Nanoparticle Formulations for the Improvement of Symptomatic Treatments of Neurodegenerative Disorders

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Abstract

Neuronanomedicine merges nanotechnology and neuroscience in the pursuit of engineering therapeutic interventions for neurological disorders, including Alzheimer's disease (AD) and Parkinson's disease (PD). While no nanoparticle-based drug delivery systems (NDDSs) are yet approved for use for targeting the central nervous system, this review critically analyses the development of NDDSs for the improvement of currently approved therapeutics for the symptomatic treatment of AD and PD. It showcases how NDDSs can help therapeutic payloads overcome existing limitations, such as insufficient drug accumulation in the brain and limited effectiveness, by enhancing their pharmacokinetics, bioavailability, brain penetration and accumulation, and overall therapeutic efficacy through drug encapsulation, manipulation of nanoparticle properties, and nanoparticle surface functionalisation. However, we also draw attention to widespread issues in the field that impede progress, including the poor selection of *in vitro* models and the inadequate design of pre-clinical *in vivo* studies. We further advocate for greater standardisation of study design and reporting requirements in the future, which would likely enhance outcomes and expedite the translation of neuronanomedicines.

Keywords: neurodegeneration; dementia; Alzheimer's disease; parkinsonism; nanomedicine; nanoparticle-based drug delivery systems; drug delivery

Introduction

Neurodegenerative disorders are characterised by the abnormal misfolding and aggregation of proteins, which leads to progressive degeneration of neurons and a decline in cognitive and/or physical function.[1] Alzheimer's disease (AD) and Parkinson's disease (PD) are the two most common neurodegenerative disorders, with global estimates of up to 57.4 million and 8.5 million cases, respectively.[2-3] The likelihood of developing either AD or PD increases from the age of 65, and, therefore, the prevalence of these diseases is expected to dramatically rise due to an aging population, with estimates predicting AD prevalence of 152.8 million cases by 2050.[3-5] The diagnostic criteria for AD includes progressive decline in cognitive function, such as visuospatial and language abilities, memory loss, and impaired reasoning.[6] In addition, changes in behaviour are observed, including atypical mood shifts and social withdrawal,[6] along with alterations in personality, such as reduced enthusiasm and energy.[7] Conversely, PD is clinically characterised by its cardinal motor features, including bradykinesia (slowness of movement and reaction times), resting tremor, muscle rigidity, and postural instability.[8] Importantly, however, PD also involves a plethora of non-motor features, including cognitive dysfunction, sleep disorders, autonomic dysfunction, and sensory symptoms, such as loss of smell.[9] In addition to health and social impacts, for both patients and caregivers, these diseases also present economic impacts, either directly from the medical costs of admission into nursing homes or hospice care, or indirectly, through a loss of income by either the patient or caregiver's inability to work.[10-11] In 2019, the global financial cost of AD and related dementias was estimated to be USD $2.8 trillion, which is predicted to rise to USD $16.9 trillion by 2050.[12] There does not appear to be a recent evaluation of the global financial cost of PD; however, the yearly cost is estimated to be USD $51.9 billion in the US.[11]

Despite the huge health, social and economic burdens associated with AD and PD, there is a shortage of therapeutic interventions. Concerningly, those that are available suffer from critical limitations. For instance, the majority of drugs developed for neurological diseases do not accumulate within the brain at pharmacologically sufficient concentrations.[13-14] In fact, systemically administered therapeutics typically undergo first pass metabolism and therefore have short half-lives. Further, the blood brain barrier (BBB) is known to prevent 95% of FDA-approved drugs penetrating and accumulating in the brain.[13] They also fail to meaningfully mitigate AD/PD progression or reverse pathology, with most therapeutics only alleviating symptoms. Furthermore, the majority of the approved therapeutics are only effective for short periods of time, and not for all patients.[15-18] Additionally, most current therapies are associated with severe side effects, including fainting and seizures.[14] Therefore, exploration of alternate therapeutic strategies is urgently needed.

Advancements in the nascent field of neuronanomedicine may provide alternative strategies to overcome at least some of these limitations, leading to improved AD/PD patient outcomes.

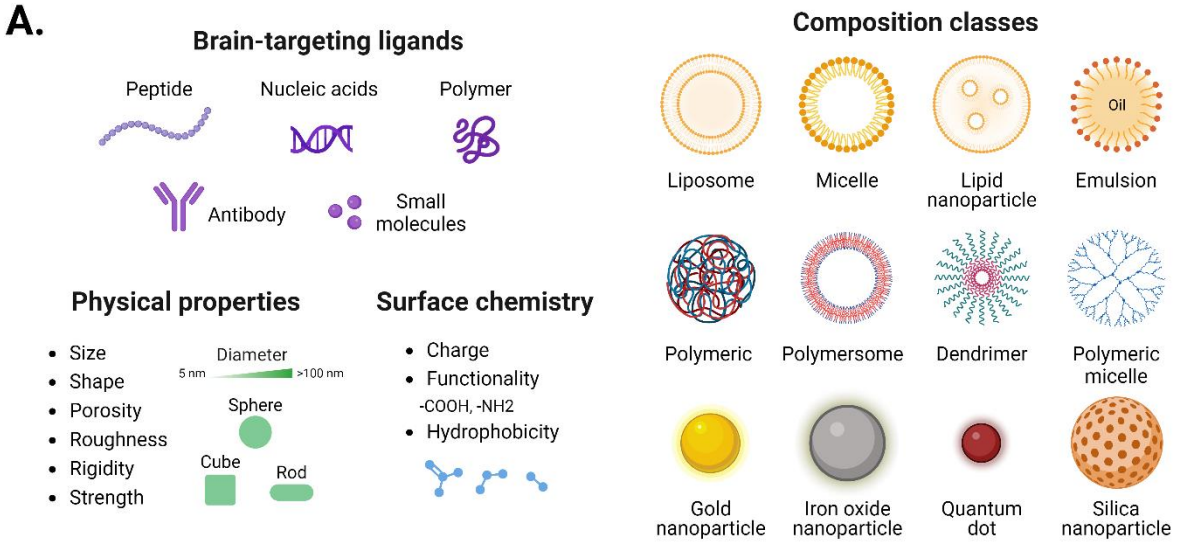
Neuronanomedicine endeavours to develop nanoparticle-based drug delivery systems (NDDSs) that enhance the safety and efficacy of therapeutic agents for neurological disorder treatment.[19] Nanoparticles (NPs) are submicroscopic particles (<100nm in size) with unique physicochemical properties and can be composed of different materials (e.g., polymer, metal, lipid). NPs can be engineered to package therapeutics, improving their stability, and helping them to overcome (or evade) biological and physiological barriers, and targeting them to their intended site-of-action. Encouragingly, a number of these NDDSs have already been approved by the FDA for therapeutic use against a variety of conditions, such as cancer (i.e., cancer nanomedicine).[20]

Importantly, NDDS facilitate the delivery of drugs to the brain. Firstly, they are able to mitigate first pass metabolism, which enhances their drug pharmacokinetic, bioavailability, and biodistribution profiles.[21-22] Moreover, they can mediate BBB crossing. The BBB is a highly selective physiological barrier situated between the parenchyma of the brain and the lumen of central nervous system (CNS) blood vessels, comprised primarily of brain capillary endothelial cells lined by astrocytic end-feet and pericytes in the basal lamina.[23] While gases, water, and small lipid-soluble molecules (<400 Da) are able to diffuse passively across the BBB, larger molecules require transcellular active transport systems.[24] Engineered NDDS have been shown to successfully package and deliver drugs across the BBB into the brain *via* different transportation mechanisms, including receptor-, carrier-, adsorptive-, and cell-mediated transcytosis (**Figure 1B**).[25-26] BBB-penetration is, in part, aided by the physical properties of a NP, such as size, shape and aspect ratio, with rod-shaped NPs showing higher accumulation in the brain endothelium compared to spherical NPs[27-28] and smaller NPs (20-100 nm) displaying better brain permeability than larger NPs, while also avoiding premature renal clearance.[29-30] Additionally, NPs can be modified to enhance their ability to cross the BBB through surface modification, chemical functionalisation or coating with certain surfactants or ligands (**Figure 1A**).[31-33] These engineering flexibilities, combined with their prolonged circulation time and ability to penetrate the BBB, make NPs an ideal candidate for CNS-targeted drug delivery. Targeted drug delivery, as opposed to systemic drug delivery, may overcome current limitations, ameliorating off target side effects, and requiring lower dosages.

Alternate administration routes for NDDSs have also been gaining traction recently, in particular, intranasal (IN) administration. This minimally invasive route delivers NPs directly to the brain, bypassing systemic clearance and issues associated with BBB penetration.[34] Further, transdermal patches containing drug-loaded NPs offer an administration route with several potential benefits, including bypass of first pass hepatic clearance, sustained release, and ability of self-application.[35] Additionally, BBB membrane disruption using ultrasound to facilitate nanoparticle, and thus drug, delivery across the BBB is also showing promising advances.[36]

Despite its potential, neuronanomedicine remains largely underdeveloped, with no approved NDDSs for use in neurodegenerative disorders to date.[37-38] Nevertheless,

an increasing number of studies have investigated the capacity of NDDSs to improve therapeutic strategies for AD/PD. We recently reviewed disease-modifying NDDSs engineered to target underlying AD/PD pathology, including abnormal protein pathologies (e.g., amyloid beta (Aβ) plaques, hyperphosphorylated tau (pTau), and misfolded alpha-synuclein (α-syn)).[39] Therefore, this review will focus on the current therapeutic landscape for AD/PD and associated limitations, before discussing the development of NPs designed to enhance the delivery of FDA-approved drugs for the *symptomatic* treatment of AD/PD that have reached *in vivo* preclinical evaluation in disease models (**Table 1)**.



