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Levodopa-loaded nanoparticles for the treatment of Parkinson's disease

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ABSTRACT

Parkinson's disease (PD) is a neurodegenerative disorder characterized by degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) resulting in dopamine (DA) deficiency, which manifests itself in motor symptoms including tremors, rigidity and bradykinesia. Current PD treatments aim at symptom reduction through oral delivery of levodopa (L-DOPA), a precursor of DA. However, L-DOPA delivery to the brain is inefficient and increased dosages are required as the disease progresses, resulting in serious side effects like dyskinesias. To improve PD treatment efficacy and to reduce side effects, recent research focuses on the encapsulation of L-DOPA into polymeric- and lipid-based nanoparticles (NPs). These formulations can protect L-DOPA from systemic decarboxylation into DA and improve L-DOPA delivery to the central nervous system. Additionally, NPs can be modified with proteins, peptides and antibodies specifically targeting the blood-brain barrier (BBB), thereby reducing required dosages and free systemic DA. Alternative delivery approaches for NP-encapsulated L-DOPA include intravenous (IV) administration, transdermal delivery using adhesive patches and direct intranasal administration, facilitating increased therapeutic DA concentrations in the brain. This review provides an overview of the recent advances for NP-mediated L-DOPA delivery to the brain, and debates challenges and future perspectives on the field.

1. Parkinson's disease

1.1. Epidemiology

Parkinson's disease (PD) is a neurodegenerative disorder where loss of dopaminergic neurons in the substantial nigra pars compacta (SNpc) results in dopamine (DA) deficiency [1]. After Alzheimer's disease (AD), PD is the second most prevalent neurodegenerative disease worldwide, with 35–100 per 100,000 individuals/year new cases [2], affecting 0.1–0.3% of the population in total [3]. PD mainly manifests in the elderly population, with the prevalence increasing almost 10-fold when comparing the 50–59 age group with the 70–79 age group, and is slightly more prevalent in males compared to females [3]. Due to a general aging of the population, global PD prevalence is expected to double by the end of 2030 [4]. The progressiveness of the disease, in combination with the lack of a cure, lead to a life expectancy that ranges between 6.9 and 14.3 years, with a median of 12.6 years, after PD diagnosis [3,5].

1.2. Symptoms and diagnosis

Patients suffering from PD often show motor and non-motor symptoms (NMS), which are, together with disease progression, highly variable between different patients [6]. The earlier stages of PD are hardly noticeable, where small inconveniences, also called prodromal features, including constipation, complications during rapid eye movement sleep and shoulder pain are the main symptoms [6,7]. However, as the disease progresses, which may take up to 10 years from first symptoms to diagnosis, NMS start to develop, including: olfactory loss (problems with sense of smell), sleep disorders (e.g. daytime sleeping), autonomic dysfunction (e.g. irregularities in urination and blood pressure variability), psychiatric disturbances (e.g. depression and anxiety) and cognitive impairment (e.g. dementia, problems with attention span) [6]. During later stages of PD, motor symptoms including bradykinesia (progressive deterioration of speed and size of movements), rigidity (resistance to passive movements of for example joints), tremor (involuntary rapid movement in rest) and postural instability (complications

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with balance and posture) start to develop [6]. In the absence of a unique diagnostic tool, PD diagnosis criteria include: 1) presence of 2 or more motor symptoms including bradykinesia, tremor at rest and rigidity, 2) presence of 1 or more NMS and 3) response to levodopa (L-DOPA) treatment [6–9]. However, approximately 60–70% of dopaminergic neurons have already died by the time PD diagnosis is confirmed [9].

1.3. Pathology and risk factors

The initial pathological feature of PD is loss of dopaminergic neurons in the SNpc, resulting in DA deficiency which progressively worsens over time [2]. This neurodegeneration is associated with accumulation of α-synuclein (α-syn, *SNCA*) aggregates within the dopaminergic neurons of the SNpc [2,10,11]. Under physiological conditions these α-syn aggregates are cleared, but due to a defect in the ubiquitin proteasome system (UPS) they accumulate in intracellular inclusions termed Lewy Bodies (LBs) instead. This results in impaired lysosomal function and autophagy, which can also be caused by mutations in the leucine-rich repeat kinase 2 (*LRRK2*) gene [2,12–14]. These LBs are known to interfere with cellular functions, increase cellular stress and eventually cause cell death. Additionally, mitochondrial dysfunctions attributed to mutations in the parkin RBR E3 ubiquitin protein ligase (*PARKIN*) and PTEN-induced kinase 1 (*PINK1*) genes, result in increased reactive oxygen species (ROS) production [2]. ROS contribute to increased cellular stress and neuronal cell death, ultimately leading to DA depletion within the SNpc [2,15–17]. While cell death leading to PD is believed to be mainly localized within the dopaminergic neurons of the SNpc, recent research has illustrated that as PD progresses, LB formation and neurodegeneration spread to other brain regions, including the cerebral cortex, optic bulb and the autonomic nervous system [18]. In addition to the genetic factors which are responsible for 5% of familial PD cases, environmental factors like exposure to certain pesticides have also been linked to increased risk of PD development [12,19]. Several studies have demonstrated that increased exposure to the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) resulted in rapid and significant degeneration of dopaminergic neurons, ultimately leading to PD phenotypes [7,8,18]. The mechanism behind MPTP-driven neurodegeneration is irreversible inhibition of mitochondrial complex I, resulting in mitochondrial-dependent apoptosis within the SNpc [2]. These observations support the now common believe that PD is caused by a combination of genetic predisposition and external factors, which mainly impact processes associated with mitochondrial biogenesis and clearance as well as lysosomal clearance of *SNCA* [2]. Additionally, other risk factors including head trauma, diabetes, hypertension and cancer have been linked with PD, however the underlying mechanisms are unknown [20].

2. Current treatments for PD

2.1. Overview of available PD treatments

Due to the inability to slow, stop or reverse the progression of dopaminergic neuronal degradation, current PD treatments focus on the reduction of both motor symptoms and NMS [6]. The 4 main treatment strategies include: physical therapy (e.g. physiotherapy, treadmill exercise and flexibility training) [21,22], rehabilitating therapy (e.g. speech therapy), pharmacological therapy (e.g. L-DOPA) and surgery (e.g. deep brain stimulation, DBS) [6,13]. Because of the high variability in symptom severity per patient, one or more of the above-mentioned treatments are generally applied. Moreover, due to the risk of the procedure, surgical treatments like DBS are only applied when other treatment options fail as a result of induced tolerance or severe motor symptom fluctuations [6]. Out of the 4 main treatment strategies, pharmacological therapy is most effective at treating motor symptoms and is therefore nearly always included in treatment of PD. Although intravenous (IV) administration by means of infusion is the most effective method for sustained and constant systemic drug levels, most drugs are delivered via the oral route because of patient compliance [23,24]. There are three main classes of drugs that are used in PD therapy: 1. L-DOPA, a precursor of DA that increases DA levels within the SNpc, 2. DA agonists (e.g. apomorphine and ropinirole), which are drugs that act on DA receptors on the post-synaptic terminal, 3. monoamine oxidase type B (MAOB) and catechol *O*-methyltransferase (COMT) inhibitors, (i.e. selegiline and entacapone) that inhibit DA degradation and catabolism [13]. Early-stage PD treatment is usually started with relatively mild drugs including MOAB and COMT inhibitors [18]. These drugs are administered daily and can cross the blood-brain barrier (BBB) thereby allowing them to reach the brain where they inhibit DA degradation. This approach preserve available DA storages and increase overall DA concentrations, resulting in slight motor symptom reduction with little to no side effects [19,25]. However, as PD progresses and motor symptom severity increases, these inhibitors are unable to sufficiently suppress disease symptoms, and therefore DA agonists are often added to the treatment regime. DA agonists, like apomorphine and ropinirole, are small drugs with lipophilic properties, allowing them to readily cross the BBB [13]. DA agonists are able to mimic DA function through activation of D1-like and D2-like receptors in various brain regions and are therefore moderately effective at the reduction of motor symptoms [26]. Depending on the type of DA agonist prescribed, oral administration or transdermal patches are used [12]. While DA agonists generally show a higher efficacy compared to MAOB and COMT inhibitors, they can cause several side effects including nausea, hallucinations, sleep disorders, impulse control disorders and psychosis [6,12,27].

