Chapter 3
Surface-Modified PLGA Nanoparticles for Targeted Drug Delivery to Neurons

Tejal A. Mehta, Neha Shah, Khushali Parekh, Namdev Dhas, and Jayvadan K. Patel

Abstract The major challenge for the treatment of neuronal diseases is the inability of therapeutic moieties to cross the blood–brain barrier (BBB) and nasal mucosal barrier. The therapeutic moieties for the treatment of brain diseases lack targeting due to its non-specificity toward receptors located at BBB and Pgp efflux mechanism. This results in impeding its ability to reach to maximum effective concentration. Many of these therapeutic moieties possess dose-limiting systemic side effects which along with complex dosage regimens hinder patient compliance and result in discontinuation of treatment. A number of drug delivery and drug-targeting systems have been investigated to increase drug bioavailability and the fraction of the drug accumulated in the targeted area, in order to minimize drug degradation and loss, as well as to reduce harmful side effects. Among all, PLGA NPs have been achieved fascinating properties as a carrier system owing to its biodegradable, biocompatible, and easy functionalization properties. Most importantly PLGA is available in various ratios which can be helpful for tuning the entrapment/loading of therapeutic moieties in NPs. Intranasal delivery has come to the forefront as a method that can bypass the BBB and target drugs directly to the brain as an alternative to invasive methods. The objective of this chapter is to provide a broad overview on current strategies for brain drug delivery and its applications. It is hoped that this chapter could inspire readers to discover possible approaches to deliver drugs into the brain. After an initial overview of the BBB and intranasal route in both healthy and pathological conditions, this chapter revisits, according to recent publications, some questions that are controversial, such as whether nanoparticles by themselves could cross the BBB and whether drugs are specifically transferred to the brain by actively targeted nanoparticles. Furthermore, in this chapter, various conjugation strategies for attaching targeting moieties to the surface of nanocarrier have been included. Current non-nanoparticle strategies are also

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reviewed, such as delivery of drugs through the permeable BBB under pathological conditions and using noninvasive techniques to enhance brain drug uptake.

Keywords Surface-modified nanoparticles · Neurons · CNS diseases · Nasal drug delivery · Targeting strategies

1 Introduction

Despite the tremendous advancement in the field of medicine and pharmacology, there is rise in the prevalence of several central nervous system (CNS) diseases. In the recent years, the treatments available for the CNS diseases are symptomatic and are usually unable to generate quality of life and alter or repair damage. Recently, a vast amount of research took place in the development of drugs for CNS diseases. The progress in the various brain disease treatments have developed new avenues in the form of targeted delivery of the prepared formulation for the specific delivery to the neurons. The average time utilized to develop an innovative therapeutic moiety ranges from 12 to 16 years.

1.1 Neuronal Diseases

The brain, spinal cord, and nerves make up the nervous system. Together they control all the workings of the body. When something goes wrong with a part of this system, symptoms are observed like trouble moving, speaking, swallowing, breathing, or learning. There are more than 600 neurologic diseases. Major types include [1] the following.

Etiology	Examples of disease
Diseases caused by faulty genes	Huntington's disease, muscular dystrophy
Faulty development of nervous system	Spina bifida
Neuro degenerative diseases (nerve cells are damaged or die)	Parkinson's disease, Alzheimer's disease
Diseases of the blood vessels that supply the brain	Stroke
Seizure disorders	Epilepsy
Cancer	Brain tumors
Infections	Meningitis

Neurological disorders affect millions of people worldwide. Statistics says that there are more than six million people who die due to brain stroke each year; approx. 80% of these deaths take place in low- and middle-income countries. It is estimated that >50 million people have epilepsy worldwide. Globally, there are more than 47.5 million people with dementia. With 7.7 million new cases every year, Alzheimer's

disease stands on the top among the most common causes of dementia and may contribute to 60–70% of cases [2].

1.2 Introduction to Various Neuronal Diseases

1.2.1 Alzheimer's Disease (AD)

AD is one of the most common forms of dementia, with more than 24 million cases worldwide [3]. The main characteristics of this disease are memory loss, decreased cognitive functions, and thereby physical functions also. It eventually leads to the death of patient due to loss of neuronal cells. The disease can manifest itself in two forms: (1) familial AD, which is caused by genetic causes usually, and (2) sporadic AD which is caused by environmental factors. The major areas of the brain affected by AD are the neocortex, which is principally involved in the processing of sensory information, and limbic system, which plays a key role in the control of emotions, instinctive behavior, learning, and short-term memory (Fig. 3.1).

The basis of disease is still unclear, but its main assurance is accumulation of beta-amyloid (Aβ peptide) plaque [4]. Over the years, studies on various animal models have provided valuable information in understating the pathogenic mechanism of AD. The pathology of AD is better explained by dividing it into three major segments, viz., positive lesions (lesions related to accumulation), negative lesions (due to losses), and inflammatory lesions (the ones due to the reactive processes). Other important histopathological characteristic of AD covers neurofibrillary tangles (NFTs). NFTs are found inside neurons and are made up of paired helical filaments of hyperphosphorylated microtubule-associated protein tau (MAPT). Intracellular accumulation of NFTs may cause dysfunction of the normal cytoskeletal structure of neurons with consequent death. Distribution of plaques (Aβ) and neurofibrillary tangles (NFTs) is not even across the brain during AD but are confined to disposed neural systems [4].

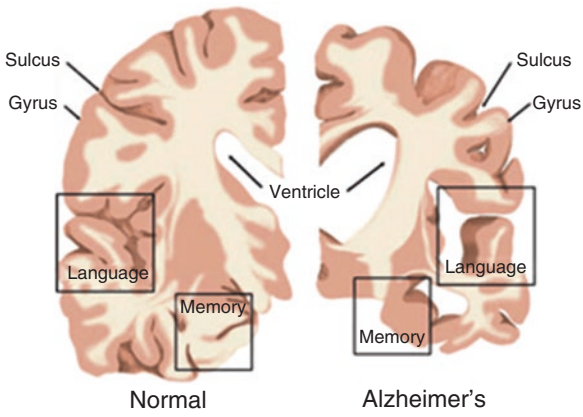

Fig. 3.1 Structure of brain in normal and Alzheimer's disease condition (Source: mybrainx.com)

The roles of important protein and its precursor peptide, amyloid-β protein precursor, have been widely investigated because of the presence of beta-amyloid in the senile plaques [5]. Currently, all the treatments for AD are only able to improve symptoms but cannot lead to the regression of the disease or to its complete cure. Many new treatment regimens for AD have been proposed, but the development of specific drug delivery system which can target the brain and also inhibit the neurodegeneration is still required.

1.2.2 Parkinson's Disease (PD)

PD is one of the neurodegenerative diseases of CNS which weakens motor skills, cognitive processes, and other functions too. Clinical symptoms of PD include bradykinesia, hypokinesia, and rigidity kind of motor and non-motor symptoms. These symptoms are known as Parkinsonism, which is essential for clinical diagnosis in PD. Various studies reflect that major PD symptoms are due to nigral dopamine deficiency-related dysfunction of the basal ganglia [6]. The exact cause of the disease is unknown. Great reduction in the activity of dopamine-secreting cells in pars-compacta region of substantia nigra (Fig. 3.2) is observed which is responsible for the symptoms of PD [7].

