**A Systematic Review of Secretome-Based Therapies for Alzheimer's Disease: Bridging the Preclinical and Clinical Gap**

**Table S1.** Characteristics of Animal Studies

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No.** | **Types of Intervention** | **Author** | **Year** | **Animal model** | **Age/ BW** | **Sex** | **Sample size** | **Exposure**  | **Grouping** |
| 1 | Secretome | Kim *et al.* | 2018 | 5XFAD mice | 6 mo | NR | 9 | - | 1 = CTRL: MEMα-administrated 5XFAD2 = MSC: hUCB-MSC-administered 5XFAD |
| 2 | Santamaria *et al.* | 2021 | WT & APP/PS1 mice | 12 & 22 mo | male | 129 | - | 1 = 12-month-old APP/PS1 mice received one IV injection of either PBS, MSC-CS or MSC-UCS2 = 12-month-old APP/PS1 mice and age-matched WT received one IV injection of PBS or MSC-CS3 = 12-two-month-old APP/PS1 mice received one IV injection of MSC-CS4 = 22-month-old APP/PS1 and age-matched WT underwent a repeated IN treatment regimen5 = 22-month-old and 25-month-old APP/PS1 and age-matched WT mice underwent a repeated IN treatment regimen with MSC-CS |
| 3 | Hijroudi *et al.* | 2022 | BALB/c mice | 8 wk | male | 36 | Aβ1-42 was injected into the ICV space using stereotaxic surgery | 1 = Control2 = AD + vehicle3 = AD + NSCs-CM |
| 4 | Mo *et al.* | 2023 | WT & 5XFAD mice | 11 wk | male | 24 (5xFAD mice) | - | 1 = WT group 2 = 5×FAD AD mouse model 3 = CNSC-SE-treated 5×FAD mice 4 = MSC-treated 5×FAD mice  |
| 5 | Exosomes | Ding *et al.* | 2018 | APP/PS1 mice | 7 mo | male | 36 | - | 1 = Control 2 = hucMSC-exosomes |
| 7 | Micci *et al.* | 2019 | C57BL/6 J and Nestin-δ-HSV-TK mice  | 6 - 8 wk | male and female | 20 | NR | 1 = PBS 2 = Exosomes3 = PBS + Aβo4 = Exosomes+Aβo |
| 8 | Cui *et al.* | 2019 | APP/PS1 mice | 7 mo | NR | 39 | - | 1 = PBS (AD)2 = exosomes derived from MSCs (MSC-Exo) 3 = RVG-conjugated MSC-Exo (MSC-RVG-Exo) |
| 9 | Reza-Zaldivar *et al.* | 2019 | C57BL/6 mice | 7 - 8 wk | NR | 48 | Aβ aggregates (Aβ1–42) were administered in the dentate gyrus bilaterally in 14 days | 1 = Control2 = AD3 = exosomes4 = MSC |
| 10 | Chen *et al.* | 2021 | WT and J20 mouse model of AD | 9 mo | NR | 24 | - | 1 = WT-PBS2 = WT-Exosomes3 = Tg-PBS4 = Tg-Exosomes |
| 11 | Poltavtsesa *et al.* | 2021 | NMRI mice | 6 mo | male | 23 | - | 1 = Sham operated + Saline2 = Sham operated + Exosomes3 = Olfactory bulbectomized + Saline4 = Olfactory bulbectomized + Exosomes |
| 12 | Zhdanova *et al.* | 2021 | NMRI mice | 6 mo | male | 23 | - | 1 = Sham operated + Saline2 = Sham operated + Exosomes3 = Olfactory bulbectomized + Saline4 = Olfactory bulbectomized + Exosomes |
| 13 | Liu H *et al.* | 2022 | APP/PS1 mice | 2 mo  | male | 40 | - | 1 = PBS (AD)2 = exosomes derived from ADSCs (Exo)3 = hypoxia-pretreated ADSCs (HExo)4 = circ-Epc1- expressing ADSCs (circ-Epc1-Exo) |
| 14 | Liu S *et al.* | 2022 | C57BL/6 mice | 4 wk | male | 24 | STZ was injected into the lateral ventricle of mice by the autosampler in the STZ group (dose of 0.3 mg/kg, speed of 0.5 μL/min, the volume of 1 μL/side, and the STZ was dissolved in ACSF and prepared for current use). | 1 = Control2 = model group3 = exosomal lateral ventricle injection (Lv) group4 = exosomal caudal vein injection (Cv) group |
