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### Recommended Citation

Rabiee, Navid; Ahmadi, Sepideh; Afshari, Ronak; Khalaji, Samira; Rabiee, Mohammad; Bagherzadeh, Mojtaba; Fatahi, Yousef; Dinarvand, Rassoul; Tahriri, Mohammadreza; Tayebi, Lobat; Hamblin, Michael R.; and Webster, Thomas J., "Polymeric Nanoparticles for Nasal Drug Delivery to the Brain: Relevance to Alzheimer's Disease" (2021). *School of Dentistry Faculty Research and Publications*. 490.

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# Polymeric Nanoparticles for Nasal Drug Delivery to the Brain: Relevance to Alzheimer's Disease

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## **Abstract**

Currently, Alzheimer's disease (AD) accounts for more than half of all dementia cases. Although genetics, age, and environmental factors affect the disease, the cause of AD is not yet fully known. Various drugs have been proposed for the prevention and treatment of AD, but the delivery of these therapeutic agents to the brain is difficult. The blood–brain barrier prevents systemic drugs from accessing the central nervous system and designing a suitable system to overcome this barrier has attracted much attention. The intranasal pathway, given its proximity to the brain, provides a great opportunity for drug delivery. Understanding the physiological characteristics of the nose can be useful in selecting the appropriate carrier and material. Some of the emerging vehicles used for nose-to-brain delivery of anti-AD drugs are natural (such as chitosan) and polymeric (such as poly(lactic-co-glycolic acid) and polyethylene glycol) nanoparticles (NPs). This review discusses the hypotheses for AD pathogenesis and highlights recent advances in the applications of natural and polymeric NPs for treatment. The fundamental and applied aspects of this approach for nasal drug delivery to the brain are reviewed here with thoughts on what is needed for the field to mature also provided.

# 1 Introduction

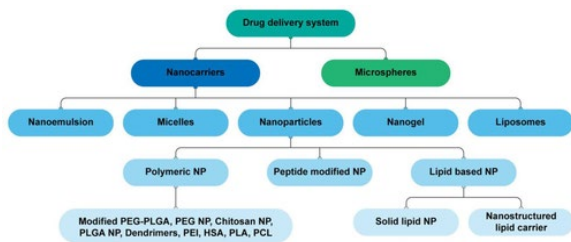
The growth of the world population and increasing life expectancies have led to a greater prevalence of neurological diseases, and so far, more than 600 different types of neurological disorders have been diagnosed. Among these, Alzheimer's disease (AD) is considered to be the most common cause of cognitive impairment throughout the world, currently affecting more than 35 million people.<sup>[1, 2]</sup> The progression of AD is slow and relentless, and it is even possible that the disease actually starts 20 years or more before symptoms emerge.<sup>[3-7]</sup> Oral medications are the most common methods for treating AD, but prescription drugs have been proven to have only a limited impact on the treatment and prognosis of AD.

Different types of natural and synthetic drugs in drug/gene delivery systems have been used based on their physicochemical properties. However, there are some obstacles that should be addressed carefully before any treatment commences. The United States Food and Drug Administration (FDA) has approved drugs for AD treatment that act as cholinesterase inhibitors, including donepezil, rivastigmine, galantamine, and tacrine. Currently, most of the approved oral drugs are administered as tablets, but suffer from disadvantages such as poor absorption from the digestive tract, difficulties in reaching the brain, and lack of effectiveness at the recommended dose; this means that patients frequently discontinue long-term treatment. Only donepezil has been approved for all stages of AD, while other medications are only effective for mild-to-moderate stages. These drugs also have side effects that include nausea, vomiting, and loss of appetite. The first cholinesterase inhibitor to be approved was tacrine, which is now rarely prescribed due to possible liver damage.<sup>[4, 8-10]</sup> One of the most important issues that could improve AD and other neurological disorder treatments includes developing effective brain drug delivery systems.<sup>[11, 12]</sup> Basically, the brain is protected by the blood–brain barrier (BBB), which prevents potentially toxic molecules from crossing from the blood stream into the cerebrospinal fluid, where they could damage the brain. However, the BBB also controls the entrance and exit of both internal and external beneficial factors.<sup>[13-15]</sup> There are various ways to overcome the BBB in order to deliver specific drugs to the brain. One of these approaches is to take advantage of the nasal route which is a channel for drug delivery directly into the brain.<sup>[16]</sup> Both intravenous and intranasal administration have been suggested for the delivery of specific drugs to the central nervous system.<sup>[17]</sup> However, the amount of drug delivered via the nasal route to the brain is less than 0.1% of the administered dose, so there is much debate about the effectiveness of this method, and clearly much more work needs to be done if nasal drug delivery to the brain reaches fruition.<sup>[18]</sup>

The administration of drugs alone (without any carrier) through the nose reduces their absorption rate, especially for peptides, proteins, and nucleic acids that are highly sensitive to harsh environments. For this reason, suitable nanocarriers that can be loaded with drugs to prevent damage to and from the nasal environment, and also to facilitate penetration through the nasal mucosa to the brain to increase the drug dosage that reaches brain cells, are needed.<sup>[19, 20]</sup>

Drug delivery mechanisms from the nasal route to the brain depend on carrier properties such as size, surface charge, mucociliary clearance process, and possible surface modification,<sup>[12]</sup> where nanoparticles (NPs) in particular have shown much promise. These NPs can be liposomes, dendrimers,

micelles, or polymeric NPs, all of which are capable of carrying and delivering drugs to the target site (Figure 1).<sup>[21]</sup>



**Figure 1** Schematic classification of nanocarriers for drug delivery to the brain.

Polymeric NPs (especially those which are biodegradable) are often considered superior to other drug delivery systems used for the central nervous system because of their ability to control drug delivery to the target location, as well as their capability to be loaded with a wide range of drugs. Polymeric NPs have several additional advantages, including their high surface-to-volume ratio that allows for a lower frequency and dose of drug administration. Their small size, better absorption by cells, improved accumulation of the drug at the target site, make such carriers suitable for intranasal drug delivery, and improve patient satisfaction.<sup>[22, 23]</sup>

In this review, we cover several hypotheses regarding the pathogenesis of AD. Central nervous system drug delivery via the intranasal route using polymeric NPs for AD treatment is also discussed with thoughts provided on how the field can advance to clinical applications.

## 2 Alzheimer's Disease

AD is a degenerative brain disease that is clinically diagnosed by symptoms of memory loss as well as related cognitive impairment including poor judgment and decision making, language disorders, loss of temper, and mood disorders. These problems are caused by the loss or destruction of neurons that are involved in cognitive functions within the brain. Neurons in other parts of the brain are eventually damaged or destroyed, leading to difficulties in performing basic functions of the body, such as walking and swallowing. Ultimately, the brain will be gradually but completely disabled, and death occurs at an average of 9 years after initial diagnosis. Causes of AD were thought to be largely related to environmental and lifestyle factors, but the present thinking has suggested that the contribution from genetic variability could be as high as 70%.<sup>[24]</sup> Alpha-2 macroglobulins, presenilins-1 and -2, and apolipoprotein E (ApoE) belong to the important category of the genes involved in the development of AD.<sup>[25]</sup> Below, we summarize some hypotheses that have been proposed to account for the progressive destruction of cortical neurons from AD, and also a comparison between the hypotheses are provided in **Table 1**.

**Table 1.** A comparison between the mentioned hypotheses

The hypothesis	Definition	Strategies	Commercialization strategies	Status
Amyloid hypothesis	Results from A $\beta$ peptide extracellular deposits as well as neuronal loss at a large scale <sup>[57]</sup>	Targeting $\beta$ - and $\gamma$ -secretase to reduce A $\beta$ production <sup>[58]</sup>	The first active AD vaccine (AN1792) for active A $\beta$ -immunotherapy developed by ELIAN <sup>[59, 60]</sup>  These antibodies in the clinics failed: Crenezumab (Genentech), <sup>[61, 62]</sup> bapineuzumab (Pfizer/Johnson & Johnson), <sup>[63]</sup> solanezumab (Eli Lilly), <sup>[64, 65]</sup> ponezumab (Johnson & Johnson) <sup>[66, 67]</sup>	Challenging
Tau hypothesis	The neurodegeneration process is a cause of tau aggregation and impairing the axons from neurons <sup>[68]</sup>	Utilizing tau vaccination <sup>[69]</sup>  Tau aggregation blocking <sup>[70]</sup>  Manipulating phosphatases and kinases <sup>[71]</sup>  Stabilizing microtubules <sup>[72, 73]</sup>	In the clinics, these strategies failed: tau-targeted vaccines (AADvac-1 and ACI35), passive vaccines (ABbv-8E12 and RG6100) <sup>[74, 75]</sup> and tau aggregation blocker (TRx0237) <sup>[76-78]</sup>	Challenging
Cholinergic and oxidative stress hypothesis	The neurotransmitters used by cholinergic neurons including acetylcholine (ACh) are responsible for critical transmittance information, therefore, damages to cholinergic neurons lead to critical causes <sup>[79, 80]</sup>  In the pathogenesis of AD, oxidative stress has several important roles including protein nitration, augmentation of protein oxidation, lipid peroxidation, and glycoloxidation <sup>[81, 82]</sup>	Inhibiting cholinesterase <sup>[83, 84]</sup>	The first anti-AD drug related to cholinesterase inhibitor was Tacrine, which was withdrawn from the global market <sup>[85-87]</sup>	Challenging

Vascular dysfunctions and NMDA receptors	NMDA receptors are involved in memory function and synaptic plasticity. Excessive stimulation of NMDA receptors by glutamate causes excitotoxicity	Activating memory receptors and their functions, and leads to excitotoxicity	—	Challenging
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## 2.1 Amyloid Hypothesis

In essence, this hypothesis is the principal theory widely considered to be the cause and consequently target for the treatment of AD. However, therapies that have been clinically tested based on the amyloid hypothesis, for lowering beta-amyloid levels and removing amyloid plaques, have not been very effective.<sup>[26, 27]</sup> Beta-amyloid peptide (A $\beta$ ) is a peptide containing about 40 amino acids made through sequential cleavage of the amyloid  $\beta$  precursor protein (APP) by  $\beta$ -secretase (BACE 1) and  $\gamma$ -secretase (a complex containing presenilin 1, PSEN1). Aggregation of these A $\beta$  peptides leads to the formation of fibrils and senile plaques outside the neurons. In 1984, researchers succeeded in identifying and purifying amyloid peptides in the cerebrospinal fluid of AD patients.<sup>[3, 28-30]</sup> A $\beta$  peptides first accumulate inside cells and then are transported to the extracellular matrix.<sup>[31]</sup> A mutation detected in the APP gene at chromosome 21 supports this hypothesis. Also, other mutations that cause AD have been identified in the PSEN1 and PSEN2 genes on chromosome 14.<sup>[32, 33]</sup> Studies have also shown that beta-amyloid can be identified in the olfactory epithelium, and this is related to neural degeneration in AD patients<sup>[34, 35]</sup> (Table 1).