Pathologically, the disease is characterized by the accumulation of alpha-synuclein protein forming inclusions called Lewy bodies [8]. Braak's group had proposed "pathogen theory" which is reasonable somewhat for considering the major gastrointestinal (GI) dysfunctions during PD and prevalence of the same in vegetarians [9]. According to this theory, assumption was made that an "unknown

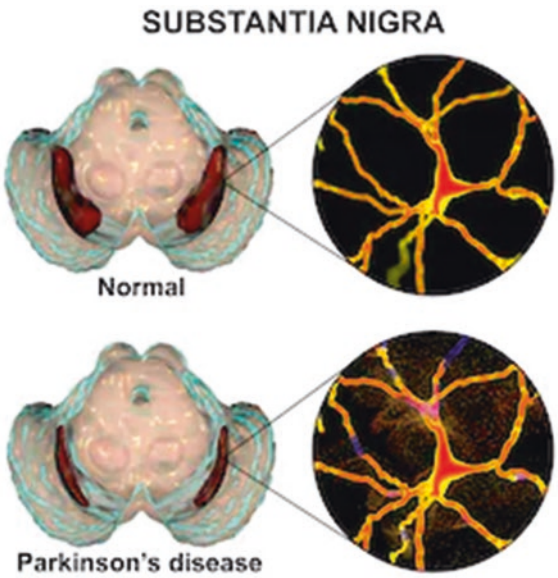

Fig. 3.2 Substantia nigra during normal and Parkinson's disease condition (Source: shutterstock.com—1049278997)

pathogen" pierces the gut wall and enters the central nervous system (CNS) by retrograde transport through the vagus nerve to cause PD [10]. Use of levodopa (L-DOPA) has made current treatments effective for management of motor symptoms. Dopamine agonists and monoamine oxidase B (MAO-B) inhibitors are also useful as dopamine cannot cross blood–brain barrier (BBB) [11]. Drug delivery to the brain remains limited because of the blood–brain barrier (BBB). For example, out of the total L-DOPA administered, only 5–10% is able to cross the blood–brain barrier. Various enzymes metabolize the remaining dopamine, causing side effects, such as nausea, dyskinesias, and stiffness [12].

1.2.3 Brain Cancer/Brain Tumors

Brain tumors can be divided into two major groups: (a) primary brain tumors, which originated from brain tissues, and (b) secondary brain tumors, which result from the metastatic spreading of cancer cells and originated in other regions. Examples of primary brain tumors are gliomas, central nervous system lymphomas (both of which originate from brain parenchyma), meningiomas, and pituitary adenomas (extraparenchymal tumors) [13]. Gliomas can originate from neural stem cells, progenitor cells, or dedifferentiated mature neural cells transformed into cancer stem cells. This classification is based on both their origins in astrocytomas, oligodendroglioma, and mixed oligoastrocytoma. It is also based on their aggressiveness in grades ranging from 2 to 4.

Currently, the use of surgical approaches and/or radiotherapy is most common for brain tumors that not only present a large amount of risk for the patient but are also unable to competently treat more hostile form of gliomas [14]. Such pharmacological treatments should be developed which are able to target cancer cells and destroy brain tumor stem cells as well, in order to prevent a repopulation effect. This will help in preventing all the processes involved in tumor metastatic spreading, including angiogenesis and cell extravasation (Fig. 3.3).

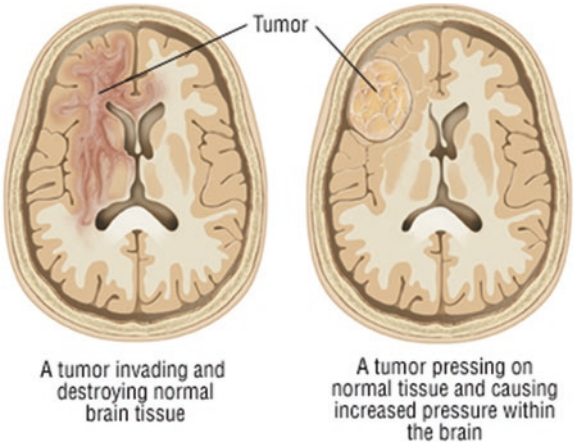

Fig. 3.3 Various types of brain tumors (Source: drugs.com)

Accomplishing successful treatment for CNS diseases remains a vast avenue for research. Till date, a variety of clinical medications for CNS diseases give just constrained change in results and are regularly joined by extreme symptoms. For example, patients determined to have glioblastoma just have a middle survival time of 14 months following surgical resection, radiation, or associative chemotherapy. The trouble in accomplishing enhanced result for CNS disorders originates from the failure to convey therapeutically relevant doses of the therapeutic to diseased cells or regions. The blood–brain barrier (BBB) and nasal mucosa act like the main impediment that keeps most deliberately administrated drugs from entering the CNS. Therefore, before starting with various approaches for targeting drugs to CNS, we need to discuss various obstacles which may hinder the drug to reach CNS to specific sites.

1.3 Blood–Brain Barrier (BBB)

The term "histohematic barrier" was first introduced by LS Stern in 1929. The term "blood–brain barrier" ("Blut-Hirn-Schranke") was coined by Lewandowsky in 1900 [15]. BBB is the subtle network of blood vessels having tightly packed endothelial cells which separates the brain from the circulatory system. BBB is also pericytes, vascular smooth muscle cells (VSMC), neurons, astrocytes, and perivascular macrophages [16]. It is an extremely selective obstacle between blood flow and CNS which plays a crucial role in the homeostasis of the brain by regulating the way of various substances [17]. By virtue of its ability to exclude the passage of certain compounds, the BBB defines compounds as either centrally or peripherally acting [18]. This barrier has less affinity for hydrophilic substances, charged molecules, proteins, and peptides; hence, they are unable to cross the barrier, whereas lipophilic drugs like antidepressants, anxiolytics, and many hormones can cross the barrier with ease.

Besides the brain capillaries, peripheral capillaries also exist. The main difference is the lack of fenestration and intercellular pores, due to the presence of interendothelial tight and adherens junctions, limiting the transport via paracellular pathways [16]. The physical barrier is because of the presence of adherens junctions (AJs) and tight junctions (TJs) between neighboring endothelial cells. TJs comprise of transmembrane proteins located within the paracellular space, preventing paracellular transport by virtually filling the space. Cytoplasmic proteins (zonula occludens [ZO] 1, 2, and 3 and cingulin) are also present which are bound to the actin cytoskeleton [19, 20].

Molecules like proteins, enzymes, and nutrients can travel in and out of the CNS through various pathways. Patients suffering from neurological disorders required prolonged dosing, leading to side effects in nontargeted organs. Majority of drugs which are useful in the treatment of neurological disorders cannot cross the BBB, and hence their potential is reduced as effective therapeutic agent. This left the society with limited treatment options for the patients. Therefore, the development of

noninvasive transport of drug to the brain is highly needed for neurological disorders and brain tumors requiring chronic therapy.

The rate-limiting factors of BBB include the presence of reticuloendothelial system across the BBB which causes protein opsonization. Several approaches have been engaged to improve the drug delivery across the BBB. Nanoparticles (NPs), being of the range from 1 to 1000 nm, are the solid colloidal particles utilized as carrier for drug delivery. Currently NPs are found to have a tremendous advantage over the other methods available for drug delivery across the BBB. Because of its size and functionalization characteristics, nanoparticles are able to penetrate and facilitate the drug delivery through the barrier. Nanomaterials in combination with therapeutic agent can be modified using various mechanisms and strategies for targeted use. Examples of delivery systems are liposomes, polymeric nanoparticles, non-viral vectors, etc. [21]. Nanomaterials are efficacious enough to improve the safety and efficacy of drug delivery devices in brain targeting. Nano-engineered devices are found to be delivering the drugs at cellular levels through nano-fluidic canals. Use of nanotechnology may reduce the need of invasive procedures for delivery of therapeutics to the CNS.

1.4 Nasal Drug Delivery to Brain

Nasal route is an alternative route for those drugs which are difficult to administer orally. This route is being explored by various researchers and proven promising for brain targeting. This route has gained potential attraction for delivering the neurotherapeutics by evading the blood circulation through IN route. Delivering drug by IN route reduces the systemic exposure and hepatic/renal clearance [22, 23]. Olfactory and trigeminal nerves are involved in this pathway, and it has achieved an importance as it can deliver a large range of therapeutic agents [23, 24].