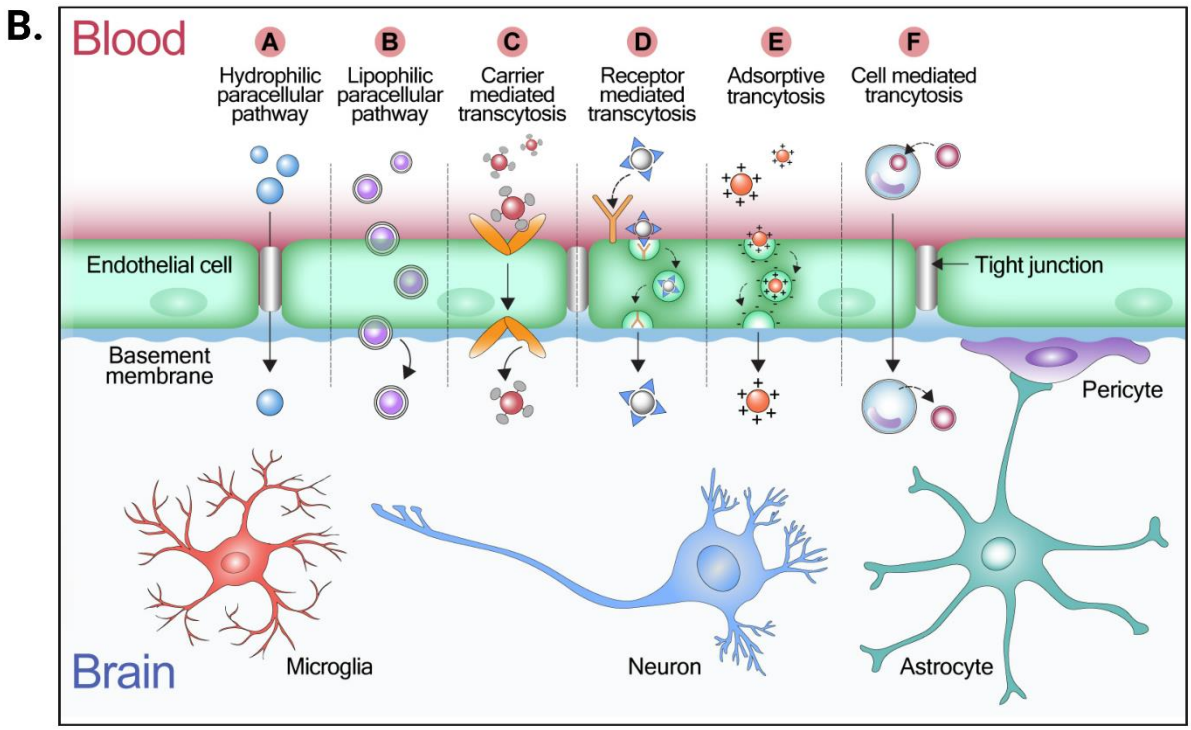


Figure 1: (A) Design and engineering options for nanoparticles in neuronanomedicine development. **(B)** Various mechanisms for nanoparticles to cross the BBB.

Harnessing nanoparticles to enhance current AD therapeutics

The symptoms of AD are largely attributed to the degeneration of cholinergic neurons in the basal forebrain, which can decrease from ~500,000 to less than 100,000 throughout the progression to advanced stages of the disease, with cognitive deficits only detectable when ~30% of cholinergic neurons have already degenerated.[40-41] The loss of cholinergic neurons reduces levels of the neurotransmitter acetylcholine (ACh), disrupting cortical neurotransmission, a process which is critical in the regulation of learning and memory.[40] Evidence also suggests ACh is involved in neurogenesis, and therefore its decreased levels in AD may contribute to cognitive deficits by reducing the formation of new neurons.[42] Similarly, changes in glutamatergic signalling, particularly at the level of the N-methyl-D-aspartate (NMDA) receptor, are also of critical importance to the pathology of AD. Glutamate is an excitatory neurotransmitter that binds with NMDA receptors and enables the signal pathway necessary for maintaining synaptic plasticity and regulation of learning and memory.[43] However, in AD, an extracellular excess of glutamate can overstimulate NMDA receptors, which allows high levels of calcium ions to enter neurons, leading to the loss of synaptic function and excitotoxicity.[44] This, in turn, results in a gradual decline of cognitive function and memory.[44]

Acetylcholinesterase inhibitors (AChEIs)

Currently, the gold standard therapy for AD is the use of AChE inhibitors (AChEIs), which prevent the enzyme acetylcholinesterase (AChE) from breaking down ACh, thereby increasing its synaptic availability and attenuating the cognitive deficits associated with cholinergic neuron loss.[45] The AChEIs donepezil (Aricept®), rivastigmine (Exelon®), and galantamine (Razadyne®) are prescribed to patients to slow down the decline of cognitive function for mild-to-moderate AD, with an increased dose of donepezil also approved for use in moderate-to-severe AD.[46] However, a meta-analysis of 80 clinical trials found only a modest benefit of AChEIs using the Mini-Mental State Examination (MMSE) score.[17] In addition, these modest effects were temporary, lasting for a maximum of 12–24 months.[18] Furthermore, these drugs are only administered in oral formulations (in the case of rivastigmine, a skin patch is newly available).[18] This can result in overstimulation of both central and peripheral cholinergic nerves, leading to gastrointestinal reactions, including nausea, diarrhea, vomiting, and weight loss.[18, 47] These adverse off-target effects limit the tolerable dosage of AChEIs to a range less than that required for meaningful clinical effect, further limiting their utility.[48] In the search for a solution to these challenges, NDDSs have been designed and developed to safely target the delivery of AChEI drugs, ensuring therapeutic quantities reach the brain.

Donepezil (Aricept®)

As donepezil is administered orally, the most common adverse side-effects are gastrointestinal, due to its rapid absorption through the gastrointestinal wall[49], with other less common side-effects, such as urinary incontinence, also experienced due to increased peripheral ACh.[50] To reduce off-target effects, Krishna *et al.* loaded donepezil into polymeric methoxy poly(ethylene glycol)–polycaprolactone (mPEG-PCL) nanospheres.[51] mPEG-PCL copolymers are biocompatible and biodegradable, and have displayed controlled release profiles.[52] Apolipoprotein E3 is a lipoprotein that binds to the hydrophobic region of $A\beta_{1-42}$, decreasing its aggregation and promoting its clearance, and was adsorbed onto the surface of the NPs with polysorbate 80 (ApoE3-DNP).[51] *In vitro* cellular uptake assessed in undifferentiated human neuroblastoma SH-SY5Y cells demonstrated a higher delivery of ApoE3-DNP NPs (88.48%) compared to free donepezil (54.97%), as well as clearance of $A\beta_{1-42}$ fibrils, reduced neurotoxicity, and restoration of cell viability. These beneficial effects translated *in vivo,* with $A\beta_{1-42}$ induced AD rat models showing improved memory retention in both the Morris water maze (MWM) test and passive avoidance tasks when intracerebroventricularly injected with ApoE3-DNP NPs (5 mg/kg) compared to free donepezil (5 mg/kg). Furthermore, histopathological and biochemical analysis of the hippocampus in these rats revealed reduced neuronal loss, AChE activity, $A\beta_{1-42}$ and pTau levels, oxidative stress and neuroinflammation, as well as increased levels of brain-derived neurotrophic factor (BDNF) in the ApoE3-DNP NP treated group.[51] While these *in vivo* results are encouraging, it is important to note that since ApoE3-DNP NPs were injected directly into the brain, bypassing the BBB, conclusions cannot be drawn about the potential of the NP to pass the BBB in more conventional administration routes.

In a different approach, donepezil was loaded into apoA-I-reconstituted high-density lipoprotein (rHDL/Do) to allow passage using scavenger receptor class B type 1 (SR-B1) found on BBB endothelial cells for receptor-mediated transcytosis **(Figure 2A)**.[53] The use of HDL additionally facilitates extracellular Aβ binding and encourages microglial internalisation and endo/lysosomal degradation, resulting in dual action of the NP. Enhanced BBB passage was demonstrated *via* intravenous (IV) administration of rHDL (1 mg/kg) into $A\beta_{1-42}$ induced AD mice models, with a 4-fold increase of rHDL in the brain, extensive distribution in the dentate gyrus of the hippocampus, a region involved in memory formation, and lower accumulation in peripheral organs compared to lipoprotein without rHDL (Lipos/DiR; 1 mg/kg) **(Figure 2B & C)**. Not only did rHDL administration improve brain penetration, it also appeared to improve its pharmacokinetic properties by prolonging its circulation time. In support of this, after administration of free donepezil (2.5 mg/kg) into wild-type (Sprague Dawley) rats, nearly no donepezil was detectable in plasma after 24 h, and elimination half-life was 6.27 h. However, after administration of rHDL/Do, donepezil was still detected 96 h after injection and had an elimination half-life of 13.67 h **(Figure 2D)**. Further, similar to ApoE3-DNP NPs, rHDL/Do treatment led to improved cognitive function in rodent models, with $A\beta_{1-42}$ induced AD mice intravenously treated with rHDL/Do performing better than the free donepezil group on the MWM test **(Figure 2E)**, with concomitant

inhibition of hippocampal AChE activity, reduced pathology in the hippocampus and cortex as evidenced by reduced neuronal damage (shrinkage, nuclear condensation, fragmentation, improved Nissl body loading area), and reduced Aβ deposition.[53]

