dis. 2020. https://doi.org/10.1016/j.nbd.2020.104814.
21. Cheng Z, Li M, Dey R, Chen Y. Nanomaterials for cancer therapy: current progress and perspectives. J Hematol Oncol. 2021. https://doi.org/10.1186/s13045-021-01096-0.
22. Huang K-W, Hsu F-F, Qiu JT, Chern G-J, Lee Y-A, Chang C-C, et al. Highly efficient and tumor-selective nanoparticles for dual-targeted immunogene therapy against cancer. Sci Adv. 2020. https://doi.org/10.1126/sciadv.aax5032.
23. Zhang Y, Bi J, Huang J, Tang Y, Du S, Li P. Exosome: a review of its classification, isolation techniques, storage, diagnostic and targeted therapy applications. Int J Nanomed. 2020. https://doi.org/10.2147/ijn.s264498.
24. Fan Z, Jiang C, Wang Y, Wang K, Marsh J, Zhang D, et al. Engineered extracellular vesicles as intelligent nanosystems for next-generation nanomedicine. Nanosc Horiz. 2022. https://doi.org/10.1039/d2nh00070a.
25. de Lázaro I, Mooney DJ. Obstacles and opportunities in a forward vision for cancer nanomedicine. Nat Mater. 2021. https://doi.org/10.1038/s41563-021-01047-7.
26. Nguyen TT, Dung Nguyen TT, Vo TK, Tran NM, Nguyen MK, Van Vo T, et al. Nanotechnology-based drug delivery for central nervous system disorders. Biomed Pharmacother. 2021. https://doi.org/10.1016/j.biopha.2021.112117.
27. Zhang Z, Yang X, Song Y-Q, Tu J. Autophagy in Alzheimer's disease pathogenesis: therapeutic potential and future perspectives. Ageing Res Rev. 2021. https://doi.org/10.1016/j.arr.2021.101464.
28. Hampel H, Hardy J, Blennow K, Chen C, Perry G, Kim SH, et al. The amyloid-β pathway in Alzheimer's disease. Mol Psychiatry. 2021. https://doi.org/10.1038/s41380-021-01249-0.
29. Wang C, Zong S, Cui X, Wang X, Wu S, Wang L, et al. The effects of microglia-associated neuroinflammation on Alzheimer's disease. Front Immunol. 2023. https://doi.org/10.3389/fimmu.2023.1117172.
30. Andrade-Guerrero J, Santiago-Balmaseda A, Jeronimo-Aguilar P, Vargas-Rodríguez I, Cadena-Suárez AR, Sánchez-Garibay C, et al. Alzheimer's disease: an updated overview of its genetics. Int J Mol Sci. 2023. https://doi.org/10.3390/ijms24043754.
31. Ashok A, Singh N, Chaudhary S, Bellamkonda V, Kritikos AE, Wise AS, et al. Retinal degeneration and Alzheimer's disease: an evolving link. Int J Mol Sci. 2020. https://doi.org/10.3390/ijms21197290.
32. Koh H, Lee S, Lee H, Min JW, Iwatsubo T, Teunissen C, et al. Targeting microRNA-485–3p blocks Alzheimer's disease progression. Int J Mol Sci. 2021. https://doi.org/10.3390/ijms222313136.
33. Janelidze S, Teunissen CE, Zetterberg H, Allué JA, Sarasa L, Eichenlaub U, et al. Head-to-head comparison of 8 plasma amyloid-β 42/40 assays in Alzheimer disease. JAMA Neurol. 2021. https://doi.org/10.1001/jamaneurol.2021.3180.
34. Machhi J, Yeapuri P, Lu Y, Foster E, Chikhale R, Herskovitz J, et al. CD4+ effector T cells accelerate Alzheimer's disease in mice. J Neuroinflammation. 2021. https://doi.org/10.1186/s12974-021-02308-7.
35. Eshraghi M, Adlimoghaddam A, Mahmoodzadeh A, Sharifzad F, Yasavoli-Sharahi H, Lorzadeh S, et al. Alzheimer's disease pathogenesis: role of autophagy and mitophagy focusing in microglia. IJMS. 2021. https://doi.org/10.3390/ijms22073330.
36. Fan H, Tang H-B, Chen Z, Wang H-Q, Zhang L, Jiang Y, et al. Inhibiting HMGB1-RAGE axis prevents pro-inflammatory macrophages/microglia polarization and affords neuroprotection after spinal cord injury. J Neuroinflammation. 2020. https://doi.org/10.1186/s12974-020-01973-4.
37. Liu L, Liu J, Bao J, Bai Q, Wang G. Interaction of microglia and astrocytes in the neurovascular unit. Front Immunol. 2020. https://doi.org/10.3389/fimmu.2020.01024.
38. Wang M, Pan W, Xu Y, Zhang J, Wan J, Jiang H. Microglia-mediated neuroinflammation: a potential target for the treatment of cardiovascular diseases. J Inflamm Res. 2022. https://doi.org/10.2147/jir.s350109.
39. Deng Q, Wu C, Parker E, Liu TC, Duan R, Yang L. Microglia and astrocytes in Alzheimer's disease: significance and summary of recent advances. Aging Dis. 2023. https://doi.org/10.14336/ad.2023.0907.
40. Kleine J, Hohmann U, Hohmann T, Ghadban C, Schmidt M, Laabs S, et al. Microglia-dependent and independent brain cytoprotective effects of Mycophenolate mofetil during neuronal damage. Front Aging Neurosci. 2022. https://doi.org/10.3389/fnagi.2022.863598.
41. Rodríguez J, De Santis AJ, Dennis VA, Rodríguez AM, Giambartolomei GH. Bystander activation of microglia by *Brucella abortus*-infected astrocytes induces neuronal death via IL-6 trans-signaling. Front Immunol. 2024. https://doi.org/10.3389/fimmu.2023.1343503.