| 15 | Sheykhhasan *et al.* | 2022 | Wistar rats | 250-300 g | N/R | 40 | To induce AD model, dissolution of STZ was performed in 0.9% saline solution. Then, STZ was maintained at − 20◦C before use. STZ (3 mg/kg, twice) was injected ICV using a Hamilton syringe after perforation of the recent site.  | 1 = Control2 = AD3 = coQ104 = Exo5 = Exo+coQ10 |
| 16 | Hou *et al.* | 2023 | WT & 5XFAD mice | female | 4 mo | 24 | - | 1 = 5 ×FAD group2 = MSCs-exo group3 = co-housed group4 = MSCs-exo + Abx group |
| 17 | Pourhadi *et al.* | 2023 | Wistar rats | 250-300 g | male | 8-10/group | STZ or normal saline was injected directly into the intracerebral ventricle (ICV) using a 10 μl Hamilton syringe (gauge 30) with the polyethylene tube (AP − 0.8, ML 1.5, DV − 3.5). STZ (3 mg/kg) or the vehicle was administered at a rate of 1 μl/min. | 1 = Sham (PBS), 2 = STZ3 = STZ+Exosomes 0.74 = STZ+Exosomes 75 = STZ+Exosomes 70  |
| 18 | Li *et al.* | 2024 | WT and SKO-AD mice | 9 mo/25-25 g | male | 75 | - | WT group, SKO-AD-Veh group, AD-Veh group, SKO-AD-ex group, and AD-ex group |
| 19 | Mico-vesicles | Elia *et al.* | 2019 | APP/PS1 mice | 3 mo | male | 11 | - | 1 = Control2 = MSC-EVs |
| 20 | Losurdo *et al.* | 2020 | triple-transgenic AD mice | 7 mo | female | 8 | - | 1 = Control 2 = MSC-EVs |
| 21 | Cone *et al.* | 2021 | 5XFAD and C57BL/6J mice | 6 wk | male and female | 56 | - | 1 = NT - Saline2 = NT - EV3 = AD - Saline4 = AD = EV |
| 22 | Zhdanova *et al*. | 2022 | NMRI mice | 6 mo | male | 6 | - | 1 = Control 2 = cytochalasin B–induced membrane vesicles (CIMVs) of MSCs |

**Table S2.** In vivo study results

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| No. | Author | Year | Outcome Measures | Results | Mechanism of action |
| 1 | Kim *et al.* | 2018 | Synaptic Density Markers, TSP-1 Secretion | * Significant increase in synaptic markers SYP and PSD-95 throughout the brain.
* hUCB-MSCs can rescue synaptic density loss induced by Aβ42 peptide in vivo
 | * The protective effect of hUCB-MSCs against synaptic dysfunction (mediated by TSP-1)
* hUCB-MSCs increases the expression of NLGN1 and α2δ-1
 |
| 2 | Santamaria *et al.* | 2021 | NORT, Amyloid Plaques, Microglial Activation, Astrogliosis, Cytokine Levels | * Memory recovery in 12-month-old APP/PS1 mice was observed 7 days post a single IV injection of MSC-CS, but the improvement was not sustained beyond 14 days.
* A 30% reduction in hippocampal and cortical amyloid plaques was noted following the same treatment (decreased amyloidosis).
* Microglial activation was significantly reduced, evidenced by decreased IBA1 positivity and CD68-marked area
* The treatment did not affect astrogliosis.
* Levels of cytokines IL-1β and TNF were not significantly altered
 | * MSC-CS mimics the neuroreparative effects of MSCs through paracrine action, releasing bioactive components in response to the AD brain environment.