The probable mechanism by which drugs are transported from the nose to the brain is not yet clear, but olfactory pathway plays a vital role. This pathway consists of olfactory epithelium, lamina propria, and olfactory bulb. Entry of drugs to caudal and rostral parts of the brain is promoted in IN delivery as trigeminal nerve enters to the brainstem through pons by innervating the nasal cavity, whereas it enters to the forebrain through the cribriform plate. Drug is delivered to the rostral area of the brain by olfactory pathway where in case of trigeminal pathway, rostral but caudal, both areas of the brain are targeted. Hence, it is difficult to differentiate that intranasally administered drug is translocated to rostral area by olfactory or trigeminal pathway.

Olfactory pathway is a reliable alternative to achieve desired therapeutic effects at lower doses for treating chronic diseases while minimizing the side effects. Direct IN drug transportation to the brain is referred by transmucosal delivery of drug via olfactory or trigeminal pathway. The only route by which the brain is connected with the outside environment is intranasal route. This neural connection has gained consideration for delivery of wide variety of drug molecules by the nanoformulations. Such formulation systems for nucleotides, peptides, and proteins can be

developed to deliver them to the brain by preventing enzymatic degradation and augmenting the pharmacological properties without systemic absorption and toxicity. It was investigated in animal and human studies that different nose to brain drug delivery systems resulted successful by enhancing the nasal permeability, improving adhesion to mucous membrane, providing continuous or measured release of drug, or increasing deposition at olfactory epithelium.

Nanotechnology is a more promising drug delivery system among the advanced technologies of targeting and controlled release systems [25].

There are various strategies to target the brain which includes invasive as well as noninvasive techniques.

2 Nanoparticles as Drug Delivery Vehicles

Merits of nanoparticles as drug delivery system are controlled release of drug, reduced toxicity, improved bioavailability, and enhanced therapeutic efficacy and bio-distribution [26]. Structure of nanoparticles enables them to protect the drugs from environmental degradation due to factors like stomach acid and enzymes [27]. Size range for polymeric nanoparticles is about 10–1000 nm [28] and can be modified with different ligands such as antibodies to create a smart targeting delivery system. To cross the BBB, surfactant-coated polymeric nanoparticles of drug should be of $\leq$300 nm [29].

Food and Drug Administration (FDA)-approved therapeutic devices use poly(lactic-co-glycolic acid) (PLGA). Random ring-opening and copolymerization of two different monomers form PLGA polymer. It is a biodegradable polymer, and its hydrolysis of PLGA produces original monomers, lactic acid and glycolic acid, in the body.

PLGA shows controlled degradation by the altering the ratio of the two monomers: the lower the content of glycolide units, the more time is required. 50:50 monomer ratio in the copolymer exhibits the fastest degradation. Minimal toxicity and ability of controlling the drug release make PLGA the most suitable for drug delivery [30]. Biocompatibility for targeting drug at cellular level shows versatility of PLGA nanoparticles among various nanoparticulate systems [31]. The degradation of polymer matrix of PLGA nanoparticles releases encapsulated L-DOPA. Hence, undesirable effects observed with the conventional oral administration can be reduced [32]. Despite having various advantages of PLGA as a drug carrier, it has been found that nontargeted PLGA NPs could not possibly target to specific site in the brain as compared to targeted PLGA NPs. Taking into consideration aforementioned statement, there are various targeting ligands and various approaches of targeting to specific receptors which get overexpressed in a specific disease. In the following section, we will discuss various targeting strategies and different types of ligands which help to target specific receptors to show significant therapeutic response. Furthermore, we have also discussed various types of surface modification/conjugation strategies of ligands to PLGA NPs.

3 Dual-/Multi-targeting Strategies

Intranasal mucosa and BBB are still a barrier in the treatment of brain diseases. The vast research has been carried out for the development of targeting delivery system for brain diseases (Fig. 3.4). Nevertheless, the developed formulations were not capable enough for the treatment of diseases of the brain due to several reasons: (1) brain diseases usually occur in a particular area of the brain; thus, the conveyance of the therapeutic moiety into the brain is a critical matter when it penetrates the BBB and nasal mucosa and enters the brain. The poor targeting efficiency to the particular diseased area leads to low concentration of drug in the particular diseased area [33, 34]. (2) The dissemination of therapeutic moiety in healthy neuronal cells, which may eventually lead to neurotoxicity [35]. To reduce this difficulty, dual-/multi-targeting nanocarrier system strategies were proposed. Initially, dual-/multi-targeting strategy was employed to overcome two or more barriers present in the brain which may retard the delivery system to reach the specific diseased area. It is generalized that dual-targeting nanocarrier systems should be able to help to pass via nasal mucosa and/or BBB and then precisely bind to cells of the diseased brain. However, certain nanocarriers were also termed as "dual-targeting system" when nanocarriers were formulated for targeting two different locations (i.e., two distinct cells namely neo-vasculature and tumor cells) and in another case targeting the same cell type but at different receptors (i.e., transferrin and folate receptor on cancer cells) [35, 36]. Depending on the target site, one can particularly classify nanocarriers into various applications:

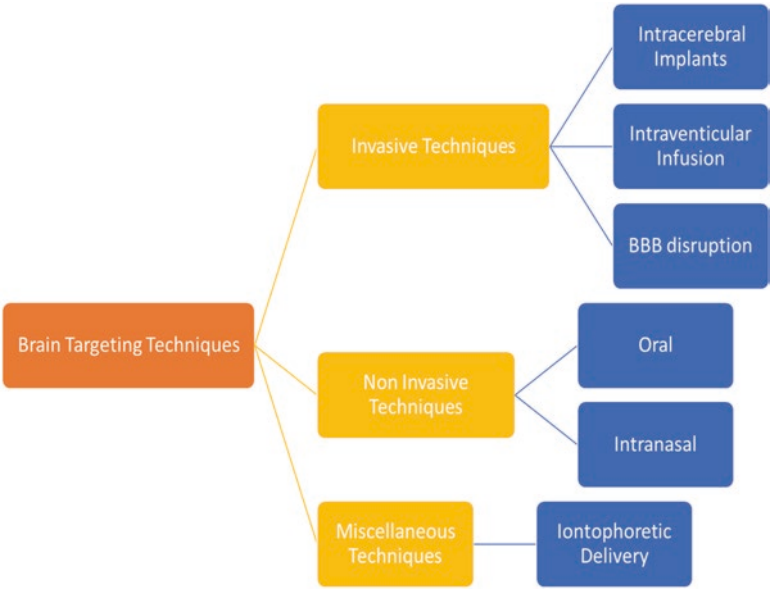

Fig. 3.4 Various approaches to target neurons

1. Targeting nasal mucosa/BBB and diseased cells utilizing unlike targeting moieties: as stated above, the earlier perception of dual-targeting delivery was passing nanocarrier via nasal mucosa/BBB and then specifically targeting to the diseased cells. To overcome this constraint, two targeting moieties were often used for constructing targeting delivery systems, one for penetration of the nasal mucosa/BBB and the other for targeting diseased cells. In the above context, Yin et al. developed sialic acid-modified selenium NPs coated with B6 peptide (B6-SA-SeNPs). Peptide B6 is known for its BBB permeability, and sialic acid has showed significance in the development of cognition and enhancement in learning and memory performance, whereas selenium NPs show synergistic action in AD in the form of antioxidants. In vitro BBB model (bEnd.3 cells) study demonstrated that B6-SA-SeNPs were efficiently crossing the BBB and were getting absorbed by PC12 cells. Furthermore, B6-SA-SeNPs not only inhibited Aβ aggregation but also disaggregated preformed Aβ fibrils into nontoxic amorphous oligomers [37]. In a similar manner, Zhang et al. developed TGN/QSH dual-conjugated H102-loaded PEG-PLA NPs (TGN/QSH-H102-PEG-PLA NPs) as a dual-targeting platform in the treatment of AD. Two targeting peptides, QSH and TGN, were conjugated to the surface of NPs for Aβ targeting and BBB transport, respectively. The maximum concentration of H102 was obtained in the hippocampi of the TGN/QSH-H102-PEG-PLA group mice 1 h after administration, which were 1.86 and 2.62 times the level of TGN-H102-PEG-PLA NPs and non-modified H102-PEG-PLA NPs, respectively [38].
2. Targeting two cell types in the diseased cells: as there can be many targeting sites for specific diseased neuronal cells, which may be responsible for pathological conditions, for example, AD has various targeting sites such as Aβ plaques, mitochondrial targeting, and tau aggregates. This indicates a researcher can target Aβ which can avoid Aβ agglomeration or disaggregate the form; Aβ plaques and other targeting moieties can be attached to target mitochondria which will help in reducing oxidative stress. As a result, the synergistic action may ameliorate the cognitive deficit. Furthermore, in case of brain tumor, there can be various sites for targeting such as blood vessels (angiogenesis) which are present in tumor and receptors present on tumor cells. In this context, targeting moiety which reduces the growth of blood vessel (anti-angiogenesis) treatment can be simultaneously be delivered with another targeting moiety which targets tumor cells which reduces tumor growth [39]. To address the abovementioned requirement, Gao et al. developed dual-functional interleukin-13 peptide (IL-13)/RGD-conjugated PEG-PCL NPs. The selection of the targeting was done with the aim of dual targeting to brain tumor. In this context, RGD would target avβ3 on neovasculature, and IL-13 would target IL13Rα2 on glioblastoma cells. The receptor labeling study demonstrated that IL13Rα2 could mediate the internalization of IL-13-modified NPs, and avβ3 could mediate the internalization of RGD-modified NPs. Furthermore, in vivo study displayed that IL-13/RGD-PEG-PLA NPs also showed high penetration ability than monomodified NPs. Additionally, CD-31 staining study demonstrated that IL-13/RGD-PEG-PLA NPs could target both neovasculature and glioblastoma cells [40].

3. Dual targeting with one ligand: there are some ligands that could directly target both nasal mucosa/BBB and diseased neuronal cells due to their receptors or transporters that are overexpressed on both nasal mucosa/BBB and diseased neuronal cell. In the above context, Ruan et al. developed DOX-loaded antigiopep-2-conjugated gold NPs as a dual-targeting nanoplatform in the treatment of brain disease. Angiopep-2 is the member of peptide family named angiopep, which are derived from the Kunitz domain of aprotinin. Additionally, angiopep-2 has high LRP binding affinity; thus, it was candidate for the dual-targeting ligand for NPs to penetrate the BBB and target the brain tumor. It was found that the release of DOX was pH sensitive owing to the hydrazine bonding between the gold NPs and DOX. Furthermore the release study demonstrated that at pH 7.4, the 48 h cumulative release was found to be 21.9%, while at pH 5 it was elevated to 88.3%. Above study revealed that the combination of therapeutic activity and dual-targeting property of angiopep-2 peptide can efficiently enhance the survival time of brain tumor-bearing mice from 19 days to 55 days [41].

3.1 Lectins

Lectins are sugar-binding proteins that are exceptionally particular for attachment to the sugar moiety. Lectins are found all through nature, in plants, i.e., seeds, and different nourishments, for example, dairy substance, and the human body [42–44]. On the cell surface of tissues, starches going about as basic parts of the human body are broadly appropriated. They are engaged with different organic procedures, for example, cell bond, irritation, and cell actuation [42, 43]. Different kinds of lectins are being bound by specific sugar moieties present of these carbohydrates. Lectins are robust proteins; they are resistant to gastric acid and digestive enzymes. They have ability to bind to stomach wall, change permeability, damage the epithelial cells, pass through the gut, and enter the blood with other proteins, which may lead to certain adverse immunological responses [45]. Taking into consideration the above statement, lectins originated from plants and animals may cause several adverse reactions to the human body. Lectins have particular targeting capability which marks them as safe and appropriate to be utilized as a prodrug agent. Recently, researchers are utilizing lectin as a targeting moiety to be targeted to specific region of disease, for example, neurological diseases. Furthermore, lectins can be conjugated to the therapeutic moiety, or it can be conjugated to nanocarrier in the form of surface functionalization. So, considering the targeting approach to the brain, lectins can overcome the BBB as well as intranasal route barrier and subsequently increase the efficiency of nanocarrier platform loaded with therapeutic moiety [43]. Wen et al. developed odorranalectin (OL)-conjugated PLGA-PEG NPs to enhance the intranasal delivery of the therapeutic moiety in the treatment of CNS diseases [46]. Recently various lectins have been approved in pharmaceutical world which includes wheat germ agglutinin (WGA) and peanut agglutinin. Among them WGA has been widely utilized in research, more specifically as a targeting moiety for nose

to brain delivery. WGA is obtained from *Triticum vulgaris*, and which has particular affinity toward sialic acid and *N*-Acetyl-glucosamine (GlcNAc). Most of the study concluded that WGA can be safely used for intranasal delivery as it does not produce any toxicity to the normal cells.

3.2 Cell-Penetrating Peptides (CPPs)

As of late, cell-penetrating peptides (CPPs) have been broadly contemplated regarding their utility in drug delivery. This makes it difficult to have a general definition covering the attributes of the distinctive CPPs found. Up until now, one can state that CPPs are short peptides (generally not surpassing 30 amino acids) that have the ability to pervasively cross cell membranes with extremely constrained toxicity, by means of energy-independent (direct membrane translocation) and/or energy-dependent (endocytosis pathways such as clathrin-mediated endocytosis, caveolae/lipid raft-mediated endocytosis and macropinocytosis) mechanisms, without the need of a chiral recognition by particular receptors [47, 48]. Albeit a few unique criteria have been proposed for the classification of CPPs, in view of their sequences, origin, function, or mechanism of uptake, no unified scientific classification of these peptides directly exists. Depending on the origin, CPPs can be categorized as natural and artificial peptides. Additionally, based on the bonding between CPP and cargoes/nanomaterial, CPPs can be classified as non-covalent (may consist of electrostatic interactions/hydrophobic interactions) and covalent (may consist of disulfide or thioester bond). Furthermore, as per their physicochemical properties, they can be simply sorted into three fundamental classes, that is, amphiphilic, hydrophobic, and cationic peptides [49]. Cellular uptake of CPP can be affected by various factors such as concentration of CPP/cargo/nanomaterial-CPP conjugates and size. Additionally CPPs can be decorated over the nanocarriers to achieve target-specific delivery. In this context, vast amount of research has been carried out on CPP as a brain-targeting agent following intranasal route. Additionally, as far as intranasal route is concerned, CPPs, when decorated over the NPs, can efficiently transfer the therapeutic moiety across nasal mucosa followed by brain targeting. They endorse transport of therapeutic moiety via olfactory pathway to the brain [50–52]. Among CPPs, low molecular weight protamine (LMWP), Tat protein, and penetratin are therapeutically important in transmucosal delivery of loaded therapeutic agent. LMWP is considered to be safer as compared to protamine as it is nonantigenic and has low toxicity [53, 54]. The LMWP-CPPs could be used to improve IN transport of therapeutic moiety to CNS. It was considered that conjugates of LMWP-NPs were successfully transported to the brain by noninvasive IN administration. In this context, Xia et al. developed a coumarin-6-loaded LMWP-conjugated PEG-PLA NPs. The in vivo results revealed that intranasally administered LMWP-PEG NPs were detected in the rat olfactory tract, cerebrum, cerebellum, and olfactory bulb and were found to be 2.68-, 2.03-, 2.55-, and 2.82-fold, respectively, as compared to that of PEG-PLA NPs. Biodistribution study also revealed that LMWP-PEG-PLA