42. Ahn YH, Tang Y, Illes P. The neuroinflammatory astrocytic P2X7 receptor: Alzheimer's disease, ischemic brain injury, and epileptic state. Expert Opin Ther Targets. 2023. https://doi.org/10.1080/14728222.2023.2258281.
43. Liu Q, Sun Y-M, Huang H, Chen C, Wan J, Ma L-H, et al. Sirtuin 3 protects against anesthesia/surgery-induced cognitive decline in aged mice by suppressing hippocampal neuroinflammation. J Neuroinflammation. 2021. https://doi.org/10.1186/s12974-021-02089-z.
44. Xiromerisiou G, Marogianni C, Lampropoulos IC, Dardiotis E, Speletas M, Ntavaroukas P, et al. Peripheral inflammatory markers TNF-α and CCL2 revisited: association with Parkinson's disease severity. Int J Mol Sci. 2022. https://doi.org/10.3390/ijms24010264.
45. Al-Khayri JM, Sahana GR, Nagella P, Joseph BV, Alessa FM, Al-Mssallem MQ. Flavonoids as potential anti-inflammatory molecules: a review. Molecules. 2022. https://doi.org/10.3390/molecules27092901.

46. Ortí-Casañ N, Zuhorn IS, Naudé PJW, De Deyn PP, van Schaik PEM, Wajant H, et al. A TNF receptor 2 agonist ameliorates neuropathology and improves cognition in an Alzheimer's disease mouse model. Proc Natl Acad Sci USA. 2022. https://doi.org/10.1073/pnas.2201137119.
47. Xu H, Gan C, Xiang Z, Xiang T, Li J, Huang X, et al. Targeting the TNF-α–TNFR interaction with EGCG to block NF-κB signaling in human synovial fibroblasts. Biomed Pharmacother. 2023. https://doi.org/10.1016/j.biopha.2023.114575.
48. Sillapachaiyaporn C, Mongkolpobsin K, Chuchawankul S, Tencomnao T, Baek SJ. Neuroprotective effects of ergosterol against TNF-α-induced HT-22 hippocampal cell injury. Biomed Pharmacother. 2022. https://doi.org/10.1016/j.biopha.2022.113596.
49. Zhang H, Wang L, Yang Y, Cai C, Wang X, Deng L, et al. DL-3-n-butylphthalide (NBP) alleviates poststroke cognitive impairment (PSCI) by suppressing neuroinflammation and oxidative stress. Front Pharmacol. 2023. https://doi.org/10.3389/fphar.2022.987293.
50. Wang L, Deng B, Yan P, Wu H, Li C, Zhu H, et al. Neuroprotective effect of ketamine against TNF-α-induced necroptosis in hippocampal neurons. J Cell Mol Med. 2021. https://doi.org/10.1111/jcmm.16426.
51. Li Y, Jin T, Liu N, Wang J, Qin Z, Yin S, et al. A short peptide exerts neuroprotective effects on cerebral ischemia–reperfusion injury by reducing inflammation via the miR-6328/IKKβ/NF-κB axis. J Neuroinflammation. 2023. https://doi.org/10.1186/s12974-023-02739-4.
52. Kim J, Joshi HP, Sheen SH, Kim K-T, Kyung JW, Choi H, et al. Resolvin D3 promotes inflammatory resolution, neuroprotection, and functional recovery after spinal cord injury. Mol Neurobiol. 2020. https://doi.org/10.1007/s12035-020-02118-7.
53. Shen S-Y, Yu R, Li W, Liang L-F, Han Q-Q, Huang H-J, et al. The neuroprotective effects of GPR55 against hippocampal neuroinflammation and impaired adult neurogenesis in CSDS mice. Neurobiol Dis. 2022. https://doi.org/10.1016/j.nbd.2022.105743.
54. Paudel YN, Angelopoulou E, Piperi C, Othman I, Shaikh MF. HMGB1-mediated neuroinflammatory responses in brain injuries: potential mechanisms and therapeutic opportunities. Int J Mol Sci. 2020. https://doi.org/10.3390/ijms21134609.
55. Zheng K, Hu F, Zhou Y, Zhang J, Zheng J, Lai C, et al. miR-135a-5p mediates memory and synaptic impairments via the Rock2/Adducin1 signaling pathway in a mouse model of Alzheimer's disease. Nat Commun. 2021. https://doi.org/10.1038/s41467-021-22196-y.
56. Sun X, Li L, Dong Q-X, Zhu J, Huang Y, Hou S, et al. Rutin prevents tau pathology and neuroinflammation in a mouse model of Alzheimer's disease. J Neuroinflammation. 2021. https://doi.org/10.1186/s12974-021-02182-3.
57. Shen S, Liao Q, Wong YK, Chen X, Yang C, Xu C, et al. The role of melatonin in the treatment of type 2 diabetes mellitus and Alzheimer's disease. Int J Biol Sci. 2022. https://doi.org/10.7150/ijbs.66871.
58. Yang J, Wise L, Fukuchi K. TLR4 cross-talk with NLRP3 inflammasome and complement signaling pathways in Alzheimer's disease. Front Immunol. 2020. https://doi.org/10.3389/fimmu.2020.00724.
59. Chen Z-R, Huang J-B, Yang S-L, Hong F-F. Role of cholinergic signaling in Alzheimer's disease. Molecules. 2022. https://doi.org/10.3390/molecules27061816.
60. Li Q, Jia C, Wu H, Liao Y, Yang K, Li S, et al. Nao Tan Qing ameliorates Alzheimer's disease-like pathology by regulating glycolipid metabolism and neuroinflammation: a network pharmacology analysis and biological validation. Pharmacol Res. 2022. https://doi.org/10.1016/j.phrs.2022.106489.