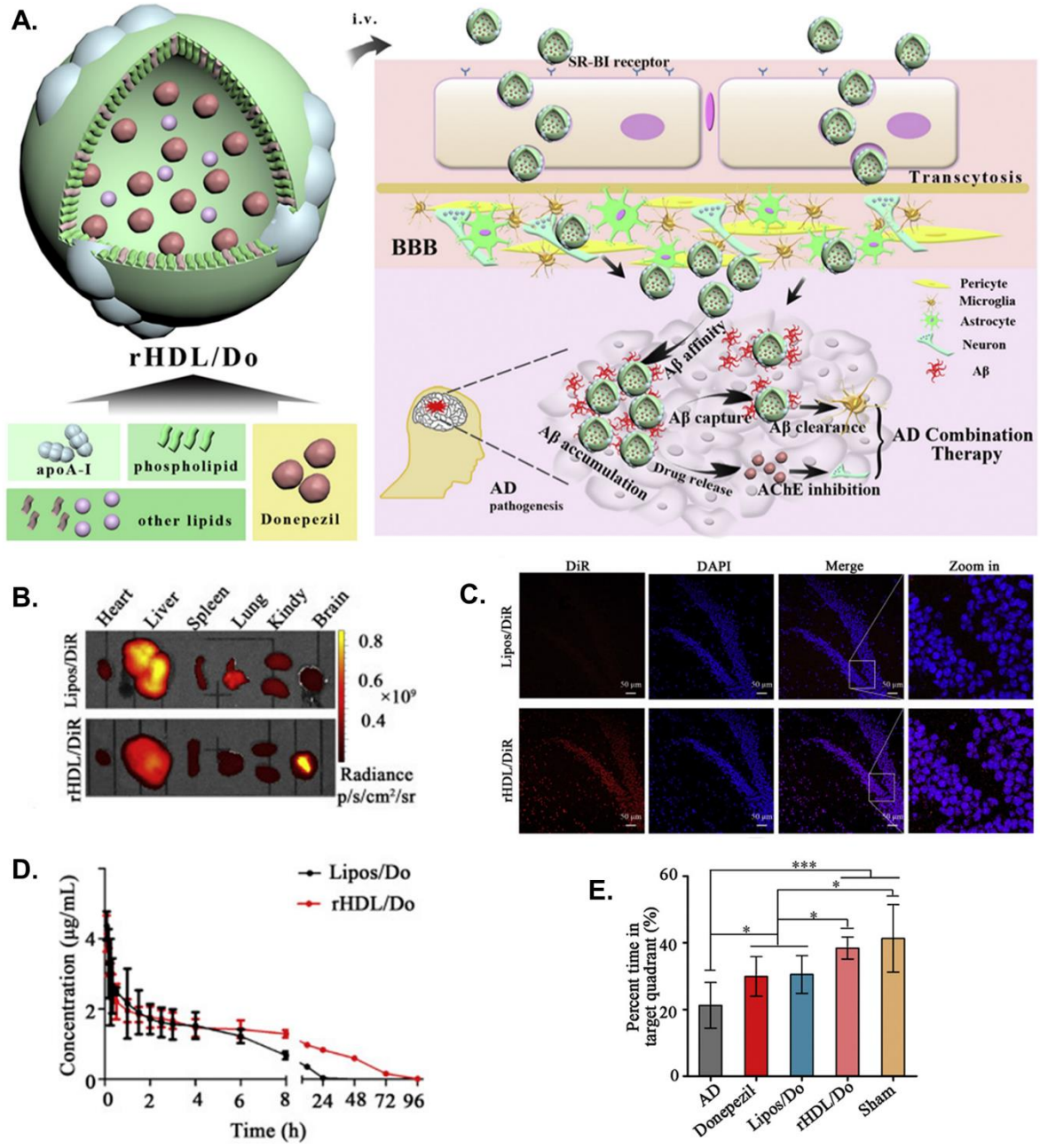


Figure 2: *In vivo* administration of donepezil-loaded rHDL NPs. (A) Reconstituted high-density lipoprotein NPs loaded with donepezil (rHDL/Do; 1 mg/kg) utilized natural lipoprotein binding for transcytosis through the BBB, and combination therapy of HDL binding to Aβ for microglial clearance and donepezil delivery. **(B)** *Ex vivo* imaging of tissues highlighted reduced peripheral accumulation and increased brain penetration of rHDL/Do NPs. **(C)** Hippocampal sections revealed higher accumulation in the dentate gyrus of rHDL/Do treated mice, a region important in memory formation. **(D)** Pharmacokinetic profile of NPs illustrating rHDL/Do's prolonged circulation time. **(E)** AD mice underwent behavioural testing in the form of the Morris

Water Maze. rHDL/Do treated mice showed a higher percentage of time spent in the target quadrant compared to free donepezil.[53]

Interestingly, another dual action drug-loaded NP has recently been investigated for its enhanced microglial Aβ clearance.[54] Metformin, the first line pharmacological treatment for type 2 diabetes, has been shown to reduce neuropathological changes and improve memory deficits in preclinical AD models (APP/PS1,[55] streptozotocin,[56] tau-seeded PS19[57]) and has even been shown to decrease the risk of occurrence of cognitive decline in diabetic patients.[58] Therefore, metformin-phospholipid-donepezil (MPD) NPs were produced. MPDs were shown to be outstandingly stable in PBS and blood; however, when exposed to Aβ-rich PBS or blood, this resulted in the partial disassembly or aggregation of MPD, suggesting site specific release of metformin and donepezil, with >80% of both drugs released within 72 h when exposed to Aβ. *In vitro* assessment determined MPD was able to cross the BBB *via* transcytosis, which was reflected *in vivo* with wild-type mice (BALB/c nude) that received an intraperitoneal (IP) injection of fluorescently labelled MPD (20 μg [200 μl of 100 μg/ml]) showing accumulation in the brain at 24hrs. In the $A\beta_{25-35}$ AD model (C57BL/6), mice treated with MPD (7.1 mg/kg; equivalent to 1.1 mg/kg donepezil, 1.1 mg/kg metformin, and 5 mg/kg phospholipid) exhibited improved outcomes in the MWM, with ameliorated memory impairment compared to donepezil alone (1.1 mg/kg). Further, MPD inhibited hippocampal AChE activity to the same extent as free donepezil, prevented the destruction of neurons, offered a greater neuroprotective effect in the hippocampus than free donepezil, and comprehensively reduced the amount of Aβ-plaques in the cortex. This reduction in plaques was determined to be a result of enhanced microglial phagocytosis through the lysosomal pathway.

Çinar *et al*. also recently investigated Aβ-targeting donepezil-loaded NPs administered *via* IV injection to reduce the side effects and increase efficiency of donepezil.[59] Donepezil-loaded poly(lactic-co-glycolic acid)-block-poly (ethylene glycol) (PLGA-*b*-PEG) nanoparticles were previously found to induce fibril destabilisation and facilitate crossing in an *in vitro* model of the BBB.[60] Donepezil-loaded PLGA-*b*-PEG NP administration (15 μg/kg) resulted in improved behavioural changes in Aβ-induced rats. Short-term memory and anxiety-like behaviour, assessed by the novel object recognition (NOR) and elevated plus maze (EPM) tests, respectively, were improved compared to free donepezil; however, there was no difference in long-term memory, as assessed by NOR (24 h between familiarisation and testing phases) and MWM outcomes, between the donepezil-loaded PLGA-*b*-PEG and free donepezil groups. Further, donepezil-loaded PLGA-*b*-PEG NPs inhibited AChE activity to a greater degree than free donepezil.[59]

Intranasal administration is also being investigated as an alternate route due to its ease of administration, as well as its direct access to the brain. To facilitate intranasal delivery, donepezil was loaded into human serum albumin (HSA) NPs.[61] HSA is biocompatible, biodegradable, and non-immunogenic,[62] and thus advantageous as a

NDDS. The presence of metal ions in the brain may be associated with AD pathogenesis by contributing to the aggregation of Aβ,[63] therefore, the metal ion chelator clioquinol was co-loaded with donepezil for combination therapy. The NPs were coated with the transcriptional activator protein (TAT) transmembrane peptide to enhance brain accumulation and the lipid monosialotetrahexosylganglioside (GM1) which has high binding affinity to Aβ to facilitate targeted delivery[64]. Upon intranasal administration to the β-amyloid precursor protein/presenilin-1 (APP/PS1) AD mouse model, NPs with both TAT and GM1 functionalisation demonstrated enhanced accumulation and retention in the brain 96 h after administration compared to free dye, or formulations displaying a single functionalisation of TAT or GM1. Moreover, NP-treated mice (10 mg/kg HSA dose) displayed significantly improved learning and space exploration in MWM compared to saline-treated controls and performed better compared to groups treated with only HSA or free donepezil or clioquinol, as well as reduced Aβ deposition and neuronal damage, and improved AChE regulation.