2.2. L-DOPA treatment

During the later stages of PD, either due to a decrease in treatment efficacy or severity of side effects caused by DA agonists, PD treatment regimens are switched to oral L-DOPA [13]. Currently, oral L-DOPA is still the golden standard for PD treatment as it shows the highest efficacy for motor symptom reduction [28]. After systemic uptake, L-DOPA can cross the BBB and reach the brain, where it can be converted to DA by aromatic L-amino acid decarboxylase (AAAD), also known as DOPA decarboxylase (DDC) [27]. This L-DOPA to DA conversion mainly takes place within the presynaptic terminals of the dopaminergic neurons in the SNpc, resulting in an increased DA concentration in these neurons, significantly reducing motor symptoms [27,28]. However, while L-DOPA displays improved motor symptom reduction compared to the other 2 pharmacological drugs, there are several disadvantages to L-DOPA treatment. L-DOPA is a hydrophilic compound that is rapidly degraded by enzymes within the gastrointestinal (GI) tract and decarboxylated by DDC during hepatic first pass metabolism as well as in the systemic circulation, resulting in a half-life of approximately 50 min [29,30]. This rapid conversion of L-DOPA within the systemic circulation not only results in low oral L-DOPA bioavailability (approximately 30%) and low percentages of the L-DOPA reaching the brain (approximately 1%), but also leads to DA exposure to the rest of the body, causing several adverse effects [31]. The most common side effects of short-term L-DOPA treatment include dizziness, headaches, vomiting and insomnia, while long-term L-DOPA treatment often results in involuntary movements and dyskinesias, severely decreasing quality of life and patient compliance [32,33]. To counteract these systemic adverse side effects and increase L-DOPA bioavailability, the currently prescribed L-DOPA formulations consist of L-DOPA that is co-administered with carbidopa [34]. Carbidopa is a peripheral amino acid decarboxylase inhibitor which inhibits L-DOPA conversion by DDC in the systemic circulation, while being unable to cross the BBB and enter the brain [13,29]. This L-DOPA/carbidopa co-administration therefore not only results in a reduction of adverse effects due to reduced systemic DA concentrations, but also increases L-DOPA half-life from 50 min to 1.5 h, leading to increased brain uptake from 1% to

approximately 5–10% [28,35]. To further increase L-DOPA half-life, current prescribed PD treatments like RYTARY™ (formerly known as IPX066) utilize a combination of carbidopa and levodopa in immediate release capsules as well as extended-release capsules [36,37]. While these treatments are very advantageous in the earlier stages of PD, administered in starting doses of 23.75 mg carbidopa/96 mg L-DOPA 3 times a day, the required dose increases as PD progresses and can reach 612.5 mg carbidopa/2450 mg L-DOPA per day depending on disease severity [36]. This necessary increase in treatment dose is a result of progressive neurodegeneration. Whereas in the earlier PD phases the dopaminergic neurons can store L-DOPA, as PD progresses and more dopaminergic neurons die this buffer function is lost, resulting in depleted brain DA storage. Eventually brain DA concentrations will then resemble blood DA ones, resulting in so-called "L-DOPA tolerance" [38]. Therefore, higher and more frequent L-DOPA/carbidopa dosages are required to induce symptom relief as PD progresses [38]. Due to this dosage increase, the "on-off phenotype" is developed, where DA levels spike just after treatment and are low between two consecutive treatments [28]. It is now believed that these swings in DA concentrations between treatments are causative for severe adverse effects like dyskinesias, stressing the importance of a continuous and constant DA supply [30]. The severity of these adverse effects increases with time, ultimately surpassing the beneficial effect of the treatment. Recent research indicated that L-DOPA/carbidopa treatment sustained long-term benefits in only 20% of patients after 2 years, while >75% of these patients experienced serious adverse events [28,32].

2.3. The BBB

To reduce side effects and improve patient compliance, increased L-DOPA bioavailability and brain delivery is required in order to minimize free systemic DA. One major hurdle for the effective and targeted delivery of any therapeutic compound to the brain is the BBB, which is responsible for the discontinuation of approximately 95% of potential therapeutic molecules for treatment of brain disorders [39]. The BBB is the gatekeeper of the CNS and maintains a strictly controlled brain microenvironment by selection of molecules that can enter the brain [31]. The BBB consists of multiple cell types, including unfenestrated endothelial cells (ECs) which are connected through tight junctions (TJs), pericytes, astrocytes and microglial cells [40]. While the BBB is highly selective, certain molecules like nutrients and amino acids (AAs) are able to cross it through two main pathways: the transcellular pathway and the intracellular pathway [40]. Due to the high number of TJs and adherens junctions (AJs), transcellular transport is mainly utilized by small hydrophobic molecules (MW <400 Da), while intracellular transport is mainly employed by hydrophilic macromolecules [41,42]. Examples of intracellular transport across the BBB are the carrier-mediated transport (CMT) of glucose through glucose transporters GLUT1 and GLUT3, receptor-mediated transcytosis (RMT) of larger macromolecules through for example the low-density lipoprotein receptor, and adsorptive-mediated transcytosis (AMT), allowing transport of charged proteins through electrostatic interactions between the proteins and the ECs [40,42]. Additionally, recent research has illustrated that BBB permeability is altered in diseases involving inflammatory, traumatic or degenerative conditions becoming disrupted and allowing the passage of more and larger molecules [39,43,44]. While the exact mechanisms of BBB disruption are still unknown, disrupted EC junctions are believed to be at the base of this phenomenon [39]. Though the high selectivity of the BBB limits the effective L-DOPA delivery to the brain, insight into the mechanisms responsible for the facilitation of BBB transport in combination with altered BBB permeability in PD, opens a window for precise L-DOPA targeting to the CNS, with the potential to increase L-DOPA bioavailability and reduce free systemic DA [1,39].

3. Nanoparticles for improved L-DOPA brain delivery

3.1. Nanoparticle composition

Improved L-DOPA targeting to the CNS as well as protection from systemic conversion by AAAD is required to decrease the L-DOPA dosage, increase bioavailability and reduce systemic side effects [44]. The use of nanoparticles (NPs) for the encapsulation and targeting of PD drugs to the BBB or the CNS has been explored in the past decades [44–46]. NPs are small colloidal nano-sized carriers (usually between 10 and 200 nm in size) that can either be of inorganic, or organic origin or a hybrid of both [47]. Inorganic NPs, usually consist of metals or quantum dots and display low batch-to-batch variability. They are easily controlled in size, easy to modify and to track using different imaging techniques and are therefore mainly utilized for imaging rather than drug delivery [13,44]. Organic NPs can consist of virtually all biological products, but usually contain either lipids, polymers or proteins. Unlike inorganic NPs, organic NPs display high biocompatibility, low toxicity and are easily modified for better BBB targeting [13]. Organic NPs can successfully encapsulate L-DOPA, thereby preventing its systemic degradation and increasing its circulation time. This potentially results in increased brain uptake through the BBB, leading to a decreased required L-DOPA dose [40]. Additionally, organic NP properties can be tailored to increase specific BBB targeting and facilitate targeted uptake into the CNS [13]. First, NP size is an important feature to overcome the BBB, where BBB penetration decreases as NP size increases [44]. Secondly, zeta potential (surface charge) strongly influences the biological fate of NPs. A negative zeta potential increases NP circulation time while reducing protein absorption, and a positive zeta potential facilitates AMT across cellular barriers through interactions with negatively charged plasma membranes [13,44]. Although positively charged NPs have been associated with increased brain uptake, positive charges which are too high have been linked with immediate BBB toxicity [44,48]. Thirdly, NP hydrophobicity impacts the pathway of NP passage across the BBB, where hydrophobic NPs tend to utilize the receptor/carrier mediated paracellular pathway, and the hydrophilic NPs employ transcellular diffusion [13,47]. Therefore, NP biomaterial composition should be carefully considered and optimized to ensure appropriate size, hydrophilicity and zeta potential to facilitate BBB penetration.