61. Yu X, Bai Y, Han B, Ju M, Tang T, Shen L, et al. Extracellular vesicle-mediated delivery of circDYM alleviates CUS-induced depressive-like behaviours. J of Extracell Vesicle. 2022. https://doi.org/10.1002/jev2.12185.
62. Dave KM, Stolz DB, Manickam DS. Delivery of mitochondria-containing extracellular vesicles to the BBB for ischemic stroke therapy. Exp Opin Drug Deliv. 2023. https://doi.org/10.1080/17425247.2023.2279115.
63. Villa A, De Mitri Z, Vincenti S, Crippa E, Castiglioni L, Gelosa P, et al. Canine glioblastoma-derived extracellular vesicles as precise carriers for glioblastoma imaging: targeting across the blood-brain barrier. Biomed Pharmacother. 2024. https://doi.org/10.1016/j.biopha.2024.116201.
64. You Q, Liang F, Wu G, Cao F, Liu J, He Z, et al. The landscape of biomimetic nanovesicles in brain diseases. Adv Mater. 2023. https://doi.org/10.1002/adma.202306583.
65. Liang H-B, Chen X, Zhao R, Li S-J, Huang P-S, Tang Y-H, et al. Simultaneous ischemic regions targeting and BBB crossing strategy to harness extracellular vesicles for therapeutic delivery in ischemic stroke. J Control Release. 2024. https://doi.org/10.1016/j.jconrel.2023.12.021.
66. Reginald-Opara JN, Svirskis D, Paek SY, Tang M, O'Carroll SJ, Dean JM, et al. The involvement of extracellular vesicles in the transcytosis of nanoliposomes through brain endothelial cells, and the impact of liposomal pH-sensitivity. Mater Today Bio. 2022. https://doi.org/10.1016/j.mtbio.2022.100212.
67. Verweij FJ, Balaj L, Boulanger CM, Carter DRF, Compeer EB, D'Angelo G, et al. The power of imaging to understand extracellular vesicle biology in vivo. Nat Methods. 2021. https://doi.org/10.1038/s41592-021-01206-3.
68. Huang M, Zheng M, Song Q, Ma X, Zhang Q, Chen H, et al. Comparative proteomics inspired self-stimulated release hydrogel reinforces the therapeutic effects of MSC-EVs on Alzheimer's disease. Adv Mater. 2023. https://doi.org/10.1002/adma.202311420.
69. Zhang W, Uyemura R, Zhong K, Guo R, Zhong L. Current advances and future perspectives on mesenchymal stem cell-derived extracellular vesicles in Alzheimer's disease. Aging Dis. 2024. https://doi.org/10.14336/ad.2023.1206.
70. Jiao F, Gao F, Liu Y, Fan Z, Xiang X, Xia C, et al. A facile "one-material" strategy for tandem enrichment of small extracellular vesicles phosphoproteome. Talanta. 2021. https://doi.org/10.1016/j.talanta.2020.121776.
71. Cone AS, Yuan X, Sun L, Duke LC, Vreones MP, Carrier AN, et al. Mesenchymal stem cell-derived extracellular vesicles ameliorate Alzheimer's disease-like phenotypes in a preclinical mouse model. Theranostics. 2021. https://doi.org/10.7150/thno.62069.
72. Puhm F, Boilard E, Machlus KR. Platelet extracellular vesicles. Arterioscler Thromb Vasc Biol. 2021. https://doi.org/10.1161/atvbaha.120.314644.
73. Ma F, Vayalil J, Lee G, Wang Y, Peng G. Emerging role of tumor-derived extracellular vesicles in T cell suppression and dysfunction in the tumor microenvironment. J Immunother Cancer. 2021. https://doi.org/10.1136/jitc-2021-003217.
74. Sailliet N, Ullah M, Dupuy A, Silva AKA, Gazeau F, Le Mai H, et al. Extracellular vesicles in transplantation. Front Immunol. 2022. https://doi.org/10.3389/fimmu.2022.800018.
75. Nowak M, Górczyńska J, Kołodzińska K, Rubin J, Choromańska A. Extracellular vesicles as drug transporters. Int J Mol Sci. 2023. https://doi.org/10.3390/ijms241210267.
76. Al Halawani A, Mithieux SM, Yeo GC, Hosseini-Beheshti E, Weiss AS. Extracellular vesicles: interplay with the extracellular matrix and modulated cell responses. Int J Mol Sci. 2022. https://doi.org/10.3390/ijms23063389.
77. Marar C, Starich B, Wirtz D. Extracellular vesicles in immunomodulation and tumor progression. Nat Immunol. 2021. https://doi.org/10.1038/s41590-021-00899-0.
78. Jeppesen DK, Zhang Q, Franklin JL, Coffey RJ. Extracellular vesicles and nanoparticles: emerging complexities. Trends Cell Biol. 2023. https://doi.org/10.1016/j.tcb.2023.01.002.
79. Rädler J, Gupta D, Zickler A, Andaloussi SE. Exploiting the biogenesis of extracellular vesicles for bioengineering and therapeutic cargo loading. Mol Ther. 2023. https://doi.org/10.1016/j.ymthe.2023.02.013.
80. Shay TF, Sullivan EE, Ding X, Chen X, Ravindra Kumar S, Goertsen D, et al. Primate-conserved carbonic anhydrase IV and murine-restricted LY6C1 enable blood-brain barrier crossing by engineered viral vectors. Sci Adv. 2023. https://doi.org/10.1126/sciadv.adg6618.
81. Chen H, Tang X, Li J, Hu B, Yang W, Zhan M, et al. IL-17 crosses the blood–brain barrier to trigger neuroinflammation: a novel mechanism in nitroglycerin-induced chronic migraine. J Headache Pain. 2022. https://doi.org/10.1186/s10194-021-01374-9.