Rivastigmine (Exelon®)

Along with adverse gastrointestinal side-effects, rivastigmine oral treatment is also limited by poor BBB penetration due to its hydrophilic nature and a short half-life (<2 h) caused by extensive first-pass metabolism, thus requiring frequent dosing that may impede patient compliance.[65] Therefore, to improve brain delivery and sustained release, rivastigmine was loaded into mPEG-PCL (Riv-NPs).[66] *In vitro* release studies, where free drug or Riv-NPs were loaded into dialysis bags and the drug concentration measured at specified timepoints, found free rivastigmine was released completely at 2 h, whereas Riv-NPs were able to mediate sustained drug release over 8 h. Similarly, intravenous administration of Riv-NPs (2 mg/kg) in wild-type (Wistar Albino) rats demonstrated a 3.7-fold higher concentration of rivastigmine in the brain after 1 h, a 2.3-fold increase in blood terminal half-life, and a 22% decrease in total clearance compared to administration of free rivastigmine (2 mg/kg). This improved retention may be attributed to the effect of surface PEG groups, which can reduce clearance by the immune system, thereby increasing drug survival while in circulation.[66] Importantly, the improved pharmacokinetics of rivastigmine were further reflected *in vivo*, with intraperitoneal injection of Riv-NPs (2 mg/kg) into the scopolamine-induced amnesia rat model showing improved spatial memory on the MWM compared to rats treated with free rivastigmine (2 mg/kg).[66]

Improving the brain delivery of rivastigmine was investigated by Gajbhiye *et al*. by loading rivastigmine into polymeric PLGA-*b*-PEG NPs conjugated with ascorbic acid (RSM-PLGA-*b*-PEG-Asc).[67] Sodium dependent-vitamin C transporters (SVCT2) are found in choroid plexus neuroepithelial cells and take part in the transport of the reduced form of Asc across the BBB;[68] therefore, the conjugation of Asc to NPs can facilitate brain targeting and BBB passage. This was demonstrated by a ~10 and ~6 times higher cellular absorption of rivastigmine in SVCT2-expressing NIT/3TC cells after 3 h compared to free rivastigmine and non-targeted RSM-PLGA-*b*-PEG,

respectively. Further, scopolamine-induced rats treated with intravenously administered RSM-PLGA-*b*-PEG-Asc (1.5 mg/kg equivalent of rivastigmine) demonstrated significantly improved cognitive performance in the radial arm maze and lower AChE activity compared to non-treated controls, free rivastigmine, and non-targeted RSM-PLGA-*b*-PEG treated groups, highlighting the enhanced efficacy of brain-targeted functionalised NPs.

Targeted BBB delivery and passage was similarly utilised by Kaboli *et al.* by modifying the surface of PCL-PEG-PCL triblock co-polymers with valine-conjugated chitosan to target the large amino acid transporter 1 (LAT-1) for carrier-mediated transport (NCs@VLCS).[69] The safety of the NPs were confirmed *in vivo* by administering Wistar rats 120 mg/kg of NCs@VLCs intraperitoneally, with no noticeable changes in body weight or physical signs, and no histopathological changes compared to controls 24 h and 14 d post administration. Rivastigmine was co-loaded with quercetin, an antioxidant with anti-inflammatory properties (RV/QT-NCs@VLCS), and intraperitoneally administered to scopolamine-induced rats (equivalent to 1 mg/kg rivastigmine). Compared to other rivastigmine groups (i.e., free RV, RV/QT-NCs, RV-NCs@VLCS), RV/QT-NCs@VLCS-treated rats demonstrated the most improved memory in MWM, along with increased antioxidant activity as measured by glutathione (GSH) levels and normalised hippocampal gene expression of AChE, BDNF, and TNF-α. Altogether, these results indicated the combined effect of dual-therapy delivered in a brain-targeting NP resulted in enhanced therapeutic efficacy.

Liposomes are another type of NP with numerous advantageous properties including biocompatibility, biodegradability, non-toxicity, and the ability to improve the stability and solubility of encapsulated drugs.[70] Intranasal and oral rivastigmine-loaded liposomal and PLGA formulations have been compared for their pharmacokinetic profile, bioavailability, half-life, and clearance rate, as well as their *in vivo* effect in both the acute scopolamine-induced amnesia and chronic colchicine-induced AD models.[71] Compared to oral rivastigmine (2.5 mg/kg), of all the formulations and routes tested, nasal liposomal rivastigmine (2 mg/kg) showed the best pharmacokinetic profile, with improved bioavailability, shorter clearance rate, and increased half-life. Further, nasal liposomal rivastigmine was the most effective formulation in improving memory deficits in both models in the MWM and passive avoidance tests, as well as inhibiting AChE activity.

Galantamine (Razadyne®)

Galantamine displays low brain penetration and retention due to its poor lipophilicity, meaning that higher doses are required, which in turn leads to strong undesirable side effects and low patient compliance.[72] To overcome such limitations, galantamine has been loaded into self-assembled nanomicelles composed from the lipid, distearoyl phosphatidyl ethanolamine (DSPE), and PEG (OPT-GAL-MNMs), due to their brain targeting properties.[73] Compared to free galantamine, wild-type Wistar rats orally administered with OPT-GAL-MNMs (10 mg/kg) showed a 2.28-fold increase of

galantamine in plasma AUC, a 2.08-fold increase in mean residence time (MRT), and a 4.80-fold increase in time taken to reach maximum drug concentration (t_{max}), demonstrating enhanced galantamine retention. In the brain, OPT-GAL-MNMs also displayed a 5.40-fold increase in maximum galantamine levels at 8h and a 4.31-fold increase in AUC compared to administration of free galantamine, indicating enhanced brain penetrance. This may be due to DSPE-PEG facilitated immune system avoidance, resulting in increased circulation time, which provides a higher chance of brain uptake.[73] Further, Aβ-induced AD rat models (ICV-administered β-Amyloid) showed enhanced cognitive performance in the MWM, as well as reduced levels of lipid peroxidation and nitrite, and increased levels of glutathione when treated with orally administered OPT-GAL-MNM (10 mg/kg) compared to free galantamine (10 mg/kg), indicating a reduction in oxidative stress.[73]

Utilising their NP formulation as previously described for rivastigmine (RSM-PLGA-*b*-PEG-Asc)[67], Gajbhiye *et al*. instead loaded the NP with galantamine (GLM-PLGA-*b*-PEG-Asc).[74] Similar to their other study, the intravenous administration of GLM-PLGA-*b*-PEG-Asc (1.5 mg/kg equivalent of galantamine) resulted in enhanced biodistribution of galantamine to the brain compared with free galantamine and non-targeted NPs, which was corroborated by significantly improved cognitive performance in both the MWM and radial arm maze, as well as lower AChE activity, following scopolamine injection in rats.

To bypass the BBB and increase brain bioavailability, polymeric NPs comprised of mucoadhesive polyacrylic acid (PAA) and sodium taurodeoxycholate loaded with galantamine hydrobromide (PAA NPs) have been administered intranasally to LPS-induced AD model mice.[75] Interestingly, both oral and intranasal delivery (both 4 mg/kg equivalent of galantamine) of PAA NPs resulted in improved outcomes on the Y-maze test, compared to untreated mice, and to a higher degree than mice treated with free galantamine. Further, intranasal delivery of PAA NPs reduced brain content of the inflammatory markers NF-κB and IL-1β, increased the microglial anti-inflammatory marker TGF-β, and decreased the activated astrocyte marker GFAP, all to a greater extent than free galantamine administered either orally or intranasally, as well as orally administered PAA NPs. Unfortunately, intranasal administration was associated with mucosal ulceration with massive inflammatory infiltrate following 3 days of treatment. This appeared to resolve and heal by 7 days of continuous treatment, however, this is likely to affect patient compliance.

Intranasal delivery of galantamine was similarly investigated by El-Ganainy *et al*. by binding to chitosan NPs (G-NP).[76] Chitosan is biocompatible, biodegradable, non-toxic, and an ideal NP for intranasal delivery due to its mucoadhesive properties minimising its clearance and therefore facilitating enhanced drug absorption.[77] Healthy rats received G-NP administered intranasally, or free galantamine administered either intravenously, orally, or intranasally, with all treatments containing 3 mg/kg equivalent of galantamine. G-NP-treated groups demonstrated superior AUC brain levels compared to all groups treated with free galantamine and displayed

enhanced brain retention with an elimination half-life of 7.56 h compared to 1.4, 2.2, and 3.9 h for free galantamine administered intravenously, orally, and intranasally, respectively. In the scopolamine-induced amnesia model, G-NP-treated rats demonstrated restored exploratory behaviour but did not show an improvement in short-term memory dysfunction in the Y-maze. However, they did show an improvement in spatial memory and memory retention in the MWM. Further, this improvement was more pronounced compared to groups treated with free galantamine administered either orally or intranasally. Further, treatment with G-NP showed the most significant decline in AChE level and activity, Aβ1–42 levels, and histopathological degeneration, and higher suppression of Notch-1 (associated with neuronal injury) compared to free galantamine.

Together, these studies demonstrate how encapsulation of AChEIs into NDDSs can overcome issues associated with traditional drug delivery by increasing their bioavailability and brain penetration, resulting in enhanced therapeutic efficacy. However, while slow and sustained release may increase bioavailability in the brain, without functionalisation for controlled or targeted release, it also presents the risk of drug delivery to non-target, healthy tissue. Moreover, greater in-depth investigation needs to be conducted into dosage and treatment regimes, as well as newly arising side-effects, such as skin or mucosal irritation.