3.2. Organic NP modifications to increase BBB penetration

In addition to the possibility to modify NP composition in order to control parameters like size, hydrophobicity and zeta potential, organic NPs are often modified with ligands at their surface, which aims to increase BBB targeting and therefore penetration [44]. These ligands can be classified into 3 different types based on the mechanism they facilitate: 1. Ligands that directly target receptors or carriers located on the BBB. These ligands often involve antibodies, peptides or proteins ligated to the surface of the NP specifically targeting receptors known to be overexpressed on the BBB [49]. Through direct interaction between NP-coupled ligands and receptors, either RMT or CMT is facilitated, increasing BBB penetration (Fig. 1C/D) [13]. Commonly targeted receptors that are upregulated by the BBB and initiate RMT include transferrin receptors, insulin receptors, leptin receptors, low-density lipoprotein receptors and lactoferrin receptors [50]. Additionally, the brain's requirement for energy can also be exploited to increase NP uptake, for instance through NP modification with glucose specifically targeting GLUT-1 mediated transport across the BBB into the CNS [49,51,52] 2. Ligands that increase NP hydrophobicity and charge. These ligands are either built into the NP or modified at the surface and increase either hydrophobicity or zeta potential [44]. Examples include NPs coated with amphiphilic peptides to increase hydrophobicity, or altered NP composition to increase zeta potential [44,53]. CNS uptake is then stimulated through either lipophilic transcellular transport (Fig. 1A), paracellular transport (exclusively for smaller NPs size)

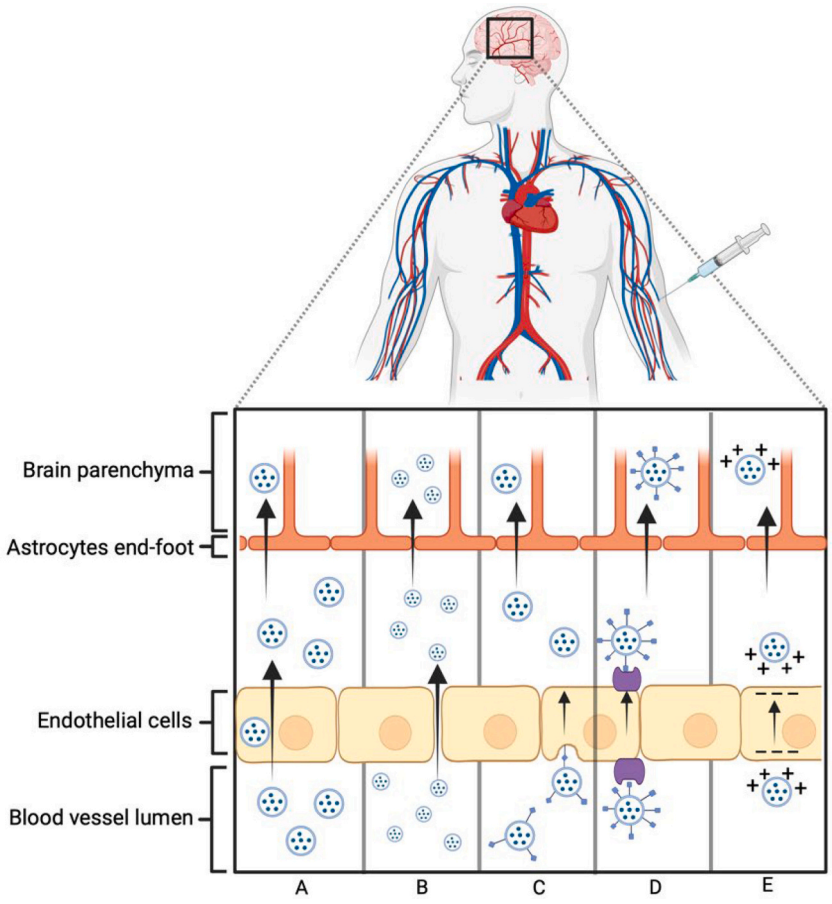

Fig. 1. NP modifications which increase BBB penetration. Different NP modifications aim to increase BBB penetration exploiting several mechanisms. NP lipophilicity can be increased to increase lipophilic transcellular transport of larger NPs (A) or hydrophilic paracellular transport of smaller NPs (B). Protein and antibody modifications specifically targeting receptors and carriers on the BBB facilitate receptor-mediated transcytosis (RMT) (C) or carrier-mediated transcytosis (CMT) across the BBB (D), while increased NP zeta potential improves AMT (E).

(Fig. 1B) or AMT (Fig. 1E) [44]. 3. Ligands and other molecules that disrupt the BBB and are either conjugated to the NPs or are co-administered, aiming at temporary disruption of TJs and AJs connecting the ECs of the BBB [40]. These include cell penetrating peptides (CPPs), hyperosmotic agents, surfactants and AAs [40,42]. While being an effective method, TJs can only be opened to a certain extent, so exclusively small NPs (<20 nm) can utilize this pathway [40,41]. Additionally, these ligands are aspecific and might become neurotoxic if used on a long-term basis, therefore bringing additional limitations and risks compared to the ligands mentioned in points 1 and 2 [40,41].

3.3. Lipid-based and polymeric NPs

While there are virtually endless configurations for organic NPs, lipid nanoparticles (LNPs) and polymer-based NPs are mostly studied because of their high biocompatibility, stability, low toxicity, potential to customize to control biological fate (e.g. targeting to the BBB) and drug release capability [13]. Due to the nature and composition of these NPs, polymers and polymeric micelles are mostly used for delivery of hydrophobic drugs, while liposomes and LNPs are more suitable for the delivery of hydrophilic drugs and oligonucleotides [13]. Even though there are limited NP-based treatments currently on the market, numerous lipid and polymeric NPs have been developed and investigated and are currently tested in clinical pipelines.

3.3.1. Polymeric NPs

Polymeric NPs are composed of one or more synthetic or natural polymer(s) which are assembled to form vesicles that are biocompatible, biodegradable and exhibit controlled and sustained release properties [40,54]. The simplest type of polymeric NP is the nanocapsule, in which a drug is encapsulated by a single polymer vesicle [54]. While there are practically endless polymeric NP *co*nfigurations, the most widely investigated FDA approved polymers include poly(ethylene glycol) (PEG), poly(trimethylene carbonate) (PTMC), poly(lactic-co-glycolic acid) (PLGA) and chitosan, due to their sustained-release properties in combination with their low toxicity and favorable safety profiles [40,55]. Examples of currently explored *co*-polymer-based NPs for brain delivery of PD drugs are PEG-PTMC NPs, developed by Wang and co-workers [55]. They demonstrated that PEG-PTMC NPs, 78 nm in size with a surface charge of approximately −10 mV, could be efficiently loaded with PD drugs which showed a partial rapid release over 4 h in vitro, as well as sustained release properties for up to 48 h. In vivo pharmacokinetic experiments in rats demonstrated a significantly increased plasma concentration as well as brain concentration of the PD drug Ginkgolide B (GB), compared to free drug after oral administration, which was sustained for up to 48 h [55]. More sophisticated copolymers include the utilization of amphiphilic copolymers, which can assemble into NPs consisting of a hydrophilic shell and a hydrophobic core, called polymeric micelles. These polymeric micelles are generally stable, can be modified to facilitate targeting to the BBB, and enable sustained drug release which can be tailored to respond to external stimuli [41,55]. Liu et al. studied polymeric micelles for the use of increased and sustained brain delivery, through the generation of PEGylated micelles modified with cell penetrating transactivator of transcription (TAT) peptides [56]. These micelles self-assembled into NPs of 180 nm or smaller, showed efficient drug loading and illustrated an in vitro sustained drug release for 6 h in PBS at body temperature. Moreover, increased cellular uptake in an in vitro human astrocytic model was also found upon addition of these micelles. Interestingly, these PEGylated micelles showed significantly increased BBB targeting and brain delivery after IV administration in rats, which was visualized by imaging of fluorescein 5-isothiocyanate (FITC)-loaded micelles and compared to free injected

FITC [56]. Although none of the polymeric NPs have received FDA approval yet for treatment of PD, some of these polymers are already applied in other treatments, where they are used to coat NPs, stabilize proteins and facilitate controlled hormone release in the treatment of for example prostate cancer [54,57,58]. The main advantage of polymeric NPs over other carrier systems is the variety of available polymers in combination with the possibility to modify their surfaces. This allows fine-tuning of NP composition to accurately control NP properties like size, hydrophilicity, surface charge, circulation time, drug release profile, degradation rate, and stimuli to external responses [54,55,59]. However, polymeric NP disadvantages comprise toxicity from degradation products, premature or incomplete drug release upon in vivo administration, batch-to-batch variation and difficulties in upscaling production [54,55,60]. Additionally, specific antibodies are formed against polymers used to coat the NP surface, such as PEG, upon multiple administrations. These antibodies induce faster clearance, leading to shortened circulation times. This process is referred to as the accelerated blood clearance (ABC) phenomenon [61–63].