NPs after intranasal administration could be delivered to the CNS along both trigeminal nerve and olfactory nerve pathways [55]. Furthermore, Yan et al. developed insulin-loaded Tat-conjugated PLGA NPs to overcome the barriers like nasal mucosa penetration, intracellular transport along the olfactory neurons, and diffusion across the heterogeneous brain compartments. The results demonstrated that Tat-conjugated PLGA NPs can efficiently deliver the insulin to the brain following intranasal route, showing the total brain delivery efficiency of 6% [56]. Additionally, Yang et al. developed rivastigmine-loaded CPP-conjugated liposomes for intranasal administration for brain targeting. It was found that the concentration of rivastigmine loaded with liposomes was higher as compared to free rivastigmine [57]. Kamei et al. hypothesized that CPP could be used to enhance the drug delivery to the brain. For the same, the researcher developed an insulin solution co-administered with penetratin and was intranasally administered to mice. The results demonstrated that insulin co-administered with D- and L-penetratin reached the distal region of the brain from the nasal cavity, including the cerebellum, cerebral cortex, and brainstem. In particular, D-penetratin could intranasally deliver insulin to the brain with a reduced risk of systemic insulin exposure [58]. In addition to intranasal route, CPP can be used as a targeting moiety to cross blood–brain barrier (BBB) and bypass effectively the Pgp in the BBB. For example, they have enhanced doxorubicin transport into the rat brain up to 30-fold. This approach is efficient on the grounds that CPPs use adsorptive-mediated endocytosis to enter the brain. It is triggered by electrostatic interactions between the negatively charged cell membranes of brain endothelial cells and positively charged moieties of the protein. In this context, Lu et al. developed CPP-conjugated BSA NPs to evaluate brain-targeting efficiency. It was found that the permeability of CPP-BSA NPs was 7.76-fold higher than that of BSA NPs [59]. Additionally, Qin et al. developed doxorubicin-loaded Tat-conjugated liposomes for glioma therapy [60].

3.2.1 Glutathione (GSH)

GSH is a natural, hydrophilic, tripeptide molecule which performs antioxidant function against reactive oxygen species and toxic metabolites of the cell [61]. The idea of glutathione-coupled nanoparticles rose up out of the way that the glutathione is the most abundant antioxidant agent existing in the human body; apart from that it additionally acts as an endogenous ligand for glutamate receptors, i.e., *N*-methyl-D-aspartate (NMDA) and 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors which are abundantly expressed in the brain, and consequently it experiences receptor-mediated endocytosis [62]. Additionally, GSH has the ability to interact with transmembrane proteins located in the brain that are involved in the active transport of various materials across BBB [63]. Additionally, glutathione also regulates the permeability of BBB through its action on tight junction proteins (occludin and claudin) of BBB [64, 65]. Among these, Pgp is involved in ATP-coupled reactions in transporting materials conjugated to GSH across membranes. Henceforth, glutathione-coupled nanocarriers establish a promising drug delivery

system for transport of various neurotherapeutics to the brain by surpassing BBB [66]. In this context, Gaillard et al. developed methylprednisolone-loaded GSH-conjugated liposomes for enhanced brain delivery [67]. Results demonstrated that treatment with GSH-PEG liposomes was significantly more effective compared to PEG liposomes. Furthermore, DOX-GSH-PEG liposomes are in clinical trials for the pharmacokinetics, tolerability, and safety in the treatment of brain metastasis or recurrent malignant glioma and solid tumors, which clearly demonstrated clinical significance of GSH-PEG liposomes as a nanocarrier for brain-targeted drug delivery. Furthermore, Patel et al. developed hydrophilic fluorescent marker-loaded GSH-conjugated BSA NPs for brain-specific drug delivery. The study revealed that the permeability of GSH-BSA NPs across the monolayer of MDCK-MDR1 endothelial tight junction was shown significantly higher as compared to BSA NPs and fluorescein sodium solution. Additionally, GSH-BSA NPs exhibited higher uptake by neuroglial cells as compared to BSA NPs and fluorescein sodium solution. Intravenously administered GSH-BSA NPs showed threefold higher fluorescein sodium carried to the brain as compared to BSA NPs [62].

4 Strategies for Targeted Nanoparticles

Various methods/chemistries have been employed to link ligands with reactive groups of the surface of the nanocarriers, and the methods can be classified into covalent and non-covalent conjugations. Common covalent coupling among the other methods involve chemistries such as (1) carbodiimide chemistry, (2) Michael addition, and (3) “click” chemistry.

4.1 Carbodiimide Chemistry

This technique is the most utilized for the conjugation of nanocarrier with the targeting moiety. The merit of this chemistry depends on its simplicity, and the required functional groups present on the targeting moiety and nanocarrier (e.g., amine group, carboxylic acid, and so on), to accomplish the coupling. This results in low possibility to lose ligand-specific activity [68]. Nevertheless, the presence of multiple functional groups in the targeting moiety can put forth a drawback trying to confine multi-site attachment or to control the targeting moiety alignment at the surface of the nanocarrier [69]. The amide bond formation takes place in two subsequent steps. Amid the first, the activation of carboxylic acid groups present on the surface of nanocarrier is carried out by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). This reagent is generally referred as carbodiimide which can shape

diverse chemical structures. EDC has great solubility in water which empowers direct utilization of EDC in aqueous solutions without addition of any organic compounds. These conditions are appropriate for the attachment of bioactive particles to the carrier surface. The o-acylisourea intermediate formed during the actuation of the carboxylic acid is vulnerable to quick hydrolysis [70]. Thusly, an excess amount of carbodiimide must be utilized to finish the reaction, which can modify the colloidal stability of the subsequent nanocarriers because of the poor solubility of the o-acylisourea. This can be fathomed utilizing *N*-hydroxysuccinimide (NHS) or *N*-hydroxysulfosuccinimide (sulfo-NHS). The ester intermediate of the latter has a better solubility. Albeit still susceptible to hydrolysis (which is anyway slower than o-acylisourea middle of the road), NHS esters as a rule prompt higher coupling efficiency [69].

The above-described information was connected with the activation of the carboxylic group on the surface of nanocarrier. Alternative method is associated with the activation of primary amine of nanocarrier. This process takes place when homo-bifunctional dithio-bis(succinimidyl propionate) (DSP) is used [71]. The DSP was used in the synthesis of drug carrier which was applied in the targeted breast cancer therapy [72]. The DSP activates the amine groups of nanocarriers. Additionally, application of NHS allows the formation of ester during the reaction with monoclonal antibody (trastuzumab). This methodology was very efficient and selective throughout cancer gene therapy [73].

4.2 Michael Addition

Michael addition is the second widely applied approach for conjugating targeting moiety and nanocarrier which involves in particular thiol-maleimide coupling strategy. Maleimides used in Michael addition exhibit slower hydrolysis rate than o-acylisourea intermediates in carbodiimide chemistry. Besides, at near neutral pH, maleimides react with thiols in a highly selective and efficient way resulting into stable thioether bond [68, 69]. The reaction runs quickly and under mild conditions, at the room temperature, and in aqueous solution. Formed thioether bond is stable within 24 h in human serum even in the presence of reducing agent, for example, DTT. To circumvent this limitation, thiol group can be obtained either by reducing existing disulfide bonds (which can be however detrimental for ternary structures of certain peptides/proteins) or by using hetero-bifunctional cross-linking agents such as *N*-succinimidyl-*S*-acetylthioacetate (SATA) or *N*-succinimidyl-3-(2-pyridyldithio) propionate (SPDP) [74]. Application of the system containing the thioether bond guarantees the high selectivity of delivery and the long time of distribution.