N-methyl-D-aspartate (NMDA) receptor antagonists

Memantine is an NMDA receptor antagonist that targets AD symptoms by binding to the NMDA receptor and blocking its interactions with excess glutamate.[78] By blocking the interaction between NMDA receptors and glutamate, memantine lowers calcium influx and excitotoxicity, thereby reducing cell death and improving cognitive function.[44] However, despite widespread use of this drug to treat moderate-to-severe AD, meta-analyses of placebo-controlled clinical trials have found memantine to have low therapeutic efficacy and thus limited clinical benefits.[79-80] Like AChEIs, memantine is delivered by oral administration and is therefore subject to unwanted first-pass metabolism and poor brain bioavailability.[81]

To overcome this, memantine was loaded into polymeric PLGA NPs and coated with PEG to reduce immune system clearance, thereby prolonging blood circulation and increasing bioavailability in the brain after oral administration.[82] The *in vitro* release profile of PEG-PLGA NP formulation had an equilibrium dissociation constant (K_D) almost 2-fold higher than free memantine, demonstrating its slower, sustained release. Furthermore, *in vivo,* it was found to accumulate in the hippocampus of C57BL/6J wild-type mice. In addition, APP/PS1 AD mice treated with the memantine-loaded NPs (30 mg/kg/day) showed enhanced cognitive performance on the MWM, as well as a significant reduction in both Aβ plaques and inflammation in the cortex, when compared to free memantine-treated mice (30 mg/kg/day).[82] In an alternative approach, memantine was targeted to the brain by loading into a polymer-based PAMAM dendrimer conjugated with lactoferrin (MEM-PAMAM-Lf).[83] Lactoferrin

transports iron across the BBB, and thus its conjugation allows NP passage through receptor-mediated transcytosis. Intravenous administration (1 mg/kg equivalent of memantine) of MEM-PAMAM-Lf into wild-type Sprague Dawley rats demonstrated significantly improved pharmacokinetic properties, with a 2.17-fold higher maximum concentration (C_{max}), 4.73-fold higher residual time of drug in the body, 10-fold higher bioavailability, and significantly higher brain uptake and retention compared to free memantine (1 mg/kg). Functional outcomes were also improved, with $AlCl_3$-induced Swiss albino mice intraperitoneally treated with MEM-PAMAM-Lf (2 mg/kg) performing better on the object recognition memory test (ORM) compared to those treated with free memantine (2 mg/kg).[83]

Recently, using a different approach, Shivananjegowda *et al*. co-loaded solid lipid nanoparticles (SLNs) with memantine hydrochloride (MeHCl) and Tramiprosate (TMPS), which binds to $A\beta_{1-42}$ and prevents fibrillation.[84] *In vitro* release studies revealed the free drugs were released within 3 h, while MeHCl/TMPS SLNs showed prolonged release over 48 h, as well as improved pharmacokinetics, with C_{max} 1.4-fold higher. $AlCl_3$-induced Wistar rats also showed improved spatial memory assessed *via* the MWM following oral treatment with MeHCl/TMPS SLNs (10 mg/kg), with a corresponding decrease in hippocampal Aβ levels, as compared to free drugs, or SLNs with a single drug (10 mg/kg).

To completely avoid first pass metabolism, Kaur *et al*. have developed memantine (mem)-loaded PLGA NPs for intranasal delivery, circumventing the oral route and directly accessing the brain.[85] Intranasally delivered Mem PLGA NPs showed higher uptake in the brain, as compared to either orally or IV delivered Mem PLGA NPs, as well as either oral or intranasal free memantine in wild type Sprague Dawley rats. In the scopolamine-induced rat model, rats treated with Mem PLGA NPs (0.1 mg/kg) showed improved cognitive function, which were similar to that of non-AD control rats.

Overall, although relatively few studies have been conducted to date using NDDSs for memantine delivery, evidence from early studies suggests that enhanced brain delivery can be achieved using this strategy, resulting in enhanced drug pharmacokinetics and improved therapeutic outcomes. Further, the specific encapsulation of memantine within NPs appears to enable its sustained release, which is advantageous as it allows a stable amount of memantine to be made bioavailable within the brain over time. This may reduce adverse effects caused by drug fluctuations and decrease administration frequency, which can ultimately improve patient compliance.

Nanoparticles to enhance current PD therapeutics

The cardinal motor symptoms of PD are associated with the loss of dopaminergic neurons within the substantia nigra *pars compacta* (SNc), leading to a subsequent decrease of dopamine levels within the striatum.[86] These decreased levels of dopamine lead to an imbalance within the basal ganglia circuits critical for control of

normal voluntary movement, resulting in delayed and uncoordinated movement and the distinctive tremors associated with PD.[87] Concerningly, the percentage of dopaminergic neurons lost at the onset of motor symptoms is estimated to already be 31%,[88-89] with a corresponding loss of ~70-80% in total dopamine levels in the striatum, limiting the efficacy of current therapeutic strategies.[90-91] Reduced dopamine levels also lead to abnormalities in multiple other neurotransmitter systems, such as glutamate, ACh, and γ-aminobutyric acid (GABA), which further contribute to symptom presentation, especially the non-motor symptoms of PD (for reviews, see Barone[92] and Brichta *et al*.[93]).

L-DOPA and Dopamine

The current gold standard therapy for the cardinal motor symptoms of PD is dopamine replacement therapy (DRT). The administration of exogenous dopamine itself to PD patients is problematic, as dopamine is a water-soluble hydrophilic molecule that is unable to cross the BBB; thus, its metabolic precursor 3,4-dihydroxy-L-phenylalanine (L-DOPA) is most commonly utilised as a therapeutic for PD.[94] L-DOPA actively traverses the BBB *via* the large amino acid transporter (LAT)-1 and is converted into dopamine by DOPA decarboxylase, increasing dopamine concentrations in the brain, restoring synaptic plasticity within the DA-denervated striatum and relieving motor symptoms.[95-96] While oral L-DOPA therapy achieves stable symptomatic control within the first few years of use, long-term treatment can result in severe side-effects, including involuntary hyperkinetic movements known as L-DOPA-induced dyskinesias.[95] While still poorly understood, L-DOPA-induced dyskinesia may be at least partially caused by the intermittent change of L-DOPA concentration in the blood from variable gastrointestinal absorption following use of the orally administered drugs.[95] Therefore, strategies that facilitate the controlled and continuous delivery for improved bioavailability of L-DOPA could potentially alleviate these complications.

Another complication encountered in the use of L-DOPA as a therapeutic is that large amounts are subject to premature peripheral degradation into dopamine by DOPA decarboxylase in the bloodstream prior to reaching its site-of-action in the brain.[97] Consequently, high doses of L-DOPA must be administered to patients to achieve therapeutic efficacy, often resulting in additional adverse side-effects, such as nausea and orthostatic hypotension.[97] Co-administration of a DOPA decarboxylase inhibitor, such as carbidopa (i.e. Sinemet®) or benserazide, can improve the pharmacokinetics of L-DOPA, thereby limiting L-DOPA dosage and reducing the potential side-effects associated with high dosing.[97] However, recent evidence suggests the use of these inhibitors may paradoxically induce peripheral DOPA decarboxylase and potentially contribute to L-DOPA fluctuation.[98] Importantly, the use of an NDDS could minimise peripheral L-DOPA metabolism by protecting it *via* encapsulation, eliminating the need for the use of DOPA decarboxylase inhibitors.

Several studies have already investigated this potential. In a study by Nie and colleagues, L-DOPA was loaded into tannic acid/polyvinyl alcohol (TA/PVA/L-DOPA) NPs.[99] *In vitro* release profiles showed that free L-DOPA displayed a burst release within ~4 h; conversely, due to hydrogen bonding, L-DOPA from TA/PVA/L-DOPA NPs demonstrated extended release for up to 48 h. TA/PVA/L-DOPA NPs were administered subcutaneously (10 mg/kg) in the reserpine rat model of PD at the back of the neck for brain-lymphatic vasculature transport. The group treated with TA/PVA/L-DOPA NPs showed both reduced motor symptoms and increased striatal levels of dopamine compared to the group treated with free L-DOPA.[99] Similarly, Vong and colleagues achieved sustained release of L-DOPA using PEG-*b*-P(l-DOPA(OAc)$_2$) (Nano^{DOPA}), whereby physiological enzymes cleaved the peptide bonds within the formulation, slowly releasing L-DOPA into the bloodstream.[100] When injected intraperitoneally (127 µmol/kg) into wild-type ICR mice, free L-DOPA levels were high 0.5 h after administration, but returned to baseline level within 1 h; conversely, after administration of Nano^{DOPA} (105 mg polymer/kg; equivalent of 127 µmol L-DOPA/kg), levels were comparatively lower at the 0.5 h point and plasma levels were maintained up to 12 h following administration. Further, plasma AUC of L-DOPA was ~8-fold higher and less L-DOPA was found in the liver using Nano^{DOPA}, demonstrating greatly improved bioavailability of L-DOPA. Nano^{DOPA} treatment also resulted in improved functional outcomes, with MPTP-induced PD mice exhibiting reduced motor dysfunction, as measured by improved outcomes in the grid walk test, improved resting tremor score and reduced latency time in the narrow beam test, following intraperitoneal injection of Nano^{DOPA}(64 mg polymer/kg; equivalent of 76 µmol L-DOPA/kg). Notably, unlike the effect seen with free L-DOPA (76 µmol/kg), mice administered a high dose of Nano^{DOPA} for a longer period (127 µmol L-DOPA/kg for 17 days) did not develop dyskinesia; however, much longer studies are required to ascertain whether this NDDS can reduce the problematic side effects associated with extended L-DOPA treatment.[100]

Excitingly, some NDDSs may hold the key to overcoming a reliance on L-DOPA for PD treatment altogether by instead facilitating the passage of dopamine itself into the brain. In one such study, dopamine was loaded into albumin/PLGA NPs (ALNP-DA) due to the ability of albumin to cross the BBB *via* receptor-mediated transcytosis, which was confirmed by verifying accumulation of ALNP in the striatum and hippocampus after intraperitoneal injection (10-20 mg/animal) into wild-type Swiss mice.[101] In the 6-OHDA mouse model of PD, ALNP-DA-treated mice (10 or 20 mg/animal ALNP equivalent to 70 or 140 µg of dopamine, respectively) displayed improved motor symptoms on the rotarod, adhesive removal, and apomorphine-induced rotation tests compared to L-DOPA-treated (70 µg/animal) mice.[101] However, dopamine levels within the striatum were not measured to confirm site-specific drug release from the NPs in this study.