3.3.2. Lipid-based NPs

Lipid-based NPs comprise carriers composed of one or more types of lipids. The most used and studied lipid-based NPs are liposomes, consisting of a bilayer of phospholipids with a hydrophilic aqueous core, micelles, consisting of a single layer of phospholipids with an aqueous core, and solid lipid NPs (SLNs) containing a solid hydrophobic core [41,54,60]. Liposomes and micelles are mostly used for the encapsulation and delivery of hydrophilic compounds entrapped in their aqueous core. However, hydrophobic and lipophilic payloads could also be loaded to some extent within their hydrophobic lipid (bi)layer(s) [40]. Compared to polymeric NPs, lipid-based NPs are more biocompatible and show decreased toxicity, and several liposomal formulations are currently on the market for applications including treatment of specific cancers, delivery of viral vaccines and treatment of fungal diseases [64,65]. Additionally, because of their relatively small size and composition, they can readily pass the BBB without any functional modifications, through either the hydrophobic transcellular pathway or the lipophilic paracellular pathway (Fig. 1) [60]. Additionally, these lipid NPs are more cost effective and easier to scale up compared to polymer-based NPs [13,45,54,60]. Functional liposome modifications can aid direct targeting to the BBB through liposome modifications with BBB-specific antibodies or ligands to facilitate RMT and CMT as described in section 3.2 [55]. Examples of surface modifications employed for targeting of the BBB include specific antibodies, mannose and the CPPs penetratin and rabies virus glycoprotein (RVG) peptide [66]. Additionally, the circulation time of lipid-based NPs can be increased through surface modifications including PEG coating and modifications neutralizing liposome charge, disguising liposomes from the reticuloendothelial system (RES), creating so-called “stealth liposomes” [41,67,68]. Although these modifications are essential for effective liposome-mediated L-DOPA delivery to the brain, they also induce specific antibody production leading to accelerated clearance, as described in 3.3.1. While recent research has demonstrated that encapsulation of PD drugs in NPs can improve circulation kinetics and targeting to the CNS, there are some shortcomings and questions to be answered regarding this strategy. First, there is an ongoing debate whether entire NPs can penetrate the BBB or whether the drug is released in the BBB before reaching the CNS [39,41,44,69,70]. Additionally, oral administration of NPs is very challenging. The major hurdles that orally administered NPs face are digestion within the GI tract, EC barriers and TJs, as well as extensive first pass metabolism in case of reaching the circulation [71]. The natural barriers result in the excretion and degradation of 85–90% of orally administered NPs, with only 2–3% of orally administered drug reaching the bloodstream after 30 min, highlighting the inefficiency of this delivery route [72]. Hence, modifications to protect NPs in the GI tract and to facilitate crossing of cellular barriers are required to optimize oral NP delivery [23]. To circumvent these issues, most polymeric and lipid-based NP formulations are designed for IV administration [68]. However, current NP formulations improve drug release for no more than a few days at best, which would result in frequent hospital visitations for PD patients, thereby drastically reducing patient compliance [55]. Therefore, alternative strategies are required to enable delivery of PD drugs to the CNS.

4. Transdermal patches

Although the previously described oral and IV administration routes are able to increase drug delivery to the CNS and reduce PD symptoms, these routes are limited by either low oral bioavailability or frequent hospital visits, respectively [31]. An alternative administration route entails the delivery of PD drugs through transdermal patches. These patches facilitate sustained drug release through the skin, thereby reducing treatment frequency while enabling self-administration by PD patients and increasing patient compliance. Obaidat et al. designed L-DOPA loaded xanthan gum and Carbopol 971 transdermal patches, which were lined with b-cyclodextrin to increase L-DOPA stability. These patches were shown to provide sustained L-DOPA release for up to 6 h in vitro, with no in vivo data shown [73]. Similarly, Nair et al. formulated polyvinylpyrrolidone transdermal patches loaded with L-DOPA/carbidopa and spread on a polyester release liner. Transdermal delivery of L-DOPA and carbidopa by transdermal patches in healthy rats illustrated increased L-DOPA plasma concentrations compared to transdermal delivery of naked L-DOPA, sustaining for up to 8 h [74]. While these papers illustrated the possible advantages of transdermal L-DOPA delivery, therapeutic concentrations of systemic L-DOPA were significantly below therapeutic range, and steady-state drug concentrations were not maintained [74]. A likely explanation is the instability of L-DOPA, causing degradation within the transdermal patch as well as after systemic absorption, resulting in low L-DOPA brain concentrations [29,30]. Additionally, the transdermal delivery of naked L-DOPA is limited by poor permeability through the skin [75] and high BBB selectivity, as described previously. A possible solution could be the encapsulation of L-DOPA in NPs, protecting L-DOPA from degradation and decarboxylation both in the transdermal patch and after systemic absorption, while enabling modifications for improved BBB targeting [44]. Therefore, Sintov et al. designed a self-assembling nanomicellar hydrogel system loaded with 2% L-DOPA and 1% carbidopa for transdermal delivery. In vivo pharmacokinetic studies in healthy rabbits revealed that L-DOPA plasma levels of this self-assembling nanomicellar hydrogel peaked at about 0.6 μg/ml after 12 h, which was fully cleared after 24–28 h. Interestingly, the self-assembling nanomicellar liquid patch resulted in 0.8–1 μg/ml peak plasma concentrations after daily patch application, which increased to 3–4.5 μg/ml when applied twice-daily, reducing dosing frequency while simultaneously avoiding fluctuating plasma L-DOPA concentrations, which are linked with severe side effects as described earlier [75]. While no further publications describe the transdermal delivery of NP-encapsulated L-DOPA, the NP-encapsulated transdermal delivery of other PD drugs has been studied. Nikhil et al. described the generation of selegiline-loaded polymeric PLGA NPs embedded in ethylene vinyl acetate transdermal films, which were transdermally delivered in reserpine-induced PD rats [76]. In vivo pharmacokinetic studies showed increased selegiline half-life and mean residence time after transdermal delivery in PLGA NPs compared to plain drug, and a 13-fold increase in area under the curve (AUC) when comparing transdermal PLGA NP delivery to IV administration, being detectable for up to 72 h after transdermal delivery. Additionally, biodistribution studies illustrated a 137% increase in brain drug-targeting efficiency and a 27% increase in brain drug-targeting potential. In vivo experiments in reserpine-induced PD rats illustrated a slight reduction of cataleptic activity after transdermal delivery of selegiline PLGA NPs, with no alterations in an open field test [76]. In another study, the authors embedded rasagiline mesylate loaded polymeric PLGA NPs in gellan gum transdermal films and observed undetectable

initial rasagiline mesylate concentrations after transdermal delivery, which increased over time and showed sustained release for 72 h, peaking at 24 h, with stable brain-drug concentrations for up to 70 h [77]. Behavioral tests in reserpine-induced PD rats showed reduced cataleptic activity for up to 24 days as well as increased number of crossovers and overall movement in an open field test after transdermal PLGA NP delivery. Mechanisms explaining functional improvement include brain dopamine concentration restoration, prevention of neuronal damage caused by oxidative stress and inhibition of dopamine catabolizing MAOB enzyme [77]. While the usage of polymeric NPs is effective, Prabhu et al. generated curcumin-loaded solid lipid NPs that were transdermally delivered in a dissolvable microneedle patch [78]. Neuroprotective studies in PD mice illustrated significantly decreased degree of bradykinesia in a pole test and improved motor coordination and balance ability in a rotarod test, while showing no signs of skin irritation or sensitivity [78]. These studies suggest that transdermal delivery of NP-encapsulated L-DOPA is feasible, however limited evidence of therapeutic L-DOPA concentrations reaching the brain exists, likely as a result of poor skin permeability and L-DOPA instability as described before.