82. Hersh AM, Alomari S, Tyler BM. Crossing the blood-brain barrier: advances in nanoparticle technology for drug delivery in neuro-oncology. IJMS. 2022. https://doi.org/10.3390/ijms23084153.
83. Iyaswamy A, Thakur A, Guan X-J, Krishnamoorthi S, Fung TY, Lu K, et al. Fe65-engineered neuronal exosomes encapsulating corynoxine-B ameliorate cognition and pathology of Alzheimer's disease. Sig Transduct Target Ther. 2023. https://doi.org/10.1038/s41392-023-01657-4.
84. Qian K, Bao X, Li Y, Wang P, Guo Q, Yang P, et al. Cholinergic neuron targeting nanosystem delivering hybrid peptide for combinatorial

mitochondrial therapy in alzheimer's disease. ACS Nano. 2022. https://doi.org/10.1021/acsnano.2c05795.
85. Gerke C, Zabala Gutierrez I, Méndez-González D, la Cruz MCI, Mulero F, Jaque D, et al. Clickable albumin nanoparticles for pretargeted drug delivery toward PD-L1 overexpressing tumors in combination immunotherapy. Bioconjugate Chem. 2022. https://doi.org/10.1021/acs.bioconjchem.2c00087.
86. McNamara NB, Munro DAD, Bestard-Cuche N, Uyeda A, Bogie JFJ, Hoffmann A, et al. Microglia regulate central nervous system myelin growth and integrity. Nature. 2022. https://doi.org/10.1038/s41586-022-05534-y.
87. Brown A-L, Wilkins OG, Keuss MJ, Hill SE, Zanovello M, Lee WC, et al. TDP-43 loss and ALS-risk SNPs drive mis-splicing and depletion of UNC13A. Nature. 2022. https://doi.org/10.1038/s41586-022-04436-3.
88. Depp C, Sun T, Sasmita AO, Spieth L, Berghoff SA, Nazarenko T, et al. Myelin dysfunction drives amyloid-β deposition in models of Alzheimer's disease. Nature. 2023. https://doi.org/10.1038/s41586-023-06120-6.
89. Carson RE, Naganawa M, Toyonaga T, Koohsari S, Yang Y, Chen M-K, et al. Imaging of synaptic density in neurodegenerative disorders. J Nucl Med. 2022. https://doi.org/10.2967/jnumed.121.263201.
90. Kent SA, Miron VE. Microglia regulation of central nervous system myelin health and regeneration. Nat Rev Immunol. 2023. https://doi.org/10.1038/s41577-023-00907-4.
91. Schweighauser M, Arseni D, Bacioglu M, Huang M, Lövestam S, Shi Y, et al. Age-dependent formation of TMEM106B amyloid filaments in human brains. Nature. 2022. https://doi.org/10.1038/s41586-022-04650-z.
92. Root J, Merino P, Nuckols A, Johnson M, Kukar T. Lysosome dysfunction as a cause of neurodegenerative diseases: lessons from frontotemporal dementia and amyotrophic lateral sclerosis. Neurobiol Dis. 2021. https://doi.org/10.1016/j.nbd.2021.105360.
93. Rodriguez S, Hug C, Todorov P, Moret N, Boswell SA, Evans K, et al. Machine learning identifies candidates for drug repurposing in Alzheimer's disease. Nat Commun. 2021. https://doi.org/10.1038/s41467-021-21330-0.
94. Jiao H-S, Yuan P, Yu J-T. TMEM106B aggregation in neurodegenerative diseases: linking genetics to function. Mol Neurodegeneration. 2023. https://doi.org/10.1186/s13024-023-00644-1.
95. Jeske R, Bejoy J, Marzano M, Li Y. Human pluripotent stem cell-derived extracellular vesicles: characteristics and applications. Tissue Eng Part B Rev. 2020. https://doi.org/10.1089/ten.teb.2019.0252.
96. Chiozzini C, Ridolfi B, Federico M. Extracellular vesicles and their use as vehicles of immunogens. Methods Mol Biol. 2022. https://doi.org/10.1007/978-1-0716-2341-1_13.
97. Guan F, Xiang X, Xie Y, Li H, Zhang W, Shu Y, et al. Simultaneous metabolomics and proteomics analysis of plasma-derived extracellular vesicles. Anal Methods. 2021. https://doi.org/10.1039/d1ay00060h.
98. Davidson SM, Boulanger CM, Aikawa E, Badimon L, Barile L, Binder CJ, et al. Methods for the identification and characterization of extracellular vesicles in cardiovascular studies: from exosomes to microvesicles. Cardiovasc Res. 2022. https://doi.org/10.1093/cvr/cvac031.
99. Wang SE. Extracellular vesicles and metastasis. Cold Spring Harb Perspect Med. 2019. https://doi.org/10.1101/cshperspect.a037275.
100. Yu D, Mei Y, Wang L, Zhao Y, Fan X, Liang D, et al. Nano-seq analysis reveals different functional tendency between exosomes and microvesicles derived from hUMSC. Stem Cell Res Ther. 2023. https://doi.org/10.1186/s13287-023-03491-5.
101. Shefler I, Salamon P, Mekori YA. Extracellular vesicles as emerging players in intercellular communication: relevance in mast cell-mediated pathophysiology. IJMS. 2021. https://doi.org/10.3390/ijms22179176.
102. Abyadeh M, Alikhani M, Mirzaei M, Gupta V, Shekari F, Salekdeh GH. Proteomics provides insights into the theranostic potential of extracellular vesicles. Adv Protein Chem Struct Biol. 2024. https://doi.org/10.1016/bs.apcsb.2023.08.001.
103. Mathieu M, Martin-Jaular L, Lavieu G, Théry C. Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication. Nat Cell Biol. 2019. https://doi.org/10.1038/s41556-018-0250-9.
