Biomolecules and Biomedicine
ISSN: 2831-0896 (Print) | ISSN: 2831-090X (Online)
Journal Impact Factor® (2024): 2.2
CiteScore® (2024): 5.2
www.biomolbiomed.com | blog.bjbms.org

REVIEW

Yuan et al: Gut vesicles and Alzheimer's disease

Gut microbiota-derived extracellular vesicles in Alzheimer's disease – Immunomodulatory mechanisms, biomarkers, and therapeutic opportunities: A review

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DOI: https://doi.org/10.17305/bb.2025.13213

ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that poses a growing global health challenge. Beyond traditional hallmarks such as amyloid-β (Aβ) deposition, tau hyperphosphorylation, and neuroinflammation, the gut–brain axis (GBA) has emerged as a significant modulator of AD pathogenesis. Among gut-derived mediators, microbiota-derived extracellular vesicles (mEVs) transport bioactive cargo across epithelial and vascular barriers, thereby linking intestinal dysbiosis to neurodegeneration. This narrative review synthesizes experimental, translational, and early clinical evidence regarding the immunomodulatory roles of gut mEVs in AD. We examine how mEVs may traverse compromised intestinal and blood–brain barriers, activate microglia and astrocytes, and influence Aβ and tau metabolism, thereby integrating peripheral and central immune interactions. Based on this evidence, we propose the "microbiota–EV–immune–neuro axis" as a conceptual framework that connects gut dysbiosis with AD-related neurodegeneration. The review also highlights emerging data on mEV signatures as minimally invasive biomarkers and explores their potential as therapeutic targets or delivery vectors. While current evidence is preliminary and methodologically heterogeneous, mEVs are increasingly recognized as both indicators and potential modulators of AD pathophysiology, emphasizing the need for standardized, longitudinal, and interventional studies.

Keywords: Gut microbiota-derived extracellular vesicles, Alzheimer’s disease, neuroinflammation, immune regulation, gut-brain axis, microbiota-immune-neuro axis.

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia, marked by progressive cognitive decline and neurodegeneration[1]. Despite major advances in neuroscience, the precise mechanisms driving AD remain incompletely understood. Traditional hypotheses have centered on amyloid-β (Aβ) accumulation, tau hyperphosphorylation, synaptic dysfunction, and chronic neuroinflammation, all of which contribute to neuronal degeneration and cognitive impairment[2]. Yet, current therapies that target these canonical pathways have yielded limited clinical benefits, suggesting that additional systemic factors may influence disease onset and progression.

In recent years, the gut-brain axis (GBA) has gained prominence as a bidirectional network integrating intestinal microbiota, immune function, and neural signaling[3]. Disturbances in gut microbial composition have been associated with systemic inflammation, metabolic imbalance, and neurodegeneration, pointing toward a broader immunometabolic basis of AD[4]. Among the various mediators linking the gut and brain, microbial extracellular vesicles (mEVs), nanosized particles secreted by gut bacteria containing lipids, proteins, and nucleic acids, have emerged as a novel and biologically active mode of communication. These vesicles can traverse epithelial and vascular barriers, enter systemic circulation, and potentially influence central immune responses. Their capacity to modulate inflammation, oxidative stress, and neuronal homeostasis positions them as potential contributors to the pathophysiology of AD.

Growing experimental and clinical evidence now supports a role for mEVs in bridging gut dysbiosis and neuroinflammation. Preclinical studies demonstrate that bacterial vesicles can cross the blood-brain barrier (BBB) and activate microglia through toll-like receptor pathways, while early human data indicate that mEV profiles in feces and plasma differ between individuals with AD and cognitively normal controls. Collectively, these findings suggest that mEVs may act as both messengers and mediators within a "microbiota-EV-immune-neuro" network.

Therefore, the aim of this review is to summarize current evidence regarding the immunomodulatory roles of gut mEVs in AD pathogenesis. We discuss how these vesicles influence immune regulation, amyloid and tau metabolism, and neuroinflammatory cascades, while also considering their diagnostic and therapeutic potential.

METHODS

Search strategy

This review was conducted to summarize and evaluate current evidence on the immunomodulatory functions of gut microbiota-derived extracellular vesicles (mEVs) in Alzheimer's disease. A comprehensive literature search was performed on PubMed, Web of Science, Embase, and Scopus for publications up to July 2025. The following keywords combination was used: "Alzheimer's disease" or "dementia," "gut microbiota" or "intestinal microbiome," and "extracellular vesicles," "bacterial outer membrane vesicles," or "microbiota-derived vesicles." These were further combined with terms related to "neuroinflammation," "immune modulation," or "microglia."

Reference lists of eligible articles and recent reviews were manually screened to identify additional relevant studies. Only peer-reviewed journal articles were considered. Publications were not restricted by language at the initial stage.

Study selection

All retrieved records were imported into a reference management software, and duplicates were removed. Their titles and abstracts were screened to exclude studies found irrelevant, such as those not directly related to Alzheimer's disease or those focusing solely on host-derived exosomes. Full-text evaluation was then performed to confirm eligibility.

Studies were included if they examined extracellular vesicles produced by gut or commensal microbes and explored their roles in Alzheimer's disease or neurodegenerative processes linked to inflammation, amyloid or tau metabolism,

blood-brain barrier function, or neuronal injury. Both experimental and human studies were considered eligible, including in vivo, in vitro or ex vivo mechanistic studies, and observational or interventional clinical research.

Studies were excluded if they (1) did not involve microbial extracellular vesicles; (2) lacked Alzheimer's-related outcomes; (3) were conference abstracts, commentaries, or editorials without original data; or (4) provided insufficient methodological information to assess study quality. Disagreements during the selection process were resolved through discussion among the authors.