* Decrease in amyloidosis
* Reduce neuroinflammation through decreasing microglial activation
 |
| 3 | Hijroudi *et al.* | 2022 | Passive Avoidance Test and MWM test, RT-PCR, ELISA, Western blot, Double-IF Staining (BrdU/Nestin and BrdU/NeuN co-expressing cells), Nissl staining  | * Improved memory retention in AD mice treated with NSCs-CM, by increased step-through latency compared to untreated group.
* Reduced escape latency and increased time spent in the target quadrant for AD mice treated with NSCs-CM
* There was a significant increase in the expression levels of PI3K, Akt, MAPK, ERK, Wnt3a, β-Catenin, and GSK3β genes in NSCs-CM group compared to untreated AD mice
* NSCs-CM increased levels of BDNF and NGF
* Reduction in Aβ plaque formation in the brains of AD mice treated with NSCs-CM
* Increased cells co-expressing BrdU/Nestin and BrdU/NeuN in NSCs-CM group.
* Decreased neurotoxicity and cell death in the hippocampus of NSCs-CM group.
 | * NSCs ability to form neurospheres and express the stem cell marker nestin
* NSCs-CM modulated the Wnt/β-catenin signaling pathway (neuroprotection and neurogenesis)
* Support neuronal survival and function based on levels of BDNF and NGF
* Reduce Aβ plaque formation
* Improved neural tissue integrity
 |
| 4 | Mo *et al.* | 2023 | Neural markers (NEUN, vGLUT, and MAP2), growth factors (BDNF, GDNF, and VEGF), IF staining, Multielectrode array recording, RT-PCR,Western blot | * Intranasal delivery of iPSC-derived CNSC-SE improved spatial memory and cognitive impairments in 5xFAD mice.
* CNSC-SE-treated group showed significant improvement in behavioral performance in the Barnes maze, with increased time in the target zone and reduced error rate compared to AD group.
* CNSC-SE treatment resulted in a similar pattern of movement to WT mice, with fewer erroneous entries.
* Significant decrease in APP in CNSC-SE-5xFAD mouse brains compared to the AD group.
 | * iPSC-derived CNSC-SE promoted cortical neuron differentiation in vitro
* CNSC-SE increased neuronal network activity and action potential bursts
* CNSC-SE reduced amyloidosis and neuro-inflammatory proteins in 5xFAD mouse brain (anti-amyloid and anti-inflammatory effects)
 |
| 5 | Ding *et al.* | 2018 | Behavior Test Modified MWM test, IF Staining, Quantitative RT-PCR, ELISA, Western Blot | * Mice treated with hucMSC-exosomes showed improved performance in the MWM test with shorter mean escape latency compared to control.
* Reduced microglial activation and increased alternative activation in the hucMSC-exosome group.
* Decreased Aβ plaques in the cortex and hippocampus of treated mice
 | * Change microglial activation states and reduced inflammation
* Decrease in Aβ40 and Aβ42 levels implicated in plaque formation and AD pathology
* The presence of exosome markers CD63 and CD9 indicated successful isolation and potential delivery of therapeutic contents
 |
| 6 | Micci *et al.* | 2019 | Electrophysiological Assessments, NORT, Synaptosomes Preparation | * Significant percentage changes from the initial average baseline fEPSP slope
* Mice study demonstrated the ability to discriminate between familiar and novel objects
* Significant reduction in Aβ oligomer binding to hippocampal synaptosomes treated with NSC-exo compared to those treated with PBS or MN-exo
 | * Synaptic plasticity indicates changes in synaptic strength following the conditioning stimulus
* Protective effect of NSC-exo against Aβ oligomer-induced synaptic vulnerability
 |
| 7 | Cui *et al.* | 2019 | MWM test, Thioflavin-S staining, ELISA, IF staining  | * MSC-RVG-Exo treatment reducted amyloid plaque deposition in both cortex and hippocampus compared to MSC-Exo treatment.
* MSC-RVG-Exo treatment resulted in lower concentrations of soluble Aβ40 and Aβ42, and insoluble Aβ40 and Aβ42 in the brain.