5. Intranasal delivery of PD drugs

5.1. Intranasal delivery routes

While the previously described transdermal and IV administered NPs focus on targeting to – and facilitation of crossing the BBB, an alternative strategy is to avoid the BBB altogether [13]. Over the past decade, increasing evidence supports the existence of a more direct delivery route between the nose and the CNS [27,79]. Direct nose-to-brain delivery is a non-invasive and easy to self-administer pathway which circumvents some of the major flaws of IV administration or oral and transdermal delivery [59]. These advantages include, evasion of degradation in the GI tract and hepatic first-pass metabolism [72], and reduction of free systemic drugs and DDC inhibitors [59]. Upon intranasal administration, drugs are deposited on the respiratory and olfactory epithelium, and can be absorbed via either the nose-to-blood-to-brain pathway or the direct nose-to-brain pathway [59,80]. For the nose-to-blood-to-brain pathway, the drug deposited on the respiratory epithelium can be absorbed through the fenestrated nasal epithelial cells of the well-vascularized lateral walls of the nasal cavities [81]. From there, the drug can enter the peripheral circulation, after which it may pass to the CNS in case the drug can cross the BBB or the more permeable blood-cerebrospinal fluid barrier (BCSFB) [59,81]. Alternatively, drugs deposited in the olfactory region of the nasal cavity can be directly transported to the CNS in a matter of minutes via the olfactory or trigeminal nerves, which are the only direct connection between the brain and the rest of the body [81,82]. These direct nose-to-brain routes can be crossed via intracellular and extracellular transport pathways [81,82]. During the intracellular route, therapeutics are internalized within the olfactory and trigeminal neurons via endocytosis, after which they are transported within the endosome towards the neuronal axons where they are released in the olfactory bulb via exocytosis before finally reaching the brain stem (Fig. 2) [59,81]. During the extracellular route, drugs penetrate the TJs of the olfactory epithelium and trigeminal nerve, after which they migrate through the paracellular space of the nasal epithelium along the length of the neuronal axon before reaching the CNS (Fig. 2) [59,79]. After reaching the brain stem, therapeutics are either absorbed in blood/lymphatic vessels or further distributed to other parts of the brain through the perivascular pump driven by arterial pulsation or transported back to the nasal cavity via P-glycoprotein (P-gp) efflux proteins (ATP-binding cassettes present in cell membranes able to export foreign substances) [83–85]. Unlike the systemic route, direct nose-to-brain delivery facilitates fast transport to the CNS, reaching the target site within minutes [59]. Salameh and coworkers demonstrated the presence of labeled insulin in and around the olfactory bulb only 5 min after nasal administration in rats, with the insulin reaching all parts of the brain within 30 min [86]. Additionally, Chao and coworkers demonstrated the rapid effect of intranasally administered L-DOPA on PD rats, where intranasal L-DOPA treatment illustrated mild reductions of modeled PD symptoms like turning behavior, foot slips and motor asymmetry 10 to 20 min after treatment administration,

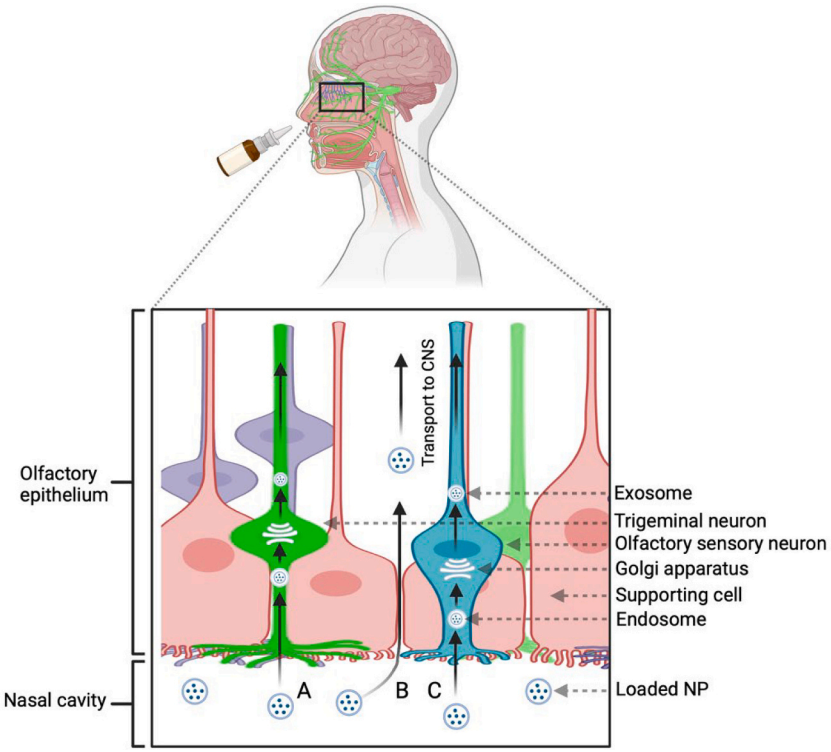

Fig. 2. Mechanisms of intracellular and extracellular nose-to-brain transportation. During the intracellular pathway, therapeutics enter the trigeminal (A) and olfactory sensory neurons (C) through endocytosis, after which they travel within the endosome, through the Golgi Apparatus, towards the neuronal axon where they are released in the olfactory bulb through exosomes. During extracellular transport (B), drugs translocate through TJs connecting the trigeminal and olfactory sensory neurons and supporting cells, after which they move through the paracellular space along the neuron axon, through the subarachnoid space before reaching the CNS.

which could be sustained for approximately 60 min [87].

5.2. NPs for improved nose-to-brain delivery

Direct intranasal administration of PD drugs has been demonstrated to result in mild symptom relief. Nevertheless, these benefits are minor and short-lived due to several problems encountered for this delivery method. First of all, particles and molecules deposited on the olfactory epithelium which are smaller than 10 μm are trapped in the nasal mucosa and are cleared within minutes, giving therapeutics limited time to be absorbed [82]. This rapid mucociliary clearance in combination with the relatively small surface area of the olfactory epithelium only allow small volumes of drug administration (25–200 μL in humans). Moreover, the active enzymatic degradation of deposited compounds by peptidases and proteases results in limited drug absorption into the CNS, and therefore exerts limited and short-lived therapeutic benefits [81,84]. For this reason, current research studying intranasal delivery of PD drugs focuses on the encapsulation of these drugs using NPs, protecting them from enzymatic degradation, increasing retention time on the olfactory epithelium, facilitating sustained release, stimulating transport across the olfactory and trigeminal nerves, and protecting drug from P-gp efflux proteins after CNS penetration [81,88]. As described in section 3, there are many different NP formulations currently under investigation, including polymeric NPs, lipid nanocarriers and mucoadhesive agents [81]. While the pros and cons of the different NP formulations have been described in detail in section 3, there are some additional considerations when employing NPs for intranasal delivery. An important characteristic for intranasal delivery is NP size, where a small size (10–90 nm) is associated with increased brain uptake through the rapid extracellular olfactory and trigeminal nerve pathways [88]. pH is an additional important characteristic for intranasal drug absorption and safety, with the human nasal mucosal pH ranging between 5.5 and 6.5, the pH of NP formulations should be in this range to facilitate improved transportation through nasal mucosa into the olfactory and trigeminal nerves [64]. Finally, total protein concentration in cerebral spinal fluid is 50–100 times lower than in blood plasma [89]. This can result in reduced adsorption of proteins to NPs and therefore decrease or delay immune recognition.