## 2.2 Tau Hypothesis

Based on this hypothesis, AD begins with the accumulation of the tau protein. Research has shown that there is an association between beta-amyloid and a high degree of phosphorylation of the tau protein and this is related to the regulator of calcineurin 1 (RCAN1). It was proposed that the A $\beta$  peptide stimulates RCAN1 transcription, which induces hyperphosphorylation of Tau. RCAN1 is expressed in various parts of the brain and its over-activation causes brain damage.<sup>[36]</sup> Adult neurons contain microtubules consisting of three tau proteins called MAP1A, MAP1B, and MAP2, and their task is to form and stabilize microtubules. The degree of phosphorylation regulates the biological activity of tau. Excessive phosphorylation of tau proteins causes the microtubules to form tangles inside the neurons that result in neuronal cell death<sup>[37, 38]</sup> (Table 1).

## 2.3 Cholinergic and Oxidative Stress Hypothesis

Based on this hypothesis, several factors that have an impact on cholinergic abnormalities may contribute to a different range of AD cognitive abnormalities; in this case, choline transport alteration, muscarinic and nicotinic receptor expression, the release of acetylcholine, as well as axonal transport have all contributed to this issue.<sup>[39]</sup> The basal forebrain cholinergic complex contains cholinergic neurons that have cerebral cortex and hippocampus projections.<sup>[40]</sup> In the advanced stages of AD, drastic cortical cholinergic innervation loss has been shown.<sup>[41, 42]</sup> In addition, there is proof for cholinergic dysfunction triggered by  $\beta$ -amyloid, which affects NGF signaling,  $\alpha$ 7 nicotinic acetylcholine receptors, tau phosphorylation, and increases acetylcholinesterase.<sup>[43]</sup> The relative success of acetylcholinesterase inhibitors supports this hypothesis.<sup>[44]</sup> Increased amounts of ROS and free radicals (termed oxidative stress) can cause damage to the cortical neurons in AD patients.<sup>[45]</sup> A $\beta$  peptides can trigger oxidative stress possibly mediated by the methionine residue at position 35.<sup>[46]</sup> On the other hand, oxidative stress can cause mitochondrial dysfunction which in turn can produce even more ROS in a vicious circle.<sup>[47]</sup> External metals (such as Fe<sup>2+</sup>, Al<sup>3+</sup>, and Cu<sup>2+</sup>) have been reported to accumulate in AD brains and produce reactive oxygen species (ROS). The accumulation of A $\beta$  produces more hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) that is catalyzed by Fe<sup>2+</sup> and Cu<sup>2+</sup> (Fenton chemistry) in the presence of oxygen to form hydroxyl radicals<sup>[48]</sup> (Table 1).

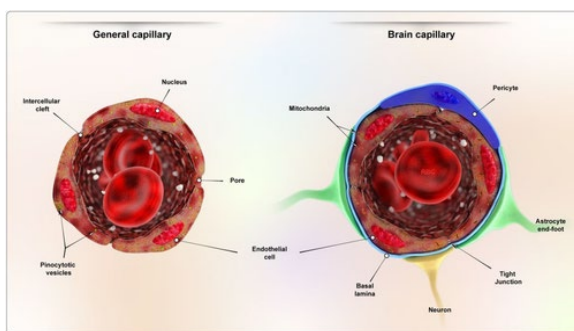
## 2.4 Vascular Dysfunction and NMDA Receptors

Patients that suffer from irregular heartbeats and hypertension experience increasing rates of AD incidence due to the hypothesis of “micron strokes”.[49] This phenomena is because of red blood cell emboli or micrometer sized cholesterol crystals, which would play a role as “seeding points,” in a healing response, for amyloid plaque growth.[50] One of the main routes of vascular dementia is certainly vascular dysfunction; however, based on recent publications, it plays an important role in AD too.[51] These micro-hemorrhages have been correlated with the formation of plaques[52] and they certainly act as triggers to activate the innate immune system in cerebral vessels, and could be indicative of BBB breakdown sites considered to be one of the early markers of cognitive dysfunction.[53] NMDA receptors are involved in memory function and synaptic plasticity. Excessive stimulation of NMDA receptors by glutamate causes an excessive influx of  $Ca^{2+}$  known as excitotoxicity, and ultimately neuronal apoptosis.[54] With excessive stimulation of the NMDA receptor, homocysteine can produce extracellular ROS.[55, 56]

## 3 Central Nervous System Drug Delivery

Different drugs are used to treat disorders of the central nervous system, such as psychosis, mood disorders such as depression, anxiety, seizure disorders (epilepsy), Alzheimer's disease, Parkinson's disease, central pain, as well as brain tumors.[88]

Blood capillaries in the brain are structurally different from those in other body systems, and these structural differences prevent the permeability of substances in the blood of cerebral capillaries and extracellular fluid to the brain tissue. This permeability barrier, which includes the capillary endothelium, is known as the BBB. This phenomenon was first described by Ehrlich in 1885 when he discovered that intravenous injected dyes could cause discoloration in most organs other than the brain.[89] The BBB is one of the major barriers to deliver drugs to the brain. This structural difference between the endothelium of the cerebral and non-cerebral capillaries is consistent with the presence of endothelial tight junctions (**Figure 2**).[90, 91]



**Figure 2** The comparison between capillaries in general organs and the brain.

Non-cerebral capillaries have gaps between the endothelial cells, which permit soluble molecules up to a certain size to move easily through them by passive diffusion. In the cerebral capillaries, the endothelium has tight junctions that prohibit movement through intercellular pathways. Pinocytosis occurs to some extent in the endothelium of the brain capillaries, which allows access of nutrients to the brain. The blood vessels of the brain include pericytes located on the outside of the endothelium (Figure 2). The tight junctions between endothelial cells in the brain create an effective barrier to trans-

endothelial diffusion, so the permeation of therapeutic molecules is highly restricted. Above 98% of drugs with small molecular structures do not have the ability to reach the brain, while this increases to 100% for macromolecular drugs. Apart from nutrients, only small hydrophobic molecules (<500 Daltons) have the ability to cross the BBB to provide an effective concentration in the brain. Moreover, the BBB has an overall hydrophobic nature that does not allow for the passage of hydrophilic drugs. There are also drug efflux pumps present in the BBB, such as P-glycoprotein, that acts to pump out potentially toxic foreign molecules.<sup>[92]</sup> The BBB also may inhibit the passage of nanomaterials with a diameter of more than 200 nm, and does not allow cellular endocytosis of nanomaterials >30 nm in diameter.<sup>[13, 17, 21, 88, 93]</sup> Solid lipid NPs (SLN) are a suitable lipid-based nanocarrier which contains a hydrophobic lipid core, in which the drug can be dispersed or dissolved. These particles with small sizes (40–200 nm) have the ability to cross tight endothelial cells of the BBB and also the reticuloendothelial system.<sup>[94, 95]</sup> Polymeric NPs with 50–200 nm sizes, as controlled drug delivery systems, increase the oral bioavailability of hydrophobic drugs. This has been obtained by high half-lives, low toxicity, and improved capability of the NPs to cross the BBB.<sup>[95]</sup> Some of the groundbreaking research studies regarding the delivery of drugs and other types of therapeutics to the brain via the BBB are listed in **Table 2**.

**Table 2.** Some of the groundbreaking research studies related to delivery of therapeutics via the BBB

The type of nanoparticle	The size of nanoparticle [nm]	Polymer/stabilizer/ targeted ligand	The type of surfactant	Tested therapeutic	Results	Ref.
Polymeric NP	200	Poly-methylmethacrylate	Poloxamer 338 Polaxamine 908 Polaxamer 188 Polaxamer 407 Polysorbate-80	Radiolabeled NPs	No uptake increase BMEC no increase About 10% increase About 17.5% increase About 15.1% increase	[96]
	650	Transferrin receptor antibody PEG	NA	Caspase-3-inhibitor	Decreased infarct volume	[97]
	270	Butylcyanoacrylate (PBCA)/dextran 70 kDa	Polysorbate-80	Doxorubicin	6 mg g <sup>-1</sup> (brain) at 2–5 h vs zero without carrier	[98]
	230	Butylcyanoacrylate (BCA)/dextran 70 kDa	Polysorbate-80	Tubocurarine	Epileptic-form spikes on EEG	[99]
	230	Polybutylcyanoacrylate (PBCA)/dextran 70 kDa	Polysorbate-80	Dalagrin	Analgesia study: increased analgesia effect by about 50%	[100]
	80–288	Polybutylcyanoacrylate (PBCA)/dextran 70 kDa, polysorbate-85	No coating	Amitriptyline	Increased brain AUC >50%	[101]
	190	Polybutylcyanoacrylate (PBCA)/dextran 70 kDa, polysorbate-85	Polysorbate-80	Dalagrin, kyotorphin	Analgesia study: increased	[101]

					latency by more than 50%	
	260	Butylcyanoacrylate (BCA)/dextran 70 kDa, polysorbate-85	Polysorbate-80	Valproic acid	No increase in brain concentrations	[102]
	60	Polybutylcyanoacrylate(PBCA)late	Polysorbate-80	Dalagrin	Analgesia study, increased latency by 50%	[103]
	50	<i>N</i> -butyl-2-cyanoacrylate	Polysorbate 80	Clioquinol	High specificity of A $\beta$ plaque and high brain retention and uptake of drug	[104]
	150	Poly lactic- <i>co</i> -glycolic acid (PLGA)	Trimethylated chitosan	Coenzyme Q10	Improved uptake to brain region, reverse behavior performance	[105]
	118	Poly lactic acid (PLA)	NA	Neuroprotective peptide	Improved drug uptake to brain and accumulation	[106]
Lipid NP	120	—	NA	Adenosine	Increased neurological deficit scores and decreased infarct volume	[107]
	90	Stearic acid	Epikuron 200	Doxorubicin	Levels 1/4 of plasma after 4 h vs zero in	[108]

					brains without NP carriers	
	200	Soybean oil	Poloxamer 188	Camphotericin	Increased brain AUC–10.4 fold	[109]
Polysaccharide core	196	Maltodextrin	Lipid coating— dipalmitoyl phosphatidylcholine	Albumin	27-fold increase in transport across in vitro BBB model	[110]