4.3 The "Click Chemistry"

The term click chemistry (CC) was developed by Kolb's group and describes the coupling of small molecules and heteroatoms in the form of R-X-R [75]. Reactions belonging to "click chemistry" group share several very important features: (1) a very high efficiency in terms of both conversion and selectivity; (2) mild experimental conditions; (3) a simple workup; (4) little or no by-products; (5) readily available reagents; and (6) easily removed solvent, for example, water [71]. The main method of the purification of final product is crystallization or distillation [75]. Within CC, one can find four different types [75, 76]: (1) cycloaddition, for example, Huisgen catalytic cycloaddition; (2) nucleophilic substitution chemistry, for example, ring opening of heterocyclic electrophiles; (3) carbonyl chemistry of the "nonaldol" type, for example, formation of ureas, thioureas, and hydrazones; and (4) addition to carbon–carbon multiple bonds, for example, epoxilation and dihydroxylation. The major type of "click chemistry" reaction is the cycloaddition which has received remarkable attention in many fields such as bioconjugations and polymer functionalization. This process creates 1,2,3-triazole by the Huisgen 1,3-dipolar cycloaddition reaction between an azides and terminal alkynes (CuAAC) in the presence of the catalyst, copper (I) [71, 77, 78]. The principle drawback of the CuAAC reaction is the necessary removal of the Cu-based catalyst after the coupling. The use of Cu ligands and organic scavengers is however possible to remove most of the catalyst.

5 Work Explored by Various Researchers for Targeting of PLGA NPs to Brain in Different Neuronal Diseases

Various researchers have worked on PLGA nanoparticles for brain targeting. The details are compiled in the Table 3.1.

6 Patents and Clinical Trial Status

Nanotechnology is being explored and has shown very promising results these days. Many nano-based products like curcumin, EGFR, ferumoxytol, etc. are under clinical trials, and initial phase studies are already completed. PLGA nanoparticles are also very well explored in research for neuronal targeting, and it is expected that they too will pass the clinical trial phase and products will be there in the market in the near future. With the enhancement in targeted NP-related research, a lot of attention has been given for commercialization of such nanoparticles which led to the filing of patents related to these NPs. Various types of patents have been filed related to these NPs by different agencies including independent researchers and pharmaceutical companies. The filed patents have been summarized in Table 3.2.

Table 3.1 PLGA NPs for neuron targeting

Architecture	Targeting ligand	Therapeutic moiety	Preparation method	Particle size	Neuronal disease/ disorder	In vitro/In vivo model	Application	Outcome	Ref.
Teniposide-PLGA NPs	–	Teniposide	Single emulsion solvent evaporation	121 nm	Neuroblastoma	U87MG cells	Uptake mechanisms of NPs	• Cytotoxicity study demonstrated that drug-loaded PLGA NPs showed significant cytotoxicity, whereas blank NPs showed no cytotoxicity within 24 h • Endocytosis inhibition test showed that NP internalization within cells took place via clathrin-mediated endocytosis and macropinocytosis	[79]
Carbamazepine (CBZ)/verapamil-PLGA/poloxamer 188 NPs	–	CBZ, verapamil	HPH followed by solvent evaporation	130–150 nm	Epilepsy	Male Wistar rats	The effect of Pgp inhibitor (verapamil) on anticonvulsant effect of CBZ and its nanoformulation in the rat model	• It was found that verapamil significantly increased the anticonvulsant effect of CBZ and reduced its effective dose by at least 30% • CBZ-loaded poloxamer 188-coated PLGA NPs enabled 30-fold increase of its anticonvulsant effect as compared to free drug	[80]

(continued)

Table 3.1 (continued)

Architecture	Targeting ligand	Therapeutic moiety	Preparation method	Particle size	Neuronal disease/ disorder	In vitro/In vivo model	Application	Outcome	Ref.
Tet-1-curcumin-PLGA NPs	Tet-1	Curcumin	Single emulsion solvent evaporation	150–200 nm	Alzheimer's disease	GI-1 glioma cells, LAG cell line	Study anti-amyloid activity and antioxidant activity of curcumin-PLGA NPs	• The Tet-1 neuropeptide-conjugated PLGA NPs demonstrated that in vitro targeting efficiency was enhanced greatly • The curcumin-loaded PLGA NPs can be used as a potential tool in treating AD with respect to its anti-amyloid and antioxidant property	[81]
g7-FITC-albumin-PLGA NPs	g7	FITC-albumin	Double emulsion technique	254–261 nm	Lysosomal storage disorders	In vivo: C57BL/6 Idua knockout (ko) mouse and wild-type mouse	Targeted delivery platform loaded with high molecular weight molecules in lysosomal storage disorders	• In vivo study demonstrated that the g7-FITC-albumin-PLGA NPs were able to cross BBB and were distributed in brain parenchyma both in ko and wild-type mice, with higher efficiency in ko mice • g7-FITC-albumin-PLGA NPs followed the clathrin pathway to enter the neuronal cells	[82]

RVG29-PEG-PLGA-docetaxel	RVG29	Docetaxel	Nanoprecipitation method	110 nm	Glioma	C6, bEnd3 and HeLa cells	Brain-targeted drug delivery platform in the treatment of glioma	• The in vitro cellular uptake study results demonstrated that RVG29-conjugated NPs showed better targeting property as compared to nontargeted NPs • RVG29-PEG-PLGA NPs showed better BBB penetration in an in vitro model and were selectively accumulated in transcranial glioma tissue	[83]
Tf-PLGA NPs, RVG (rabies virus glycoprotein)-PLGA NPs, Tf-BSA NPs RVG-BSA NPs	Tf RVG	Oxytocin	Multiple emulsion solvent evaporation, Coacervation /nanoprecipitation method	100–278 nm	Autism spectrum disorder	Dendritic cells	Nanoformulations for sustained release and brain targeting	• The study demonstrated that the prepared formulation was found to be safe as it did not produce immunogenic and cytotoxic response • The release study revealed that BSA NPs showed faster initial burst of release compared to PLGA NPs, in addition to later sustained release	[84]

(continued)

Table 3.1 (continued)

Architecture	Targeting ligand	Therapeutic moiety	Preparation method	Particle size	Neuronal disease/ disorder	In vitro/In vivo model	Application	Outcome	Ref.
PEG-PLGA-epigallocatechin-3-gallate NPs	PEG	Epigallocatechin-3-gallate	Double emulsion method	169 nm	Temporal lobe epilepsy	In vitro: astrocytes, bEnd.3, and PC12 cells In vivo: 1-month-old wild-type C57BL/6J mice	Development of nanoplatform for the treatment of epilepsy	• Cytotoxicity assays showed that formulation was safe for delivery • Behavior test demonstrated that NPs reduced most number of episodes of epilepsy and their intensity as compared to free drug • Neurotoxicity and immunohistochemistry studies confirmed a decrease in neuronal death and neuroinflammation	[85]

Pep-apoE-PLGA NPs Lipocalin-type prostaglandin-D-synthase (L-PGDS)-PLGA NPs	Pep-apoE, L-PGDS	–	Single emulsion solvent evaporation method followed by conjugation with carbodiimide chemistry	198 nm	Brain diseases	DCs	Novel functionalization strategies for brain targeting	• The study revealed that targeted PLGA NPs, but not nontargeted PLGA NPs, were found in cerebral cortex parenchyma after 2 h of IV in mice • It was also found that NPs were mostly internalized by neurons and microglia • The finding showed that functionalization strategies were found to be safe as they did not elicit adverse immune response	[86]

(continued)

Table 3.1 (continued)