5.2.1. Polymeric NPs for intranasal delivery

One of the most studied types of drug delivery vehicles for intranasal delivery are polymeric NPs. Their main functions include drug protection, increase stability, and improve drug transport into the brain [81]. The wide range of available polymers to control NP properties and the possibility for functional modifications potentially facilitate specific brain delivery through this route [59]. Examples of polymeric NP modifications include modification of PEG-PCL micelles with CPP TAT, aiming to increase NP transport through the extracellular and intracellular olfactory and trigeminal neural pathways [90]. Kanazawa et al. demonstrated a 5-fold increase in drug uptake in the brain after intranasal NP delivery compared to IV administration, which was significantly further improved after TAT modification, showing a peak increase in NP concentration in the olfactory bulb 15 min after intranasal administration, and in the entire brain after 1 h [90]. Another study investigated a similar approach by modifying L-DOPA-loaded PEG-PLGA NPs with wheat germ agglutinin (WGA), to increase direct NP transport across the neural pathways [27]. The authors demonstrated therapeutic concentrations of DA within the brain and marginal free systemic DA in a PD mouse model while showing NP tolerance and low short-term toxicity [27]. However, previous research has shown the induction of minor toxicity and oxidative stress after short-term treatment with polymeric NPs modified with lectins (like WGA) and CPPs, although lacking data on safety of longer-term treatment [88,91]. Moreover, Liu and coworkers modified PEG-PCL NPs with lactoferrin, which is an iron-binding protein mainly expressed in respiratory epithelial cells and neurons, to enhance endocytosis-mediated brain uptake and direct translocation to the CNS. [88]. The authors discovered that lactoferrin-modified NPs resulted in increased coumarin-6 delivery to the cerebrum, cerebellum, olfactory tract, olfactory bulb and hippocampus when compared to unmodified "naked" NPs in healthy rats, which could be detected for up to 8 h, while showing reduced drug levels in the blood [88]. Interestingly, tracking the labeled lactoferrin-modified NPs demonstrated that the entire NP was translocated to the CNS [88]. Most studies do not specifically focus on increasing NP penetration into the CNS but rather aim to increase NP retention and residence time on the olfactory epithelium through NP modification with chitosan. Chitosan has been illustrated to display mucoadhesive properties as well as a positive effect on epithelial membrane permeability [29,81]. Dimiou and coworkers designed a self-assembling N-palmitoyl-*N*-monomethyl-*N*,*N*-dimethyl-*N*,*N*,*N*-trimethyl-6-O-glycolchitosan polymeric NP (GCPQ) loaded with L-DOPA, which displayed a significant increase in brain DA levels compared to unmodified L-DOPA after intranasal administration in rats [1]. Remarkably, they discovered that the brain DA concentrations kept increasing for 2 h after intranasal administration, highlighting the benefits of the mucoadhesive properties in combination with sustained drug release from polymeric NPs [1]. Similarly, Ahmad et al. designed chitosan-modified PLGA NPs which demonstrated a 2-fold increase in rat brain L-DOPA concentrations compared to free L-DOPA [29].

5.2.2. Lipid-based NPs for intranasal delivery

Alternatively, lipid-based NPs are under investigation as vehicles for intranasal drug delivery. Lipid-based NPs have shown the ability to penetrate epithelial cells more easily due to lipophilic properties, causing no cellular damage and necrosis in the nasal mucosa or the CNS [85,92]. However, efficient drug loading and major systemic bioavailability after intranasal delivery are challenges to overcome [85,92]. To better control the problems with drug loading, SLNs are currently the most studied lipid-based NPs for intranasal delivery [64,85]. Pardeshi and coworkers developed ropinirole hydrochloride loaded SLNs with surface-modified stearylamine-induced cationic charge for improved SLN loading and stability [93]. Intranasal administration in a PD mouse model demonstrated significant reduction in PD symptoms, such as tremors and immobility, compared to oral formulations, even at lower dosages [93]. Similarly, another study described stearic acid- and lecithin-coated SLNs for the intranasal delivery of astaxanthin [94]. Biodistribution studies in healthy rats elucidated a 2-fold increase in brain astaxanthin levels compared to IV administration, which could be maintained for approximately 4 h. Nevertheless, a significant percentage of astaxanthin could be found in the peripheral blood, lungs, kidneys, liver and particularly the intestine, underlining the high systemic bioavailability and subsequent excretion [94]. In order to overcome high systemic bioavailability, Gartziandia et al. prepared lipid-based NPs consisting of Precirol ATO5, Dynasan 114 and Miglyol lipids, which were coated with chitosan after SLN formation [95]. Biodistribution studies after intranasal delivery of the chitosan-coated SLNs in mice illustrated the presence of labeled SLNs in the olfactory bulb and the rest of the brain [95]. However, a significantly higher percentage of SLNs distributed to the lungs and the circulation when compared to the olfactory tract and olfactory bulb, highlighting the current problems with lipid-based NPs and the need for more specific targeting to the brain [95]. To improve brain targeting of lipid NPs by improving cellular barrier penetration, Yang and coworkers generated rivastigmine-loaded liposomes, which were surface-modified with PEG-coupled CPPs for intranasal delivery [96]. These NPs showed increased CPP-liposome penetration in an in vitro BBB cell model [96]. Additionally, a significantly increased rivastigmine concentration was found in the plasma, hippocampus, cortex and olfactory region of healthy rats using CPP-liposomes compared to unmodified liposomes, indicating that both the olfactory pathway and the systemic pathway contributed to rivastigmine targeting to the CNS [96]. Finally, increased rivastigmine concentrations could be distinguished in all brain regions and plasma when comparing

intranasally administered and IV injected CPP-liposomes with IV injected free rivastigmine [96]. Table 1 and 2 provide an overview of studies published in the past decade investigating L-DOPA-loaded polymeric- and lipid-based NPs in vitro and in vivo, respectively.

Table 1
In vitro research published in the past decade studying polymeric- and lipid-based NP encapsulation of L-DOPA.

NP composition	Size + Zeta potential	Outcome parameters	Reference
Polymeric Glutathione (GSH) coated NH2–Poly (ethylene oxide) (PEO)–PCL NPs	128.6 ± 1.2 nm +11.0 ± 0.4 mV	Drug loading efficiency 12% ± 1.4%, encapsulation efficiency 3.6% ± 0.4%, stable after freeze-drying, low cytotoxicity in Vero and PC-12 cells, and compatible with blood.	[114]
Polymeric PLGA + PVA NPs	173.1–500.6 nm Zeta potential unspecified	Optimal NPs: 5% (*w/v*) PLGA, 6% (w/v) PVA and 700 rpm solvent removal, drug loading efficiency 62.19%, size 256.2 nm, stable backbone structure, porous outer layer.	[115]
Phosphatidylethanolamine, cardiolipin and phosphatidic acid/ cholesterol (2:1) liposomes	0.5 μm to 2 μm Zeta potential unspecified	Drug loading efficiency 61.4%, stable for at least 5 days when stored at 37 °C and − 20 °C. Drug release dependent on pH and temperature: initial burst followed by plateau. Cell viability decreased significantly in SH-SY5Y and 3 T3-L1 cell lines after treatment with L-DOPA loaded liposomes.	[116]
Egg phosphatidylcholine Cholesterol, and stearylamine (5:4:1 M ratio) liposomes	302 nm - 2432 nm −27.56 mV - 29.50 mV	Drug loading efficiency 43% when ascorbic acid was co-loaded for increased L-DOPA stability (degradation started after 12 days storage at 4 °C). Drug release profiles showed a burst release of 80% after 3 h, followed by a gradual release, reaching 100% after 6 h.	[117]