### 3.1 Intranasal Drug Delivery

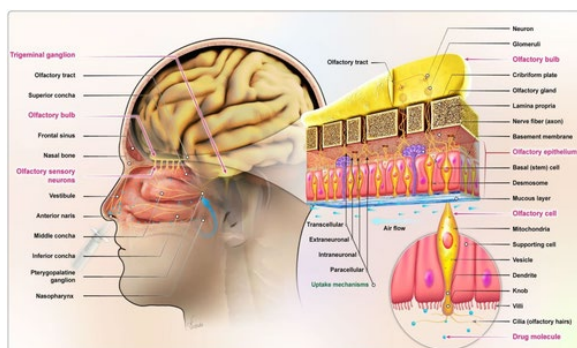
The delivery of drugs or biological therapeutic agents (proteins, peptides, oligonucleotides, stem cells, and viral carriers) through the nasal passage to the central nervous system is of high interest to researchers. The intranasal route is a non-invasive and rapid method for direct drug delivery from the nasal mucosa to the brain, with the aim of treating central nervous system diseases with minimal contact with systemic blood circulation.<sup>[90]</sup>

The nerve pathways of the olfactory and the trigeminal nerves are located within the nasal cavity, are connected to the brain, and are of particular importance. The olfactory nerves begin at the olfactory epithelium and end with the olfactory bulge, while the end of the trigeminal nerve is in the respiratory epithelium, which is less important than the olfactory route. The nasal system also contains blood vessels, cerebrospinal fluid, and a lymphatic system, which plays an important role in the transfer of molecules from the nasal cavity to the brain. The precise formulation, the physicochemical properties of the drug, and the type of nanocarrier employed all affect the efficiency of the intra-nasal route.<sup>[41, 111-113]</sup>

#### 3.1.1 Nasal Anatomy

The nose is used for breathing (inhalation and exhalation) and externally consists of two symmetrical nostrils that are rich in blood vessels, and lined with mucosa and a thick layer of mucus. The mucosa is responsible for trapping and transferring external particles first to the nasopharynx and then to the esophagus and stomach. The nasal cavity is divided into two halves by the middle nose blade. The respiratory region forms a major part of the nasal cavity consisting of three parts: upper, middle, and lower.<sup>[112, 114-116]</sup> The nasal cavity provides a relatively large area (about 160 cm<sup>2</sup>) for possible absorption of drugs. The metabolic activity in the nose is lower than the digestive tract, which contains enzymes, proteins, and peptides. This is another factor that makes this route more attractive than the oral route. Compared with the oral route, the nasal pathway prevents the destruction of the drug in the stomach, intestines, or liver, before it reaches the circulatory system.<sup>[88, 117]</sup>

The epithelium in the olfactory region is non-ciliated and contains olfactory nerve endings. The remaining surface of the nasal cavity is covered with a mucosal epithelium consisting of basal cells, goblet cells, and columnar cells that all can either be ciliated or non-ciliated (**Figure 3**).<sup>[88]</sup> Drugs delivered in this way can be absorbed either by the epithelium of the respiratory tract or by the epithelium of the olfactory region.<sup>[118]</sup>



**Figure 3** Schematic of the olfactory and trigeminal nerve pathways in transferring therapeutics from the nasal pathway to the brain.

### **Respiratory Region**

If the drug is deposited on the respiratory epithelium, it can be directly absorbed by the circulatory system and reach the central nervous system, if it is able to cross the BBB.

One of the pathways for the drug to reach the brain via the nasal cavity includes the trigeminal nerve, which has two branches in the epithelium of the respiratory region, and has been less studied (trigeminal nerve route). In this route, the drug is transmitted through the respiratory epithelium to the brain stem and can reach the caudal and rostral parts of the brain (Figure 5).<sup>[113, 119, 120]</sup> The respiratory region inhabits the largest area of the nasal cavity covering the respiratory area with the large surface area according to the existence of cells that have numerous microvilli. This area, with a high density of blood vessels, makes this area the large site of drug absorption into the systemic circulation.<sup>[121]</sup> However, there is a requirement for optimization of this route and understanding of dose following nasal drug administration and controlled nanocarrier sizes for CNS targeted therapy.

### **Olfactory Region**

The olfactory region route is the essential pathway for intranasal drug delivery. The olfactory region covers only about 10% of the total nasal surface area and is the closest to the brain tissue. The olfactory epithelium is covered with a dense mucous membrane that is about 95% water, 2% myosin, and is about 5 mm thick. Drugs deposited on the olfactory epithelium can be transmitted to the central nervous system directly, and in particular, the cerebrospinal fluid or the central core brain tissue, through paracellular or intracellular transmission via olfactory nerves or olfactory epithelial cells.<sup>[111, 114, 122-124]</sup>

The olfactory epithelium consists of three types of cells: nerve cells, progenitor cells, and supporting cells, all of which are attached to each other via tight junctions. The neurons begin in the olfactory bulge in the brain and end in the olfactory epithelium within the nasal cavity where they detect odor molecules and send information to the brain.<sup>[125]</sup>

The expression of some saccharide residues (such as *n*-acetylglucosamine and l-fucose) in the olfactory mucosa is much higher than the respiratory mucosa. Therefore, agglutinin-type molecules can be used in delivery vehicles to increase drug delivery to the brain because they have the ability to bind to saccharide groups and stimulate endocytosis.<sup>[126]</sup>

Drugs are transmitted to the olfactory bulge after being transported along the nerves, and accumulate therein. In the olfactory epithelium, drugs can be absorbed via three different pathways: 1) an intercellular pathway where the drug passes through the tight junctions; 2) an olfactory nerve transport pathway where the drug enters the neurons via endocytosis or pinocytosis and passes along the axons to reach the olfactory bulge; and 3) an intracellular pathway that has been observed in the olfactory epithelium, to be exact between the sustentacular cells.<sup>[127-130]</sup>

The diameter of the neuronal axons is between about 100 and 700 nm, and it may take hours or days to fully transfer the material to the olfactory bulge after nasal administration.<sup>[131]</sup>

Due to the high activity of the olfactory nerves, their lifespan is estimated to be between 30 and 60 days, after which time they undergo apoptosis, and newly formed neurons are created to replace the apoptotic ones.<sup>[132-134]</sup> After the neurons and epithelial cells are replaced, a delay occurs in the



reformation of the tight junctions, which increases the permeability to drugs for a specified time.<sup>[135, 136]</sup>

In addition to its advantages, intranasal drug delivery also has some limitations. For example, hydrolytic enzymes in the nose are part of the host defense mechanisms. These include carboxylesterases, aminopeptidases, aldehyde dehydrogenase, carbonic anhydrase, glutathione S-transferase, and glucuronyl transferase.<sup>[137, 138]</sup> For example, degradation of proteins and peptide drugs is carried out by aminopeptidases, as proteolytic enzymes, which act as a barrier against the absorption of peptide drugs. This enzyme catalyzes the cleavage of amino acids from the amino terminus of proteins/peptides.<sup>[139]</sup> Carboxylesterases (CEs) are a significant class of enzymes for the biotransformation of drugs and they are responsible for the hydrolysis of carboxylesters into carboxylic acid and alcohol. This enzyme can play an important role in the bioactivation of prodrugs or inactivation of soft drugs.<sup>[140, 141]</sup>

The nose has only a limited capacity to retain fluid (less than 200  $\mu\text{L}$ ). If this is exceeded, the fluid either leaks out through the nostrils or is transferred into the esophagus. Therefore, a high concentration (obtained by increasing the solubility of the drug in the formulation) is required in order for the drug to remain in the nose long enough to be absorbed.<sup>[112]</sup>

The most important natural obstacle to drug delivery via the nasal cavity is mucociliary clearance. This process is not very efficient for rapidly absorbed drugs, but for those drugs that are more slowly absorbed due to their physicochemical properties, it can be limiting.<sup>[88, 117]</sup> The mucous membrane has two layers: 1) the surface epithelium and 2) a connective tissue called the submucosa. The surface of the epithelial layer is covered with mucus containing glycoproteins, enzymes, and inorganic salts. The viscous mucus is produced by: a) goblet cells in the surface epithelium and b) epithelial glands within the connective tissue. The cilia that are located on the surface epithelium beat with a speed of up to 1000 bpm, and act as a mucosal cleansing system to remove particles that have been inhaled. This process reduces the lifetime of the drug within the nasal cavity, lessening its chance of absorption. The forward movement in the respiratory region is much faster than in the olfactory region. The mean residence time for the respiratory region is only 15–20 min, while in the olfactory region it can be as long as a few days.<sup>[122, 142, 143]</sup> In addition, other factors may interfere with cilia movement or the rheological properties of the mucus, such as the common cold, which can also affect absorption.<sup>[144, 145]</sup> Viscosity enhancers and mucoadhesive substances can be used to increase the lifetime of drugs within the nasal cavity to improve drug absorption.<sup>[146]</sup> The submucosa is rich in blood vessels, and drugs can directly reach the bloodstream. Therefore, paying attention to factors that affect blood circulation, such as conditions of physical and mental stress, infections, smoking, and environmental conditions, such as temperature and humidity, is also important.<sup>[145, 147]</sup>

In the epithelial cellular membrane, there are also efflux pumps that pump drugs from the inside of the cells into the lumen, and may contribute to poor absorption of the drug. P-glycoprotein is one of the most abundant efflux pumps because it not only exists in the liver, intestines, and central nervous system, but also in some parts of the nasal epithelium that contain ciliated cells and the submucosal section of the olfactory region.<sup>[148-152]</sup>

As previously mentioned, the properties of the nasal cavity, such as high enzymatic activity, quick clearance, efflux pumps, and poor mucosal permeability, decrease the efficient delivery of therapeutic agents to the brain. In order to increase the efficacy of drug delivery and absorption of drugs through the nasal cavity, solutions have been proposed.