104. Sardar Sinha M, Ansell-Schultz A, Civitelli L, Hildesjö C, Larsson M, Lannfelt L, et al. Alzheimer's disease pathology propagation by exosomes containing toxic amyloid-beta oligomers. Acta Neuropathol. 2018. https://doi.org/10.1007/s00401-018-1868-1.
105. Elsherbini A, Qin H, Zhu Z, Tripathi P, Crivelli SM, Bieberich E. In vivo evidence of exosome-mediated Aβ neurotoxicity. Acta Neuropathol Commun. 2020. https://doi.org/10.1186/s40478-020-00981-y.
106. Villemagne VL, Doré V, Burnham SC, Masters CL, Rowe CC. Imaging tau and amyloid-β proteinopathies in Alzheimer disease and other conditions. Nat Rev Neurol. 2018. https://doi.org/10.1038/nrneurol.2018.9.
107. Saman S, Kim W, Raya M, Visnick Y, Miro S, Saman S, et al. Exosome-associated tau is secreted in tauopathy models and is selectively phosphorylated in cerebrospinal fluid in early Alzheimer disease. J Biol Chem. 2012. https://doi.org/10.1074/jbc.m111.277061.
108. Fiandaca MS, Kapogiannis D, Mapstone M, Boxer A, Eitan E, Schwartz JB, et al. Identification of preclinical Alzheimer's disease by a profile of pathogenic proteins in neurally derived blood exosomes: a case-control study. Alzheimers Dementia. 2014. https://doi.org/10.1016/j.jalz.2014.06.008.
109. Liang L-G, Kong M-Q, Zhou S, Sheng Y-F, Wang P, Yu T, et al. An integrated double-filtration microfluidic device for isolation, enrichment and quantification of urinary extracellular vesicles for detection of bladder cancer. Sci Rep. 2017. https://doi.org/10.1038/srep46224.
110. Zhang Y, Jin X, Liang J, Guo Y, Sun G, Zeng X, et al. Extracellular vesicles derived from ODN-stimulated macrophages transfer and activate Cdc42 in recipient cells and thereby increase cellular permissiveness to EV uptake. Sci Adv. 2019. https://doi.org/10.1126/sciadv.aav1564.
111. Gupta D, Wiklander OPB, Görgens A, Conceição M, Corso G, Liang X, et al. Amelioration of systemic inflammation via the display of two different decoy protein receptors on extracellular vesicles. Nat Biomed Eng. 2021. https://doi.org/10.1038/s41551-021-00792-z.
112. Ye Q, Xu H, Liu S, Li Z, Zhou J, Ding F, et al. Apoptotic extracellular vesicles alleviate Pg-LPS induced inflammatory responses of macrophages via AMPK/SIRT1/NF-κB pathway and inhibit osteoclast formation. J Periodontol. 2022. https://doi.org/10.1002/jper.21-0657.
113. Han JM, Song H-Y, Jung J-H, Lim S, Seo HS, Kim WS, et al. *Deinococcus radiodurans*-derived membrane vesicles protect HaCaT cells against H2O2-induced oxidative stress via modulation of MAPK and Nrf2/ARE pathways. Biol Proced Online. 2023. https://doi.org/10.1186/s12575-023-00211-4.
114. Pirisinu M, Pham TC, Zhang DX, Hong TN, Nguyen LT, Le MT. Extracellular vesicles as natural therapeutic agents and innate drug delivery systems for cancer treatment: recent advances, current obstacles, and challenges for clinical translation. Semin Cancer Biol. 2022. https://doi.org/10.1016/j.semcancer.2020.08.007.
115. Du S, Guan Y, Xie A, Yan Z, Gao S, Li W, et al. Extracellular vesicles: a rising star for therapeutics and drug delivery. J Nanobiotechnol. 2023. https://doi.org/10.1186/s12951-023-01973-5.
116. Blondeel J, Gilbo N, De Bondt S, Monbaliu D. Stem cell derived extracellular vesicles to alleviate ischemia-reperfusion injury of transplantable organs. A Systematic Review. Stem Cell Rev and Rep. 2023. https://doi.org/10.1007/s12015-023-10573-7.
117. Yoo MH, Lee A-R, Moon K-S. Characteristics of extracellular vesicles and preclinical testing considerations prior to clinical applications. Biomedicines. 2022. https://doi.org/10.3390/biomedicines10040869.
118. Zhao M, van Straten D, Broekman MLD, Préat V, Schiffelers RM. Nanocarrier-based drug combination therapy for glioblastoma. Theranostics. 2020. https://doi.org/10.7150/thno.38147.
119. Hernando S, Santos-Vizcaíno E, Igartua M, Hernandez RM. Targeting the central nervous system: from synthetic nanoparticles to extracellular vesicles—focus on Alzheimer's and Parkinson's disease. WIREs Nanomed Nanobiotechnol. 2023. https://doi.org/10.1002/wnan.1898.
120. Wu L-P, Wang D, Li Z. Grand challenges in nanomedicine. Mater Sci Eng, C. 2020. https://doi.org/10.1016/j.msec.2019.110302.
121. Ribovski L, Hamelmann NM, Paulusse JMJ. Polymeric nanoparticles properties and brain delivery. Pharmaceutics. 2021. https://doi.org/10.3390/pharmaceutics13122045.
122. Liu X, Xia T, Fang Y, Zuo H, Dong X, Xu P, et al. Overcoming the blood–brain barrier by using a multistage exosome delivery system to inhibit central nervous system lymphoma. Nanomed Nanotechnol Biol Med. 2022. https://doi.org/10.1016/j.nano.2022.102523.
123. Maher R, Moreno-Borrallo A, Jindal D, Mai BT, Ruiz-Hernandez E, Harkin A. Intranasal polymeric and lipid-based nanocarriers for CNS drug

delivery. Pharmaceutics. 2023. https://doi.org/10.3390/pharmaceutics15030746.