Architecture	Targeting ligand	Therapeutic moiety	Preparation method	Particle size	Neuronal disease/ disorder	In vitro/In vivo model	Application	Outcome	Ref.
hGDNF-polyamidoamine (PAMAM)-PEG-Lf NPs	Lf	hGDNF	Synthesis using Michael addition reaction	196 nm	Parkinson's disease	In vivo: Male Sprague Dawley rats	Platform for neuroprotection in a 6-hydroxydopamine-lesioned Parkinson's model	• The neuroprotective evaluation demonstrated that increase in the number of injections of NPs ameliorated locomotor activity, reduced dopaminergic neuronal loss, and enhanced monoamine neurotransmitter levels • Study also revealed that five injections of Lf-modified NPs loaded with hGDNF exhibited much more powerful neuroprotection than a single injection	[87]
83–14 mAb-polyacrylamide (PAA)-cardiolipin (CL)-PLGA-rosmarinic acid (RA) and curcumin (CUR)	83–14 mAb	RA and CUR	Emulsification followed by solvent displacement method	100–500 nm	Alzheimer's disease	In vitro: SK-N-MC cells	Development of promising platform for pharmacotherapy to permeate the BBB and reduce the fibrillar Aβ-induced neurotoxicity	• Study revealed that the increase in concentration of 83–14 mAb enhanced the permeability coefficient of RA and CUR using NPs •	[88]

mAb-CUR-PLGA NPs	mAb	CUR	Nanoprecipitation method	250 nm	Glioblastoma	In vitro: DKMG/ $EGFR_vIII$, DK-MG^{low}	In vitro photodynamic therapy on human glioblastoma cell line	• The experimental study demonstrated that mAb-CUR-PLGA NPs were able to show more effective photodynamic toxicity (56% vs. 24%) on the DKMG/ $EGFR_vIII$ cells as compared to CUR-PLGA NPs	[89]
GSH-PEG-CUR-PLGA NPs	GSH	CUR	Nanoprecipitation method followed by functionalization using click chemistry	149–180 nm	AD	In vitro: SK-N-SH cells	Investigation of the internalization pathway in neuronal cells	• Presence of GSH on the surface of NPs exhibited neuroprotective property against acrolein • GSH-PEG-CUR-PLGA NPs showed higher neuronal internalization as compared to free CUR	[90]

(continued)

Table 3.1 (continued)

Architecture	Targeting ligand	Therapeutic moiety	Preparation method	Particle size	Neuronal disease/ disorder	In vitro/In vivo model	Application	Outcome	Ref.
MNPs-T7-PTX/ CUR-PLGA NPs	MNPs, T7	PTX/CUR	Single emulsion solvent evaporation method followed by Michael addition	130 nm	Brain tumor therapy	In vitro: U87, bEnd.3 cells In vivo: Balb/c nude mice	Dual-targeting platform for brain tumor targeting	• The combination of drug yielded synergistic effects on inhibition of tumor growth via the mechanisms of apoptosis induction and cell cycle arrest, showing significantly increased efficacy than single use of each drug • Dual-targeting effect yielded over tenfold increase in cellular uptake studies and over fivefold enhancement in brain delivery compared to the nontargeted NPs	[91]
Alexa fluor-labeled VEGF-loaded liposomes	Alexa fluor	VEGF	Synthesized using conjugation of Alexa fluor with DSPE followed by rehydration process for liposomes	<200 nm	Ischemia	In vivo: C57BL/6J mice	Liposomes for ischemia tissue by controlling blood vessel permeability	• The study demonstrated that IV-administered liposomes target ischemic site via EPR effect in early stage of ischemia and maintain permeability of vessels for longer period of time (7 days) as compared to delivery of empty liposomes	[92]

Irinotecan hydrochloride/ metformin-PLGA NPs	–	Irinotecan hydrochloride/ metformin	Single emulsion solvent evaporation method	216 and 300 nm	Glioblastoma	U-87 MG cells	Effect of irinotecan hydrochloride/ metformin-PLGA NPs on in vitro/in vivo studies of glioblastoma	• The study demonstrated that 1 mM and 2 mM doses of metformin and metformin-loaded PLGA NPs, respectively, significantly reduce the volume of extracted cancer	[93]
p38 SiRNA-PLGA NPs	–	P38 SiRNA	Double emulsion method	153.1 nm	Neuropathic pain	In vitro: BV2, HT22, and U87MG cells	Effect of p38 SiRNA-PLGA NPs as a drug delivery platform in neuropathic pain in rats by inhibiting microglia activation	• The study revealed that p38 SiRNA-PLGA NPs significantly reduced mechanical allodynia as well as microgliosis in the spinal dorsal horns of SNL rats, consistent with a downregulation of p38-related proinflammatory mediators	[94]
Chitosan-Eugenol-PCL NPs	–	Eugenol	Double emulsification-solvent evaporation method	224 nm	Cerebral ischemia		Quantification and targeted delivery of eugenol-loaded NPs via intranasal route in the treatment of cerebral ischemia	• The toxicity studies revealed that the nanoformulation was found to be safe • Chitosan-eugenol-PCL NPs was found to effectively enhance the bioavailability drug in the brain when administered in rat	[95]

(continued)

Table 3.1 (continued)

Architecture	Targeting ligand	Therapeutic moiety	Preparation method	Particle size	Neuronal disease/ disorder	In vitro/In vivo model	Application	Outcome	Ref.
Lamotrigine-pluronic F127/ Carbopol 974P gel	–	Lamotrigine	Cold method	–	Epilepsy	In vivo: Male CD-1 mice	Development of nose to brain delivery platform for the treatment of epilepsy	• Biodistribution studies demonstrated that there is higher concentration of lamotrigine in olfactory bulb as compared to frontal cortex • It was also revealed that directly transfer of lamotrigine to the brain was done via olfactory neuronal pathway	[96]
CPP-chitosan-PLGA/NLC NPs	CPP	–	Melt emulsification technique Double emulsion solvent evaporation method	200 nm	CNS diseases	In vitro: Olfactory cell monolayer	Study transport of NPs across olfactory cell monolayer	• It was found that 0.7% of PLGA NPs was able to cross olfactory cell monolayer, whereas 8% and 22% of NLC and chitosan-coated NLC were transported across olfactory cell monolayer respectively • Additionally addition of CPP significantly enhanced their transport i.e. 46%	[97]

Chitosan-PLGA NPs; PLGA NPs	–	–	Solvent displacement method	250 nm	Nose to brain delivery in the treatment of CNS disease	In vivo: male Wistar rats	Study of effect of surface charge of the NPs on brain subregion localization	• Study demonstrated that both surface-charged NPs had reached the brain and persist in the brain for 48 h after intranasal administration • Surface charge on the NPs affects the pathways involved in their translocation from the nasal cavity to CNS • Furthermore, in brief, positively charged NPs followed the trigeminal pathway, whereas negatively charged NPs followed olfactory pathway	[98]
Chitosan-L-pGlu-(1-benzyl)-L-His-L-ProNH2 (NP-355)/L-pGlu-(2-propyl)-L-His-L-ProNH2 (NP-647)-PLGA NPs	–	NP-355 and NP-647	Emulsion solvent evaporation	110 nm and 163.6 nm	Epilepsy		Delivery platform for antiepileptic TRH analogs in the treatment of epilepsy via nose to brain delivery	• The toxicity studies revealed that NP-355- and NP-647-loaded PLGA NPs were found to be safe • The ability of these NPs to reach the brain was evaluated by utilizing quantum dots as fluorescent probes	[99]

(continued)

Table 3.1 (continued)

Architecture	Targeting ligand	Therapeutic moiety	Preparation method	Particle size	Neuronal disease/ disorder	In vitro/In vivo model	Application	Outcome	Ref.
Rivastigmine-mucoadhesive thermosensitive in situ gel	–	Rivastigmine	Cold method	–	AD	Ex vivo transnasal study	Rivastigmine-mucoadhesive thermosensitive in situ gel platform for targeting to brain via nasal route in the treatment of AD	• The optimal RV in situ gel (PF127 and 1% Carbopol 934) showed significant transnasal permeation (84%) which was reflected in better distribution to the brain (0.54 %ID/g), when compared to RV IN solution (0.16 %ID/g) and RV IV intravenous solution (0.15 %ID/g)	[100]
Lf-Rotigotine-NPs	Lf	Rotigotine	Nanoprecipitation method	200 nm	Parkinson's disease	In vivo: Sprague Dawley male rats	Study of neuroprotective effect of Lf-Rotigotine-NPs and its biodistribution and pharmacodynamics using nose to brain route	• Lf-R-NPs more efficiently supplied rotigotine to the brain (with a greater sustained amount of the drug delivered to this organ and with more effective targeting to the striatum) than R-NPs • Furthermore, Lf-R-NPs significantly alleviated nigrostriatal dopaminergic neurodegeneration in the rat model of 6-hydroxydopamine-induced PD	[101]