Data extraction and synthesis

Each included study was reviewed to extract information on publication year, study design, model system, microbial source of vesicles, experimental or clinical context, and main outcomes. For animal and cell studies, data on vesicle preparation, dose, and routes of administration were recorded together with reported effects on immune and neural parameters. For human studies, participant characteristics, biological samples analyzed (plasma, feces, cerebrospinal fluid), and identified vesicle biomarkers were summarized. Due to heterogeneity among study designs and outcome measures, results were summarized narratively rather than statistically pooled. The synthesis was organized thematically according to the major biological mechanisms discussed in the main text, including vesicle transport across biological barriers, activation of innate immune pathways, modulation of neuroinflammation, and implications for diagnostic or therapeutic development, which allowed comparison across experimental and human findings while highlighting methodological consistencies and limitations.

Quality assessment

The methodological quality of the included literature was evaluated using criteria appropriate to study type. For in vitro and ex vivo work, emphasis was placed on the characterization of vesicle preparations, purity validation, and functional assays supporting mechanistic conclusions. Human studies were assessed based on their cohort designs, focusing on sample selection, confounding control, and measurement

reliability.

Studies judged to have major methodological deficiencies or incomplete reporting were discussed separately in the review but were not included in the mechanistic synthesis. This approach ensured that the conclusions of the present review were based on the most reliable and reproducible evidence currently available.

Ethics approval

As this work synthesized data from previously published studies, no new ethical approval or patient consent was required.

PATHOLOGICAL FEATURES AND IMMUNE MECHANISMS OF ALZHEIMER'S DISEASE

Classical pathological processes

AD is neuropathologically defined by two major hallmark proteinopathies: the extracellular accumulation of Aβ peptides forming senile plaques, and the intracellular aggregation of hyperphosphorylated tau protein into neurofibrillary tangles[5]. Aβ arises from the sequential enzymatic cleavage of amyloid precursor protein (APP), whereas tau functions as a microtubule-associated protein essential for maintaining cytoskeletal stability and axonal transport[6]. Disruption in the normal turnover or post-translational regulation of these proteins induces conformational changes that confer neurotoxicity, leading to progressive neuronal dysfunction and death[7].

According to the widely accepted amyloid cascade hypothesis, excessive Aβ production or inefficient clearance results in its accumulation within the extracellular space, where it aggregates into plaques that impair synaptic signaling and activate neuroinflammatory cascades[8]. In parallel, tau undergoes abnormal phosphorylation, detaches from microtubules, and assembles into insoluble fibrils[9]. The resulting tangles disrupt intracellular trafficking and contribute to cytoskeletal collapse, synaptic failure, and neuronal degeneration[10].

Although amyloid plaques and neurofibrillary tangles remain diagnostic cornerstones of AD, growing evidence indicates that early synaptic dysfunction, mitochondrial impairment, and programmed neuronal apoptosis may occur well before overt structural pathology becomes apparent[11]. These early molecular disturbances are now recognized as critical events in the initiation and amplification of the neurodegenerative process that defines AD [12].

The central role of neuroinflammation

Neuroinflammation is now recognized as a central pathological feature of AD, rather than a secondary consequence of neuronal injury[13]. Microglia, the resident immune cells of the central nervous system, are rapidly activated by Aβ plaques and hyperphosphorylated tau aggregates. Once activated, these cells release pro-inflammatory mediators such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and reactive oxygen species[14]. The sustained presence of these mediators contributes to neuronal stress, synaptic dysfunction, and the establishment of a self-perpetuating inflammatory environment. Chronically activated microglia also display reduced phagocytic efficiency, thereby impairing Aβ clearance and exacerbating plaque accumulation[15].

Recent studies suggest that the inflammatory activation observed in AD is not confined to the central nervous system. Peripheral immune signals, including circulating cytokines and pathogen-associated molecular patterns, can modulate central immune tone and microglial reactivity[16]. This crosstalk is facilitated through the BBB, a selectively permeable interface that normally preserves central immune privilege[17]. Under pathological conditions such as aging, metabolic disorders, or chronic systemic inflammation, BBB permeability becomes compromised, allowing peripheral immune components to access the brain parenchyma, thereby allowing a bidirectional loop in which systemic inflammation enhances microglial activation, which in turn amplifies neuroinflammation within the CNS[18].

This concept of peripheral-to-central inflammatory signaling provides a mechanistic basis for exploring gut-mediated immune influences on the brain. mEVs

may exploit these compromised barriers to deliver immunogenic molecules and modulate neuroinflammatory pathways. Understanding this gut-immune-brain communication axis may reveal novel therapeutic opportunities that target both systemic and central drivers of AD pathology.

STRUCTURAL AND FUNCTIONAL CHARACTERISTICS OF GUT MICROBIOTA-DERIVED EXTRACELLULAR VESICLES

Gut mEVs are nanoscale, bilayered particles secreted by both Gram-negative and Gram-positive bacteria[19]. In Gram-negative species, outer membrane vesicles (OMVs) bud outward from the bacterial outer membrane, whereas Gram-positive bacteria generate vesicles through mechanisms that involve localized cell wall remodeling and turgor-driven release. These vesicles typically measure between 20 and 250 nm in diameter and are enclosed by negatively charged lipid bilayers that incorporate characteristic bacterial surface components such as lipopolysaccharide (LPS), lipoteichoic acids, and outer membrane protein A (OmpA)[20, 21].

Beyond their structural organization, mEVs carry diverse molecular cargos, including proteins, lipids, nucleic acids, and metabolites, which collectively determine their biological activity. These cargos enable mEVs to participate in microbe-host communication, immune modulation, and metabolic and neuroactive signaling. Of particular relevance to AD, vesicular cargos such as LPS and microbial RNA fragments are increasingly recognized as potent activators of innate immune pathways, linking gut dysbiosis with neuroinflammatory cascades (Table 1).