6. Discussion

6.1. Discussion

The current most prescribed PD treatment consists of a combination of oral L-DOPA and carbidopa, which significantly decreases PD symptoms, particularly in the earlier stages of PD [29]. Nevertheless, this treatment struggles with low oral bioavailability, extensive degradation in the gastro-intestinal (GI) tract, hepatic first pass metabolism, systemic decarboxylation into DA and low L-DOPA accumulation in the CNS due to the BBB [29,31]. The progressive dopaminergic degeneration requires increasing L-DOPA dosages as PD progresses, resulting in elevated systemic DA concentrations causing major side effects like dyskinesias after long-term treatment [28]. To increase L-DOPA bioavailability and protect L-DOPA from systemic degradation, current research increasingly focuses on the encapsulation of L-DOPA into NPs. [44,47]. These NPs can be surface-modified to increase targeting to- and penetration of the BBB, thereby improving drug delivery to the CNS [56,66]. However, orally delivered NPs are still subject to extensive degradation in the GI tract and liver, and struggle with intestinal absorption [71], while IV injected NPs reduce patient compliance due to frequently required hospital visitations [55]. Transdermal L-DOPA delivery offers easy self-administration and a more sustained release profile, but struggles with L-DOPA degradation, low drug plasma concentrations and insufficient brain targeting due to the BBB [75]. Additionally, the poor L-DOPA solubility in combination with the occurrence of drug crystallization within the patches decreases overall L-DOPA release and skin permeability thereby further complicating L-DOPA delivery through this route [74,75]. These complications are the most likely reason that recent research studying transdermal delivery on PD drugs focuses on dopamine agonists and other neuroprotective therapeutics rather than L-DOPA. Interestingly, intranasal delivery of NP-encapsulated L-DOPA and other antiparkinsonian drugs, offers an alternative as a non-invasive delivery strategy. This method provides a direct pathway between the nose and brain via the olfactory and trigeminal nerves, reducing exposure to the systemic circulation and the BBB [59,81]. Direct nose-to-brain delivery has shown potential to reduce symptoms within minutes which can be sustained for several hours while reducing free systemic DA and associated side effects [82]. The type of NP formulation employed for this should be carefully considered, as both polymeric and lipid-based NPs have their strengths and weaknesses. Although lipid-based NPs are relatively cheap, easy to scale up and non-toxic, they struggle with efficient L-DOPA entrapment, reproducibility, impurities and mainly distribute drugs to the lungs and systemic circulation rather than the brain after intranasal delivery in rodents [85,92,96–98]. Therefore, creating commercial scale batches of high-quality lipid NPs that target the brain after intranasal administration with high consistency is a lengthy process. Polymeric NPs are easy to modify and show improved control over NP characteristics like size, zeta-potential and hydrophobicity, and have been illustrated to efficiently localize to the CNS after intranasal delivery with low systemic exposure [81]. However, the main problem with polymeric NPs is safety and nasal mucosal/brain toxicity of the *co*-polymers and their frequently used surface modifications (e.g. lectins) upon frequent administration [88,91]. Functional surface modifications of NPs, including lactoferrins and CPPs, have been shown to potentially facilitate targeting to the BBB and other cellular barriers, and induce penetration/transport [96]. However, the presence of these functional groups, together with other parameters including shape, aggregation, surface structure and charge, affect the toxicity of the polymeric NP. As a result, upscaling of polymeric NP must only be performed after thoroughly assessing the toxicology of the NPs with mentioned parameters [98]. It is important to consider that brain degeneration is not limited to dopaminergic neurons. Inflammation and angiogenesis are involved in the pathophysiology of PD and are reported to induce increased permeability of the BBB [99]. This improves NP transport across these barriers and thereby likely contributes to

Table 2
In vivo research published in the past decade studying polymeric- and lipid-based NP encapsulation of L-DOPA.

Model system	Administration route	NP composition	Size + Zeta potential	Outcome parameter	Reference
L-DOPA-induced dyskinesia Wistar rats	Subcutaneous	Polymeric PLGA + PA NPs	500 nm Zeta potential unspecified	Gradual reduction in involuntary movements and apomorphine-induced rotations (± 50%) sustained for 20 days	[118]
Reserpine-induced PD Wistar rats	Intravenous + subcutaneous	Polymeric tannic acid (TA)/ polyvinyl alcohol (PVA) NPs	± 57 nm −9.8 - –14.8 mV	2-fold increase in brain DA and 4-fold increase in brain tyrosine hydroxylase, significantly increased scores in motor movement tests and 30% increase in superoxide dismutase activity compared to control.	[119]
6-hydroxydopamine (6-OHDA)-induced PD Wistar rats	Intranasal	Polymeric PLGA NPs	250 ± 50 nm Zeta potential unspecified	Significant Improvement in multiple motor coordination performance tests (±90%), which was sustained for approximately 3 months and maintained after discontinuation of treatment.	[120]
MPTP-induced PD rats	Intranasal	Polymeric WGA-conjugated PLGA NPs	383.7 ± 66.94 nm −20.8 ± 3.63 mV	Biodistribution studies showed augmented dopamine brain delivery at all time points after intranasal WGA-conjugated PLGA NP delivery compared to blood. Significantly improved locomotor activity after treatment	[27]
Healthy adult male Sprague-Dawley rats	Intranasal	Polymeric GCPQ NPs	72.0 ± 5.0 nm +40.5 ± 2.1 mV	17-fold increased blood plasma L-DOPA levels after intranasal delivery of polymeric GCPQ NP compared to crystalline L-DOPA, peaking after 2 h and no detectable plasma DA. Brain DA concentrations increased significantly after NP encapsulation, increasing over time, while DA concentrations were too low to quantify for crystalline L-DOPA.	[1]
Healthy male Wistar rats	Intranasal	Polymeric PLGA NPs and Chitosan NPs	553 ± 52 nm +46.2 mV	Intranasal delivery of C2 chitosan NPs showed a maximum L-DOPA plasma concentration with a bioavailability of 45%, whereas the bioavailability for soluble L-DOPA maximally was 27%.	[29]
Healthy Wistar rats	Intranasal	Polymeric chitosan NPs incorporated in a thermo-reversible gel	164.5 ± 3.4 nm Zeta potential unspecified	Biodistribution studies of intranasally administered L-DOPA-encapsulated chitosan NPs in saline showed 75% recovery in the brain after 15 min which dropped to 29% after 4 h, while recovery for chitosan NPs in thermos-reversible gel was 26% after 15 min dropping to 19% after 4 h.	[121]
6-OHDA-induced hemilesioned PD mice	Intravenous	Tristearin + Lecithin lipid-based nanocarriers	161.9 ± 0.8 nm Zeta potential unspecified	IV delivery of lipid nanocarrier encapsulated L-DOPA resulted in slight improvement in 6-OHDA-induced parkinsonian disabilities similar to IV administered free L-DOPA, lasting for up to 24 h after NP encapsulation and 6 h for free L-DOPA.	[122]
MPTP-induced PD mice	Intravenous	Mesoporous silica NP core coated with lactoferrin modified lipid bilayer	226.8 ± 5.4 nm Zeta potential unspecified	Biodistribution studies showed a significant increase in lactoferrin modified NPs in the brain compared to unmodified NPs upon IV injection, peaking after 90 min. Motor function significantly improved in MPTP-induced PD mice after treatment with lactoferrin modified NPs whereas free L-DOPA and L-DOPA/curcumin did not.	[123]
MPTP-induced PD mice	Intraperitoneal	Chlorotoxin-modified HSPC/Chol/DSPE-PEG stealth liposomes	106.8 ± 3.01 nm 0.375 ± 0.09 mV	Biodistribution studies of L-DOPA-loaded stealth liposomes showed increased percentages of DA in SNpc and striata compared to control in healthy and more pronounced in MPTP mice. Similar findings for DA metabolite DOPAC. Rotarod tests in MPTP-induced PD mice showed improved motor function after treatment with L-DOPA loaded chlorotoxin-modified stealth liposomes and free L-DOPA and unmodified liposomes did not.	[124]
6-OHDA-induced PD Sprague-Dawley rats	Intragastric	Chitosan-coated liposomes	Not specified	Treatment with L-DOPA loaded chitosan-coated liposomes significantly decreased abnormal involuntary movements compared with free drug for 21 days. Phospho-ERK1/2, phospho-Thr34, DARPP-32 and FosB/ΔFosB levels in rat striatum significantly decreased after L-DOPA liposome treatment compared to free L-DOPA treatment.	[125]
Healthy New Zealand white rabbits	Transdermal	self-assembling nano-micellar system	5–45 nm Zeta potential unspecified	In vivo pharmacokinetic studies in healthy rabbits revealed that L-DOPA plasma levels peaked after 12 h, and were fully cleared after 24–28 h. A self-assembling nanomicellar liquid patch resulted in increased peak plasma concentrations after daily patch application, which further increased when application was switched to twice-daily	[75]

augmented drug delivery to the brain [100]. However, in advanced stages of PD, the brain requires a constant supply of DA to attenuate PD symptoms [55]. Because of the required long-term and frequent treatment, research into brain toxicity and inflammation at the nasal level is essential to push NP formulations from animal models to the clinic [82,101].