The use of enzymatic inhibitors can inhibit enzyme-induced degradation, thereby increasing the stability of therapeutic drugs at the site of absorption.<sup>[153]</sup> Aminopeptidases and serine proteases are the most common enzymes that exist in the lung. As a result, due to the large number of enzymes in the lungs, drugs are metabolized before reaching the target.<sup>[154]</sup> For that reason, enzymatic inhibitors can protect drugs against degradation within the nasal cavity, olfactory routes, and lung. Some of these protease inhibitors studied in nasal drug delivery as absorption enhancers are leupeptin, bacitracin, and phosphoramidon.<sup>[155-157]</sup> However, formulation-based NPs, and the modulation of environmental pH, also relate to the regulation of protease activity. It should be noted that the interface of these enzymes with olfactory routes are not direct, and those enzymes make an impact on nasal drug delivery with an indirect approach.<sup>[158, 159]</sup>

Permeation enhancers have been developed as a potential formulation that improves the permeability of therapeutic agents across membranes. The most common permeation enhancers studied for nasal drug administration include tight junction modulators, surfactants, cationic polymers, and cyclodextrins.<sup>[160]</sup> Surfactants are amphiphilic molecules having both lipophilic and hydrophilic residues, and can be classified as phospholipids, salts of fatty acids, bile salts, etc. This molecule can increase the absorption with various mechanisms; these include avoiding enzymatic degradation of the drugs or opening of tight junctions.<sup>[160, 161]</sup> Cationic polymers, as absorption enhancers, interact with mucosal barriers and increase the absorption of hydrophilic molecules through tight junction modification due to positive charges. For example, the interaction between cationic polymers with insulin with negative charges showed high effective insulin absorption.<sup>[160, 162]</sup>

According to some studies, co-administration of rifampicin, as a P-glycoprotein efflux inhibitor, may play an important role in increasing drug uptake in the brain through the nasal cavity route. The pharmacokinetics of a drug may be changed when co-administered with compounds which prevent P-glycoprotein activity.<sup>[163, 164]</sup>

The employment of NP-based formulation delivery systems to increase nasal absorption of therapeutic agents can improve nasal delivery. NPs can increase nasal drug delivery and drug absorption because they are capable of protecting the encapsulated drug from degradation and from P-gp efflux pumps.<sup>[159, 165]</sup> In recent years, various types of nano and micro carriers have been studied for nasal drug delivery. NPs could help drugs pass through the nasal cavity while maintaining their payload from enzymatic degradation and increasing their retention time, resulting in improved drug concentration at the targeted area. The design and synthesis of the nanocarriers can be tuned, according to biocompatibility, drug loading, particle size, and zeta potential.<sup>[146]</sup> The BBB inhibits the passage of NPs of a larger size and direct delivery of the nanocarriers with average sizes of  $\leq 200$  nm could be accomplished through the olfactory and respiratory routes,<sup>[166]</sup> although it is important to mention that NPs with smaller sizes (20-200 nm) have the ability to cross the BBB and also the reticuloendothelial system,<sup>[94]</sup> but as NPs increase in size, most drug molecules can be accommodated and their release is slow. Although smaller NPs have a higher volume-to-surface ratio and they provide effective drug

delivery, they can aggregate and rapidly release a drug since they adhere to the edge surface of the particles.<sup>[166]</sup> In addition, with a decreasing particle diameter less than 500 nm, the deposition increases in most organs, such as the lungs.<sup>[167]</sup> **Table 3** summarizes some advantages and limitations of olfactory and trigeminal nerve pathways in transferring therapeutic agents from the nasal pathway to the brain.

**Table 3.** Summary of the advantages and limitations of olfactory and trigeminal nerve pathways in transferring drugs to the brain

	<b>Advantages</b>	<b>Limitations</b>
Respiratory region route	<ul style="list-style-type: none"> <li>• Most permeable region due to large surface area</li> <li>• Non-invasive method and rapid absorption</li> <li>• Direct drug delivery to the brain bypassing the blood–brain barrier</li> </ul>	<ul style="list-style-type: none"> <li>• Limitation in particle size</li> <li>• Need a limited volume of drugs with high concentration</li> <li>• Low CNS delivery for peptide/proteins</li> </ul>
Olfactory region route	<ul style="list-style-type: none"> <li>• Non-invasive method and rapid absorption</li> <li>• Direct drug delivery to the brain bypassing the blood–brain barrier</li> <li>• Desired therapeutic effects at lower doses</li> </ul>	<ul style="list-style-type: none"> <li>• Enzymatic degradation of drugs by different nasal enzymes</li> <li>• Need a limited volume of drugs (less than 200 <math>\mu</math>L) with high concentration</li> <li>• Low CNS delivery for proteins</li> <li>• Surfactants used as a catalyst may cause the membrane to break at high concentrations</li> </ul>

#### 4 Use of Polymeric Nanocarriers for AD Drug Delivery via the Nasal Route to the Brain

Given the advances made in the field of nanotechnology, new opportunities have been created for drug delivery to treat central nervous system diseases. The construction of a suitable carrier and selection of the appropriate polymer depend on the route of administration and the possible fate of the NPs.

The size of the polymeric NPs is by far the most important factor that affects their performance as drug delivery vehicles. Smaller NPs are much better for mucus penetration, and for transport along neuronal pathways compared to larger particles, resulting in higher efficiency of drug delivery. Mistry et al. studied chitosan-coated polystyrene (C-PS) with diameters of 100 to 200 nm and found that particles of 100 nm were detected inside olfactory epithelial cells, but none were found in the olfactory bulge. This result showed that an appropriate NP diameter for axonal transport is less than 100 nm in mice.<sup>[168]</sup> Also, besides the size, the electrical charge of the NPs is an important parameter that affects carrier performance. In general, the nasal mucous membrane has a negative charge, so that positively charged polymers or NPs can bind to it via electrostatic interactions, impairing bioadhesive properties. Zeta potential is an indicator of stability of NP suspensions and has values that range from +100 to –100 mV. Zeta potential indicates the potential difference between the NPs and the solvent.

Alternatively, this is also sometimes referred to as a particle's surface charge. This, in turn, can be indicative of the particles' suspension stability and tendency to agglomerate.<sup>[169, 170]</sup> However, the Zeta potential can have a positive or negative sign (depending on the surface chemistry) and it is typically assumed that an absolute value greater than positive 30 mV or less than negative 30 mV can be indicative of a stable suspension. In fact, a high electrostatic repulsion due to high particle charge is needed to prevent NP agglomeration.<sup>[169]</sup> NPs should have a zeta potential values of  $\geq$  positive 30 mV in order to increase static repulsion and facilitate the interaction between the NPs and the mucosal cells. In this regard, natural and synthetic polymeric NPs have recently been used as nose-to-brain delivery systems for AD treatment. Therefore, in the following sections, we discuss different polymeric NPs which have recently been used for the treatment of AD. In addition, based on the literature, in **Table 4**, a list of different promising NPs were compared in terms of their advantages and disadvantages, but only polymeric NPs are discussed in detail in the below sections for the treatment of AD. Compared with other nanocarriers, the advantages of polymeric NPs include high loading capacity, high stability, decreased drug toxicity, easy surface modification, and controlled drug release, which make these nanoparticles suitable for numerous drug delivery applications.

**Table 4.** A comparison between different forms of nanocarriers for nasal drug delivery in terms of their advantages and disadvantages

Types of NPs	Advantages	Disadvantages	Examples
Peptide/protein NPs	Delivery of large therapeutics, including insulin	Possible cytotoxicity regarding surface modification	Bovine serum albumin (BSA) NPs <sup>[171]</sup>
Polymeric NPs	Enhancing the residence time of the olfactory region, reducing the clearance of mucocilliary	Possible nasal irritation, possible mucosal damage, possible cytotoxicity regarding surface modifications as well as crosslinks	PLGA, PEG, PEI, polyvinyl alcohol (PVA) <sup>[172, 173]</sup>
Lipid-based nanocarrier	High tunability, easy chemical modification on the surface, low cellular toxicity, encapsulation method possibilities	Low entrapment efficiency as well as difficult to optimize size	Liposome <sup>[174]</sup>
Nanogels	Enhanced and improved drug delivery through the nasal epithelium, easy surface modification, acceptable entrapment efficiency	Unknown mechanism of BBB penetration, possible aggregation in the nasal epithelium	Poly((2-dimethylamino)ethyl methacrylate) (PDMAEMA) nanogels, <sup>[175]</sup> P(DEAEMA-co-TBMA- <i>g</i> -PEGMA) nanogels <sup>[176]</sup>

Micelles	Easy modification of ligands on the surface for targeted delivery of drugs/genes	Need to use surfactants that may cause interfering problems as well as cytotoxicity	PEG–polylactic acid, <sup>[177]</sup> PCL–P2VP, Core(Laur)PEG micelles <sup>[178]</sup>
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#### 4.1 Chitosan-Based Nanoparticles

Chitosan is a linear copolymer obtained by the deacetylation of chitin (poly-*N*-acetylglucosamine) and comprises a certain number of *N*-acetyl d-glucosamine and  $\beta$ -linked d-glucosamine units. Chitin is a natural polysaccharide with a chemical formula of  $C_8H_{13}NO_5$  that is found in abundance in the shells of crustaceans or from certain fungi. Chitosan has extensive applications in the fields of drug delivery and tissue engineering.<sup>[179, 180]</sup> The source of chitin and the process for its conversion to chitosan affect its physical and chemical properties.<sup>[181]</sup> The length of the chitosan polymer chains and its average molecular weight can be controlled by enzyme-mediated decomposition or acid hydrolysis.<sup>[182]</sup>