124. Gao Y, Chu C, Jablonska A, Bulte JWM, Walczak P, Janowski M. Imaging as a tool to accelerate the translation of extracellular vesicle-based therapies for central nervous system diseases. WIREs Nanomed Nanobiotechnol. 2020. https://doi.org/10.1002/wnan.1688.
125. Shi S, Ou X, Cheng D. Nanoparticle-facilitated therapy: advancing tools in peripheral nerve regeneration. Int J Nanomed. 2024. https://doi.org/10.2147/ijn.s442775.
126. Szabat-Iriaka B, Le Borgne M. Brain safety concerns of nanomedicines: the need for a specific regulatory framework. Drug Discovery Today. 2021. https://doi.org/10.1016/j.drudis.2021.06.011.
127. Pottoo FH, Sharma S, Javed MN, Barkat MA, Harshita AMS, Naim MJ, Alam O, Ansari MA, Barreto GE, Ashraf GM, et al. Lipid-based nanoformulations in the treatment of neurological disorders. Drug Metab Rev. 2020. https://doi.org/10.1080/03602532.2020.1726942.
128. Zingale E, Bonaccorso A, Carbone C, Musumeci T, Pignatello R. Drug nanocrystals: focus on brain delivery from therapeutic to diagnostic applications. Pharmaceutics. 2022. https://doi.org/10.3390/pharmaceutics14040691.
129. Rather HA, Almousa S, Craft S, Deep G. Therapeutic efficacy and promise of stem cell-derived extracellular vesicles in Alzheimer's disease and other aging-related disorders. Ageing Res Rev. 2023. https://doi.org/10.1016/j.arr.2023.102088.
130. Zhou Y, Bhatt H, Mojica CA, Xin H, Pessina MA, Rosene DL, et al. Mesenchymal-derived extracellular vesicles enhance microglia-mediated synapse remodeling after cortical injury in aging Rhesus monkeys. J Neuroinflammation. 2023. https://doi.org/10.1186/s12974-023-02880-0.
131. Labusek N, Ghari P, Mouloud Y, Köster C, Diesterbeck E, Hadamitzky M, et al. Hypothermia combined with extracellular vesicles from clonally expanded immortalized mesenchymal stromal cells improves neurodevelopmental impairment in neonatal hypoxic-ischemic brain injury. J Neuroinflammation. 2023. https://doi.org/10.1186/s12974-023-02961-0.
132. Kowalski RG, Ledreux A, Violette JE, Neumann RT, Ornelas D, Yu X, et al. Rapid activation of neuroinflammation in stroke: plasma and extracellular vesicles obtained on a mobile stroke unit. Stroke. 2023. https://doi.org/10.1161/strokeaha.122.041422.
133. Wu W-C, Tian J, Xiao D, Guo Y-X, Xiao Y, Wu X-Y, et al. Engineered extracellular vesicles encapsulated bryostatin-1 as therapy for neuroinflammation. Nanosc Roy Soc Chem (RSC). 2022. https://doi.org/10.1039/d1nr05517h.
134. Tang T-T, Wang B, Lv L-L, Liu B-C. Extracellular vesicle-based nanotherapeutics: emerging frontiers in anti-inflammatory therapy. Theranostics. 2020. https://doi.org/10.7150/thno.47865.
135. Huyan T, Li H, Peng H, Chen J, Yang R, Zhang W, et al. Extracellular vesicles–advanced nanocarriers in cancer therapy: progress and achievements. IJN. 2020. https://doi.org/10.2147/ijn.s238099.
136. Chen C, Wang J, Sun M, Li J, Wang H. Toward the next-generation phyto-nanomedicines: cell-derived nanovesicles (CDNs) for natural product delivery. Biomed Pharmacother. 2022. https://doi.org/10.1016/j.biopha.2021.112416.
137. Szwedowicz U, Łapińska Z, Gajewska-Naryniecka A, Choromańska A. Exosomes and other extracellular vesicles with high therapeutic potential: their applications in oncology, neurology, and dermatology. Molecules. 2022. https://doi.org/10.3390/molecules27041303.
138. Chen C, Sun M, Wang J, Su L, Lin J, Yan X. Active cargo loading into extracellular vesicles: highlights the heterogeneous encapsulation behaviour. J of Extracellular Vesicle. 2021. https://doi.org/10.1002/jev2.12163.
139. Oliveira Silva R, Counil H, Rabanel J-M, Haddad M, Zaouter C, Ben Khedher MR, et al. Donepezil-loaded nanocarriers for the treatment of Alzheimer's disease: superior efficacy of extracellular vesicles over polymeric nanoparticles. IJN. 2024. https://doi.org/10.2147/ijn.s449227.
140. Liu Y, Gong Y, Xie W, Huang A, Yuan X, Zhou H, et al. Microbubbles in combination with focused ultrasound for the delivery of quercetin-modified sulfur nanoparticles through the blood brain barrier into the brain parenchyma and relief of endoplasmic reticulum stress to treat Alzheimer's disease. Nanoscale. 2020. https://doi.org/10.1039/c9nr09713a.
141. Gothwal A, Kumar H, Nakhate KT, Ajazuddin, Dutta A, Borah A, et al. Lactoferrin coupled lower generation PAMAM dendrimers for brain targeted delivery of memantine in aluminum-chloride-induced Alzheimer's disease in mice. Bioconjugate Chem. 2019. https://doi.org/10.1021/acs.bioconjchem.9b00505.
142. Grabska-Kobyłecka I, Szpakowski P, Król A, Książek-Winiarek D, Kobyłecki A, Głąbiński A, et al. Polyphenols and their impact on the prevention of neurodegenerative diseases and development. Nutrients. 2023. https://doi.org/10.3390/nu15153454.