Once released, mEVs can interact with host systems through several routes. They are readily internalized by intestinal epithelial cells via endocytosis or membrane fusion, influencing epithelial permeability and mucosal immune tone [22]. Under pathological conditions, including chronic inflammation, metabolic stress, or aging, the intestinal barrier becomes more permeable, allowing vesicles to translocate into systemic circulation. Within this context, circulating mEVs act as long-range communication vectors between the gut and peripheral organs, and potentially the

central nervous system, thereby mediating the molecular dialogue that underlies GBA dysfunction in AD [23].

IMMUNOMODULATORY MECHANISMS OF mEVs IN ALZHEIMER'S DISEASE

From gut to brain: How mEVs may reach and disrupt the central nervous system

In AD, the pathophysiological relevance of mEVs extends beyond general immunomodulation to include a plausible role in linking intestinal dysbiosis with central neuroinflammation and protein-homeostasis disruption. Under conditions of gut barrier compromise, such as aging, low-grade inflammation or epithelial tight-junction dysregulation, mEVs released by commensal or opportunistic gut microbes can gain access to the systemic circulation[22]. Once in circulation, mEVs may interact with the vascular endothelium, including the BBB. Increased permeability of the BBB, which has been observed in both aging and AD, could potentially facilitate the passage of mEVs into the CNS via paracellular leakage or transcytosis[24]. Preclinical data indicate vesicles derived from gut bacteria access distal organs, carrying outer-membrane LPS, lipoproteins, nucleic acids and small RNAs as bioactive cargo[25].

Once in the bloodstream, mEVs may interact with the vascular endothelium of the BBB via multiple putative pathways. One route involves paracellular passage through endothelial tight junctions when expression of claudin-5 and occludin is downregulated, a phenomenon observed in aging and neurodegenerative states[26]. A second route is receptor-mediated transcytosis: mEV surface ligands (for example bacterial lipoproteins or LPS) may bind endothelial TLR4/CD14/MD-2 complexes, triggering MyD88/NF-κB signalling, increased vesicle internalisation (via clathrin/caveolin) and release into the abluminal side of the BBB[27]. In some models vesicle exposure also appears to increase endothelial vesicle-secretion and local inflammation, thereby amplifying barrier leakiness[28].

After entering the brain parenchyma, mEVs engage resident glial and neuronal cells. Microglia detect vesicle-bound LPS or lipoprotein cargo via TLR4 (and potentially TLR2) which triggers MyD88-dependent NF-κB activation, production of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6) and reactive oxygen species[29]. This activated microglial phenotype can reduce phagocytic clearance of Aβ and contribute to synaptic pruning and neuronal damage[30]. Astrocytes, when exposed to mEVs, may respond by increasing complement (C3) expression and chemokine release (e.g., CCL2) that recruits peripheral immune cells and exacerbates neuroinflammatory circuits[31].

In addition, mEV cargo may directly influence Aβ and tau metabolism. Small RNA and miRNA-like fragments within vesicles have been predicted to target mRNAs encoding APP, β-secretase (BACE1), presenilin-1 (PSEN1) and tau-kinases such as GSK-3β and CDK5 [32]. Moreover, vesicle endocytosis by neurons may induce mitochondrial dysfunction, increase intracellular calcium, activate stress-kinase cascades (e.g., JNK/p38), and thereby promote tau hyper-phosphorylation and subsequent aggregation[33]. These dual modes, namely immune-mediated reduction of Aβ clearance and direct modulation of protein-processing gene networks, create a plausible mechanistic bridge between gut-microbiota vesicles and hallmark AD pathology.

Taken together, available evidence outlines a coherent mechanistic sequence linking intestinal dysbiosis to central pathology. Disruption of the gut barrier allows increased release and systemic spread of bacterial vesicles, which can interact with the vascular endothelium and penetrate the BBB. Once within the brain, these vesicles activate microglia and astrocytes, amplify neuroinflammatory signaling, and disturb the balance of Aβ and tau metabolism through both immune and post-transcriptional pathways. Although many of these findings remain based on experimental models and human data are still limited, the emerging evidence provides a plausible biological framework connecting the gut microbiota with neurodegeneration in AD.

Peripheral-central immune feedback loops

In addition to their direct effects within the central nervous system, gut-mEVs appear to modulate peripheral immune homeostasis in a manner that may feed back into neuroinflammatory processes. Dysbiosis of the gut microbiota alters the balance of T-helper 17 (Th17) cells and regulatory T cells (Tregs), with a shift toward Th17-dominant responses being linked to increased systemic inflammation and impaired immune control[34]. Although most work to date has focused on microbial metabolites (short-chain fatty acids, ATP) rather than vesicles, emerging evidence suggests that mEVs may deliver bacterial antigens, lipoproteins or small RNAs to antigen-presenting cells in the gut-associated lymphoid tissue, thereby promoting Th17 differentiation (via IL-6/IL-23 signalling) and suppressing Treg development (via reduced IL-10/TGF-β)[35]. Once launched, this peripheral pro-inflammatory milieu may influence central immune dynamics through multiple routes.

Systemic inflammatory mediators, including IL-17, IL-1β, TNF-α, and microbial-derived antigens carried within mEVs or released secondary to vesicle-stimulated immune activity, can affect the integrity of the BBB and endothelial signalling[35]. Endothelial cells exposed to IL-17 or bacterial vesicles up-regulate adhesion molecules (VCAM-1, ICAM-1) and secrete chemokines (CCL2), facilitating leukocyte trafficking and increasing permeability. mEVs themselves may interact with endothelial TLR4 or RAGE receptors, activating NF-κB and the NLRP3 inflammasome[36], thereby further increasing barrier permeability and enabling peripheral immune effectors or further vesicular traffic into the CNS. This mechanism establishes a bidirectional feedback loop: peripheral immune activation influences central glial responses, which in turn amplify systemic inflammation via cytokine spill-over.