Lf-N-trimethylated chitosan-Huperzine A-PLGA NPs	Lf	Huperzine A	Emulsion solvent evaporation method	153.2 nm	AD	In vitro: 16HBE, SH-SY-5Y cells; In vivo: KM mice	Nanoformulation as a platform in the treatment of AD	• Lf-N-trimethylated chitosan-Huperzine A-PLGA NPs showed lower toxicity on 16HBE cells as compared to Huperzine A solution • Lf-N-trimethylated chitosan-Huperzine A-PLGA NPs showed higher accumulation as compared to nontargeted NPs, i.e., N-trimethylated chitosan-Huperzine A-PLGA NPs in 16HBE and SH-SY-5Y cells • In vivo imaging results showed that Lf-TMC NPs exhibited a higher fluorescence intensity in the brain and a longer residence time than nontargeted NPs	[102]

Table 3.2 Patents on nanoparticles for neuronal cell targeting

Application number	Title	Applicant	Status
JP2018065862	Drug-loaded polymeric nanoparticles and methods of making and using the same	Pfizer	Pending
WO2018146599 (A1)	Polymeric nanoparticles encapsulating a combination of natural bioactive trans-resveratrol (rsv) and celastrol (cl), a process for the preparation and use thereof in the treatment of prostate cancer	Univ DEGLI STUDI DI SASSARI	Pending
WO2018073740 (A1)	Therapeutic polymeric nanoparticles comprising lipids and methods of making and using same	Pfizer	Pending
US2017326085 (A1)	Glycolic acid and/or D-lactic acid for the treatment of neurodegenerative diseases	De max-planck-gesellschaft zur foerderung der wss e v	Pending
RU2009148718 (A)	Pharmaceutical composition for neurodegenerative diseases of hydrogenated pyrido(4,3-b)indole, method for preparing and based drug		Pending
US2014234402 (A1)	Intravenous infusion of curcumin and a calcium channel blocker	Signpath PHARMA INC.	Pending
5,955,506	Benzamides for neurodegenerative disorder treatment	Centaur PHARMACEUTICALS, INC.	September 21, 1999
7,081,345	Use of a polypeptide for detecting, preventing, or treating a pathological condition associated with a degenerative, neurological, or autoimmune disease	Biomerieux STEHLYS	July 25, 2006
7,255,874	Biocompatible polymers and adhesives: compositions, methods of making, and uses related thereto	Closure MEDICAL CORPORATION	August 14, 2007
7,262,189	Benzothiazine and benzothiadiazine derivatives, method for preparing same and *pharmaceutical compositions* containing same	Les LABORATOIRES SERVIER	August 28, 2007

(continued)

Table 3.2 (continued)

Application number	Title	Applicant	Status
7,273,618	Method for administering agents to the central nervous system	Chiron CORPORATION	September 25, 2007
7,445,931	Compositions and methods for enrichment of neural stem cells using ceramide analogs	Medical COLLEGE OF GEORGIA RESEARCH INSTITUTE	November 4, 2008
7,888,066	Methods for identifying substances for the treatment of Alzheimer's disease	Mount SINAI SCHOOL OF MEDICINE	February 15, 2011
8,288,444	Iontophoretic delivery of curcumin and curcumin analogs for the treatment of Alzheimer's disease	Codman & SHURTLEFF, INC.	October 16, 2012
8,329,719	Neuroprotective agents for the prevention and treatment of *neurodegenerative diseases*	Lixte BIOTECHNOLOGY, INC.	December 11, 2012
8,609,652	Method of administering a methylene blue-curcumin analog for the treatment of Alzheimer's disease	DePuy SYNTHES PRODUCTS, LLC	December 17, 2013
8,758,778	Polymeric nanocarriers with a linear dual-response mechanism and uses thereof	The REGENTS OF THE UNIVERSITY OF CALIFORNIA	June 24, 2014
8,778,904	Methods and compositions for treating diseases, disorders, or injuries of the CNS	Quark PHARMACEUTICALS, INC	July 15, 2014
8,835,387	Histidyl-tRNA synthetases for treating autoimmune and inflammatory diseases	Pangu BioPharma LIMITED	September 16, 2014
8,871,212	Amyloid-beta polypeptide vaccine	H. Lundbeck a/s	October 28, 2014
8,999,927	Glial cell line-derived neurotrophic factor (GDNF) compositions and use thereof	The UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES (WASHINGTON, DC)	April 7, 2015
9,295,689	Formulation and delivery of *plga* microspheres	Moderna THERAPEUTICS, INC.	March 29, 2016
9,486,559	Methods of treatment with a bioresorbable scaffold for neurologic drug delivery	Abbott CARDIOVASCULAR SYSTEMS INC	November 8, 2016

(continued)

Table 3.2 (continued)

Application number	Title	Applicant		Status
9,555,071	Methods and compositions for the treatment of axonal and neuronal degeneration	Nguyen; THIEN	Potomac	January 31, 2017
9,579,300	Nanoparticle and polymer formulations for thyroid hormone analogs, antagonists, and formulations thereof	NanoPharmaceuticals LLC		February 28, 2017
9,687,553	Polymeric nanocarriers with linear dual response mechanism	The REGENTS OF THE UNIVERSITY OF CALIFORNIA		June 27, 2017
9,827,353	Cross-linked fatty acid-based biomaterials	Atrium MEDICAL CORPORATION		November 28, 2017
9,913,877	Methods and compositions for the treatment of axonal and neuronal degeneration	Ewaleifoh; OSEFAME NGUYEN; THIEN		March 13, 2018
10,028,971	Compositions and methods for treating psychiatric disorders	Gosforth CENTRE (HOLDINGS) PTY LTD		July 24, 2018
10,034,918	Therapeutic use of a growth factor, metrnl	Hoba THERAPEUTICS APS		July 31, 2018
10,040,783	Prostaglandin receptor ep2 antagonists, derivatives, compositions, and uses related thereto	Emory UNIVERSITY		August 7, 2018

7 Concluding Remarks and Future Perspectives

In summary, therapeutic formulations fabricated owing to nanotechnology development have been extensively used in the treatment of CNS diseases. Although various formulations such as liposomes, micelles, nanostructured lipid carrier, dendrimers, nanotubes, and others are used in the treatment of various CNS diseases, PLGA-based polymeric-based nanostructures have been extensively studied. The literature of various studies demonstrated that despite BBB, intranasal route can be an effective route for brain targeting. PLGA NPs exhibits various advantages like better drug entrapment, ease in surface functionalization of ligands. PLGA NPs also has application in simultaneous diagnosis and therapy (theranostic). Surface functionalization of PLGA NPs and the attachment of the targeting moiety incorporate useful information and progress in this field. Additionally, the type of chemistry/conjugation of the nanocarrier with the ligands depends on the type of functional group present on both, i.e., ligand and nanocarrier. Dual-/multi-targeting strategy was employed to overcome two or more barriers present in the brain which may retard the delivery system to reach specific diseased area. It is generalized that dual-targeting delivery systems should function to penetration through nasal mucosa and/

or BBB and then specifically binds to cells of diseased brain. Until now, none of the ligand-targeted nanomedicines have been approved probably due to two major aspects: ambiguous active-targeting effect in CNS diseases and the difficulties encountered in large-scale and reproducible production while maintaining the activity of targeting ligands. Therefore, future studies should focus more on human CNS biology (e.g., by establishing models that are more close to CNS-specific disease). Besides, more attention should be paid to the design, production, and control aspects of dual-targeted nanomedicines to facilitate possible clinical translation.

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