Various biocompatible and biodegradable polymers have been investigated for controlled release properties and intranasal delivery to the brain. Among these polymers, chitosan has shown particular advantages such as bioadhesive properties, reduced mucosal clearance, low toxicity, non-allergenic, absorbency, and suitability for the delivery of hydrophilic drugs. Chitosan shows an increased residence time in the olfactory region, partly because of its mucoadhesive properties, and partly because its natural polysaccharide structure can interact with saccharide groups already present on the mucosa. It shows increased penetration through the mucosa by widening the tight gaps between epithelial cells. Chitosan is thought to disturb intercellular tight junctions, therefore increasing the permeability of the epithelium. According to its precise properties, it can take the form of NPs, microparticles, or hydrogels.<sup>[183-189]</sup>

Chitosan has a pKa value of about 6.5, so chitosan has a slightly positive charge in physiological conditions, and is insoluble in water and organic solvents. However, chitosan can be dissolved in dilute acids that convert the glucosamine units into protonated amines, which are soluble in water ( $R-NH_3^+$ ).<sup>[190, 191]</sup> After protonation of the amine groups in chitosan, this cationic polymer can be soluble in aqueous solutions by the addition of some acids; however, for clinical as well as in vitro and in vivo experiments, we need to increase the hydrophilic groups on the surface of chitosan to increase its solubility as well as increase the adherence to the mucosal surface.<sup>[192, 193]</sup> The interaction between the positive amino groups of chitosan and the mucosal negative sialic acid groups, or other groups in the mucosa that have a negative charge, causes mucosal adhesion.<sup>[194, 195]</sup>

Various methods such as ionic gelation, microemulsion diffusion, and polyelectrolyte complex formation have been used to produce chitosan NPs. Among these, the ionic gelation method is preferred due to its lack of use of high temperatures, organic solvents, and its simplicity.<sup>[188, 196-199]</sup> In the past, this method has been called an “ionic-induced gel,” and it can produce both NPs and microparticles. Nevertheless, it has some limitations such as inappropriate surface morphology, high dispersion index of particle size, and the lack of suitable sites for surface modification.<sup>[200-202]</sup> The ionic gelation method is based on the electrostatic interactions between cationic chitosan and negatively charged molecules, such as sodium tripolyphosphate, gum katira, and sulphobutyl-ether-beta-

cyclodextrin, which results in the formation of a hydrogel composed of NPs or microparticles useful in drug delivery.<sup>[203-205]</sup>

Feng et al. in 2012 constructed a nasal spray including chitosan NPs for the intranasal delivery of bFGF (basic fibroblast growth factor), as a peptide/protein drug in male Sprague–Dawley (SD) rats.<sup>[206]</sup> bFGF is an important neurotransmitter factor, composed of 154 amino acids. Due to its various effects on promoting axonal branch formation and stimulating the proliferation of neural precursors, this factor has very good potential for the treatment of central nervous system disorders and neurodegenerative diseases. The uptake measured in the brain revealed that the area under the curve (AUC<sub>0–12 h</sub>) of bFGF in the cerebrum, olfactory bulb, hippocampus, and cerebellum were, respectively, 2.38, 2.47, 2.19, and 2.56 times that of bFGF delivered by intravenous injection. AUC<sub>0–12h</sub> is the area under the curve from time 0 to 12h after the administration of drugs. The values were 1.11, 1.95, 1.40, and 1.93 times higher than that found using an intranasal bFGF solution alone, respectively. Additionally, the bFGF NP nasal spray gradually improved spatial memory impairment, even at lower doses. In this case, the NPs containing half of the dose (20 µg kg<sup>-1</sup>) of the bFGF solution recovered levels of choline acetyltransferase and acetylcholinesterase activity to the control level, as well as alleviated the degeneration of neuronal structures in the rat hippocampus, thus, demonstrating neuroprotective effects in the central nervous system.

Targeting of rivastigmine-loaded chitosan NPs (CS-RHT NPs) synthesized by the ionic gelation technique to the brain in Wistar rats was reported in 2012.<sup>[207]</sup> The cumulative drug release profile through the nasal mucosa was about 89% over a day indicating that a sustained and controlled release profile was achieved. The encapsulation efficiency was typically about 85%, and the reproducibility of the preparation was satisfactory. Biodistribution and pharmacokinetic studies in Wistar rats showed values for the nose-to-brain direct transport (DTP)% and drug targeting efficiency (DTE)%, which indicated direct nose-to-brain transport not seen with the RHT solution. A qualitative biodistribution study using CLSM also revealed the existence of nose-to-brain transport as compared to the RHT solution.

In 2014, another research group studied the treatment of AD via intranasal administration of a donepezil-loaded chitosan nanosuspension by an ionic cross-linking method in Sprague–Dawley rats.<sup>[208]</sup> The nanosuspension had an average size of 140–200 nm with an acceptable polydispersity index based on the related publications. This method was able to lower the high dosage and improve the bioavailability of the drug by delivery through olfactory nerves, thus, prolonging the action of donepezil. Additionally, in vivo studies confirmed a lack of mortality, hematological changes, and variations in body weight or toxicity in animals.

In 2015, Hanafy et al. reported the preparation of cationic NPs (GH-CX-NP) using the ionic gelation method and complexation of cationic chitosan together with a cationic drug, galantamine hydrobromide, to produce a neurotherapeutic agent that could be transported through the intranasal pathway for the treatment of AD.<sup>[209]</sup> The results from fluorescent imaging microscopy showed that significant amounts of drugs accumulated in different regions of the brain after just 1 h of intranasal administration. The NPs were found to be localized intracellularly as a result of clathrin-dependent endocytosis.

One year later, the same group showed that the intranasal administration of the chitosan-based NPs led to a significant decrease in AChE protein levels and enzyme activity in rat brains, compared to the oral and nasal GH solutions.<sup>[209]</sup> No evidence of cytotoxicity or histopathological abnormalities was observed. The NPs were again found to be intracellularly located within brain neurons.

Memantine is an amantadine derivative which acts as a psychoanaleptic anti-dementia drug. It is an antagonist of *N*-methyl-d-aspartate receptors clinically used for the treatment of moderate to severe AD. Chitosan NPs have been examined for the intranasal delivery of memantine.<sup>[210]</sup> These NPs were prepared by ionic gelation of chitosan using TPP, and then memantine hydrochloride was loaded onto the NPs. In the optimized formulation, the diameter of the NPs was 129 nm after stirring for 4 h at 1000 rpm and using a 2:1 concentration ratio of chitosan to TPP. According to their results, the compatibility between chitosan and memantine hydrochloride was shown to be acceptable and the stability of the nanopatform was enough for further formulations. Besides, the zeta potential of the formulation was 54 mV, which showed high polydispersity and stability. The intranasal formulation of memantine-loaded chitosan NPs could be a unique nasal drug delivery of memantine hydrochloride for the treatment of the AD.

In another study, Elnaggar et al. prepared ionically cross-linked chitosan-TPP NPs for the intranasal delivery of piperine (a phytopharmaceutical with reported neuroprotective potential) for treating AD in vivo (Wistar rats [180–220 g, 8 weeks old]) and in vitro.<sup>[4]</sup> The NPs were synthesized using the ionic gelation method with poloxamer 188 as a stabilizer. The addition of poloxamer 188 did not change the overall size of the particle; however, it reduced the zeta potential that led to improved polydispersity. The drug loading mechanism was attributed to the entrapment of the drug by piperine encapsulation, and an in vitro release study showed a sustained release of above 90% of the drug after 24 h via the diffusion and degradation processes affecting the polymeric component. Also, the performance of the drug-nanocarrier in comparison with donepezil for the treatment of AD was measured with biochemical tests. The results confirmed that the NPs were as effective as donepezil alone.

Recently, a study investigated the bioavailability of galantamine in the brain after intranasal drug delivery using thiolated chitosan NPs prepared by a modified ionic gelation method.<sup>[211]</sup> The nanocarrier was compared to the nasal and oral delivery of the galantamine solution alone, measuring pharmacodynamic activity as well as biochemical properties. The results showed improvements in memory in a mouse model of induced amnesia after the intranasal administration of galantamine-loaded thiolated chitosan NPs showing the relevance of nose-to-brain delivery compared to the conventional drug oral route for the treatment of AD. In addition, some of the groundbreaking approaches toward drug delivery through the BBB via chitosan-based nanoparticles are listed in **Table 5**.

**Table 5.** Some of the groundbreaking approaches toward drug delivery through the BBB via chitosan-based nanoparticles

Ligand	Surface functionalization approach	Ref.
Magnevist	Chemical conjugation	[214]
Transferrin	Covalent linkage between dimethylsuberimidate and palmitoylated groups on the surface of chitosan nanoparticles	[215]

Transferrin monoclonal antibodies	Chemical conjugation between chitosan, PEG, and biotin linkages	[216]
Rabies virus glycoprotein 29-Cys peptide	MAL-PEG-NHS linker	[217]

Risperidone is an atypical antipsychotic drug used to treat certain mental/mood disorders such as schizophrenia, bipolar disorder, and irritability associated with autistic disorders. Clinical trials have shown that risperidone has a small but significant beneficial effect against aggression and, to a lesser extent, for psychosis in patients with AD. A study investigated the intranasal delivery of risperidone to reduce the side effects of AD treatment through preparation of a risperidone nanoemulsion (RNE) and a mucoadhesive nanoemulsion (RMNE).<sup>[212, 213]</sup> The RNE was prepared by emulsification of Capmul MCM (oil phase), Tween 80 (surfactant), and a propylene glycol/transcutol mixture as a co-surfactant, and was followed by the addition of chitosan to increase the mucoadhesive properties of the RMNE. They observed superior bioavailability and drug absorption of the RMNE due to the mucoadhesive properties of chitosan. Importantly, the scintigrams of rats demonstrated the accumulation of the formulation within the brain administered via the intranasal route. The highest accumulation of radioactivity was seen in the brain following intranasal administration of RMNE as compared to the intravenous administration of RNE. Additionally, some radioactivity was also seen in the esophagus and in the abdominal region.