On this basis, we propose the “microbiota-Evs-immune-neuro axis” as a conceptual framework for AD progression: gut microbial vesicles prime peripheral immune responses (shifting Th17/Treg balance), systemic inflammation and vesicle traffic compromise barrier and vascular signalling, and the resultant glial activation

and neuroinflammation accelerate protein-opathy (Aβ and tau) and neurodegeneration. Although direct evidence of mEV-mediated Th17/Treg modulation in AD remains nascent, the convergence of vesicle biology, immune-axis dysregulation and neurodegenerative pathology supports this integrative model.

EXPERIMENTAL EVIDENCE LINKING mEVs TO ALZHEIMER'S DISEASE

Evidence from animal models

Animal studies have provided important preliminary insights into how mEVs may influence cognitive function and neuropathological changes under controlled experimental conditions. In one investigation, systemic administration of OMVs derived from *Escherichia coli* in mice led to measurable cognitive impairment accompanied by increased hippocampal amyloid deposition and enhanced microglial activation[37]. These findings suggest that bacterial EVs can cross physiological barriers and engage neuroinflammatory mechanisms. These observations indicate that bacterial vesicles are capable of crossing physiological barriers and initiating neuroinflammatory cascades within the central nervous system.

Similarly, mEVs derived from dysbiotic microbiota have been shown to induce pro-inflammatory phenotypes in glial cells when applied to murine brain slices or following intracerebroventricular injection. In germ-free mouse models, colonization with bacterial strains that secrete vesicles enriched in LPS or miRNA-like molecules has been associated with elevated expression of cytokines such as IL-6 and TNF-α in both peripheral and central tissues[38]. These results collectively suggest that mEVs may act as mediators linking microbial imbalance to immune dysregulation, neuroinflammation, and pathological protein aggregation characteristic of AD.

Despite these advances, several limitations should be recognized when interpreting data from animal models. Many studies employ supraphysiological doses of vesicles that likely exceed natural exposure levels, thereby limiting biological relevance. Furthermore, considerable variability in vesicle purification methods and

source materials introduces uncertainty regarding purity and reproducibility. Behavioral assays used to assess cognitive outcomes in rodents also have inherent constraints and may not adequately capture the multifactorial and progressive nature of human AD. Taken together, current evidence from animal models should therefore be viewed as indicative rather than conclusive, providing a foundation for more physiologically relevant investigations in the future.

Preliminary findings in human studies

In humans, evidence implicating mEVs in AD remains at an exploratory stage, largely due to methodological difficulties in isolating and accurately characterizing bacterial vesicles from complex clinical samples. Nevertheless, several cross-sectional investigations have reported distinct alterations in fecal EV profiles between individuals with AD and cognitively healthy controls, including changes in lipid composition, inflammatory activity, and RNA cargo content[39, 40].

In addition, specific microbial miRNA fragments detected in plasma-derived EVs have been shown to correlate with systemic inflammatory markers and cognitive performance scores, although these associations do not yet establish causality [22]. A small-scale clinical study further observed that AD patients exhibited elevated circulating EVs expressing bacterial markers such as LPS-binding protein (LBP), a finding that may reflect increased gut permeability or enhanced microbial translocation across mucosal barriers[41].

Despite these promising observations, several factors continue to limit interpretation. Human studies generally involve small cohorts and cross-sectional designs, which restrict statistical power and preclude causal inference. Moreover, confounding variables, including age, diet, medication exposure, and comorbid conditions, are often insufficiently controlled, potentially obscuring true biological associations. Methodological inconsistency in vesicle isolation and profiling also remains a major concern; techniques such as ultracentrifugation, immunoaffinity capture, and polymer-based precipitation produce variable yield and purity, complicating reproducibility and inter-study comparison. These limitations are

summarized in Table 2, which outlines representative findings from animal and human investigations alongside their respective methodological constraints.

Taken together, current human data provide only preliminary yet biologically meaningful indications that mEVs may participate in AD pathophysiology. Although definitive mechanistic evidence is lacking, the convergence of animal and human observations supports the plausibility of mEVs acting as both mediators and biomarkers of neurodegenerative processes, underscoring the importance of longitudinal and interventional studies to clarify their causal relevance in disease progression.

DIAGNOSTIC AND THERAPEUTIC POTENTIAL OF mEVs IN ALZHEIMER'S DISEASE

As AD research increasingly adopts systems-level approaches, mEVs have emerged as important mediators of communication between the gut and brain. Their ability to circulate systemically, interact with immune and endothelial pathways, and be readily detected in accessible biofluids makes them promising candidates for early diagnosis, therapeutic modulation, and engineered delivery in AD.

Biomarker potential

mEVs hold strong potential as non-invasive biomarkers. Their lipid bilayer encapsulation protects diverse molecular cargo, such as microbial RNAs, lipids, and surface proteins, ensuring stability under physiological conditions[42], and they can be recovered from plasma, feces, and cerebrospinal fluid[43]. In practice, plasma and fecal sampling are the most scalable for serial testing. Methodological progress has improved analytical specificity: for example, selective recovery of LPS-positive bacterial EVs from feces and plasma has been demonstrated using orthogonal characterization (nanoparticle tracking analysis, immunogold TEM, flow cytometry, super-resolution microscopy, and 16S profiling), offering a route to enrich microbiota-derived signals from mixed vesicle pools[44].

Emerging clinical and translational evidence supports the diagnostic utility of extracellular vesicle (EV) profiling in AD. A 2024 study of plasma EV-derived mRNA in 82 subjects (healthy controls, mild cognitive impairment, and AD) reported a diagnostic model with an area under the ROC curve exceeding 0.98 [45]. Although this analysis evaluated total plasma EVs, rather than microbiota-derived vesicles specifically, it provides an important methodological blueprint that can be adapted for microbiota-EV-focused assays. Recent workflow reviews further confirm that EVs are accessible biomarker vehicles in neurodegenerative disease and outline vital steps, from pre-analytical sample handling to library preparation and data integration, which are directly applicable to mEV investigations[46, 47].