* MSC-RVG-Exo treatment significantly attenuated the expression of GFAP (reduced astrocyte activation)
* MSC-RVG-Exo treatment improved spatial learning and memory in APP/PS1 mice
 | * Exosomes derived from MSCs were tagged with RVG peptide to target the CNS.
* The RVG modification enhanced the engraftment of exosomes in the cortex and hippocampus.
* Targeted exosomes facilitated the clearance of Aβ plaques and reduced astrocyte activation (improve cognitive function)
 |
| 8 | Reza-Zaldivar *et al.* | 2019 | MWM test, NORT, IF staining (DCX and PSA-NCAM markers) | * Both exosome and MSC treatments reduced cognitive impairment in AD mouse model
* Exosome treatment stimulated neurogenesis in the SVZ. Similar effects were observed with MSC treatment.
 | * MSCs and Exosomes mediate effects through paracrine activity.
* Exosomes promote neurogenesis and reduce cognitive impairments, which may internalize and degrade Aβ oligomers, secrete antioxidant enzymes, anti-inflammatory cytokines, and neurotrophic factors.
 |
| 9 | Chen *et al.* | 2021 | Glucose Metabolism, NORT, Amyloid Plaque, Astrocyte Activation, Neuronal Memory and Synapse-Related Genes | * MSC-exosomes treatment resulted in a significant increase in [18F] FDG uptake in both the whole brain and specific brain regions
* Significant improvement in long-term recognition memory following MSC-exosomes treatment
* MSC-exosomes regulated the phase of neurons and astrocytes in the brain of AD mice
 | * Decreased the expression of Aβ in a human neural cell culture model with familial AD mutations
* Restored the expression of neuronal memory/ synaptic plasticity-related genes
* Modulated the phase of neurons and astrocytes in the brain
* Exosomal miR-29a upregulated memory/ synaptic plasticity-related genes by HDAC4
 |
| 10 | Zhdanova *et al.* | 2021 | MWM test and localization of exosomes | * Improved performance in the MWM, with animals spending more time and making more visits to the target sector, indicating enhanced spatial memory.
* Labeled exosomes were found in the hippocampus and neocortex 4 hours after intranasal administration, areas crucial for learning and memory and affected by AD.
 | * Exosomes expressed typical markers CD9, CD63, and CD81, which demonstrate high therapeutic efficacy.
* Intranasal administration allows direct delivery to the brain, bypassing the BBB.
* Exosomes facilitate intercellular communication by transferring bioactive compounds to target cells.
 |
| 11 | Poltavtsesa *et al.* | 2021 | Spatial memory and localization of exosomes | * Exosomes prevented spatial memory deterioration in OBE model.
* Significant differences showed in factor detection between control and treated groups.
* Fluorescently labeled exosomes were found in the brain tissue after IV administration
* Exosomes localized in the hippocampus and neocortex.
 | * Penetrate the BBB and reach the hippocampus and temporal cortex (learning and memory)
* The therapeutic effect is likely due to the transfer of proteins, nucleotides, amino and fatty acids, mRNA, and microRNA from exosomes to recipient cells, facilitating intercellular communication.
* Exosomes may exert their effects without the need for immunological compatibility with the recipient tissue, unlike MMSCs.
 |
| 12 | Liu H *et al.* | 2022 | MWM test, RT-PCR, Luciferase reporter assays, IHC, IF, ELISA | * Mice treated with circ-Epc1-containing ADSC exosomes showed reduced escape latency and increased platform crossing numbers in the spatial probe test
* Circ-Epc1-containing ADSC exosomes decreased neuronal damage.
* TUNEL staining in brain tissue sections indicated a decrease in neuronal apoptosis in mice treated with circ-Epc1-containing ADSC exosomes.
* The presence of circ-Epc1 in ADSC exosomes facilitated the shift of microglial polarization from M1 to M2 in the hippocampus
* Decreased IL-1β, IL-6, and TNF-α levels cytokines
 | * High-throughput sequencing identified circEpc1 as a crucial component in hypoxia-pretreated ADSC exosomes for improving cognitive functions.
* Luciferase reporter assays revealed TREM2 and miR-770-3p as downstream targets of circ-Epc1.