Several studies on chitosan-based NPs show that they are promising nano carriers for targeted nose-to-brain drug delivery route in order to treat nervous system diseases. This particle caused mucosal adhesion by having a positive charge and fostering the interaction between the positive amino groups of chitosan and the groups of negative mucosal cialic acid, or other negative groups, and showed increased penetration. However, further studies are required, such as toxicity evaluation of these NPs for long-term use.

#### 4.2 PLGA-Based Nanoparticles

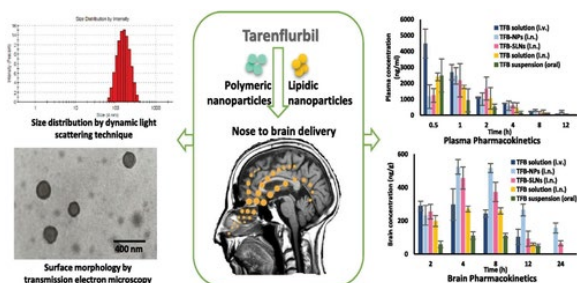
PLGA is one of the most commonly used polymers for the preparation of pharmaceutical carriers with biodegradability, biocompatibility, ability for mucoadhesion, controlled drug release, and the ability to encapsulate various types of drugs.<sup>[218, 219]</sup>

Because of the poor bioavailability of olanzapine (OZ), Seju et al. tested PLGA NPs for OZ drug loading for sustained nose-to-brain OZ drug release for brain targeting. OZ acts as a second-generation antipsychotic drug that can bind to serotonin (5-HT<sub>2c</sub>) and D<sub>2</sub> receptors. These NPs were synthesized by a nanoprecipitation method, and had a size of 91.2 ± 5.2 nm together with a high drug entrapment ratio. According to the results of this study, a burst release and then a sustained drug release was observed in vitro. The ex vivo results showed 59% release of the drug from PLGA nanoparticles after about 3 h. In vivo studies in a rat model demonstrated a significant increase in the uptake of the drug in the brain by intranasal NP delivery, in comparison with the drug used alone. As a result, delivery of the drug to the brain directly using intranasal release of NPs could be helpful in the treatment of central nervous system diseases, especially AD.<sup>[219]</sup>



In another study, PLGA NPs were prepared by solvent displacement, with an average particle size of <300 nm to increase brain drug bioavailability. Musumeci et al. reported that the intranasal administration of oxcarbazepine (OXC) loaded into PLGA NPs decreased the time required for a therapeutic effect to 1 h compared to 24 h for the drug alone by monitoring the reduced numbers of seizures in rats. Fluorescence molecular tomography showed the transfer of a NP-loaded dye and the accumulation of NPs in the brain. They demonstrated a non-invasive nose-to-brain delivery of OXC and a higher drug accumulation in the brain.<sup>[220]</sup>

The delivery of tarenflurbil (TFB, a  $\gamma$ -secretase modulator) using novel polymeric carriers administered via the intranasal route both in vivo SD rats and in vitro models was reported by the Muntimadugu group in 2016.<sup>[221]</sup> Since the TFB drug failed in phase III clinical trials conducted on patients, which was attributed to poor brain penetration, the synthesis of efficient nanocarriers that could overcome this limitation is important. For this purpose, they used various strategies for the synthesis of the NPs. The first involved polymeric NPs (TFB-NPs) synthesized by the emulsification solvent diffusion technique with an appropriate surfactant, and the other one used solid lipid NPs (TFB-SLNs) prepared by the emulsification solvent evaporation technique utilizing glyceryl monostearate, soy lecithin, and stearic acid. The particle size of the nanocarriers was found to be <200 nm in both cases, allowing transcellular transport across the olfactory axons whose diameter was  $\approx$ 200 nm and then a direct path to the brain. TFB-NPs and TFB-SLNs resulted in 64% and 57% entrapment efficiencies, respectively, and achieved protection of the drug from chemical and biological degradation within the nasal cavity. Among the NPs, TFB-NPs exhibited comparatively high efficiency in delivering the drug (% drug targeting efficiency [%DTE] and drug transport percentage [DTP]) to the brain. Higher %DTE (287.24) and DTP (65.18) values were detected for TFB-NPs than TFB-SLNs with the %DTE being 183.15 and the DTP being 45.41. However, both TFB-NP and TFB-SLN as delivery systems could be good candidates for overcoming the poor penetration of TFB across the BBB, leading to its failure in stage III clinical trials. Finally, an in vitro release study showed that the drug release followed a biphasic release pattern with an initial burst for 2 h followed by a sustained release (up to two days) for both of the synthesized NPs (**Figure 4**).



**Figure 4** The intranasal delivery of TFB with PLGA NPs with an average size of 118.7 nm and a Zeta potential of  $-31.18$  mV. Reproduced with permission.<sup>[221]</sup> Copyright 2020, Elsevier.

In addition to the many advantages of a polymeric NP delivery system for the intranasal route, a greater portion of the administered delivery platform reached systemic circulation where it can be opsonized releasing the components from where it reaches the BBB to face the P-gp efflux pumps. Some molecules, such as Cur, can prevent these pumps. In 2018, the Hathout group compared two bio-similar natural molecules, curcumin (Cur) and bisdemethoxycurcumin (BDMC), utilizing several bio/chemoinformatic tools to optimize a potential nose-to-brain delivery route for AD drugs.<sup>[222]</sup> These drugs were formulated in PLGA NPs and delivered into the brain through the intranasal route. This

permitted reaching the BBB and the interaction of the PLGA-drugs with targets linked to the treatment of AD. The results showed the superiority of Cur over BDMC to improve the treatment of AD.

Coating of chitosan on PLGA NPs can increase the therapeutic ability through intranasal delivery since the chitosan can bind to the ciliary or mucous membrane improving NP retention over the surface.<sup>[223, 224]</sup> Kaur et al. used PLGA NPs with a chitosan coating to increase mucoadhesive properties. The mean diameter of the non-loaded PLGA NPs was 110.7 nm, whereas for the chitosan coated NPs, it was 163.6 nm. This biodegradable chitosan-PLGA drug was synthesized in the form of a nanoformulation as a therapeutic assay for the effective intranasal delivery of drugs to the brain of SD rats and Balb/c mice through olfactory and trigeminal neural pathways. The capability of these NPs to reach the brain was assessed through utilizing florescent quantum dot encapsulating NPs. The in vivo results revealed that the recovery increased after intranasal delivery of the encapsulated drugs over the un-encapsulated drugs. In vitro toxicity studies showed that the drug formulations were safe and they were very suitable for drug delivery to the brain.<sup>[223]</sup>

Tong and colleagues used a PLGA-chitosan NP-based system to improve the pharmacokinetic profile of desvenlafaxine (a selective serotonin and norepinephrine reuptake inhibitor) in the brain. These NPs were prepared by solvent emulsion evaporation. The size of the NPs was about 151 nm, but the NPs loaded with the drug had larger sizes.<sup>[225]</sup> The results indicated a high increase in DVF uptake into the brain by DVF PLGA-CN NPs. These NPs also effectively controlled and reversed depression in mice. There is still a need for a more rigorous investigation to verify their performance in animal models and their delivery exclusively via the olfactory route.

In 2018, Meng and co-workers reported that huperazine A (HupA, a naturally occurring sesquiterpene alkaloid) could be loaded into mucoadhesive PLGA NPs synthesized using emulsion–solvent evaporation. These NPs were surface modified by lactoferrin (Lf)-conjugated *N*-trimethylated chitosan to produce HupA Lf-TMC NPs for the efficient intranasal delivery of HupA to the brain for AD treatment.<sup>[226]</sup> The HupA Lf-TMC NPs had an average particle size of about 153 nm, zeta potential of +35.6 mV, drug entrapment efficiency of 73%, and a sustained release in vitro over 2 days. The HupA Lf-TMC NPs showed improved mucin adsorption compared to the PLGA NPs (Lf-TMC NPs 86.9% ± 1.8%, PLGA NPs 32.1% ± 2.5%). In vivo imaging results showed a higher fluorescence intensity of Lf-TMC NPs within the brain and a longer residence time than non-targeted NPs. Also, the Lf-TMC NPs facilitated the distribution of HupA in the brain, and the values of the drug targeting index in the mouse olfactory bulb, cerebrum (with hippocampus removed), cerebellum, and hippocampus were 2.0, 1.6, 1.9, and 1.9, respectively.

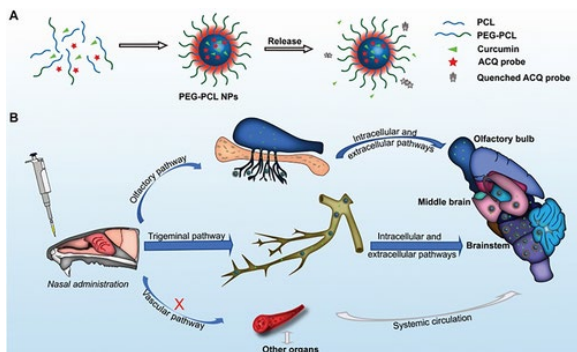
In general, PLGA NPs can improve drug penetration across various barriers, such as the BBB and nasal mucosa. Thus, this polymer has been applied as a drug delivery system to increase the bioavailability and water solubility of drugs. PLGA is resistant to salt and pH instability by producing spherical particles with a negative charge and a smooth surface, and is able to slow the release of the drug during polymer hydrolysis. However, the absence of mucoadhesiveness is one of the limitations of these particles, which limits their use in nasal drug delivery. However, the modification of the PLGA NPs surface with a mucoadhesive polymer can overcome this problem. The coating of chitosan on the PLGA NPs could improve stability of the macromolecules, given a positive surface charge, and increase cellular adhesion at the targeted site. These mucoadhesive delivery platforms improve the delivery

time of therapeutics to the targeted site, thus, reducing the effective dose of the drug as well as reducing the side effects.