Recent mechanistic studies have strengthened the link between bacterial vesicle biology and AD-related pathology. In vivo work has shown that gut-derived bacterial EVs (bEVs) enriched in LPS can cross the BBB, activate microglia, and trigger complement and mechanosensory pathways that contribute to synaptic injury and neuronal loss [48]. These findings provide a biological rationale for detecting disease-associated mEV signatures in peripheral fluids such as blood or stool. Similar advances in biosensing and microfluidic technologies suggest that EV-based point-of-care diagnostic systems are now technically feasible. Recent work using surface-plasmon resonance and nanoelectronic biosensors has achieved sensitive detection of AD-related EV cargo, supporting the potential for similar approaches to be adapted for microbiota-derived vesicle targets once capture ligands and antibody panels are standardized [49].

Comprehensive analyses of microbiota-derived vesicles continue to refine our understanding of cargo composition and host interaction patterns, highlighting the diversity of outer-membrane ligands, lipids, and small RNAs that mediate immune and neuroinflammatory signaling [25]. These studies provide important guidance for selecting candidate molecular markers in future mEV-based assays.

However, a major practical challenge in translating these findings lies in accurately distinguishing bacterial vesicles from the far more abundant host-derived EVs present in human biofluids. Since their size and density distributions overlap,

reliable separation requires targeted enrichment for bacterial markers such as LPS or outer-membrane proteins, combined with proteomic or genomic confirmation[50]. In addition, the lack of harmonized omics workflows, from sample collection and RNA extraction to normalization and data integration, introduces substantial variability across laboratories[51]. Thus, establishing standardized reference materials and cross-platform validation schemes could be essential to ensure reproducibility and comparability of future biomarker data.

Although recent advances have expanded the understanding of microbiota-derived vesicles, several practical challenges continue to limit their clinical translation. Isolation methods remain inconsistent, with techniques such as ultracentrifugation, size-exclusion chromatography, and immunoaffinity capture yielding variable recovery and purity across laboratories. The molecular composition of mEVs is also highly context-dependent, influenced by dietary factors, medications, microbial diversity, and circadian fluctuations, which complicates the definition of stable disease signatures. Diagnostic specificity poses an additional obstacle, as certain RNA and lipid profiles overlap with those seen in other chronic inflammatory or metabolic disorders. Overcoming these barriers will require harmonized pre-analytical workflows, enrichment approaches capable of distinguishing bacterial vesicles from host-derived counterparts, such as LPS-directed immunocapture, and the integration of multi-omics datasets to enhance analytical reproducibility. Once these elements are standardized, mEV-based biomarkers could progress from exploratory findings to clinically validated diagnostic tools, complementing the broader extracellular-vesicle platforms already being developed for neurodegenerative disease research.

Therapeutic targeting strategies

mEVs influence AD pathology through their secretion, systemic transport, and interaction with host immune and neural pathways, each representing a potential therapeutic target.

From the microbial standpoint, modulating gut composition to reduce the release of pro-inflammatory vesicles has shown promising results. Certain bacterial strains, particularly *Bacteroides fragilis* and *Escherichia coli*, are prone to release LPS-enriched vesicles that intensify systemic inflammation and microglial activation[28]. A recent study demonstrated that gut-derived bacterial vesicles carrying LPS could cross the BBB, activate microglia, and engage complement signalling in AD models[48]. Thus, modulating gut microbial communities, such as enhancing probiotic strains or increasing dietary fibre to shift vesicle content toward anti-inflammatory profiles, is therefore a plausible strategy.

Another approach is to limit the systemic dissemination of mEVs by strengthening the intestinal barrier or BBB. A microfluidic platform study found that microbial-derived exosomes and metabolites influence neuron growth and synaptic plasticity in GBA chip systems, suggesting barrier integrity matters for vesicle entry into the CNS [52]. Nutritional or hormonal interventions that reinforce epithelial or endothelial junctions may help reduce vesicle load in circulation, though direct human evidence is still lacking.

At the host level, interfering with vesicle-host receptor interactions offers another therapeutic angle. Blocking pattern-recognition receptors (such as TLR4 or NOD2) that detect bacterial vesicle cargo may suppress downstream immune activation[53]. For example, a recent study on probiotic-derived EVs highlights how vesicle cargo modulates immune signalling and how engineered vesicles may bypass inflammatory triggers [54]. The concept of "functional decoupling," in which commensal microbial signals remain beneficial but pro-inflammatory vesicle-driven pathways are attenuated, is intriguing though technically difficult.

Despite these encouraging directions, most strategies remain preclinical. One major limitation is the lack of clinical trials specifically targeting mEV pathways or vesicle-based therapies in AD. Additional barriers include incomplete understanding of which vesicle subtypes are pathogenic, variability in vesicle isolation, and host-specific response differences. Future progress will depend on integrating microbiome taxonomy, detailed vesicle cargo profiling, and host immune

phenotyping into unified experimental and clinical frameworks. This integrative strategy may eventually enable rational design of vesicle-targeted or vesicle-based therapies for AD.

Engineered vesicles and probiotic-derived applications

Beyond efforts to modulate endogenous mEVs, researchers are exploring two complementary therapeutic directions: engineered vesicles and probiotic-derived vesicles.