Dopamine has also been directly incorporated into spherical polymeric NPs comprised of iron nodes and ditopic ligand 1,4-bis(imidazol-1-ylmethyl)benzene (BIX) (DA-NCPs).[102] Administration of DA-NCPs in dopaminergic (BE)2-M17 cells led to a 6-fold

higher concentration of dopamine compared to administration of free dopamine. Additionally, intracerebroventricular administration of DA-NCPs (equivalent to 50 µg dopamine) in wild-type Sprague Dawley rats resulted in distribution of the NPs to the brain, as indicated by an increase in striatal dopamine levels. Therapeutically, intranasal administration of DA-NCPs (equivalent to 50 µg dopamine) in the 6-OHDA rat model resulted in reduced apomorphine-induced rotations compared to free dopamine treated rats, likely due to their enhanced sustained release profile.[102]

Beyond conventional NDDSs that rely upon chemically synthesised NPs, there is growing interest in exploring the use of biologically-derived NPs instead. For example, extracellular vesicles, known as exosomes, are secreted by cells and transport biomolecules (i.e., nucleic acids, proteins, lipids, metabolites).[103] As endogenous biological NPs, exosomes possess enhanced biocompatibility and low immunogenicity. Recently, the natural brain targeting property of exosomes extracted from blood and loaded with dopamine (DA-blood-exo) enabled receptor-mediated transcytosis of dopamine across the BBB in Kunming mice **(Figure 3A)**.[104] DA-blood-exo's were able to successfully deliver dopamine to the striatum and substantia nigra (SN) **(Figure 3C)**, following intravenous injection (equivalent to 18 mg/kg free dopamine), increasing brain distribution of dopamine >15-fold after 6 h, whereas free dopamine (18 mg/kg) accumulated in the liver, lung and kidneys **(Figure 3B)**, indicating a short half-life and rapid clearance. This increased brain penetration translated to functional improvement, with intravenous injection (equivalent to 4.95 mg/kg levodopa) of DA-blood-exo's resulting in improvements in motor function, as determined by reduced amphetamine-induced rotations, as well as significantly increased striatal dopamine levels and reduced oxidative stress, in a 6-OHDA mouse model.[104] Exosomes as therapeutics is still an emerging field; however, an understanding of different exosomes, their surface proteins and their role in natural targeting and fate, as well as utilisation of modern surface engineering and particle isolation techniques, is quickly developing.[105] Thus, exosomes, and other EVs, hold exciting potential as the next generation of NDDSs for neurodegenerative disorders.

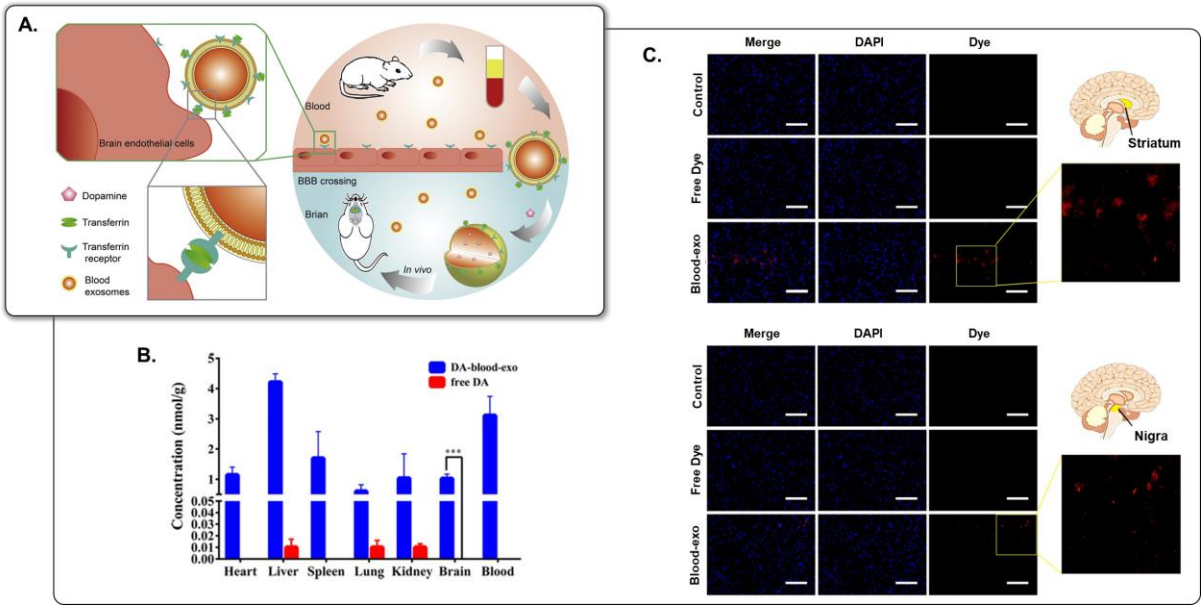


Figure 3. Blood-derived exosomes for targeted dopamine delivery *in vivo.* **(A)** Qu and colleagues developed dopamine-loaded exosomes (DA-blood-exo) that have natural brain targeting ability. **(B)** DA-blood-exo (red) were shown to be delivered to the striatum and SN *in vivo*. **(C)** Biodistribution analysis revealed greater concentrations of DA-blood-exo in all organs 6 h after infusion, and significantly higher concentration in the brain, as compared to free DA.[104]

Evidently, NDDSs can potentially overcome current L-DOPA limitations, including, most significantly, helping to avoid L-DOPA-induced dyskinesia by facilitating the continuous and controlled administration of L-DOPA, reducing the need for high dosage treatment regimens linked to adverse side-effects. Further, NDDSs can facilitate the direct brain delivery of dopamine, potentially eliminating a reliance on L-DOPA (and decarboxylase inhibitors) therapy altogether, a strategy that gradually loses its efficacy due to the progressive loss of decarboxylase in the brain of PD patients.[106]

Dopamine Agonists and Monoamine Oxidase Type B Inhibitors

Dopamine agonists, including pramipexole, ropinirole, rotigotine, and apomorphine, are drugs that alleviate PD symptoms by 'mimicking' dopamine to activate its receptors and help restore dopaminergic signalling in PD patients.[107] Monoamine oxidase type B (MAO-B) inhibitors (selegiline, rasagiline, and safinamide) are another class of anti-Parkinsonian drugs that irreversibly inhibit the MAO-B enzyme from metabolising dopamine, thereby maintaining existing dopaminergic activity in the brain.[108] Dopamine agonists and MAO-B inhibitors are often employed as an early PD treatment to delay initiation of L-DOPA treatment, or used in conjunction with lower amounts of L-DOPA to minimise its side-effects.[107] However, these treatments are mostly orally administered in tablet form, and this delivery route makes them susceptible to first-pass metabolism either in the gut or the liver; consequently, the vast majority of the drug does not enter the brain.[108-109] Due to the rapid clearance of

these drugs, patients are often required to take multiple daily doses, which can reduce patient compliance. In addition, side-effects associated with these treatments range from nausea and headaches to hallucinations and compulsive behaviour.[107] Therefore, NDDSs are being explored as a mechanism to allow alternative administration routes to deliver dopamine agonists and MAO-B inhibitors to increase their bioavailability and thus improve selective accumulation in the brain.

In line with this, Barcia *et al.* created a biodegradable PLGA NP designed to facilitate sustained release of the dopamine agonist ropinirole.[110] Intraperitoneal injection of the ropinirole-loaded PLGA NPs (equivalent to 1 mg/kg) resulted in decreased catalepsy and akinesia and improved motor balance and coordination compared to rats treated with free ropinirole (1 mg/kg) in the rotenone model of PD in Wistar rats. Further, brain histology and immunohistochemistry results indicated that treatment with ropinirole-PLGA NPs reduced ROS production, astrogliosis, and cell death in the SN.[110]

Interestingly, while not traditionally delivered through a transdermal patch, Dudhipala and Gorre combined topical delivery and solid lipid nanoparticles or nanostructured lipid carriers in hydrogel, in an attempt to improve bioavailability and reduce drug dosages of ropinirole.[111] Topical administration of these was shown to improve bioavailability by >3-fold in wild type Wistar rats. In the haloperidol-induced PD model, the nano-hydrogel-formulations restored lipid peroxidation levels, catalase activity, and dopamine levels in the striatum.