### 4.3 PEG-Coated Nanoparticles

Polyethylene glycol (PEG)-coated NPs or “PEGylated NPs” is a general approach for improving the efficiency of gene and drug delivery to target organs and cells.<sup>[227]</sup> In 2015, Zheng and co-workers developed H102 (a  $\beta$ -sheet breaking peptide) encapsulated into a PEG-based liposomal system for intranasal administration to the brain.<sup>[228]</sup> The system showed no toxicity to the nasal mucosa and consistently penetrated Calu-3 cell monolayers. The investigations revealed that H102 could be effectively delivered to the brain, and the AUC of the H102 liposomes into the hippocampus was 2.92-fold higher than that of a H102 solution control. Moreover, H102 liposomes ameliorated the impairment of spatial memory in an AD rat model, increased the activities of ChAT and IDE, and inhibited plaque deposition, even at a lower dosage compared to the H102 intranasal solution.

The intranasal transport of intact polymeric NPs, such as curcumin (Cur)-loaded polycaprolactone (PCL) NPs and PEGylated PCL-NPs via the trigeminal pathway and the olfactory pathway, and their investigation using aggregation-induced quenching (ACQ) probes and fluorescent imaging in Sprague Dawley (SD) rats (200±20 g) is shown in **Figure 5**. PCL is a biodegradable hydrophobic polymer, and therefore, can transport hydrophobic chemotherapeutics, such as Cur with a reduction of side effects. PEGylated PCL-NPs play an important role in improving biodistribution and their penetration into the brain.<sup>[229]</sup>



**Figure 5** Schematic illustration of the olfactory and trigeminal pathways for the nose-to-brain delivery of Cur-loaded PCL-NPs and PEGylated PCL-NPs. Reproduced with permission.<sup>[230]</sup> Copyright 2020, American Scientific Publisher.

In this study, the size of the synthesized NPs was about 100 nm. Cur alone was able to be transported via the olfactory bulb, but the intact NPs were not. However, the NPs did have the ability to penetrate through the mucosa and pass along the trigeminal nerve. Although the penetration of the PCL-NPs into the trigeminal nerve was greater than that of the PEGylated NPs, the PEGylated NPs did have the ability to penetrate into the middle brain and other parts of the brain structure due to better tissue transport, which could lead to effective treatment. Therefore, the trigeminal nerve could provide an effective pathway for the nose-to-brain transport of intact NPs.<sup>[230]</sup>

An in situ gel formulation for the intranasal delivery of tacrine (THA) in adult male SD rats as an animal model was reported by Qian and co-workers.<sup>[231]</sup> For this purpose, the thermosensitive polymer Pluronic F-127, chitosan, and PEG 8000 were used to prepare THA in situ gels. Measurements of the

nasal mucociliary transport time confirmed that the in situ gel formulation prolonged its retention in the nasal cavity compared to the solution form. The rats administered with nasal in situ gel indicated a higher THA concentration profile than oral administration. They demonstrated that the intranasal route improved bioavailability and reduced the side effects of tacrine.

In 2014, Zhang and co-workers demonstrated the entrapment of bFGF in PEG-PLGA NPs intermixed with *Solanum tuberosum* lectin (STL) to selectively bind to *N*-acetylglucosamine expressed on the nasal epithelial membrane. The areas under the concentration-time curve of <sup>125</sup>I-bFGF in the olfactory bulb, cerebrum, and cerebellum of rats following nasal application of STL-modified NPs (STL-bFGF-NP) were 1.79- to 5.17-fold higher in rats compared to those found with intravenous administration, and 0.61- to 2.21- and 0.19- to 1.07-fold higher in comparison with the intranasal solution and unmodified NPs, respectively.<sup>[212]</sup>

In general, PEGylation of NPs can provide a highly effective assay for increasing systemic delivery of therapeutic agents. PEG coatings can play an important role in the design of NPs for drug/gene delivery applications, which will help further studies into how the properties of PEG coatings influence NP biodistribution and the ability to penetrate into the brain, and also clearance from the body.

#### 4.4 PEI-Based Nanoparticles

PEI is a polycationic polymer with a branched backbone that has been applied in nucleic acid transfection and protein delivery.<sup>[232]</sup> Compared to the positively charged peptide sequence, PEI has a greater charge-to-mass ratio. Thus, due to this feature, it can easily be paired with carboxyl C-terminals of peptide terminals and protect peptides from peptidase attack. As a result, PEI is able to transfer large and functional proteins, such as insulin and other neurotrophic factors from the nose to the brain.<sup>[233, 234]</sup>

Lin et al. developed a peptide inhibitor V24P(10-40)-PEI that can assist with A $\beta$ 40/A $\beta$ 42 to form the aggregate morphology of the nanoparticle that leads to lower toxicity. This peptide has the sequence of A $\beta$ 10-40 and is cationized by conjugating with PEI for membrane penetration. In fact, the PEI cationized peptide improved the ability to cross the cell membrane. In vivo results showed that peptide V24P can reduce the formation of toxic amyloid fibrils in the brain of the APP/PS1 double transgenic mice and shift the process towards the formation of amorphous A $\beta$  aggregates. This nasal peptide delivery is easy to apply and could be further developed to prevent and treat AD.<sup>[235]</sup>

PEI can be a good candidate for the transmission of various drugs, especially nucleic acids and proteins across the cell membrane, and can be used in drug design to cross the BBB to the parenchyma by an intranasal route.

#### 4.5 PLA-Based Nanoparticles

Poly(lactic acid) (PLA) is a biocompatible and biodegradable polymer that has long-term safety in humans and a wide range of applications. This polymer can be degraded by various enzymatic activities in the human body and converted into its monomeric units. Another interesting fact about PLA is that it has low immunogenicity; therefore, it can be used in different types of drug/gene delivery applications.<sup>[236]</sup> PLA has been approved by the FDA for certain medical applications.<sup>[236, 237]</sup>

Cheng et al.<sup>[238]</sup> studied the delivery of neurotoxin-I (NT-I) using PLA NPs with a particle size of 60 nm in adult SD rats that are 13 weeks old. In this study, they used PLA instead of poly(butylcyanoacrylate) (PBCA) to reduce toxic effects induced by the material. The level of neurotoxin-I (NT-I) increased in the brain after intranasal administration of NT-I encapsulated by PLA NPs. The results showed that the intranasal delivery of NT-I–PLA NPs was more effective and caused a 1.8-fold increase in peptide bioavailability than intravascular administration.

Pan et al. reported the delivery of  $\alpha$ -asarone into the brain of SD rats (200  $\pm$  20 g) through lactoferrin (Lf)-modified mPEG–PLA NPs. They synthesized NPs by premix membrane emulsification and applied intranasal administration. These NPs efficiently delivered  $\alpha$ -asarone into the brain and showed high bioavailability. This study showed that Lf-mPEG–PLA NPs could increase the efficacy of brain targeting and decrease the toxicity on nasal mucosal cilia and epithelial cells.<sup>[239]</sup>

PLA has been applied to improve drug absorption by nasal mucosa and improve the paracellular absorption of most hydrophilic drugs in the nasal cavity while reducing their clearance. These properties of PLA NPs can make them suitable as a candidate for the treatment of AD.

#### 4.6 HSA Polymeric Nanoparticles

Human serum albumin (HSA) is a water-soluble and biodegradable protein that has been used widely in targeted drug/gene applications and hormone delivery because of its long half-life of approximately 19 days in the circulatory system. As a colloidal drug carrier, it can attach small molecules by its charged amino acids. NPs made of HSA have been studied for drug delivery to the brain and their potential in treatment. These NPs are capable to increase drug absorption by the nasal mucosa.<sup>[240-242]</sup>

Wong et al. improved the efficiency of HSA as a nanocarrier for the intranasal delivery of R-flurbiprofen (R-flurbiprofen [R-FP]) and the treatment of AD in C57BL/6 mice (10–12 weeks of age). In this study, C57BL/6 mice were exposed to three treatment groups, such as an intranasal R-FP solution, intranasal R-FP albumin NPs, and oral R-FP solution. The results showed that the intranasal delivery of NPs containing R-FP NPs obtained a higher brain-to-plasma ratio profile than intranasal and oral delivery of an R-FP solution only, and it was shown that these NPs are probable therapeutic agents for the treatment of AD.<sup>[243]</sup>

#### 4.7 Dendrimer-Based Nanoparticles

Dendrimers are in the class of polymeric NPs, although they have a changed structure from classical polymers, which makes them unique. They are large, 3D, single-weight molecules that have a structure consisting of a nuclei, repeating units, and various functional groups such as COONa, COOH, and NH<sub>2</sub>.<sup>[244]</sup> Due to the chemical type of core and branches, there are various types of dendrimers, such as polyamidoamine (PAMAM), carbosilane, poly-L-lysine (PLL), and polypropylene-imine (PPI). Due to the synthesis method, it is probable to control the shape and size, polydispersity, and defined surface structure (hydrophilic or lipophilic, charged or neutral) in the range of nanometers.<sup>[245]</sup>

PAMAM dendrimers are the most common class of dendrimers that are suitable in many areas such as drug and gene delivery systems and regenerative medicine. They comprise an inner alkyl-diamine core and peripheral shell containing amine branches. The great level of control over dendritic designs makes

dendrimers potential carriers in biomedical applications and effective delivery system for hydrophobic and insoluble drugs.<sup>[246, 247]</sup>

Intranasal preparations of dendrimers suggest their benefits of being cost-effective and non-invasive for effective therapeutic agent delivery. Win-Shwe et al. showed that a single dose of PAMAM dendrimers (3 or 15 µg per mouse) intranasally delivered to 8-week-old BALB/c mice, upregulated BDNF mRNA in the cerebral cortex of mice. This study proposed that the PAMAM dendrimers entered the brain through systemic circulation or olfactory nerve routes to change gene expression.<sup>[248]</sup>