Engineered or synthetic EVs are being developed as precision carriers of small RNAs, anti-inflammatory compounds, or neuroprotective peptides to specific tissues such as the intestinal mucosa or central nervous system [55]. Their lipid bilayer composition enables both stability and controlled release, while surface engineering improves tissue tropism and receptor-specific targeting. Liang et al. demonstrated that engineered exosomes modified with neuron-targeting ligands and loaded with siRNAs against tau effectively reduced hyperphosphorylated tau and neuroinflammatory cytokines in AD mouse models [56].

Similarly, synthetic EV-mimetic nanoparticles encapsulating AMPK activators have shown potential to restore mitochondrial metabolism and reduce β-amyloid aggregation in preclinical systems [57]. These findings highlight the feasibility of using EV scaffolds for targeted, biocompatible neurotherapies. Parallel work has explored vesicles naturally secreted by beneficial probiotic strains. Lactobacillus rhamnosus- and Bifidobacterium longum-derived vesicles attenuate TNF-α, IL-1β, and IL-6 release while upregulating IL-10, thus dampening microglial activation and supporting intestinal-brain homeostasis [58]. Their safety profile and ability to integrate with microbiome modulation strategies make probiotic-derived vesicles appealing for AD-related inflammation control [59]. Zhang et al. further emphasized their postbiotic potential, describing how bacterial vesicle lipids and RNA cargo can recalibrate host immune tone without systemic toxicity [54].

A newer concept, that is in vivo vesicle induction, suggests stimulating the host microbiota to generate beneficial mEVs through targeted diets or microbial consortia.

For instance, fermentable fibers and specific prebiotics enhance the release of anti-inflammatory vesicles enriched in short-chain fatty acid pathways, indirectly mitigating neuroinflammation. Verbunt et al. described this as a controllable "endogenous vesicle bioreactor" strategy that may bypass production complexity and minimize immune rejection [60]. Collectively, these approaches, from engineered EV carriers to probiotic and diet-induced vesicles, demonstrate the expanding translational landscape of mEVs in AD. They offer integrated opportunities for biomarker development, therapeutic modulation, and precision vesicle-based delivery. A structured overview of representative strategies, experimental evidence, and their comparative advantages and limitations is summarized in Table 3.

LIMITATIONS AND FUTURE DIRECTIONS

While the translational potential of gut microbiota-derived mEVs in AD is increasingly recognized, substantial limitations remain. Addressing these challenges is critical to move the field from preclinical promise toward clinical applicability.

Current limitations

Most mechanistic understanding of mEVs in AD has been obtained from cellular and animal studies. Although these models provide valuable insight into potential signaling pathways, they cannot replicate the full physiological and environmental complexity of the human GBA. Moreover, the composition of mEV cargo is highly variable and influenced by microbial species, host immune status, diet, and other external factors, making it difficult to establish consistent disease-specific patterns.

Evidence from human studies remains preliminary and largely correlative. Existing investigations generally involve small cohorts and cross-sectional designs, with limited adjustment for confounding variables such as age, medication, and comorbidities. As a result, it is still unclear whether the observed changes in mEV profiles act as drivers, consequences, or epiphenomena of Alzheimer's pathology.

Technical and methodological challenges continue to hinder progress. Reliable differentiation between bacterial and host-derived vesicles remains difficult, and

isolation and characterization procedures vary considerably among laboratories. These inconsistencies compromise reproducibility and restrict meaningful comparison across studies. In addition, many preclinical experiments employ supraphysiological vesicle doses or lack standardized reporting of experimental controls, further complicating interpretation and translation to human relevance.

Lastly, translational and regulatory barriers persist. Current frameworks governing therapeutic applications of microbial vesicles remain underdeveloped, and good-manufacturing-practice standards for large-scale, stable, and safe production remain to be established. Ethical questions related to microbiome manipulation and engineered vesicle therapy also require careful evaluation. Collectively, these limitations indicate that while mEVs represent a promising link between the gut microbiome and neurodegeneration, their causal role in AD remains uncertain. Future research should focus on improving methodological consistency, expanding well-controlled human cohorts, and establishing standardized analytical pipelines before therapeutic translation can be responsibly pursued.

Future research directions

To advance understanding of mEVs in AD, several research priorities should be performed across technical, mechanistic, and translational levels.

In the short term, improving technical consistency and methodological rigor is essential. Standardizing vesicle isolation and characterization procedures, and integrating multi-omics approaches such as metagenomics, transcriptomics, proteomics, and lipidomics, will enhance data comparability and biological interpretation. The use of advanced in vitro GBA models, including organ-on-chip systems, may complement animal studies by providing controlled environments for investigating vesicle transport and host-cell interactions.

In the medium term, strengthening human-based evidence should be a key objective. Well-designed longitudinal cohort studies focusing on at-risk populations, such as individuals with mild cognitive impairment, are needed to determine whether mEV signatures can predict cognitive decline or disease progression. Mechanistic

studies that incorporate host genetic background, including APOE4 and TREM2 variants, will help clarify how genetic susceptibility modifies vesicle-mediated signaling. In parallel, research into vesicle engineering, through microbial strain modification or synthetic vesicle platforms, should continue to develop more precise tools for mechanistic exploration and potential therapeutic applications.

Recent advances in analytic technology are helping to clarify how mEVs contribute to AD. The use of spatial transcriptomics has begun to reveal region-specific inflammatory and metabolic changes in the Alzheimer's brain that could be linked to vesicle-mediated signaling[61]. Studies using single-vesicle multi-omics have also shown that individual vesicles in human plasma and cerebrospinal fluid carry distinct RNA and protein signatures associated with disease stage, supporting their potential as precise biomarkers[62]. Therefore, the integration of these spatial and single-particle datasets with microbiome-derived information may allow mapping of gut-to-brain communication at cellular resolution. Moreover, the concept of strain-specific vesicle profiling, in which vesicle composition is matched to a person’s gut microbial strains and genetic background, is emerging as a foundation for personalized risk assessment and therapy design. Thus, establishing standardized pipelines that combine these methods across human cohorts would be an important next step toward reproducible, individualized mEV-based diagnostics and treatment strategies.