6.2. Challenges and future perspectives

Intranasal delivery of NP-encapsulated L-DOPA is arguably the most promising PD treatment option, as it enables patient self-administration in combination with therapeutic L-DOPA brain concentrations and rapid delivery. However, as stated in section 6.1, additional research into NP

toxicity at mucosal and brain level is pivotal to pushing the intranasal delivery of NPs to the clinic. Additionally, while several publications discriminated between drug delivery to different brain regions [88,90], there are currently no reports showing targeting to and/or accumulation of L-DOPA loaded NPs to the primary target site, the SNpc. Improved L-DOPA delivery specifically to the SNpc could not only improve treatment efficacy but could also drastically impact treatment toxicity and safety profiles. Another aspect that is often ignored during intranasal NP delivery-related research is the effect of the immune system in both the nasal cavity and the brain. It is known that later stage PD patients display activated innate and adaptive immune responses, particularly at affected brain sites like the SNpc [102]. The frequent L-DOPA-loaded NP treatments should not interfere with/avoid the already active immune system, which could trigger undesirable immunological reactions [82]. For example, PEGylated NPs have demonstrated to activate the immune system and trigger PEG antibody formation, resulting in faster degeneration and clearance of PEGylated NPs upon repeated administration, thereby reducing treatment efficacy [61,62,103]. Hence, future research into intranasal delivery of NPs should focus on the establishment of long-term toxicity and safety profiles as well as NP interactions with the immune system. Additionally, most of the current NP formulations under investigation solely focus on PD symptom reduction by compensating decreased DA production in the SNpc, ignoring neurodegeneration and PD progression [28]. While this effectively increases quality of life, this treatment strategy will not lead to a cure for PD [30]. Wang and coworkers described the therapeutic benefits of IV injected polymeric NPs containing the neuroprotective drug GB on PD symptom progression in mice and illustrated the neuroprotective properties against MPTP-induced PD [55]. Similarly, recent research illustrated the neuroprotective and regenerative functions of intranasally delivery brain-derived neurotrophic factor-loaded small extracellular vesicles, which improved functional behavior, neural repair, neurogenesis, fiber preservation and decreased inflammation after ischemia [104]. Beside NPs and extracellular vesicles, PD stem cell treatments have been attempted via intranasal delivery, illustrating the possibility to deliver whole cells to the brain via the direct nose to brain pathway [105]. Therefore, it could potentially benefit PD patients when L-DOPA-loaded intranasally delivered NPs are co-administered with neuroprotective drugs or stem cells, to facilitate symptom relief while simultaneously delaying/halting PD progression.

Although the intranasal delivery of polymeric- and lipid-based NPs to treat neurodegenerative diseases has been under investigation for over 20 years now, the research field is stagnating and struggles to pass the pre-clinical testing phase [81]. Even though most in vivo studies are performed in either mice or rats, it is acknowledged that significant differences exist in both brain- and nasal cavity anatomy between humans and rodents [82]. For example, the olfactory epithelium/body mass ratio is roughly 200 times smaller in humans compared to rats, and mucosal clearance takes place at different rates in different species [82]. Additionally, the anatomy and the structural arrangement of the SNpc also show vast differences when comparing humans to rodents, which results in discrepancies in DA release and distribution within the SNpc, explaining the difficulty in the translation of in vivo results to the clinic [106]. Therefore, the use of animal models which more closely resemble human anatomy, like marmoset monkey models, could be a valuable intermediate between rodent studies and human clinical trials [106]. Currently available marmoset monkey models include MPTP-induced PD, which models disease characteristics with biochemical, anatomical and behavioral resemblance to the human situation [106], and could therefore play a role in the progression of NPs for intranasal administration to the clinic and should be utilized [107].

Besides the use of mismatched animal models, lack of consideration for the methods applied for intranasal delivery also hampers the progression of these NP formulations to the clinic [82]. Charlton et al. demonstrated the impact of delivery to specific nasal regions on observed drug biodistribution. Targeted delivery specifically to the olfactory epithelium resulted in significantly increased direct nose-to-brain transport and reduced systemic absorption, compared to conventionally used uncontrolled intranasal delivery [82,108]. Despite the direct link between olfactory epithelium targeting and brain uptake, most in vivo studies ignore factors such as olfactory targeting, nasal airflow and lack of sensory reflexes during animal sedation upon intranasal administration, thereby limiting clinical relevance and decreasing reproducibility [82]. Future research should therefore carefully consider their intranasal delivery methods, to optimize brain uptake. In addition, many reports are lacking detailed information on experimental procedures used for intranasal administration. This leads to inconsistency when comparing data published by different research groups. Therefore, a more systematic and well documented approach to intranasal treatment methodology is required to allow the normalization and comparison of data. Hence, methods applied for intranasal delivery in humans are also crucial for achieving optimal therapeutic efficacy and reduction of adverse effects. Even though the olfactory and trigeminal nerves are mainly located in the upper and posterior regions of the nasal cavity, beyond the nasal valve, currently used nasal delivery devices like spray pumps and pressurized metered dose inhalers often fail to successfully target this area due to a mismatch in delivery device and nasal anatomy [82]. Instead, most of the drug is delivered either to the anterior of the nasal valve or to the lower parts of the nasal cavity, which results in drug clearance through the GI tract [82]. To improve intranasal delivery efficiency, different devices have been developed, including breath powered bi-directional nasal delivery devices [109], pressurized gas powered bi-directional delivery devices [110] and vortex based nebulizers [111], which have demonstrated to significantly increase targeting to the olfactory region in humans [82,109]. However, due to the vast differences between humans and rodents, employing the abovementioned intranasal delivery devices in the animal models generally used to study the delivery of NP-encapsulated L-DOPA is impossible. Therefore, previously mentioned PD marmoset monkey models could be an appropriate intermediate species in which intranasal spray devices can be tested, and should be used to bridge the gap from lab to clinic. Finally, improving brain delivery in PD by employing NPs is not restricted to L-DOPA. Alternative NP-based therapeutics that could benefit from improved target site delivery include novel potential synthetic drugs and oligonucleotide-based medicines [112]. Also, NP-mediated brain delivery can potentially contribute to improving therapies for other brain disorders such as Alzheimer's disease and mental disorders [113].

6.3. Conclusion

In conclusion, the NP encapsulation of L-DOPA is a promising new treatment strategy for PD which has been demonstrated to improve drug delivery to the CNS while reducing dose, treatment frequency and systemic side effects. Moreover, due to problems with oral and transdermal delivery and IV administration, intranasal NP delivery offers a non-invasive and easy to self-administer alternative which has the potential to increase therapeutic efficacy through direct brain delivery via the olfactory and trigeminal nerves, which could be of interest for various brain diseases. Nevertheless, before intranasally delivered drug-loaded NPs can be tested in patients, further research into optimal NP composition and characteristics, systemic methodology for intranasal delivery devices, more representative in vivo model systems, improved NP-mediated brain targeting and delivery, and long-term safety of NP formulations are required.

CRediT authorship contribution statement

Emile F. van Vliet: Writing – original draft. **Maarten J. Knol:** Writing – original draft. **Raymond M. Schiffelers:** Writing – review & editing. **Massimiliano Caiazzo:** Writing – review & editing, Conceptualization. **Marcel H.A.M. Fens:** Writing – review & editing,

Conceptualization.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

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