143. Yulug B, Altay O, Li X, Hanoglu L, Cankaya S, Lam S, et al. Combined metabolic activators improve cognitive functions in Alzheimer's disease patients: a randomised, double-blinded, placebo-controlled phase-II trial. Transl Neurodegener. 2023. https://doi.org/10.1186/s40035-023-00336-2.
144. Cao H, Zuo C, Gu Z, Huang Y, Yang Y, Zhu L, et al. High frequency repetitive transcranial magnetic stimulation alleviates cognitive deficits in 3xTg-AD mice by modulating the PI3K/Akt/GLT-1 axis. Redox Biol. 2022. https://doi.org/10.1016/j.redox.2022.102354.
145. Jia J, Hu J, Huo X, Miao R, Zhang Y, Ma F. Effects of vitamin D supplementation on cognitive function and blood Aβ-related biomarkers in older adults with Alzheimer's disease: a randomised, double-blind, placebo-controlled trial. J Neurol Neurosurg Psychiatry. 2019. https://doi.org/10.1136/jnnp-2018-320199.
146. Lee H, Hoe H-S. Inhibition of CDK4/6 regulates AD pathology, neuroinflammation and cognitive function through DYRK1A/STAT3 signaling. Pharmacol Res. 2023. https://doi.org/10.1016/j.phrs.2023.106725.
147. Stavrinou P, Andreou E, Aphamis G, Pantzaris M, Ioannou M, Patrikios I, et al. The effects of a 6-month high dose omega-3 and omega-6 polyunsaturated fatty acids and antioxidant vitamins supplementation on cognitive function and functional capacity in older adults with mild cognitive impairment. Nutrients. 2020. https://doi.org/10.3390/nu12020325.
148. Delbreil P, Rabanel J-M, Banquy X, Brambilla D. Therapeutic nanotechnologies for Alzheimer's disease: a critical analysis of recent trends and findings. Adv Drug Deliv Rev. 2022. https://doi.org/10.1016/j.addr.2022.114397.
149. Cunha S, Forbes B, Sousa Lobo JM, Silva AC. Improving drug delivery for Alzheimer's disease through nose-to-brain delivery using nanoemulsions, nanostructured lipid carriers (NLC) and in situ hydrogels. Int J Nanomed. 2021. https://doi.org/10.2147/ijn.s305851.
150. Séguy L, Groo A-C, Malzert-Fréon A. How nano-engineered delivery systems can help marketed and repurposed drugs in Alzheimer's disease treatment? Drug Discov Today. 2022. https://doi.org/10.1016/j.drudis.2022.02.022.
151. Hu L, Tao Y, Jiang Y, Qin F. Recent progress of nanomedicine in the treatment of Alzheimer's disease. Front Cell Dev Biol. 2023. https://doi.org/10.3389/fcell.2023.1228679.
152. Moreira DA, Santos SD, Leiro V, Pêgo AP. Dendrimers and derivatives as multifunctional nanotherapeutics for Alzheimer's disease. Pharmaceutics. 2023. https://doi.org/10.3390/pharmaceutics15041054.
153. Hanafy AS, Schoch S, Lamprecht A. CRISPR/Cas9 delivery potentials in Alzheimer's disease management: a mini review. Pharmaceutics. 2020. https://doi.org/10.3390/pharmaceutics12090801.
154. Chacko L, Chaudhary A, Singh B, Dewanjee S, Kandimalla R. CRISPR-Cas9 in Alzheimer's disease: therapeutic trends, modalities, and challenges. Drug Discov Today. 2023. https://doi.org/10.1016/j.drudis.2023.103652.
155. Usman WM, Pham TC, Kwok YY, Vu LT, Ma V, Peng B, et al. Efficient RNA drug delivery using red blood cell extracellular vesicles. Nat Commun. 2018. https://doi.org/10.1038/s41467-018-04791-8.
156. Osteikoetxea X, Silva A, Lázaro-Ibáñez E, Salmond N, Shatnyeva O, Stein J, et al. Engineered Cas9 extracellular vesicles as a novel gene editing tool. J Extracell Vesicle. 2022. https://doi.org/10.1002/jev2.12225.
157. Srinivasan K, Friedman BA, Etxeberria A, Huntley MA, van der Brug MP, Foreman O, et al. Alzheimer's patient microglia exhibit enhanced aging and unique transcriptional activation. Cell Rep. 2020. https://doi.org/10.1016/j.celrep.2020.107843.
158. Morabito S, Miyoshi E, Michael N, Shahin S, Martini AC, Head E, et al. Single-nucleus chromatin accessibility and transcriptomic characterization of Alzheimer's disease. Nat Genet. 2021. https://doi.org/10.1038/s41588-021-00894-z.

159. Jiang J, Wang C, Qi R, Fu H, Ma Q. scREAD: a single-cell RNA-seq database for Alzheimer's disease. iScience. 2020. https://doi.org/10.1016/j.isci.2020.101769.
160. Su Y, Zheng H, Shi C, Li X, Zhang S, Guo G, et al. Meningeal immunity and neurological diseases: new approaches, new insights. J Neuroinflammation. 2023. https://doi.org/10.1186/s12974-023-02803-z.
161. Liu C, Zhao X-M, Wang Q, Du T-T, Zhang M-X, Wang H-Z, et al. Astrocyte-derived SerpinA3N promotes neuroinflammation and epileptic seizures by activating the NF-κB signaling pathway in mice with temporal lobe epilepsy. J Neuroinflammation. 2023. https://doi.org/10.1186/s12974-023-02840-8.
162. Maïer B, Tsai AS, Einhaus JF, Desilles J-P, Ho-Tin-Noé B, Gory B, et al. Neuroimaging is the new "spatial omic": multi-omic approaches to neuroinflammation and immuno-thrombosis in acute ischemic stroke. Semin Immunopathol. 2023. https://doi.org/10.1007/s00281-023-00984-6.
