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

**Table S1.** Characteristics of Animal Studies

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **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 5XFAD 2 = 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-UCS 2 = 12-month-old APP/PS1 mice and age-matched WT received one IV injection of PBS or MSC-CS 3 = 12-two-month-old APP/PS1 mice received one IV injection of MSC-CS 4 = 22-month-old APP/PS1 and age-matched WT underwent a repeated IN treatment regimen 5 = 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 = Control  2 = AD + vehicle  3 = 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 = Exosomes  3 = PBS + Aβo  4 = 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 = Control  2 = AD  3 = exosomes  4 = MSC |
| 10 | Chen *et al.* | 2021 | WT and J20 mouse model of AD | 9 mo | NR | 24 | - | 1 = WT-PBS  2 = WT-Exosomes  3 = Tg-PBS  4 = Tg-Exosomes |
| 11 | Poltavtsesa *et al.* | 2021 | NMRI mice | 6 mo | male | 23 | - | 1 = Sham operated + Saline 2 = Sham operated + Exosomes 3 = Olfactory bulbectomized + Saline 4 = Olfactory bulbectomized + Exosomes |
| 12 | Zhdanova *et al.* | 2021 | NMRI mice | 6 mo | male | 23 | - | 1 = Sham operated + Saline 2 = Sham operated + Exosomes 3 = Olfactory bulbectomized + Saline 4 = 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 = Control  2 = model group  3 = exosomal lateral ventricle injection (Lv) group  4 = 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 = Control  2 = AD  3 = coQ10  4 = Exo  5 = Exo+coQ10 |
| 16 | Hou *et al.* | 2023 | WT & 5XFAD mice | female | 4 mo | 24 | - | 1 = 5 ×FAD group 2 = MSCs-exo group 3 = co-housed group 4 = 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 = STZ  3 = STZ+Exosomes 0.7  4 = STZ+Exosomes 7  5 = 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 = Control  2 = 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 - Saline 2 = NT - EV 3 = AD - Saline 4 = 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

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| --- | --- | --- | --- | --- | --- |
| 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 |