In the long term, clinical translation will depend on establishing regulatory standards, scalable production systems, and comprehensive safety evaluation. Development of GMP-compliant manufacturing protocols, reproducible efficacy testing, and thorough immunogenicity assessments will be essential for advancing vesicle-based strategies toward clinical testing. Incorporating bioethical oversight into future trial design will ensure that emerging interventions are evaluated not only for effectiveness but also for safety and ethical acceptability. Collectively, these steps could help transform current experimental insights on mEVs from exploratory findings into reliable, clinically relevant strategies for AD prevention and management.

CONCLUSION

Gut mEVs are emerging as key mediators linking the GBA to AD. By crossing biological barriers and carrying immune-active cargo, they provide a mechanistic link between gut dysbiosis, immune modulation, and central nervous system dysfunction. Current evidence, though still limited, supports two main research priorities. The first is to clarify the immunomodulatory role of mEVs in AD, particularly their involvement in peripheral-central immune communication. The second is to move from mechanistic studies toward translational applications, including their use as biomarkers and potential therapeutic tools. Thus, mEVs offer a new perspective on the systemic nature of AD and may serve as both indicators and modulators of disease processes. Continued efforts to integrate basic research with clinical studies will be essential to realize their potential in diagnosis and treatment.

Conflicts of interest: Authors declare no conflicts of interest.

Funding: This work was supported by the Center for Cognitive Science and Transdisciplinary Studies, Jiujiang University [grant number :RZYB202202, RZYB202206, RZYB202207], Health Commission of Jiangxi Province [grant number: 202311171], Jiujiang base and talent plan - high level scientific and technological innovation talent project [grant number: S2020QNZZ011], Science and Technology Program of Jiangxi Provincial Health Commission[grant number:202311158], Science and Technology Plan Project of Jiangxi Provincial Health Commission [grant number:SKJP220225877], Basic Research Program of Jiujiang Science and Technology Bureau [grant number: S2022XCTD081].

Data availability: The data used to support the findings of this study are available from the corresponding author upon request.

Submitted: September 3, 2025

Accepted: November 22, 2025

Published online: December 3, 2025

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TABLES AND FIGURES WITH LEGENDS

Table 1. Representative cargo categories of gut microbiota-derived mEVs and their biological significance

Cargo Category	Representative Molecules / Source	Putative Biological Functions
Proteins	OmpA, lysozymes, lipoproteins	Modulate epithelial barrier integrity; participate in microbial recognition
Lipids	LPS, phospholipids, lipopeptides	Trigger host immune alertness; stabilize vesicle structure
Nucleic acids	miRNA-like fragments (e.g., from E. coli, Bacteroides)	Interfere with host gene expression; may influence inflammatory gene programs
	sRNAs, tRNA-derived fragments	Internalized by host cells; regulatory functions under investigation
Metabolites	Short-chain fatty acids (SCFAs: butyrate, propionate), secondary bile acids, indole derivatives	Regulate metabolic tone; modulate immune homeostasis; affect neuroactive signaling pathways

Abbreviations: mEVs: Microbiota-derived extracellular vesicles; OmpA: Outer membrane protein A; LPS: Lipopolysaccharide; miRNA: MicroRNA; sRNAs: Small RNAs; tRNA: Transfer RNA; SCFAs: Short-chain fatty acids.

Table 2. Summary of experimental evidence linking gut microbiota-derived mEVs to Alzheimer's disease

Study Type	Model / Sample	Key Observations	Limitations	References
Animal (in vivo)	Mice injected with *E. coli*-OMVs	Cognitive deficits; ↑ hippocampal Aβ; microglial activation	High-dose exposure; species-specific effects	Wei et al., 2025; Mol Neurobiol.
Animal (ex vivo / in vitro)	Murine brain slices; glial cultures	Pro-inflammatory activation induced by dysbiotic mEVs	May not replicate in vivo physiology	Choi et al., 2022; J Alzheimer's Dis. Yang et al., 2024; BMC Microbiol.
Human (fecal EVs)	Cross-sectional cohorts (AD vs controls)	Altered lipid and RNA cargo; ↑ inflammatory potential	Small sample sizes; cross-sectional; confounders	Lin et al., 2025; Front Neurosci.
Human (plasma EVs)	AD patients vs controls	↑ LPS-binding protein–positive EVs; microbial RNAs linked to cognition	Correlative only; EV origin hard to verify	Li et al., 2024; Nature Aging

↑: Increased. Abbreviations: AD: Alzheimer's disease; Aβ: Amyloid-β; OMVs: Outer membrane vesicles; mEVs: Microbiota-derived extracellular vesicles; EVs: Extracellular vesicles; LPS: Lipopolysaccharide.

Table 3. Diagnostic and therapeutic potential of gut microbiota-derived mEVs in Alzheimer's disease: Current status, advantages, and limitations

Application Area	Strategy / Approach	Representative Evidence	Advantages	Limitations / Challenges	Maturity (TRL / Stage)	References
Diagnostics	Plasma/fecal/CSF mEV profiling (miRNAs, lipids, proteins)	Altered mEV profiles in AD vs controls; microbial RNAs linked to cognitive decline	Non-invasive; stable cargo; accessible in multiple biofluids	Heterogeneous isolation methods; small sample sizes; confounders (age, diet, comorbidities); low specificity	TRL 3–4 (Exploratory clinical association study)	Lin et al., 2025; Front Neurosci.
Therapeutics: Microbial targeting	Modulate gut microbiota composition (reduce pro-inflammatory	Animal studies showing reduced LPS-rich mEVs improve	Shifts vesicle profile; leverages microbiome	Strain-specific effects; lack of causal validation	TRL 3–4 (preclinical, in vivo proof-of-concept)	Li et al., 2024; Nature Aging.