* Overexpressing miR-770-3p or downregulating TREM2 reversed the effects of circ-Epc1 on M2 microglial polarization during lipopolysaccharide treatment, indicating their role in the modulation of microglial phenotypic transformations and inflammatory cytokine expressions.
 |
| 13 | Liu S *et al.* | 2022 | OFT, EPM test, NORT, TST, Nissl staining, IF staining, Western blot, RT-PCR | * BMSC-exos improved AD-like behaviors
* Positive correlations were observed between the duration and distance in the center in the OFT and the preference of the novel object in the NOR.
* Reduced glial cell activation, detected new neurons and measured the positive area of Aβ1−42 in the hippocampus.
* Reduced expression of IL-1β, IL-6, TNF-α, Aβ1−42, and p-Tau
* Upregulated protein expression of synapse-related proteins and BDNF
 | * BMSC-exos are associated with the regulation of glial activation and the associated neuroinflammation, as well as BDNF-related neuropathological changes in the hippocampus.
* The reduction in the expression of inflammatory cytokines such as IL-1β, IL-6, and TNF-α, along with the decrease in Aβ1−42 and p-Tau, suggests a reduction of the neuroinflammatory response.
* Potential restoration of synaptic function and promotion of neuronal survival and plasticity.
 |
| 14 | Sheykhhasan *et al.* | 2022 | MWM test, passive avoidance task, Nissl staining, ELISA, IHC  | * CoQ10-loaded exosomes derived from ADSCs-Exo significantly improved memory impairment induced by STZ in rats
* Treatment with CoQ10-loaded ADSCs-Exo led to an increase in BDNF expression in STZ-induced rats, compared to groups treated with CoQ10 or exosomes alone
* CoQ10-loaded ADSCs-Exo group showed the highest cell density and SOX2 gene expression
 | * CoQ10 helps in reducing the expression of pro-inflammatory cytokines and improving mitochondrial function
* Exosomes derived from ADSCs contribute to the therapeutic effects of CoQ10 by facilitate drug delivery to the brain, overcome the BBB, and potentially carry other beneficial molecules.
* CoQ10-loaded ADSCs-Exo treatment not only protects existing neurons but also promotes the growth and differentiation of new neurons and synapses.
 |
| 15 | Hou *et al.* | 2023 | CFC test, MWM test, HE staining, Nissl staining, IHC, ELISA, GFAP staining | * Plaque deposition was reduced in the MSCs-exo group and increased in the co-housed group compared with the 5xFAD mice
* The MSCs-exo + Abx group had lower plaque deposition than the MSCs-exo treated group.
* Aβ1-40 and Aβ1-42 levels in the hippocampus and serum of mice reduced after MSCs-exo treatment compared with the 5xFAD group.
* Co-housing increased Aβ1-40 and Aβ1-42 levels, whereas these levels reduced after Antibiotics treatment compared with MSCs-exo treatment
* AD gut microbiota removed MSCs-exo therapeutic effect.
* Antibiotics improved MSCs-exo efficacy by treating disordered gut microbiota and metabolites.
 | * MSCs-exo treats AD by promoting Aβ degradation, modulating immune responses, protecting neurology.
* Promoting axonal growth, and improving cognitive impairment.
* Gut microbiota dysbiosis may limit MSCs-exo therapy.
* Antibiotics enhance therapy by modulating gut microbiota and metabolites.
 |
| 16 | Pourhadi *et al.* | 2023 | MWM test, Congo Red Staining, IF staining, Western blot, MTT assay, flow cytometry, AO/PI staining | * STZ-induced rats showed learning deficits in the MWM test, which were dose-dependently reduced by Exosomes treatments.
* Amyloid plaque deposition was observed in the hippocampal regions in AD-model rats
* There were changes in neuronal marker expression due to Exosomes treatment.
* Confirmed protein expression alterations in response to treatments.
* Cell viability was improved in Exosomes-treated groups compared to the glutamate group.
* Increased live cell percentages and decreased apoptotic cell percentages in Exosomes groups.
* Improved cell viability in 3D culture conditions with Exosomes treatment.
 | * Improve cell viability and counteract the cytotoxic effects of L-glutamate.