In another study, Yan *et al.* loaded the dopamine agonist rotigotine into PEGylated PLGA NPs functionalised with lactoferrin (Lf-R-NPs) to enhance brain targeting.[112] Currently, rotigotine is marketed as a transdermal patch to increase its bioavailability through slow and sustained release;[107] however, its bioavailability is still limited.[113] Conversely, following intranasal administration of Lf-R-NPs (1 mg/kg rotigotine) in wild-type Sprague Dawley rats, a high and sustained accumulation of rotigotine was observed in the brain, indicating improved bioavailability. Furthermore, targeting of the striatum, the main site of DA denervation in PD, was improved compared to that achieved using NPs without lactoferrin (R-NPs), indicating that lactoferrin may facilitate enhanced targeting due to the high expression of lactoferrin receptors on the surface of neurons. Additionally, in the 6-OHDA model, rats treated with Lf-R-NPs (2 mg/kg) showed significant reductions in apomorphine-induced rotations, as well as reduced dopaminergic neuron loss, compared to either R-NPs or L-DOPA-treated rats.[112]

In an alternative approach, brain delivery of rotigotine was achieved by loading the drug into chitosan NPs, due to its mucoadhesive properties, facilitating enhanced residency time in the nasal cavity for nose-to-brain delivery.[113] Intranasal administration of these rotigotine-loaded chitosan NPs (2 mg/kg) in haloperidol-treated Sprague Dawley rats resulted in reduced catalepsy and akinesia, restored swimming ability, and enhanced antioxidant effect compared to both intranasally and orally administered free rotigotine. These enhanced therapeutic effects were also supported

by the superior pharmacokinetic behaviour of intranasally delivered rotigotine-loaded chitosan NPs, with a significantly higher brain/blood ratio at 0.5 h compared to intranasally administered free rotigotine (2 mg/kg), and compared to both orally and intravenously administered rotigotine-loaded chitosan NPs (2 mg/kg), showing the benefit of pairing intranasal delivery and NP encapsulation in increasing brain bioavailability.[113] Chitosan assisted intranasal delivery was similarly investigated using the dopamine agonist pramipexole in order to prolong its circulation and reduce its renal clearance.[114] Pramipexole-loaded chitosan NPs (P-CNs) showed sustained release of 83% of pramipexole over 24 h across goat nasal mucosa *ex vivo*. Intranasal delivery of these P-CNs (0.3 mg/kg) in rotenone-induced PD in Sprague Dawley rats resulted in significantly increased locomotor activity and decreased catalepsy compared to the orally administered tablet Pramipex®. Further, analysis of brain homogenate from P-CN treated rats revealed significantly increased dopamine levels compared to both orally administered Pramipex® and intranasally administered pramipexole without chitosan encapsulation.[114] However, the concentration of pramipexole in the brain, and its metabolism/elimination, need to be evaluated to verify the advantage of encapsulating pramipexole in chitosan.

As an alternative to dopamine agonists, MAO-B inhibitors may be employed to maintain dopamine sensitivity by irreversible inhibition of dopamine metabolism. Oral administration of the MAO-B inhibitor rasagiline is associated with gastrointestinal side-effects, a short half-life, and a bioavailability of only 36% due to extensive first-pass metabolism in the liver.[108] To overcome this, Bali *et al.* loaded rasagiline mesylate (RM) encapsulated in PLGA NPs into a gellan gum patch for transdermal delivery (RM-NP TDDS).[115] Pharmacokinetic evaluation using wild type Wistar rats showed RM-NP TDDS (0.088 mg/kg) had a much slower release profile over 72 h with C_{max} at 24 h, compared to IV or orally delivered RM-NPs (0.088 mg/kg), with C_{max} at 30 mins and 2 h, respectively. Additionally, RM-NP TDDS showed MRT of 49 h, while IV or orally delivered RM-NPs were considerably shorter with MRT 3.9 h and 5.2 h, respectively. In reserpine-induced PD rats, RM-NP TDDS (equivalent to 1 mg/kg/day rasagiline) restored reserpine-induced dopamine depletion as indicated *via* improved cataleptic activity (bar test) and improved locomotor activity in the open field test. Behavioural improvements were associated with increased dopamine and GSH levels, increased catalase activity, and reduced MAO-B levels in homogenised whole brain samples.

Similarly to rasagiline, another MAO-B inhibitor, selegiline, is also limited by >90% of orally administered drug being subject to first-pass metabolism.[116] To combat this, selegiline was formulated into a polysaccharide chitosan NP and, upon intranasal administration (1 mg/kg) into Sprague Dawley rats, found to have a 20-fold higher brain C_{max} and a 2-fold increase in AUC compared to orally administered Selgin® tablets (1 mg/kg).[117] Functional improvement was also assessed, with intranasal administration of selegiline-loaded NPs resulting in improved locomotor activity and reduced catalepsy in the rotenone-induced rat model of PD compared to orally administered Selgin®. This improvement in motor symptoms was accompanied by a

2-fold restoration of dopamine in whole brain homogenates, and improved antioxidant action.[117]

Taken together, these studies demonstrate the ability of NDDSs to increase the brain bioavailability of existing dopamine agonists and MAO-B drugs through a range of different mechanisms, such as encapsulation to allow sustained release, thereby prolonging circulation time of the drug and protecting it from first-pass metabolism, facilitation of targeted delivery to the brain using ligands for receptor-mediated transcytosis, and utilisation of the mucoadhesive properties of chitosan to minimise mucociliary clearance, allowing intranasal delivery of drugs to circumvent the BBB. While these strategies enhanced the pharmacokinetics of the drugs, the functional benefits, particularly long-term, remain to be investigated, with only some studies to date assessing functional outcomes relative to commercially-available formulations. Thus, while preliminary pre-clinical evidence is promising, key questions remain around the clinical translatability of such approaches.

Conclusion and future perspectives

In this review, we have highlighted the latest advancements in neuronanomedicine, particularly in enhancing the bioavailability of existing therapeutics for AD/PD. The potential for further functionalisation of these NDDSs holds promise for improved targeting capabilities and reduced off-target effects. However, while neuronanomedicine is an exciting and rapidly growing field, no brain-targeting NP therapies have yet received approval for use. Notably, as recently as December 2023, the FDA rejected an application for a gold-nanocrystal therapy targeting motor neuron disease (amyotrophic lateral sclerosis), another neurodegenerative disease (Clinical trial numbers: NCT04098406 and NCT05299658).

While reviewing the literature, we noted there are relatively few studies of NDDSs that have moved from *in vitro* evaluation into an *in vivo* disease model. Further, while the NDDSs highlighted above are capable of inducing molecular and biochemical changes, and/or functional improvements in disease models, several notable oversights merit investigation before advancing to further disease models or clinical translation. For instance, some studies lack in-depth exploration of uptake mechanisms, despite functionalisation for the purpose of BBB crossing. For the most part, simply reporting that uptake *in vitro* is improved in any type of brain cell, let alone a BBB model or with regard to intra-brain targeting, appears to be deemed sufficient to move to *in vivo* models. Additionally, there is a severe lack of studies that deign to investigate the effects of these formulations in female pre-clinical models. Such are the differences that Rompicherla *et al.* note that they chose to purely use male rats when investigating rivastigmine nano-formulations, as they had previously shown that male and female rats have significantly different pharmacokinetic profiles.[71, 118] In fact, of all the studies reviewed here, only 2 include female animals as part of pre-clinical therapeutic assessment, and neither discuss differences observed between the

sexes.[85, 113] Considering that 2/3 of those diagnosed with AD are women,[119] and that women develop highly-disabling treatment-related complications following treatment for PD,[120] much more work needs to be done to address this disparity.

Further, despite concerns relating to patient compliance, the administration route of choice in many of these studies remains subcutaneous, IV or IP injection, with one going as far as the clinically unrealistic treatment option of intracerebroventricular injection.[51] If possible, oral administration, or even intranasal administration, of NDDS formulations warrant further investigation.

Considering the impact that adverse side effects have on current patient compliance, it is also somewhat surprising that there are limited reports of side-effect profiles in most of these studies to date. In fact, of the 29 research articles presented here, only one reported adverse side-effects, while 2 reported no side-effects, 2 reported prevention of L-DOPA induced side-effects, and 3 reported no adverse effects, however fail to mention drug-related side-effects. In the other 21 studies, there was no mention of side-effect profiles. Moving forward, therefore, studies should consider reporting these as a secondary outcome, as this is an important indicator of potential future patient compliance.

Overall, given the limitations of a number of the above studies, we make the following recommendations for NDDS development in neuronanomedicine. Firstly, bio-nano interactions should be elucidated. Understanding BBB penetration, intra-brain uptake mechanisms, and brain targeting efficiency is important to optimise the therapeutic efficacy of NDDSs. To this end, *in vitro* models that replicate the BBB can be paired with microscopy techniques to visualise NP transport mechanisms.[121] The protein corona (PC) is the layer of proteins that coat NPs upon interaction with biological fluid and can determine the biodistribution and pharmacokinetic profile of NDDSs.[122] Notably, we recently found that cerebrospinal fluid-derived PCs enhance NP uptake by various brain cells and affect them differently compared to serum-derived PCs.[123] Despite these implications, a gap of knowledge towards PC formation in neuronanomedicine persists. An improved understanding may facilitate the manipulation of PC formation to allow more control over the biological fate of an NDDS and thus improve its therapeutic efficacy. [124] There also remains a lack of information pertaining to NP neuro-nanotoxicity, this was highlighted by the majority of studies reviewed failing to assess *in vivo* side-effects. Therefore, monitoring any potential NP toxicity, as well as NP degradation and clearance, is recommended.

Translational gaps should be bridged by carefully considering disease models when moving from bench top to patient. The majority of preclinical models do not use aged animals, despite age being the largest risk factor for AD and PD and can affect biological processes.[125] Reduced BBB integrity in aged brains can facilitate enhanced brain localisation of NPs compared to young brains.[126] However, aged cells can be more susceptible to NP toxicity.[127] Therefore, incorporation of aged models may be necessary to better understand the potential differences neuronanomedicine may have in aged individuals.

A disease-first approach, focusing on the pathophysiology of disease should be adopted rather than a formulation-first approach. By designing NPs to be responsive to stimuli from the internal disease-specific environment, targeted drug release could be enhanced while reducing off-target effects to healthy tissue. For example, the administration of Aβ-responsive metformin-phospholipid-donepezil NPs resulted in the site-specific release of metformin and donepezil when exposed to overexpressed Aβ.[54] Further, responses to neuronanomedicine may vary given the heterogeneity among patients with AD[128] and PD[129]. Therefore, stratification of patients based on specific diagnostic/prognostic profiles may help personalise treatment regimens, leading to better outcomes.

Finally, standardising neuronanomedicine with minimum reporting standards to ensure transparency, potentially leading to the establishment of neuronanomedical databases for comprehensive research and clinical data collation. Nevertheless, given their unparalleled potential to circumvent limitations associated with current therapies, NDDSs stand poised to revolutionise neuro-related disease treatment.