Katare and co-workers studied the efficacy of PAMAM dendrimers to deliver water-insoluble haloperidol through intraperitoneal and intranasal routes into the brain. PAMAM DG5.0 could increase the delivery of haloperidol after intranasal and intraperitoneal routes. The study showed up to a 100-fold increment in haloperidol solubility when applied with a dendrimer formulation. Additionally, 6.7 times lower doses of the dendrimer–haloperidol formulation were delivered through the intranasal route which created behavioral responses in comparison with those formulations delivered through the intraperitoneal route. This result showed the potential of dendrimers in increasing the delivery of water-insoluble drugs to the brain.<sup>[249]</sup>

In 2020, Igartúa and co-workers reported a combined therapy based on the tacrine (TAC) and PAMAM dendrimers (DG4.0 and DG4.5) co-administration in three experimental models to prevent the toxicity produced by the wide first-pass metabolism of the TAC drug. Tacrine as an AChE inhibitor improves the function of brain neurons in AD patients and has a good intestinal permeability due to its lipophilicity. However, due to the short half-life of its removal (1.3-3.5 hours) and the reduction of bioavailability from a high first-pass metabolism, the use of this drug has been limited. TAC treatments also lead to dose dependent hepatotoxicity and other side effects. The charge of these dendrimers is different (positive charge amine surface and negative charge for DG4.0 and DG5.0, respectively), and both dendrimers were able to incorporate the drug inside their pockets or surface. The co-administration with DG4.5 decreased toxicity of the TAC drug on a Neuro-2a cell line as an in vitro model. Co-administration of DG4.0-TAC and DG4.5-TAC decreased the hepatotoxic effects of the drug in zebrafish larvae in an in vivo model. Moreover, co-administration of DG4.0-TAC and DG4.5-TAC in human red blood cells was accomplished in an ex vivo model, although a reduction in toxicity did not reduce drug activity since the anti-acetylcholinesterase activity remained when it was co-administrated with dendrimers. The results of this study suggest the use of this combination therapy due to its low toxicity for the treatment of AD through transdermal and intranasal routes.<sup>[250]</sup>

Dendrimers can play a major role in drug delivery to the brain by having the ability to carry hydrophobic or protein drugs. However, PAMAM dendrimers showed rapid clearance from blood circulation by the phagocyte system and a high amount of administrated dendrimers accumulated in various organs. Functionalization of its surface or different parts of the dendrimers with PEG chains can increase their biocompatibility blood circulation time.<sup>[251]</sup> **Table 6** summarizes current studies of transferring therapeutic agents from the nasal pathway to the brain through polymeric NPs delivery systems.

**Table 6.** Summary of recent studies investigating polymeric NPs delivery systems for intranasal drug delivery

	<b>Types of nanocarriers/drug</b>	<b>Size of nanoparticle [nm]</b>	<b>Delivery route</b>	<b>In vivo/in vitro studies</b>	<b>Ref.</b>
Chitosan-based NPs	Chitosan/bFGF	—	Olfactory route	In vivo/male Sprague–Dawley (SD) rats	[206]
	Chitosan/donepezil	185–340	Olfactory route	In vivo/Wistar rats (aged 4–5 months)	[207]
	Chitosan/galantamine hydrobromide (GH)	140–200	Olfactory route	In vivo/ Sprague–Dawley rats	[209]
	Chitosan/memantine hydrochloride	129	Olfactory route	—	[210]
	Chitosan/piperine (PIP)	248.50	Olfactory route	In vivo/Wistar rats (180–220 g, 8 weeks old) In vitro/dialysis method	[4]
PLGA-based NPs	PLGA/olanzapine (OZ)	91.2 ± 5.2	Olfactory route	In vivo/rat Ex vivo	[219]
PLGA-based NPs PEG-coated NPs	PLGA/oxcarbazepine (OXC)	<300	—	Wistar rats	[220]
	PLGA/tarenflurbil (TFB)	<200	Olfactory route	In vitro/dialysis bag method In vivo/Sprague Dawley (SD) rats	[221]
	PLGA–chitosan/TRH analogues	110.7 nm PLGA/163.6 nm chitosan-coated NPs	Olfactory route	In vivo/(SD) rats/Balb/c mice In vitro/HaCaT cells	[223]
	PEG-PCL /curcumin (Cur)	100	Respiratory route/olfactory route	In vivo/Sprague Dawley (SD) rats (200 ± 20 g)	[230]
PEG-coated NPs PEI-based NPs	PEG/tacrine (THA)	—	Olfactory route	In vivo/Sprague Dawley (SD) rats	[231]
	PEG–PLGA/bFGF	100	Respiratory route/olfactory route	In vivo/Sprague Dawley (SD) rats	[212]

	PEG-PCL/lactoferrin (Lf)	70–90	Olfactory route	In vivo/ICR mice and SD rats In vitro/16HBE14o-cells	[252]
	PEI/R8-A $\beta$ (25–35)	—	Olfactory route	In vivo/APP/PS1 double transgenic mice	[234]
PEI-based NPs PLA-based NPs	PEI/V24P (10-40)	—	Olfactory route	In vivo/APP/PS1 double transgenic mice	[235]
	PLA/neurotoxin-I (NT-I)	60	Olfactory route	In vivo/SD rats	[238]
PLA-based NPs HAS-based NPs	PEG-PLA/ $\alpha$ -asarone	355–370	Olfactory route	In vivo/SD rats <i>Ex vivo</i>	[239]
	HAS/R-flurbiprofen (R-FP)	284.4–14.9	—	In vivo/e C57BL/6 mice (10–12 weeks of age)	[243]
Dendrimer-based NPs	PAMAM/haloperidol	10–20 nm	Olfactory route	In vivo/(groups of animals)	[249]
Dendrimer-based NPs	PAMAM/tacrine (TAC)	—	Respiratory route/ Olfactory route	In vivo/zebrafish larvae In vitro/neuro-2a cell culture Ex vivo/human red blood cell	[250]
	PAMAM/tacrine (TAC)	—	Respiratory route/ olfactory route	In vivo/zebrafish larvae In vitro/neuro-2a cell culture Ex vivo/human red blood cell	[250]

## 5 Conclusions and Future Perspectives

AD is one of the most important causes of morbidity and dementia throughout the world.<sup>[253, 254]</sup> The delivery of drugs to the brain is a major obstacle for the treatment of AD. Most drugs have only limited effectiveness due to the presence of first pass metabolism, the BBB, inadequate blood perfusion, enzymatic degradation, and other physiological barriers. Since the nose is directly connected to the brain, it can be utilized as an appropriate route for overcoming these limitations and could be a preferred delivery route compared to oral and parenteral drug administration. Despite the various



advantages of intranasal drug delivery, the nasal cavity presents several limitations for drug absorption, such as low permeability for some drugs (large and hydrophilic molecules), enzymatic degradation of drugs in the presence of different enzymes in the nasal cavity, and rapid mucociliary clearance. To obtain safe and efficient intranasal drug delivery, NPs for overcoming such nasal delivery barriers should be used.

Due to the low permeability of the blood vessels within the brain, the use of polymeric NPs may also help to bypass the BBB, delivering therapeutic agents directly into the brain. Understanding the physiological and anatomical environment can be very effective for designing suitable polymeric nanocarrier systems. Various types of nanocarriers, such as polymers, are used to facilitate drug delivery, and are capable of binding to the olfactory epithelium undergoing paracellular and intercellular transport. However, transport via the trigeminal nerve and olfactory pathways requires NPs that are smaller than 200 nm in size. In fact, NPs smaller than 200 nm are also less affected by mucociliary clearance, and are therefore more suitable for delivery via these pathways. Additionally, the encapsulation of drugs into NPs protects them from being degraded by enzymes.

NPs should display mucoadhesive properties to increase their residence time in the olfactory mucosa. Improved formulation approaches are needed to lengthen the residence time of the loaded NPs within the nasal cavity. Another important point is that the total drug dose delivered to the target area within the nose should be optimized. The total amount of the drug delivered depends on the fraction of the drug that is transmitted through the olfactory mucosa. Nevertheless, the total amount of drug delivered also depends on its transport and distribution within the brain, which appears to be dynamic.

In general, biodegradable nanocarriers, especially polymer-based NPs, suggest appropriate applications in the drug delivery system and biomedical fields. The prominent benefits of biodegradable polymeric NPs (synthetic or natural polymers) are their biocompatibility, less toxicity, biodegradability in different delivery systems, and their ability to encapsulate both hydrophilic and hydrophobic drugs. In addition, NPs that are sensitive to environmental stimuli for controlled drug release, in combination with the intranasal route of administration, are novel methods for therapeutic agent delivery into the brain. These delivery systems through intranasal administration can overcome the barrier involving the BBB, improving efficiency of drug delivery, and a reduction in drug side effects. However, the size, zeta potential, shape, and surface properties of NPs can significantly impact cellular absorption and efficiency of the treatment, especially in intranasal delivery. One of the most important drawbacks is toxicity and aggregation of NPs into the brain. The toxicity level of anionic NPs is low; however, the cationic NPs like cationic polymeric NPs can cause platelet aggregation and hemolysis.<sup>[255]</sup> Strategies to evaluate NP-based toxicity and efficacy in vitro and in vivo remains a challenge. Biocompatibility, cytotoxicity, biodegradability, and immune response of NPs need to be assessed for intranasal drug delivery. As a result, more research is needed to improve NP synthesis methods and their chemical modification which improve biological distribution and pharmacokinetics of the drug delivery system.

Distribution of an NP-based drug delivery system in the brain and its relation to an effective dose are likely to become important fields of research in the near future. Nevertheless, most investigations are currently still only in the preclinical or early clinical stages, and no nano-based systems have entered advanced clinical trials up to now. Due to the limited number of in vivo studies in animal models, as

well as the anatomical differences between animals and humans, we cannot yet reach a definitive conclusion. In fact, the field of nano-based peptide delivery is still in the early stages of development and requires more definitive studies to obtain pharmacological data to address the targeting efficiency for nanosystems to the brain.

Overall, we believe that polymeric NPs are promising carriers for the intranasal delivery of AD drugs, and could eventually emerge in the pharmaceutical industry as commercially viable improved candidates for AD treatment.

## Acknowledgements

M. R. H. was supported by U.S. NIH Grants R01AI050875 and R21AI121700.

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