	strains)	inflammation	modulation			
Therapeutics: Barrier function	Enhance intestinal barrier and BBB integrity (prebiotics, nutrition, hormonal modulation)	Animal models show improved barrier reduces systemic inflammation	Indirectly limits mEV trafficking; feasible in lifestyle interventions	Effects on EV trafficking not yet directly proven; variable in humans	TRL 2–3 (animal studies)	Wang et al., 2025; Front Pharmaco.
Therapeutics: Host receptor blocking	Inhibit TLR4/NOD2 signaling ("functional decoupling")	In vitro evidence of dampened inflammatory response to mEVs	Selective targeting of inflammatory pathways	Technical complexity; systemic immune side effects possible	TRL 4–5 (preclinical validation)	Chen et al., 2024; Cell Rep Med.
Engineered vesicles	Synthetic/engineered EVs delivering siRNAs, peptides	Preclinical models (Parkinson's, MS) show therapeutic benefit	High specificity; customizable cargo	Early-stage; safety and immunogenicity concerns	TRL 2–3 (concept / in vitro)	Zhao et al., 2024; Adv Sci.
Probiotic	EVs from	In vivo	Biocom	Limited	TRL 3–4	Verbunt et

-derived vesicles	*Lactobacillus*, *Bifidobacterium*	suppression of pro-inflammatory cytokines; cognitive benefit in AD-like models	patible; safe history of probiotic use	human validation; production scale-up issues	(Preclinical animal validation)	al., 2025; Microbiome Res Rep.
In vivo induction	Stimulate host-compatible strains to produce beneficial EVs via diet/microbial interventions	Conceptual; no direct AD-specific validation yet	Potentially safer, self-sustaining therapeutic effect	Highly speculative; mechanism not defined	TRL 1–2 (Conceptual / hypothesis-generating)	Zhang et al., 2025; J Nanobiotechnology.

Abbreviations: AD: Alzheimer's disease; BBB: Blood–brain barrier; CSF: Cerebrospinal fluid; EVs: Extracellular vesicles; mEVs: Microbiota-derived extracellular vesicles; LPS: Lipopolysaccharide; miRNAs: MicroRNAs; siRNAs: Small interfering RNAs; MS: Multiple sclerosis; TLR4: Toll-like receptor 4; NOD2: Nucleotide-binding oligomerization domain-containing protein 2; TRL: Technology readiness level.

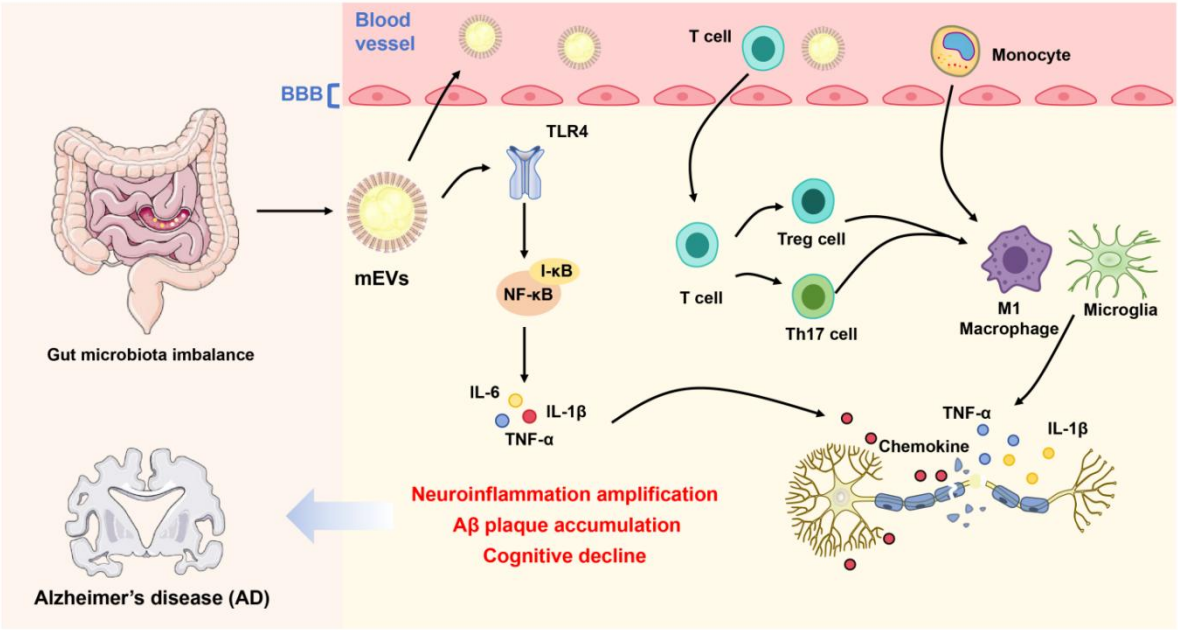

Figure 1. The immunomodulatory mechanism of gut mEVs in AD. An imbalance in gut microbiota leads to an increase in circulating mEVs, which traverse the intestinal and blood-brain barriers. This process activates TLR4-NF-κB signaling, resulting in the secretion of pro-inflammatory cytokines, including IL-6, IL-1β, and TNF-α. These inflammatory responses disrupt T-cell homeostasis (Th17/Treg), promote the activation of microglia and M1 macrophages, exacerbate neuroinflammation, and contribute to the accumulation of Aβ plaques and subsequent cognitive decline. Abbreviations: AD: Alzheimer's disease; mEVs: Microbiota-derived extracellular vesicles; TLR4: Toll-like receptor 4; NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells; TNF-α: Tumor necrosis factor alpha; Th17: T-helper 17 cell; Treg: Regulatory T cell; Aβ: Amyloid-beta.