163. Clark C, Dayon L, Masoodi M, Bowman GL, Popp J. An integrative multi-omics approach reveals new central nervous system pathway alterations in Alzheimer's disease. Alz Res Therapy. 2021. https://doi.org/10.1186/s13195-021-00814-7.
164. Lv S, Wang Q, Zhang X, Ning F, Liu W, Cui M, et al. Mechanisms of multi-omics and network pharmacology to explain traditional Chinese medicine for vascular cognitive impairment: a narrative review. Phytomedicine. 2024. https://doi.org/10.1016/j.phymed.2023.155231.
165. Visconte C, Fenoglio C, Serpente M, Muti P, Sacconi A, Rigoni M, et al. Altered extracellular vesicle miRNA profile in prodromal Alzheimer's disease. IJMS. 2023. https://doi.org/10.3390/ijms241914749.
166. Li C, Qin T, Jin Y, Hu J, Yuan F, Cao Y, et al. Cerebrospinal fluid-derived extracellular vesicles after spinal cord injury promote vascular regeneration via PI3K/AKT signaling pathway. J Orthop Transl. 2023. https://doi.org/10.1016/j.jot.2023.02.001.
167. Geng L, Xu J, Zhu Y, Hu X, Liu Y, Yang K, et al. Targeting miR-9 in glioma stem cell-derived extracellular vesicles: a novel diagnostic and therapeutic biomarker. Transl Oncol. 2022. https://doi.org/10.1016/j.tranon.2022.101451.
168. Beretta C, Nikitidou E, Streubel-Gallasch L, Ingelsson M, Sehlin D, Erlandsson A. Extracellular vesicles from amyloid-β exposed cell cultures induce severe dysfunction in cortical neurons. Sci Rep. 2020. https://doi.org/10.1038/s41598-020-72355-2.
169. George SK, Lauková L, Weiss R, Semak V, Fendl B, Weiss VU, et al. Comparative analysis of platelet-derived extracellular vesicles using flow cytometry and nanoparticle tracking analysis. Int J Mol Sci. 2021. https://doi.org/10.3390/ijms22083839.
170. Koksal AR, Ekmen N, Aydin Y, Nunez K, Sandow T, Delk M, et al. A Single-step immunocapture assay to quantify HCC exosomes using the highly sensitive fluorescence nanoparticle-tracking analysis. JHC. 2023. https://doi.org/10.2147/jhc.s423043.
171. Losurdo M, Pedrazzoli M, D'Agostino C, Elia CA, Massenzio F, Lonati E, et al. Intranasal delivery of mesenchymal stem cell-derived extracellular vesicles exerts immunomodulatory and neuroprotective effects in a 3xTg model of Alzheimer's disease. Stem Cells Transl Med. 2020. https://doi.org/10.1002/sctm.19-0327.
172. Gratpain V, Mwema A, Labrak Y, Muccioli GG, van Peschdes Rieux VA. Extracellular vesicles for the treatment of central nervous system diseases. Adv Drug Deliv Rev. 2021. https://doi.org/10.1016/j.addr.2021.05.006.
173. Hering C, Shetty AK. Extracellular vesicles derived from neural stem cells, astrocytes, and microglia as therapeutics for easing TBI-induced brain dysfunction. Stem Cells Transl Med. 2023. https://doi.org/10.1093/stcltm/szad004.
174. Chen Y, Zhang H, Hu X, Cai W, Jiang L, Wang Y, et al. Extracellular vesicles: therapeutic potential in central nervous system trauma by regulating cell death. Mol Neurobiol. 2023. https://doi.org/10.1007/s12035-023-03501-w.
175. Go V, Bowley BGE, Pessina MA, Zhang ZG, Chopp M, Finklestein SP, et al. Extracellular vesicles from mesenchymal stem cells reduce microglial-mediated neuroinflammation after cortical injury in aged Rhesus monkeys. GeroScience. 2019. https://doi.org/10.1007/s11357-019-00115-w.
176. Zhang Q, Jeppesen DK, Higginbotham JN, Franklin JL, Coffey RJ. Comprehensive isolation of extracellular vesicles and nanoparticles. Nat Protoc. 2023. https://doi.org/10.1038/s41596-023-00811-0.
177. Meng W, He C, Hao Y, Wang L, Li L, Zhu G. Prospects and challenges of extracellular vesicle-based drug delivery system: considering cell source. Drug Delivery. 2020. https://doi.org/10.1080/10717544.2020.1748758.
178. Giancaterino S, Boi C. Alternative biological sources for extracellular vesicles production and purification strategies for process scale-up. Biotechnol Adv. 2023. https://doi.org/10.1016/j.biotechadv.2022.108092.
179. Zeng Y, Qiu Y, Jiang W, Shen J, Yao X, He X, et al. Biological features of extracellular vesicles and challenges. Front Cell Dev Biol. 2022. https://doi.org/10.3389/fcell.2022.816698.
180. Gao J, Dong X, Wang Z. Generation, purification and engineering of extracellular vesicles and their biomedical applications. Methods. 2020. https://doi.org/10.1016/j.ymeth.2019.11.012.
181. Nasiri Kenari A, Cheng L, Hill AF. Methods for loading therapeutics into extracellular vesicles and generating extracellular vesicles mimetic-nanovesicles. Methods. 2020. https://doi.org/10.1016/j.ymeth.2020.01.001.
182. Staubach S, Bauer FN, Tertel T, Börger V, Stambouli O, Salzig D, et al. Scaled preparation of extracellular vesicles from conditioned media. Adv Drug Deliv Rev. 2021. https://doi.org/10.1016/j.addr.2021.113940.

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