* Reduce amyloid plaque deposition in the hippocampus.
* Influence the expression of neuronal markers (neuroregeneration or synaptic plasticity)
* The dose-dependent effects of EXOs on learning and memory suggest a potential therapeutic action in sporadic AD models
 |
| 17 | Li *et al.* | 2024 | MWM test, immunostaining, whole brain imaging, Fluorescence signal intensity analysis, Western Blot, ELISA | * NSC-derived exosomes led to reduced escape latencies and increased time spent in the target quadrant
* Enhanced expression of mitochondrial biogenesis-related proteins in multiple brain regions
* Increased fluorescence signal intensity for mitochondrial biogenesis-related proteins in selected brain regions
* Increased levels of SIRT1 and mitochondrial biogenesis-related proteins in the brains of mice treated with NSC-derived exosomes
* Significant decrease in the ratio of soluble Aβ42 to Aβ40 was observed in the SKO-AD groups, indicating a slight positive effect on Aβ levels
 | * Increased SIRT1 levels and enhanced the production of mitochondrial biogenesis-related factors.
* Inhibited astrocyte activation but did not suppress amyloid-beta production.
* Activate of the SIRT1-PGC1α signaling pathway and increase synthesis of NRF1 and COXIV (improved mitochondrial biogenesis)
* Restored abnormal protein distribution in the brain, indicating promotion of mitochondrial biogenesis.
 |
| 18 | Elia *et al.* | 2019 | Aβ Plaque and Dystropic Neurites  | * Administering BM-MSC-EVs at 3 and 5 months of age in APP/PS1 mice (before and just as clinical signs start to appear) effectively reduced pathological signatures of AD.
* Quantitative analysis showed a significant reduction in plaque area, solidity, and density, as well as a decrease in dystrophic neurite occurrence.
 | * BM-MSC-EVs carry Neprilysin, a β-amyloid degrading enzyme, suggesting a direct action on Aβ degradation.
* The EVs inherit protective, anti-inflammatory, and neurotrophic properties from their parental BM-MSCs
 |
| 19 | Losurdo *et al.* | 2020 | ELISA, Western Blot, IF staining, Analysis of microglia activation, Golgi-Cox staining, Dendritic spine analysis | * Decrease in microglia activation following the treatment with MSC-derived EVs.
* Intranasal administration of MSC-derived EVs resulted in an increase in dendritic spine density in the hippocampal CA1 pyramidal neuron, entorhinal cortex, and prefrontal cortex neurons of EV-treated mice compared to control mice.
 | * MSC-derived EVs reached the brain, reducing microglia activation and increasing dendritic spine density.
* This suggests a shift of microglia toward an anti-inflammatory phenotype, contributing immunomodulatory and neuroprotective effects
 |
| 20 | Cone *et al.* | 2021 | Cognitive Performance, Aβ plaque, GFAP and Aβ Colocalization, Behavioral Assays, IHC | * 5XFAD mice treated with hMSC-EV showed significantly improved performance in cognitive tests compared to control.
* Reduction of Aβ plaque load was observed in the hippocampus of EV-treated mice compared to control.
* There was less colocalization between GFAP and Aβ plaques in the brains of EV-treated mice compared to control.
* hMSC-EV group performed better in behavioral assays assessing learning and memory than control.
* EV-treated mice showed lower Aβ plaque load and reduced colocalization of GFAP and Aβ plaques in the hippocampus
 | * MSC-derived EVs exhibit similar immunoprotective and immunomodulatory abilities
* EVs play a role in cell-to-cell communication, carrying a diverse molecular payload
* EVs can cross most barriers, including the BBB
 |
| 21 | Zhdanova *et al*. | 2022 | localization of vesicles | * Four hours after intranasal administration, vesicles containing the fluorescent protein RFP were observed in the hippocampus and neocortex of OBX mice
 | * Microvesicles act as nanocontainers for targeted delivery of biologically active compounds or drugs to brain regions affected by neurodegeneration.
* Intranasal delivery provides a non-invasive route to the CNS, bypassing the BBB
 |