Table 1. Nanoparticle-based strategies designed to enhance outcomes of symptomatic therapies in AD and PD.

Therapy	Delivered therapeutic agent	Composition	Size (nm)	BBB-penetrating mechanism	Improved drug properties	Model	Admin route	Molecular/biochemical changes	Behavioural/motor changes	Ref
Acetylcholinesterase inhibition	Donepezil	mPEG-PCL coated with ApoE3 and polysorbate 80	123.45 ± 3.98	Physical bypass	Increased cellular uptake	Aβ1-42-induced Wistar rats	ICV	Reduced neuronal loss, AChE activity, Aβ1-42 and pTau levels, oxidative stress, and neuroinflammation, and increased BDNF levels	Improved memory retention in MWM test and passive avoidance task	[51]
		rHDL	45.8 ± 4.45	Receptor-mediated transcytosis	Increased circulation time and elimination half-life	Aβ1-42 induced ICR mice	IV	Higher AChEI efficacy and neuronal integrity, attenuated neuronal shrinkage, and decreased Aβ deposition	Improved spatial learning and memory in MWM	[53]
		Dual-drug-phospholipid inclusion complex	100	Transcytosis	Improved stability and solubility, increased circulation time, brain accumulation, and retention, targeted release	Aβ25-35-induced C57BL/6 mice	IP	Inhibited AChE activity. Increased neuroprotective effect compared to free donepezil. Increased clearance of Aβ plaques.	Improved outcomes in MWM	[54]
		PLGA-b-PEG	174 ± 12	NR	Sustained release	Aβ25-35-induced rats	IV	Inhibited AChE activity	Reduced anxiety-like behaviour in EPM, short-term memory deficits in NOR, and improved cognitive function in MWM	[59]
		Human serum albumin	~15	Physical bypass and cell membrane penetration using TAT peptide	Improved biocompatibility, and increased brain accumulation and retention	APP/PS1 mice	Intranasal	Reduced Aβ deposition and neuronal damage, and improved AChE regulation	Improved cognition performance on MWM	[61]
	Rivastigmine	mPEG-PCL	98.5 ± 2.1	NR	Sustained release, reduced immune clearance resulting in longer circulation time, and increased brain accumulation	Scopolamine-induced Wistar albino rats	IP	NR	Improved spatial memory in MWM	[66]
		PLGA-b-PEG with ascorbic acid	94.2	SCVT2 transporters – carrier-mediated	Sustained release, increased cellular absorption	Scopolamine-induced Sprague Dawley rats	IV	Reduced AChE activity	Improved cognitive performance in radial arm maze	[67]

		PCL-PEG-PCL	121.83 ± 4.58	Valine facilitates carrier-mediated transport	Sustained release	Scopolamine-induced Wistar rats	IP	Increased antioxidant activity and normalised gene expression of AChE, BDNF, and TNF-α	Improved memory in MWM	[69]
		Liposomal	343.25 ± 50.55	Physical by-pass	Improved bioavailability, lower clearance rate, increased residence time and half-life	Scopolamine-induced and colchicine-induced Wistar rats	Intranasal	Inhibited AChE activity	Improved memory deficits in MWM and passive avoidance tests	[71]
	Galantamine	Micelle (DSPE and PEG)	58.65	General amphiphilic nature and P-glycoprotein inhibitory effect increase cell internalisation	Sustained release, increased residence time and brain accumulation	β-amyloid-induced Wistar rats	Oral	Reduced oxidative stress	Enhanced cognitive performance in MWM	[73]
		PLGA-b-PEG with ascorbic acid	96.2 ± 3.5	SCVT2 transporters - carrier mediated	Sustained release, enhanced cellular uptake, improved brain accumulation	Scopolamine-induced Sprague Dawley rats	IV	Reduced AChE activity	Improved cognitive performance in MWM and Radial Arm Tests	[74]
		Polymeric particles - polyacrylic acid	185.55 ± 4.3	Physical bypass, and transporter-mediated absorption and increased membrane permeability via bile salt	Sustained release, direct drug delivery	LPS-induced Swiss mice	Intranasal	Reduced brain inflammatory cytokines, increased anti-inflammatory TGF-β, and decreased activation of astrocytes and microglia	Improved cognitive outcomes on the Y-maze	[75]
		Chitosan	201 ± 1.2	Physical bypass	Sustained release, increased brain accumulation and retention	Scopolamine-induced Spraque Dawley rats	Intranasal	Reduced AChE level and activity, Aβ1-42 levels, and histopathological degeneration, and higher suppression of Notch-1	Improved exploratory behaviour in Y-maze and memory in MWM	[76]
N-methyl-D-aspartate receptor antagonism	Memantine	Polymeric PLGA	152.6 ± 0.5 nm	NR	Sustained release, reduced immune clearance resulting in longer circulation time	APP/PS1 mice	Oral	Reduced Aβ plaques and inflammation	Enhanced cognitive recovery in MWM	[82]
		PAMAM dendrimer with lactoferrin	131.72 ± 4.73	Lactoferrin receptor-mediated transcytosis	Sustained release, increased brain uptake and bioavailability	AlCl3-induced Swiss mice	IP	No change in AChE activity	Improved recognition memory in ORM	[83]
		Solid lipid nanoparticles	159.9 ± 0.569	NR	Biocompatibility, sustained drug release, increased residence time, and	AlCl3-induced Wistar rats	Oral	Reduced Aβ levels	Improved spatial memory determined in MWM	[84]

					increased brain concentration					
		PLGA	58.04	Physical bypass	Sustained release, improved biocompatibility, increased brain uptake	Scopolamine-induced Sprague Dawley rats	Intranasal	NR	Improved spatial memory in MWM	[85]
Increase of dopamine concentration	L-DOPA	Tannic acid/polyvinyl alcohol	54	Physical bypass via SC injection at the neck to brain-lymphatic vasculature	Sustained release	Reserpine-induced Wistar rats	SC	Increased dopamine levels and improved markers of oxidative stress in the brain	Reduced motor dysfunction in pole-hold test, hanging test, and rotarod test	[99]
		PEG-*b*-poly(l-DOPA(OAc)$_2$)	52.2	NR	Sustained release and improved biocompatibility	MPTP-induced C57BL/6 mice	IP	NR	Improved performance in the grid walk test, narrow beam walk test, and resting tremor score. No induction of L-DOPA induced dyskinesia	[100]
	Dopamine	Albumin/PLGA	353	Albumin receptor-mediated transcytosis	Improved bioavailability	6-OHDA-induced Swiss mice	IP	Did not significantly restore DA neurons	Improved motor function in rotarod, adhesive removal, and apomorphine-induced rotation tests	[101]
		Polymeric (iron nodes and ditopic ligand BIX)	81 ± 4.0	Physical bypass	Improved biocompatibility, enhanced uptake, and sustained release	6-OHDA-induce Sprague Dawley rats	Intranasal	NR	Reduced motor symptoms in apomorphine-induced rotations	[102]
		Exosome	40–200	Transferrin receptor-mediated transcytosis	Improved biocompatibility and increased brain distribution	6-OHDA-induced Kunming mice	IV	Significantly increased striatal dopamine levels and reduced oxidative stress	Reduced amphetamine-induced rotations	[104]
Dopamine agonists	Ropinirole	PLGA	152.2 ± 3.1	NR	Sustained release	Rotenone-induced Wistar rats	IP	Reduced ROS production, astrogliosis, and cell death in the substantia nigra	Decreased catalepsy and akinesia and improved motor balance and coordination on rotarod tests and swim-tests	[110]
		Solid lipid nanoparticles and nanostructured lipid carriers containing hydrogel	210.6 ± 3.6	NR	Sustained release, enhanced permeation, and improved bioavailability	Haloperidol-induced Wistar rats	Transdermal	Restored lipid peroxidation levels, catalase activity and dopamine levels in the striatum	NR	[111]

	Rotigotine	PLGA with lactoferrin	118.4 ± 12.4	Physical bypass and receptor-mediated transcytosis	Targeted delivery, improved brain bioavailability, and sustained release	6-OHDA-induced Sprague Dawley rats	Intranasal	Reduction of dopaminergic neuron loss in the substantia nigra and striatum	Reduced apomorphine-induced rotations	[112]
		Chitosan	75.37 ± 3.37	Physical bypass	Improved brain bioavailability and increased residency time	Haloperidol-induced Sprague Dawley rats	Intranasal	Decreased LHD and increased catalase activity	Reduced catalepsy and akinesia, restored swimming ability	[113]
	Pramipexole	Chitosan	150–250	Physical bypass	Sustained release, increased residency time	Rotenone-induced Sprague Dawley rats	Intranasal	Increased dopamine levels in the brain	Increased locomotor activity and decreased catalepsy	[114]
Monoamine oxidase type B inhibition	Rasagiline	PLGA	221.7 ± 5.71	NR	Sustained drug release, increased half-life, enhanced bioavailability	Resperpine-induced Wistar rats	Transdermal	Increased DA, GSH and catalase, and decreased MAO-B	Improved locomotor activity as determined by catalepsy and open field tests	[115]
	Selegiline	Chitosan	165–255	Physical bypass	Sustained drug release, increased drug concentration in brain and plasma	Rotenone-induced Sprague Dawley rats	Intranasal	Restored dopamine levels and improved antioxidant action	Improved locomotor activity and catalepsy recovery	[117]

ICV = Intracerebroventricular; IV = intravenous; IP = intraperitoneal; SC = subcutaneous; NR = not reported

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Conflict of Interest

The authors declare no competing financial interest.

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